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Preliminary Investigations on *Salmonella* spp. Incidence in Meat Chicken Farms in Italy, Germany, Lithuania and the Netherlands

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Abstract: The broiler chickens, especially if intensively reared, can be considered as an important reservoir of *Salmonella* infections in humans. Many consumers assume that broiler chickens, grown under conventional commercial conditions, have higher infection levels of *Salmonella* than free-range organic chickens. The subject of this study was firstly to assess the incidence of *Salmonella* spp. In conventional chicken farms, located in different areas of Italy, Germany, Lithuania and in the Netherlands. In addition organic farms were investigated both in Italy and the Netherlands. The typification of *Salmonella* was also effected in attempt to value the distribution of the organism on the basis of the different geographical areas. The incidence of *Salmonella* in “conventional” broiler farms was 29% in Lithuania, 20% in Italy and 11% in The Netherlands, while in Germany *Salmonella* was not detected. *Salmonella* was isolated from organic broiler flocks in Italy (18, 1%) and in The Netherlands (3, 7%). Our results indicated that *Salmonella* Enteritidis and *Salmonella* Typhimurium dominated in Lithuanian broiler flocks while *Salmonella Infantis* and *Salmonella* Java were predominant in the Netherlands. *Salmonella hadar* and *Salmonella heidelberg* seemed to be prevalent in Italy.

Key words: Broilers, conventional, organic, *salmonella*

Introduction

Broiler chickens, especially if intensively reared, can be considered as an important reservoir of *Salmonella* infections for humans (EFSA, 2006). Various risk factors can be responsible for *Salmonella* infection and its spread in poultry farms: housing system, flock size, different age of chicken and season of the year. (Rose *et al.,* 1999; Heyndrickx *et al.,* 2002). Many consumers assume that the organic chickens shed less *Salmonella* than conventional broilers because of the particular system management (low density stocking, access to outside and special diets) (Bailey and Cosby, 2005). A high number of chickens in the farm can increase the chance of infection with *Salmonella*, (Mollenhorst *et al.,* 2005). On the other hand in organic farm the access to outside may increase the risk of the infection with *Salmonella* through the contact with faeces of wild birds and other animals. Cui *et al.* (2005) found that organically raised broilers had higher prevalence of *Salmonella* than broilers raised conventionally. In several studies the incidence of *Salmonella* was lower in organic than in conventional broiler farms (Heuer *et al.,* 2001; Wolf-Reuter *et al.,* 2002). However, Van Overbeke *et al.* (2006) reported no significant differences in the prevalence of *Salmonella* between organic and conventional broilers at slaughter. The subject of this study was to assess the incidence of *Salmonella* spp. in broiler farms of 4 EU countries: Italy, Germany, The Netherlands and Lithuania. In Italy and in the Netherlands besides the conventional farms, organic flocks were investigated in order to preliminary determine the diffusion of *Salmonella* spp. in this rearing condition. The typification of *Salmonella* was also effected in attempt to evaluate the distribution of the organism on the basis of the different geographical areas.

Materials and Methods

Sampling: This study was performed in 4 EU countries (Italy, Germany, Lithuania and the Netherlands). In Italy and in the Netherlands both organic and conventional farms were investigated. In Italy the investigations were performed in 11 organic flocks and in 10 conventional flocks over a period of two years. During the production cycle environmental samples consisting of 5 pools of litter, 2 pools of dust and 1 water sample were collected.

At slaughterhouse 60 caecal samples per farm, subdivided in 2 pools, were collected. In the Netherlands 18 conventional flock and 108 organic flocks were examined over a period of two years. Different numbers of dust, litter samples, water and caecal pool samples were collected from each flock (Table 1). Deliveries of organic feed were also sampled and analysed individually. In Lithuania and in Germany the investigations were performed in 27 and 22 conventional flocks respectively. In Lithuania flock samples consisting of 5 pools of litter, 2 pools of dust and 1 water sample were collected during the production cycle. Litter since it is not heat treated samples consisted of boots swabs. Five (pooled) samples of caecum per flock were taken from a broiler slaughterhouse. In Germany only caecum samples were collected and analysed as one pooled sample per flock. Dust samples were collected from the walls, fans and other surfaces and pooled into samples of approximately 25 g. Per pool Farms were supplied from local underwater reservoir in Italia and in Lithuania, while Dutch farms were all connected to public water supply. In all cases the water sampled consisted of pool of 1 litre collected from nipples.

