

Serological and Bacteriological Evaluation of *Salmonella* Status in Swine Herds

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Abstract

ŠIŠÁK F., HAVLÍČKOVÁ H., MATIAŠOVIC J., KARPÍŠKOVÁ R. (2011): **Serological and bacteriological evaluation of *Salmonella* status in swine herds.** Czech J. Food Sci., **29** (Special Issue): S102–S108.

The sera from 690 slaughtered fattening pigs from 15 farrow-to-finish swine herds (12 herds of unknown *Salmonella* status, 3 herds known as latently infected) in the Czech Republic were examined for *Salmonella* antibodies in a cross sectional study using an ELISA test. *Salmonella* seroprevalence ranging from 0% to 20% was found in 14 herds. Seroprevalence of 73.9 was found in 1 herd with previously unknown *Salmonella* status. A longitudinal study of the three previously identified latently infected herds found seroprevalence ranging from 23.9% to 83.4% in sows after farrowing. *Salmonella* findings from faeces in the farrowing sections ranged between 1.8 and 24.5, and in the environmental samples between 0 and 25. In weaned piglets, *Salmonella* findings from faeces ranged from 6.3 to 48.0, and in environmental samples from 0 to 90%. The most prevalent serotypes were *S. Derby* (56.8) and *S. Typhimurium*, phage type DT104 (18.5). The seroprevalence comparison in sows and slaughtered fattening pigs revealed variations in the course of *Salmonella* infection in swine herds.

Keywords: *Salmonella* seroprevalence; latent infection; swine herds; food safety

Salmonella transmission in swine herds is a significant health and hygiene issue concerning public health and food safety (ANONYMOUS 2003a,b). The major source of *Salmonella* contamination in pork is the fattening pigs from latently infected herds (BELOEIL *et al.* 2004). After the slaughter of the infected pigs, a high risk occurs of the pig carcass contamination with *Salmonella* from faeces, palatine tonsils and mesenteric lymph nodes, the contamination and cross-contamination occurring in the slaughter line (FOSSE *et al.* 2009). The contaminated pork and meat products account for 10–200 of human *Salmonella* outbreaks in EU, however, this value varies considerably among the member states. The prevalent occurrence of the

serotype *S. Typhimurium* and its strains, which are multiresistant to antibiotics, is the most important from the epidemiological point of view (2010). The increasing occurrence of multiresistant strains results in the antibiotic treatment failure in both humans and animals and transmission of antibiotic resistance to other bacteria (CLOECKAERT & SCHWARZ 2001). *Salmonella* detection from porcine faeces originating from asymptomatic carriers is difficult when using culture examination due to the intermittent shedding and low counts of bacteria. For these reasons serological examinations based on enzyme-linked immunosorbent assay (ELISA) have been used for the identification of *Salmonella* positive herds of fattening pigs (ANONYMOUS 2006).

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QH81062, and the Ministry of Education, Youth and Sports of the Czech Republic, Project No. ED0006/01/01 AdmireVet.

Serological monitoring by ELISA test has been used in Denmark (NIELSEN *et al.* 2001), Germany (OSTERKORN *et al.* 2001) and other countries (VAN DER WOLF *et al.* 2001; DAVIES *et al.* 2003) for the classification of fattening pig herds within the national *Salmonella* control programmes. *Salmonella* IgG antibodies have been demonstrated in blood serum and meat juices from slaughtered fattening pigs using commercial kits, because these antibodies persist long after the infection. The herds with middle and high prevalence of *Salmonella* antibodies (from 20 to 400 and more) pose a high risk of the incidence of *Salmonella* infections (ANONYMOUS 2006). In the Czech Republic, serological diagnosis of *Salmonella* infections has not yet been implemented into pig herds. However, limited pilot studies have been carried out on slaughtered fattening pigs (ŠIŠÁK *et al.* 2006) and on sow herds (ŠIŠÁK *et al.* 2007).

A substantial contribution to *Salmonella* spreading in piglet rearing and pig fattening may be ascribed to sows which play an important role in the epidemiology of *Salmonella* infection, being carriers which harbour infections in the herds for an extended period (KRANKER *et al.* 2001, 2003).

The objectives of this study were to: (1) Examine *Salmonella* seroprevalence in slaughtered fattening pigs from 15 farrow-to-finish herds using a cross-sectional study design; (2) Examine the longitudinal *Salmonella* status of 3 previously identified latently infected pig herds via serology and culture.

MATERIAL AND METHODS

Cross-sectional study in slaughtered fattening pigs. The blood samples were collected during 2008 from slaughtered fattening pigs originating from 15 different farrow-to-finish herds in the Czech Republic. Three herds were previously identified as latently infected with *Salmonella*; however, no data was available regarding *Salmonella* incidence in the remaining 12 herds. The samples were collected at the slaughterhouses selected because they received pigs from the known *Salmonella*-positive farms. At each slaughterhouse, the blood samples were collected from pigs from one latently infected herd and from four herds with unknown *Salmonella* status. In the slaughter line, the blood samples were taken at one time from 46 to 50 randomly selected fattening pigs of each herd. On the farms with latently infected herds,

fattening pigs were specifically selected so that they were the progeny of the sows which were previously found positive by serology and culture in the farrowing sections.

