

DETECTION OF *Salmonella indiana* IN BROILER PRODUCTION IN THE CZECH REPUBLIC

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ABSTRACT

Salmonella has been linked to many foodborne diseases across the world. Several control measures have been implemented to reduce *Salmonella* in poultry farms. The present study was focused on presence and serotyping of *Salmonella* in various samples from broilers. *Salmonella* isolates were obtained by standard cultivation methods. Four samples of faeces (n = 8) and twenty pooled samples of neck skins (n = 40) were found as positive. Pooled samples of chicken breast fillet (n = 20) were negative. Positive detection of *Salmonella* in the neck skins may be caused by contamination from the digestive tract or from the environment of the slaughter line. An interesting finding is that in all serotyped isolates (n = 24) only *Salmonella indiana* was present. Because *S. indiana* may pose a risk to human health, preventive measures have to be performed during the whole production chain.

Key words: poultry / broilers / microbiology / *Salmonella* Indiana / serotyping/ Czech Republic

1 INTRODUCTION

Salmonella has been linked to many foodborne illnesses across the whole world, and it is still considered to be one of the main agents causing human gastroenteritis. Poultry carcasses, poultry meat and poultry products belong to the most frequently discussed source of *Salmonella*. Contamination of poultry products can occur through the whole production chain, but until now most studies have been focused on the primary production (Rasschaert *et al.*, 2007). Several control measures have been implemented to reduce *Salmonella* contamination of poultry flocks at farm level as well as strict hygienic rules during poultry processing.

Salmonella spp. are pathogens but can frequently persist in animals as a transient member of the intestinal microbial population without causing disease; however, when host animals and their carried serotypes are consumed by humans, foodborne illness can result (Callaway *et al.*, 2008). Serotyping of *Salmonella* isolates is a useful classification method that could provide a link be-

tween the patient and the source of infection. In the EU, *S. enteritidis* and *S. typhimurium* are the most frequent serovars associated with human illness. Human *S. enteritidis* cases are mostly associated with the consumption of contaminated eggs and poultry meat, while *S. typhimurium* cases with the consumption of contaminated pork, poultry and beef meat. The most frequently reported serovars in poultry are *S. enteritidis*, *S. typhimurium*, *S. hadar*, *S. infantis*, *S. virchow* (EFSA, ECDC, 2012).

Under the European Regulation (EC 2160/2003) a special international program for reducing *Salmonella* in broilers is used from 2009 in the Czech Republic. The main aim of this program is to reduce *Salmonella* positive flocks (all *Salmonella* serotypes with public health significance and special monitoring of *S. typhimurium* and *S. enteritidis*) to less than 1%. Regulation 2160/2003 (EC 2160/2003) is focused on primary production of poultry and sets the rules for monitoring *Salmonella* directly at the farm during rearing period. The year 2010 was the second year of implementing the EU reduction target of ≤1% for *S. enteritidis* and *S. typhimurium* for broiler

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flocks. The *Salmonella* spp. prevalence was reduced from 5.0% in 2009 to 4.1% in 2010 (EFSA, ECDC, 2012).

European Regulation (EC 1441/2007) lays down preventive measures to reduce *Salmonella* in the food chain. The Regulation prescribes rules for sampling and testing, and sets limits for the presence of *Salmonella* in specific food categories and in samples from food processing. The current requirement is that the poultry meat products placed on the market during their shelf-life have to be free of *Salmonella*. In addition, process hygiene criteria require absence of *Salmonella* in 25 g of a pooled sample of neck skins.

The main objectives of the present study were: 1) to investigate the presence of *Salmonella* spp. in samples of faeces and neck skins; 2) to determine the relationship between *Salmonella* isolates from neck skins and faeces.

2 MATERIAL AND METHODS

2.1 THE POULTRY PROCESSING PLANT, FLOCK CHARACTERISTIC

The study was conducted in one large commercial poultry abattoir during autumn and winter 2011. The poultry slaughter process included unloading, manual hanging to the processing line, electrical stunning and killing. In a separated area, the carcasses were scalded at a temperature 54 ± 2 °C for 180 s and plucked. Mechanical evisceration, final washing and veterinary inspection were made separately in the next area. The plant used evaporative chilling for 70 min; the temperature in chilling tunnel was under 0 °C. Line speed was 8000–8500 birds per hour.

All broilers came from Czech farms with conventional production. The average rearing period was 36 days and the average weight was 2,1 kg. Growth-promoting antibiotics were not used during rearing period.