Microbiological analysis: *Salmonella* were isolated by standard methods (International Organization for Standardization 6579, 1998). From each pool of litter, dust and caecal content, 10 g were homogenised with approximately. 1:10 Buffered peptone water (BPW) for 60 seconds; 25 ml of each water sample were poured into a container followed by adding 225 ml of BPW. The samples were incubated at 35±2°C for 20-24 h. And then 1±0.1 ml was transferred into 10 ml Muller Kauffmann Tetrathionate Novobiocin broth (MKTTn)broth (1.0 ml in 9.0 ml TBG at 37°C for 24 h) (Modified Semisolid Rappaport Vassiliadis Agar in The Netherlands) and 0.1±0.02 ml was transferred into 10 ml Rappaport Vassiliadis Broth (RV) broth. The tubes were incubated in a water bath at 42±0.5°C for 18-24 h .The subcultures from the enrichment media were made onto Xylose Lysine Desoxycholate (XLD) agar plates and on Hectoen enteric (Rambach agar in Germany and Brilliant Green Agar in the Netherlands) agar plates and then incubated aerobically at 37°C for 20-24 hours. One loopful of inoculum was used for each plate. Presumptive colonies were inoculated onto Mc Conkey agar (Standard I agar in Germany and Brilliant Green Agar in the Netherlands), incubated at 37°C for 24 h and biochemically checked with Ureum Agar (UA), Triple Sugar Iron Agar (TSI) using commercial tests (API 20 E). Identification was performed by agglutination testing with agglutination sera. The 95% confidence intervals (CI) for the observed prevalence of *Salmonella*-positive samples were estimated by linear interpolation formula (Montgomery Ranger, 1999):

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

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

Results

All results are summarized in Table 1. In Italy 2 out of 10 conventional farms were positive for *S. hadar* and *S. heidelberg* (20%) while 2 out of 11 organic flocks were positive for *S.* Hadar (18, 1%). In particular, in conventional farms *Salmonella* was isolated from 5 out of 50 litter samples (10%; CI 1, 6-18, 3) and from 4 out of 20 caecal samples (20%; CI 2, 4-37, 5). In one farm both litter and caecal samples were *Salmonella* positive, while in the other farms it was detected only in the intestinal samples. In the organic farms *Salmonella* was isolated from 4 of 22 caecal samples,(18, 8%; CI 2, 3-33, 6). The dust and water samples were always *Salmonella* negative. The results obtained in Lithuania showed that the most infected samples were litter and caecum (25, 9%; CI 17.7-34.1 and 25%; CI 24.6-25.3 respectively) while *Salmonella* presence resulted lower in dust and water (9, 6%; CI 9.2-9.9 and 3, 8%; CI 3.5-4.0, respectively). 22 conventional flocks, collected over a period of one year gave negative results for *Salmonella* in all cases. In the Netherlands the analysis for *Salmonella* was performed in 11 conventional farms with a total of 18 flocks and in 108 flocks from 16 organic broiler farms during 2 years. With respect to organic farming a total of 181 litter samples were taken; from which 4 (2, 2%) were *Salmonella* positive. All samples from dust and environmental samples proved to be *Salmonella* negative. *S. infantis* was isolated in 1 organic feed sample from one flock, while *S. senftenberg* was found, both in a feed sample and in litter samples from another flock. No pools of caecal samples from organic chickens were *Salmonella* positive at slaughter. *Salmonella* serotypes isolated from organic flocks were: *S. infantis, S. senftenberg*, one isolate from the B group and one from the C group could not be typed completely. In Dutch conventional flocks the most infected samples resulted to be caecum (4 positive out of 36 = 11.1%). The serotype isolates were *S. infantis* in the samples from one farm (dust, litter and caeca) and S. Java from litter samples in one other farm.

