

Prevalence of *Salmonellae* and Their Resistance to Antibiotics in Slaughtered Pigs in the Czech Republic

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Abstract

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Salmonella prevalence was assessed in 816 pigs from fifteen herds which were slaughtered in ten slaughterhouses from June 2001 to December 2002. No *Salmonellae* were isolated in pigs from eight herds in four slaughterhouses. *Salmonella* prevalence in pigs originating from the other seven herds ranged from 2.0% to 12.0%. The most frequent site of *Salmonella* isolation was caecum (2.45%). This finding is statistically significant ($P < 0.01$) as compared to those obtained with mesenteric lymph nodes (0.73%) and carcass swabs (0.12%). *Salmonellae* were not found in samples from the environments ($n = 197$). A total of 27 *Salmonella* isolates were classified into serotypes *S. infantis* ($n = 8$), *S. typhimurium* ($n = 5$), *S. agona* ($n = 4$), *S. kaapstad* ($n = 4$), *S. derby* ($n = 3$), *S. bredeney* ($n = 2$), and *S. london* ($n = 1$). All five *S. typhimurium* DT 104 were resistant to the phenotype ACSSuT. Resistance genes *bla*_{PSE-1}, *floR*, *aadA2*, *sul1*, and *tetG* were identified in all pentaresistant strains. One strain of *S. derby* was resistant to gentamicin, streptomycin and sulphonamides. The other *Salmonella* isolates were sensitive to all antibiotics tested.

Keywords: slaughter pig; *Salmonella* serotype; phage type DT104; antibiotic resistance

Swine herds and pork are principal *Salmonella* sources, especially of the serotype Typhimurium and its epidemic phage types, which are often multidrug resistant (WEGENER *et al.* 1994). The occurrence of these multiresistant strains and their spread are connected with the impact of selection pressure caused by excessive use of antibiotics and growth stimulants in the herds of food animals (AARESTRUP 1999; TOLLEFSON & MILLER 2000; McDERMOTT *et al.* 2002). The widespread occurrence of multiresistant clones of *Salmonella enterica* serotype Typhimurium (*S. typhimurium*), especially of the phage type – “definitive type” DT104, in human population, foodstuffs, and in various species of domestic and wild animals is a serious problem with regard to the public health protection. In these strains, R-type ACSSuT with chromosomally

coded resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline is predominantly detected (HUMPHREY 2001; DAVIS *et al.* 2002). Worldwide upsurge of *S. typhimurium* has been observed in pig herds since mid 1990s. This serotype is in most European countries the dominant agent causing subclinical salmonellosis in pigs. The infected pigs are shedding germs through faeces during the whole fattening period and the germs are then transmitted to slaughterhouses. Contamination of swine carcasses and the slaughter line with *Salmonellae* via the intestinal content and cut lymph nodes poses a potential health risk for humans (SWANENBURG *et al.* 2001). In some European countries, statutory and voluntary programmes of monitoring *Salmonellae* based on culture and serology in slaughtered pigs and pig

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herds have been introduced (MOUSING *et al.* 1997; VAN WINSEN *et al.* 2001; STEINBACH *et al.* 2002).

In the Czech Republic in humans, pentaresistant ACSSuT *S. typhimurium* strains of phage type DT104 were isolated for the first time in 1996 (KARPIŠKOVÁ *et al.* 1999). However, the incidence of the epidemic multiresistant strains has been confirmed in cattle herds in the Czech Republic since the early 1990s (FALDYNOVA *et al.* 2003).

The objective of our pilot study was to evaluate *Salmonella* prevalence in slaughtered pigs and to analyse the isolate resistance to antibiotics. The data obtained are used for the assessment of the level of health risk posed by *Salmonella* contaminated pork.