2.2 SAMPLES COLLECTION

In total the abattoir was visited eight times between October and December 2011. The sampling plan was based on European Regulation 1441/2007, Annex 1, Chapter 2.1. (EC 1441/2007). Neck skin samples were obtained from carcasses after chilling. Fifteen neck skins (each 25 g) were sampled once a week; a total of 120 neck skins were sampled. Samples were removed using separate sterile scissors and plastic bags for each neck skin. The carcasses were then removed from the processing line, put into sterile plastic bags and saved at the cold storage room at the abattoir. One pooled faeces sample

(approximate weight of 500 g) was taken directly from the transport crates. Faeces samples and neck skin samples came from the same flock with known *Salmonella* status. All tested flocks were declared to be *Salmonella* negative. Samples of neck skins and faeces were transported to the laboratory under cooled conditions and processed immediately.

Samples of chicken breast fillets were taken only from those carcasses that were found presumptively positive (due to the suspect *Salmonella* colonies isolated from pooled neck skin samples). These types of samples were obtained directly at the laboratory from the carcasses stored at the abattoir. A total of 60 chicken breast fillets were collected.

2.3 BACTERIOLOGICAL ANALYSIS

Three pieces of neck skins were pooled to obtain a sample of at least 25 g; (40 pooled samples were analysed). Three samples of chicken breast fillet were pooled to obtain a sample of at least 25 g; a total of 20 pooled samples were analysed. From 8 flocks 25 g of faeces were analysed separately. The presence of *Salmonella* spp. was determined according to modified ISO 6579:2003 standard method. As the selective agar media, XLT4 agar and Brilliant Green Agar were used, incubated at 37 °C for 24 h. Suspect colonies were subcultured onto Blood Agar, incubated at 37 °C for 24 h, and confirmed by agglutination with poly O antiserum. After confirmation, isolates were serotyped at the National Institute of Public Health, Brno, Czech Republic. All the media used for the determination of *Salmonella* spp. were purchased from Oxoid, United Kingdom.

3 RESULTS AND DISCUSSION

In total eight samples of faeces, forty pooled samples of neck skins and twenty pooled samples of chicken breast fillet were analysed.

The presence of *Salmonella* in broiler flocks has to be excluded three weeks before slaughter. The samples of faeces are collected at the farm and analysis results have to be provided in Food chain information. All investigated flocks of broilers declared *Salmonella* negative status. But in four samples of faeces (flock numbers 2, 3, 6, 7) *Salmonella* (serotyped as *S. indiana*) was found. Determination of *Salmonella* status few weeks before slaughter is questionable, because in the time span between sampling at farm and slaughter the birds a new infection can acquire e.g. during transport (Rasschaert *et al.*, 2007). It is well known that *Salmonella* spp. is excreted intermit-

Table 1: Presence of *Salmonella* spp. in neck skin samples and samples of chicken breast fillets

Flock	No. of <i>Salmonella</i> -positive samples/total no. (%)	
	neck skins	chicken breast fillets
1	0/5 (0)	-/-
2	5/5 (100)	0/5 (0)
3	2/5 (40)	0/2 (0)
4	2/5 (40)	0/2 (0)
5	1/5 (20)	0/1 (0)
6	5/5 (100)	0/5 (0)
7	5/5 (100)	0/5 (0)
8	0/5 (0)	-/-
Total	20/40 (50)	0/20 (0)

tently, so stress during transport can lead to presence of *Salmonella* spp. in faeces. Another cause of a positive finding of *Salmonella* in faeces can be inadequate cleaning of transport crates.

The results of microbiological analyses of neck skins and chicken breast fillets are shown in Table 1. Skin samples were contaminated by *Salmonella* in six of eight tested flocks (75%). In three cases, all five pooled samples were contaminated. In the same flock, there was found *Salmonella* in faeces, too. On the other hand, all samples of chicken breast fillets were found to be negative. Twenty isolates of *Salmonella* were obtained from neck skin samples. All these isolates were serotyped as *S. indiana*. This serovar was found both in faeces and pooled neck skin samples.

Positive detection of *Salmonella* in the neck skins may be caused by contamination from the digestive tract or from the environment of the slaughter line. The fact that *Salmonella* was not found in any of the tested meat samples, although samples of neck skins were positive proves that the correct handling and hygienic rules significantly reduces the risk of *Salmonella* infection of the final consumer.

An interesting finding is the presence of only one serotype of *Salmonella* in all samples. *Salmonella indiana* is an infrequently occurring serotype, isolated mostly from poultry, which has occasionally been the etiological agent of human gastroenteritis (Beckers *et al.*, 1985). The worldwide frequency of the isolation of *S. indiana* is low. (Luque *et al.*, 2009).

4 CONCLUSION

Cross-contamination of carcasses during processing cannot be avoided. Strict separation of flocks with *Salmonella* positive and *Salmonella* negative status have to be kept together with effective cleaning and disinfection of processing line. The occurrence of *S. indiana* poses a sporadic threat to human health. Education of consumer, proper food handling, complete cooking and other security measures greatly reduce possible foodborne disease.

5 ACKNOWLEDGEMENTS

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