Longitudinal study in *Salmonella* latently infected farrow-to-finish herds. *Salmonella* status was evaluated serologically and by culture in 3 long-term latently infected farrow-to-finish pig herds in 2008 and 2009. These *Salmonella*-positive swine herds were designated I, II and III, with the capacity of 600, 800 and 1200 sows, respectively. No specific measures for *Salmonella* control had been implemented in these herds. The testing was conducted once each year on the sows after farrowing and on their offspring – weaned piglets and slaughtered fattening pigs.

Sows. The occurrence of *Salmonella* infection was monitored in sows serologically and by culture 2–3 weeks after farrowing. It was prohibited to clean and disinfect the examined sections 1 day prior to the sample collection for culture. In each herd, samples were taken in the farrowing sections from 46 sows prior to the weaning of the piglets. The samples included blood, faeces from the floor (25 g each) from the in boxes housing individual, and swabs from the stable environment from the upper side of feeders and internal walls of pens.

Weaners. Two to 3 weeks after weaning, 5 faecal samples (25 g each) were collected from the rearing units with approximately 40 weaners. A total of 47–50 faecal samples were collected from 10 units in the weaning sections of each herd. Swabs from stable environments were also collected at the same locations as in the farrowing sections.

Finishing pigs: Blood samples were collected from the batches of 46 slaughtered pigs from each herd for serological examinations. *Salmonella* seroprevalence in the sows and the slaughtered fattening pigs was compared over the investigated period of 2008 and 2009.

Serological examinations. The sera from the slaughtered fattening pigs and sows were examined for the presence of *Salmonella* antibodies in duplicates using the commercial kit SALMOTYPE® Pig Screen ELISA, according to the manufacturer's instructions (Labor Diagnostik, Leipzig, Germany). The cut-off values of the antibody concentrations for the positive samples were assessed according to the OD0 (optical density percentages) ≥ 20 .

***Salmonella* isolation, identification and subtyping.** The collected samples of the faeces and swabs from stable environments were examined

by culture ISO 6579:2002 Annex D (ISO 2002). After pre-enrichment in buffered peptone water for 18–24 h at 37°C, 100 µl of the pre-culture were applied onto the surface of modified semisolid Rappaport-Vassiliadis medium (MSRV medium OXOID CM0910) and cultured for 24 h and 48 h at 42°C. From the end of the migration zone on MSRV medium, the culture was plated, using a loop, on xylose-lysine-deosycolate agar with novobiocine and incubated at 37°C for 24 hours. After serotyping with *Salmonella* O and H anti-sera (BIO-RAD, Marnes-la-Coquette, France), the isolated strains were classified into serotypes according to Kauffmann-White's scheme. *S. Typhimurium* strains were phage typed according to ANDERSON *et al.* (1977) and *S. enteritidis* strains according to WARD *et al.* (1987) using the sets of typing phages (Health Protection Agency, London, UK).

RESULTS

Cross sectional study in slaughtered fattening pigs

The sera from 690 fattening pigs, originating from 15 different farrow-to-finish herds, were examined by ELISA test for the presence of *Salmonella* antibodies during 2008. Of the total number

of 690 sera examined, 60 (8.70) were positive at a cut-off level of OD \geq 200. Based on the seroprevalences obtained, the herds of fattening pigs were classified into 3 groups: zero or very low (< 100); low (10–200); high (> 200). Zero or very low seroprevalence was detected in 12 herds. This group included fattening pigs from herds I and II which were known to be latently infected with *Salmonella*. In two herds, low seroprevalence ranging from 10 to 200 was detected. This group contained pigs from herd III with a history of *Salmonella* infection. A very high seroprevalence (73.90) of *Salmonella* antibodies was found in only one herd with previously unknown *Salmonella* status.

Longitudinal study in *Salmonella* latently infected farrow-to-finish herds

The results of the serological examinations on the sows by ELISA and culture in 3 farrow-to-finish herds known to have been latently infected with *Salmonella*, carried out in 2008 and 2009, are summarised in Table 1.

Herd I. Salmonella seroprevalence in the sows was 54.30 (25/46) and 67.40 (31/46) in 2008 and 2009, respectively, while the sow faecal culture was positive in 2.00 (1/50) and 6.00 (3/50) of the samples. Positive faecal culture in the samples

Table 1. Serological examinations by ELISA and culture in *Salmonella* latently infected swine herds in 2008 and 2009