MATERIALS AND METHODS

Sampling. In ten slaughterhouses located in South Moravia, the Czech Moravian Highland and East Bohemia, samples from slaughtered pigs coming from 15 herds were collected in the period of June 2001–December 2002. Samples from 40 to 55 pigs of one herd were collected at each visit to the slaughterhouse. Samples of the caecum content were taken using a sterile plastic kit (Transystem AMIES, COPAN). Carcass swabs were collected prior to cooling near rectum, from external and internal parts of abdominal cavity, and thoracic and pharyngeal sites. Samples of mesenteric lymph nodes were collected into sterile plastic Petri dishes. Simultaneously, at each visit to the slaughterhouse, 10–12 environmental samples were taken, i.e. water from the scalding tank and swabs from dehairing machine, belt conveyers, splitting machine and slaughter line constructions. The collected samples were transferred to the laboratory within 4 hours and processed.

***Salmonella* isolation and identification.** The method EN ISO 6579 was used for *Salmonella* isolation from the examined samples. The samples of the caecum content and the swabs from carcasses and environment were transferred into 9 ml of buffered peptone water (BPW). The sampled mesenteric lymph nodes were surface sterilised by singeing. Amount of 1 g of tissue was then cut by sterile scissors and put into 9 ml BPW. All inoculated samples were cultivated by pre-enrichment in BPW at 37°C for 24 h. Further, 100 µl of the culture from each test tube was inoculated onto 10 ml Rappaport Vassiliadis medium (RVM) and cultivated at 42°C. After 24 and 48 h, the cultures

were inoculated onto a selective medium Xylose-Lysine-Deoxycholate Agar (XLD agar, HIMEDIA M031). The growth was evaluated following the culture at 37°C for 24 h. Pure *Salmonella* cultures were typed using agglutination with monovalent O and H anti-*Salmonella* sera (BIO-RAD).

Strain subtyping. All isolates were tested by the disc diffusion method (NCCLS, 1999) for their sensitivity to the following 14 antibiotics: Ampicillin (AMP 10 µg), Amoxycillin/Clavulanic acid (AMC 30 µg), Apramycin (APR 15 µg), Colistin (CT 10 µg), Sulphamethoxazole/Trimethoprim (SXT 25 µg), Cefotaxime (CTX 30 µg), Enrofloxacin (ENR 5 µg), Gentamicin (CN 10 µg), Neomycin (N 30 µg), Streptomycin (S 10 µg), Tetracycline (TE 30 µg), Chloramphenicol (C 30 µg), Nalidixic acid (NA 30 µg) and Sulphonamides (Su 300 µg).

Phage types of *S. typhimurium* strains were determined using a set of 30 typing phages CPHA London, UK.

Genes encoding resistance to antibiotics were identified by specific PCR reactions.

For the detection of genes coding resistance to ampicillin (*bla*_{PSE-1}), chloramphenicol (*floR*), streptomycin (*aadA2*), sulphonamides (*sul1*) and tetracycline (*tetG*), five gene specific PCR reactions were designed. The amplification products were detected by electrophoresis in 1.5% agarose gel supplemented with etidium bromide and visualised under UV light (FALDYNOVA *et al.* 2003).

Statistical analysis. The χ^2 test using Stat Plus software was used for the statistical evaluation of the *Salmonella* findings in caecum, mesenteric lymph nodes and swabs from carcasses (MATOUSKOVÁ *et al.* 1992)

RESULTS

The evaluation of *Salmonella* occurrence in pigs from 15 herds slaughtered in 10 slaughterhouses from June 2001 to December 2002 is shown in Table 1. No *Salmonellae* were isolated in pigs originating from eight herds (i.e. 53.34%) in four slaugh-

Table 1. *Salmonellae* isolated from pigs slaughtered between June 2001 and December 2002

Totally examined	Number of	
	negative	positive
15 herds	8	7
10 slaughter-houses	4	6