Discussion

Various risk factors exist for infection with *Salmonella spp.* and its diffusion in poultry farms: housing system, flock size, different age of chicken and season of the year (Angen *et al.*, 1996; Skov *et al.*, 1999). This study was intended to investigate the incidence of *Salmonella* in broiler chicken farms located in limited geographical areas of 4 different countries: Italy, Germany, Lithuania and the Netherlands. In Italy and in the Netherlands the

Table 1: *Salmonella spp.* prevalence in conventional and organic meat chicken farms in Italy, Germany, Lithuania and The Netherlands

Country	Type of production	No.of tested Sample	No.of positive flocks	No. of tested flocks	samples	No. of positive samples	CI (95%)	Serotype
Italy	Org.	Dust	11	2	22	0		*S. hadar,*
		Litter	11		55	0		
		Water	11		11	0		
		Caecum	11		22	4	2.3-33.6	
		Total:	11	2	110	4		
	Conv.	Dust	10	2	20	0		*S. hadar,*
		Litter	10		50	5	1.6-18.3	
		Water	10		10	0		
		Caecum	10		20	4	2.4-37.5	*S. heidelberg*
		Total:	10	1	100	9		
Lithuania	Conv.	Dust	27	8	52	5	9.2-9.9	*S. enteritidis,*
		Litter	27	8	108	28	17.7-34.1	*S. typhimurium*
		Caecum	27	5	104	26	24.6-25.3	
		Water	27	1	26	1	3.5-4.0	
		Total:	27	8	290	41		
The Netherlands	Conv.	Env./dust	18	1	320	1	0.3-0.9	*S. infantis,*
		Litter	18	2	386	3	0.1-1.6	*S. java*
		Caecum	17	2	36	4	10.3-21.4	
		Water	16	0	29	0		
		Total:	18	2	771	8		
	Org.	Env./dust	108	0	37	0		*S. infantis*
		Litter	108	4	181	4	0.1-4.3	*S. senftenberg*
		Caecum	16	0	160	0		S. Group B
		Feed	61	2	61	2	3.1-9.6	S. Group C
		Total:	108	5	439	6		
Germany	Conv.	Caecum	22	0	660	0		
Total per 4 countries:			196	20 (10%)	2370	75 (3.2%)		