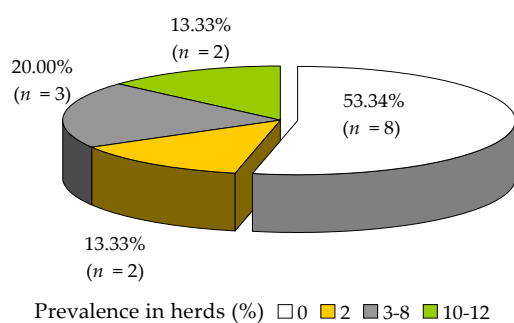


Figure 1. *Salmonella* prevalence in pigs slaughtered between June 2001 and December 2002 based on herds

terhouses. However, *Salmonellae* were found in pigs from seven herds (i.e. 46.66%) in six slaughterhouses. The herd classification based on *Salmonella* prevalence in slaughtered pigs over the investigated period is shown in Figure 1. In pigs of the above seven herds, *Salmonella* prevalence ranged from 2.0% to 12.0%. *Salmonella* prevalence of 2%, 3–8%, and 10–12% was found in two herds (13.33%), three herds (20.0%) and two herds (13.33%), respectively. The results of *Salmonella* isolation in samples of 816 slaughtered pigs are summarised in Table 2. *Salmonellae* were isolated in caecum of twenty pigs, which represents 2.45% of the total samples examined. A significant difference ($P < 0.01$) was found between this finding and *Salmonella* occurrence in mesenteric lymph nodes (0.73%) and swabs from the carcasses (0.12%). All environmental samples collected from five sites on the slaughter line were negative ($n = 197$). *Salmonella* serotypes isolated from the samples of slaughtered pigs are summarised in Table 3. The total of the twenty *Salmonella* isolates from caecum were classified

Table 2. *Salmonellae* isolated from samples of slaughtered pigs and the slaughterline environment (June 2001–December 2002)

Total number of findings/number of samples			
Cecum	MLN*	Carcass swabs	Environmental swabs
20/816	6/816	1/816	0/197
2.45%	0.73%	0.12%	0%

*mesenteric lymph nodes

into the serotypes *S. typhimurium* ($n = 4$), *S. agona* ($n =$), *S. kaapstad* ($n = 4$), *S. infantis* ($n = 3$), *S. derby* ($n = 3$), *S. bredeney* ($n = 1$) and *S. london* ($n = 1$). Six *Salmonella* isolates from mesenteric lymph nodes were classified into the serotypes *S. infantis* ($n = 5$) and *S. bredeney* ($n = 1$). One carcass isolate belonged to serotype *S. typhimurium*. All five *S. typhimurium* isolates were of the same phage type DT104. The occurrence of the resistance to antibiotics among *Salmonella* serotypes isolated from slaughtered pigs is shown in Table 4. None of the strains *S. infantis* ($n = 8$), *S. agona* ($n = 4$), *S. kaapstad* ($n = 4$), *S. bredeney* ($n = 2$) and *S. london* ($n = 1$) were resistant to the antibiotics used. All five strains of *S. typhimurium* phage type DT104 had the same antibiotic profile of resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline. One strain *S. derby* was resistant to gentamicin, streptomycin and sulphaamides. The remaining two strains of this serotype were sensitive to all antibiotics used. The resistance genes *bla*_{PSE-1}, *floR*, *aadA2*, *sul1* and *tetG* were identified in all five pentaresistant strains determined by the disc diffusion methods as ACSSuT phenotype (Figure 2).

Table 3. *Salmonella* serotypes isolated from samples of slaughtered pigs (June 2001–December 2002)

Serotype	Number of isolates		
	Caecum ($n = 20$)	MLN* ($n = 6$)	Carcass swabs ($n = 1$)
<i>S. infantis</i>	3	5	0
<i>S. typhimurium</i>	4**	0	1**
<i>S. agona</i>	4	0	0
<i>S. kaapstad</i>	4	0	0
<i>S. derby</i>	3	0	0
<i>S. bredeney</i>	1	1	0
<i>S. london</i>	1	0	0

*mesenteric lymph nodes; **phage type DT 104

Table 4. Antibiotic resistance in *Salmonella* serotypes isolated from slaughtered pigs (June 2001–December 2002)

Antibiotics	<i>S. infantis</i> <i>n</i> = 8	<i>S. typhimurium</i> DT104 <i>n</i> = 5	<i>S. agona</i> <i>n</i> = 4	<i>S. kaapstad</i> <i>n</i> = 4	<i>S. bredeney</i> <i>n</i> = 2	<i>S. london</i> <i>n</i> = 1	<i>S. derby</i> <i>n</i> = 3
AMP (10 µg)	0	5	0	0	0	0	0
AMC (30 µg)	0	0	0	0	0	0	0
APR (15 µg)	0	0	0	0	0	0	0
CT (10 µg)	0	0	0	0	0	0	0
SXT (25 µg)	0	0	0	0	0	0	0
CTX (30 µg)	0	0	0	0	0	0	0
ENR (5 µg)	0	0	0	0	0	0	0
CN (10 µg)	0	0	0	0	0	0	1
N (30 µg)	0	0	0	0	0	0	0
S (10 µg)	0	5	0	0	0	0	1
TE (30 µg)	0	5	0	0	0	0	0
C (30 µg)	0	5	0	0	0	0	0
NA (30 µg)	0	0	0	0	0	0	0
Su (300 µg)	0	5	0	0	0	0	1

DISCUSSION

The importance of pork as a source of human salmonellosis and multiresistant *Salmonella* strains is increasing in connection with European Union requirements to ensure food safety in the whole production chain using the “farm to fork” system. Swine herds have an important place in the epidemiology of salmonellosis. Latently infected herds are the major sources of *Salmonellae* which subsequently results in slaughterhouses contamina-

tion and poses health risks for humans (Directive 2003/99/EC). In most countries, relations between human cases of salmonellosis and pork contamination have not been elucidated yet. It was found in Denmark, prior to launching the national programme for *Salmonella* control in pigs and slaughterhouses in 1995, that 15–20% of cases of human salmonellosis were caused by contaminated pork. After implementation of food safety programme, *Salmonella* occurrence in pork dropped from 3.5% in 1993 to 0.7% in 2000 (NIELSEN *et al.* 2001). The

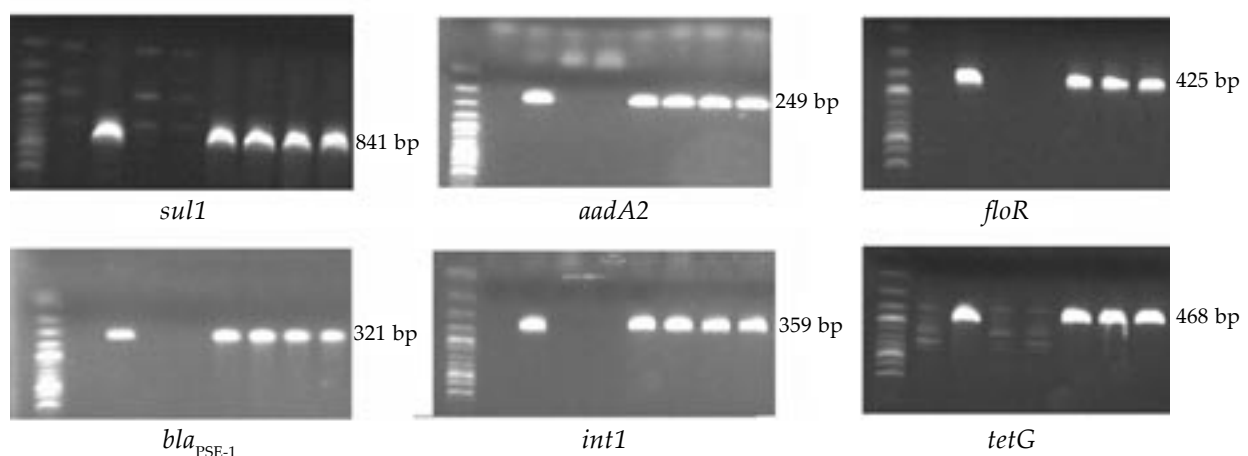


Figure 2. Gene-specific PCR in pentaresistant strains isolated from slaughtered pigs