presence of *Salmonella* was also investigated in the organic meat type chicken in order to evaluate a possible influence of the rearing conditions. Our results indicated that the incidence of *Salmonella* spp. varies in the countries investigated. The percentage of *Salmonella* isolates in conventional housing system was 29% in Lithuania, 11.1% in the Netherlands and 9% in Italy. In one Dutch farm *Salmonella* could be isolated from samples taken in broiler houses after cleaning and disinfection (data not shown). From these data a specific intervention could be started, which proved to be effective on the farms. The infection was proved to persist in faecal remains, in cracks and joints or in the feeder auger system inside the broiler houses. In this farm black beetles and larvae appeared to be a vector for transmission of *Salmonella* Java. In another study this type of transmission was also found (Bolder, 2004). In the EU Baseline study on broilers (EFSA, 2007) the results for the four countries involved in this study deviated from the results of the present paper. In the baseline study, performed on the flock basis, Germany, Italy, Lithuania and the Netherlands had respectively 15%; 28.3%; 2.9% and 7.5% of *Salmonella* positive flocks. However the data from our investigations covered only a fraction of the totals presented in the Baseline study. In Germany *Salmonella* in conventional broiler flocks was not detected but it should be underlined that the data is referred to limited sampling , though it consisted of caecal samples, always considered the best substratum for the *Salmonella* recovery. *Salmonella* isolation was also slightly inferior to expectations in Italy. In Lithuania and in the Netherlands a higher prevalence for *Salmonella* was detected in comparison with the EU Baseline study. *Salmonella* in organic broiler flocks was observed both in Italy (2 out of 11 flocks) and in The Netherlands (4 out of 108 flocks). The data is not enough extensive to make a comparison between the two rearing systems but they may give an idea in relation to the distribution of *Salmonella* in organic broiler farms. In the literature there is controversial data about the influence of housing systems for *Salmonella* infection. In several studies the incidence of *Salmonella* was lower in organic than in conventional broiler farms (Heuer *et al.*, 2001; Wolf-Reuter *et al.*, 2002; Van der Hulst *et al.*, 2004). Italian data indicated that the presence of *Salmonella* in organic broiler flocks is comparable with conventional broiler flocks and are in agreement with those reported by Van Overbeke *et al.* (2006), who did not find significant differences in the prevalence of *Salmonella* between organic and conventional broilers at slaughter. Bailey and Cosby (2005) also did not agree in assuming that the free range or organic conditions can influence the *Salmonella* presence in the chickens. The explanation of the lower *Salmonella* prevalence in Dutch organic broilers could be that organic broilers grow longer (8 week +) than conventional broilers (5-6 weeks) and are mostly slow growing brands. This could explain the absence of *Salmonella* in caecal contents of previously *Salmonella* positive flocks. The prevalence at the age of 8 weeks may have dropped below the detection level of the survey. *Salmonella* infection experiments in conventional broilers showed that even after relatively heavy *Salmonella* challenge at the early age, broilers may have cleared the infection at approximately 6 weeks of age (Bolder *et al.,* 1999; Linton *et al.,* 1985). Feed samplings in Dutch organic broiler farms were included since feed for organic broilers is usually not pelletized. If it is not heat treated it could be considered a risk factor in transmission of *Salmonella*. In this study organic feed samples proved to be *Salmonella* positive and in one occasion the same serotype was not isolated from feed and litter samples in the same flock. However monitoring results indicate that there is a negligible risk from pelletized feed. (Bolder, unpublished data). Our results showed that the most infected samples were from litter and caecum in comparison with those of dust and water. Some investigators 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). Concerning the dust samples, *Salmonella* was positive in conventional farms in Lithuania and in the Netherlands and always negative in organic farms. Although dust can be considered an excellent vector for *Salmonella*, survival in dust is limited (Davies and Breslin, 2003). Gast *et al.* (1998) suggested that infection could occur by oral ingestion of external surfaces contaminated by *Salmonella*. Different *Salmonella* serovars were identified in chicken. At EU level the most common serovar was *S.* Enteritidis which represented approximately half of the isolates (EFSA, 2007). Our results indicated that *S.* Enteritidis and *S.* Typhimurium dominated in Lithuanian broiler flocks, while in the Netherlands *S. infantis* and *S. java* were isolated. In Italy *S. hadar* and *S. heidelberg* were found to be prevalent.

These results confirm those of the EU Baseline study in broilers (EFSA, 2007) where wide variety of prevalent *Salmonella* serovars was isolated in different countries. Besides, other investigators (Byrd *et al.,* 1997; Roy *et al.,* 2002) found that *S.* Kentucky and *S.* Heidelberg were predominant serotypes isolated from poultry meat products. The investigators from Japan (Limawongpranne *et al.,* 1999) detected that *S. blockley, S. hadar* and *S. bredeney* were predominant in broilers, meanwhile *S. enteritidis* was found only in 0.9% of samples. It seems to prove that distribution of *Salmonella* serovars in the world depends on the geographical region. In addition the distribution of *Salmonella* could change year by year in relation to different factors, like the use of certain antibiotics; for instance the recent distribution of *S.* Hadar in several areas could be due to its resistance to fluoroquinolones (Tran *et al.,* 2004; Breuil *et al.,* 2000).

Conclusion: The subject of this study was primary to assess the incidence of *Salmonella* spp. in meat chicken farms of 4 EU countries: Italy, Germany, the Netherlands and Lithuania. In Italy and in the Netherlands organic flocks were also investigated in order to compare the *Salmonella* distribution in different rearing conditions. Our results cannot be conclusive, since they are referred to limited areas in the countries and only Italy and the Netherlands could produce data on both rearing systems (organic and conventional), though the results were not very comprehensive either. In the Netherlands and in Lithuania conventional housing system seems to constitute a favourable condition for *Salmonella* spread in broilers. Italian data showed that no significant differences in the incidence of *Salmonella* were found between organic and conventional broiler rearing system. The absence of *Salmonella* in caecum samplings from organic chickens in the Netherlands can be explained by the natural clearance, which consequently leads to the prevalence below detection level of the survey with the age.

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