results of this pilot study conducted in the Czech Republic confirmed that *Salmonella* occurrence in slaughtered pigs was dependent on the origin of the respective herd. No *Salmonellae* were isolated in four slaughterhouses with pigs originating from eight herds (53.34%). *Salmonella* prevalence in the remaining seven herds (46.66%) in slaughtered pigs in six slaughterhouses ranged from 2.0 to 12.0%. The evaluation of *Salmonella* prevalence in slaughtered pigs can be compared with the data from some European countries. In Denmark, 6.2% of caecal samples in slaughtered pigs were positive, caused usually by one dominant *Salmonella* serotype in the herd. *S. typhimurium* of the phage type DT12 was the most frequent serotype (69.4%). *Salmonellae* were isolated in slaughtered pigs coming from 22.2% of the herds examined (BAGGESEN *et al.* 1996). It was found in the Netherlands that 5–30% of swine herds were shedding *Salmonellae* via faeces at the end of the fattening period (EXEL & TIELEN 1999). DAVIES *et al.* (1999) found in slaughtered pigs in the UK the dominant serotype *S. typhimurium* in 11.6% in the caecum content, and in 7.0% in carcasses. In Germany, *Salmonella* prevalence was found in 3.7% of caecum samples, in 3.3% in mesenteric lymph nodes, and in 4.7% of carcass swabs. No *Salmonellae* were found in slaughtered pigs originating from about 70% of the herds examined. In the positive herds, *Salmonella* prevalence ranged from 1% to 50%. Prevalence exceeding 50% was found only in 2% of the herds examined (KÄSBOHRER *et al.* 2000). No *Salmonella* incidence was reported in Sweden, where 3388 samples of slaughtered pigs collected from five slaughterhouses during one year were examined (THORBERG & ENGVALL 2001). In Norway, *Salmonella* prevalence in slaughtered pigs was very low, 0.4% in sows and 0.1% in fattening pigs (SANDBERG *et al.* 2002). In our study on slaughtered pigs, *Salmonellae* were most frequently isolated from the caecum content (2.45%). This value is significantly higher compared to those found with mesenteric lymph nodes (0.73%) and carcass swabs (0.12%). All *Salmonella* findings were only of one type of samples, i.e. caecum, mesenteric lymph nodes, and carcass swabs collected from different slaughtered pigs. No correlation was found between *Salmonella* isolates from caecum and carcass swabs, or from the environmental samples collected in dirty and clean zones of the slaughter line.

Salmonella prevalence in slaughtered pigs, faecal samples, lymph nodes, on carcasses and in slaughterhouse environments varies largely. It depends

on the epidemic situation in particular countries, regions, and on risk factors in herds. Major risk factors for *Salmonella* spreading in fattening pigs are a low level of hygiene in herds, contaminated feedstuffs, widely-used antibiotics, *Salmonella* occurrence in the herd, stress during transportation to the slaughterhouses, and the keeping of pigs in the lairage prior to slaughter (BERENDS *et al.* 1996). Further, it depends on the season, the hygienic and technological levels in the slaughterhouses as well as on sampling and the cultivation methods (HALD & ANDERSON 2001). Caecum and colon, and regional lymph nodes, are frequent sites of *Salmonella* carriers in fattening pigs with no clinical signs (DAVIES *et al.* 1999). After the slaughtering of pigs and their evisceration, carcasses are contaminated by faeces and tissues of cut lymph nodes. At poor slaughterhouse hygiene, raw products are further cross-contaminated with *Salmonellae* (BOTTELDOORN *et al.* 2003). Although *Salmonellae* were detected in caecum and mesenteric lymph nodes in slaughtered pigs coming from contaminated herds, findings in carcasses were infrequent, and samples from the slaughter line were negative. These findings testify to high levels of hygiene and technology during pork processing. Preventive measures in slaughterhouses based on the concept HACCP can further reduce faecal contamination of carcass halves and cross-contamination of raw swine products. Thus, the potential risk of *Salmonella* transmission via swine products to humans can be minimised (BOUDRY *et al.* 2002).

S. infantis was the most frequent serotype whose strains were isolated from caecum, and in one case, from carcasses of different pigs originating from two herds and slaughtered in different slaughterhouses. In one case, *S. infantis* and *S. typhimurium* were detected in caecum of two different pigs of the same herd, slaughtered in the same slaughterhouse. The second most frequent serotype was *S. typhimurium*, whose strains were isolated from caecum and in one case from carcasses of pigs originating from two herds and slaughtered in different slaughterhouses. Findings of *S. agona* and *S. derby* in caecum were recorded in slaughtered pigs from two different herds and different slaughterhouses. Mixed infection of the serotypes *S. kaapstad* and *S. bredeney* was detected in caecum and mesenteric lymph nodes in pigs coming from one herd. Prevalence of *Salmonella* serotypes in fattening pigs and in slaughterhouses depends on the epidemiological situation in particular countries. Dominant serotypes in pigs are

S. typhimurium and *S. derby* (about 80%) in most European countries. The occurrence of other serotypes varies widely (BAGGESEN *et al.* 1996).

The resistance to antibiotics among *Salmonella* serotypes was most frequent in *S. typhimurium* isolates. The occurrence of strains *S. typhimurium* phage type DT104 of pentaresistant clone ACSSuT in slaughtered pigs originating from two herds was the most important finding with regard to human health. A specific PCR reaction identified the resistance genes *bla*_{PSE-1'}, *floR*, *aadA2*, *sul1* and *tetG* in all strains (GEBREYES *et al.* 2000; FALDYNOVA *et al.* 2003). The occurrence of multiresistant strains of the above serotype is continuously increasing in human population in the Czech Republic (ŠRÁMOVÁ *et al.* 2003). The results obtained in our pilot study will be used in the national programme of *Salmonella* monitoring and control in swine herds and slaughterhouses in the Czech Republic that will be launched in the near future.

CONCLUSION

Salmonella prevalence in slaughtered pigs depends on the origin of the respective herd. *Salmonellae* detected in caecum of slaughtered pigs were the major potential source of the pork contamination. The findings of pentaresistant ACSSuT strains *S. typhimurium*, phage type DT104, were the most important with regard to the public health. Safety practices and high hygienic standards can considerably reduce the potential health risk of pig carcasses contamination with *Salmonellae*.

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Souhrn

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Byla vyhodnocena prevalence salmonel u 816 prasat pocházejících z 15 chovů, která byla poražena od června 2001 do prosince 2002 na 10 jatkách. Salmonely nebyly izolovány u poražených prasat z osmi chovů na čtyřech jatkách. Prevalence salmonel u prasat pocházejících ze zbývajících sedmi chovů se pohybovala od 2,0 % do 12,0 %. Salmonely byly izolovány nejčastěji ve slepém střevě (2,45 %). Je to statisticky významný rozdíl ($P < 0,01$) oproti nálezům v mezenterálních mízních uzlinách (0,73 %) a střezech z vepřových půlek (0,12 %). Salmonely nebyly zjištěny ve střezech z prostředí ($n = 197$). Celkem 27 izolátů salmonel bylo zařazeno do sedmi sérovarů: *S. infantis* ($n = 8$), *S. typhimurium* ($n = 5$), *S. agona* ($n = 4$), *S. kaapstad* ($n = 4$), *S. derby* ($n = 3$), *S. bredeney* ($n = 2$) a *S. london* ($n = 1$). Všechny pět kmenů *S. typhimurium* DT104 mělo rezistentní fenotyp ACSSuT. U všech pentarezistentních kmenů byly identifikovány geny rezistence *bla*_{PSE-1}, *floR*, *aadA2*, *sul1* a *tetG*. Jeden kmen *S. derby* byl rezistentní ke gentamicinu, streptomycinu a sulfonamidům. Ostatní izoláty salmonel byly citlivé ke všem testovaným antibiotikům.

Klíčová slova: poražené prase; sérotyp salmonel; fagotyp DT104; antibiotická rezistence

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