Herd	Year	ELISA serum sows	Culture			
			faeces		environmental samples	
			sows	weaners	sows	weaners
I	2008	25/46*	1 ^a /50	4 ^a /48	0/6	0/6
		54.30	2.00	8.30	0.00	0.00
	2009	31/46	2 ^d , 1 ^a /50	3 ^a /48	1 ^a /6	1 ^b /12
		67.40	6.00	6.30	16.70	8.30
II	2008	22/46	1 ^a /56	6 ^a /50	0/16	0/6
		47.80	1.80	12.00	0.00	0.00
	2009	26/46	4 ^a , 1 ^d /50	13 ^a , 9 ^b , 2 ^d /50	2 ^a /8	5 ^b , 3 ^a , 1 ^d /10
		56.50	10.00	48.00	25.00	90.00
III	2008	11/46	1 ^b /48	11 ^c , 6 ^b /48	0/10	3 ^c /8
		23.90	2.10	35.40	0.00	37.50
	2009	37/46	10 ^a , 2 ^b /49	4 ^b , 1 ^a /47	1 ^a , 1 ^b /10	0/10
		80.40	24.50	10.60	20.00	0.00

^aS. Derby, ^bS. Typhimurium phage type DT104, ^cS. Enteritidis phage type PT8; ^dS. Infantis

*No. of positive/No. of examined samples

Table 2. Distribution of *Salmonella* serotypes isolated from samples collected in latently infected swine herds in 2008 and 2009

Herd	Year	<i>S. Derby</i>	<i>S. Typhimurium</i>	<i>S. Enteritidis</i>	<i>S. Infantis</i>
I	2008	5	0	0	0
	2009	5	1	0	2
II	2008	7	0	0	0
	2009	17	0	0	4
III	2008	0	7	14	0
	2009	12	7	0	0
Total		46 56.80	15 18.50	14 17.30	6 7.40

from the weaned piglets was 8.30 (4/48) and 6.30 (3/48). The cultures of the samples from the stable environments in the sow- and weaning-sections were negative in 2008. In 2009, the culture of the environmental samples was positive in 16.70 (1/6) of sows and in 8.30 (1/12) of the weaners.

Herd II. Seroprevalence in the sows was 47.80 (22/46) and 56.50 (26/46) in 2008 and 2009, respectively, while the sow faecal culture was positive in 1.80 (1/56) and 10.00 (5/50) of the samples. In the weaners, the faecal culture was positive in 12.00 (6/50) and in 48.00 (24/50) of the samples. The cultures from the environmental samples in the sows and weaners were negative in 2008. In 2009, the culture was positive in 25.00 (2/8) of the sow samples and 90.00 (9/10) of the weaner samples.

Herd III. Seroprevalence in the sows was 23.90 (11/46) and 80.40 (37/46) in 2008 and 2009, respectively, while the sow faecal culture was positive in 2.10 (1/48) and 24.50 (12/49) of samples.

In the weaners, the faecal culture was positive in 35.40 (17/48) and in 10.60 (5/47) of samples. The culture from the stable environments was negative in the sows in 2008 and positive in 2009 in 20.00 (2/10) of the samples. In the weaning sections, the samples were positive in 37.50 (3/8) of the samples in 2008 but negative in 2009.

A total of 81 samples were *Salmonella*-positive over the entire study period (Table 2). The most prevalent serotype was *S. Derby* (56.80; $n = 46$), which was isolated from the faeces of the sows and weaners and from the environmental swabs collected in the farrowing and rearing sections of all three herds. *S. Typhimurium* phage type DT104 was the second most frequent serotype (18.50; $n = 15$) which was isolated in herd I in 2009 from the environmental samples in the weaning sections, and in herd III from the faecal samples of the sows and weaners in both years, and from the environmental samples in the farrowing sections

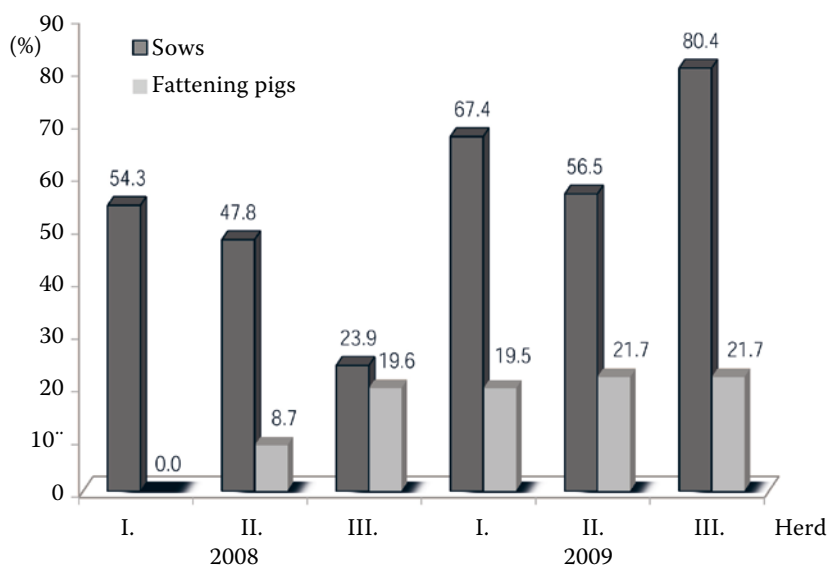


Figure 1. Comparison of seroprevalence in sows and slaughtered fattening pigs in *Salmonella* latently infected herds in 2008 and 2009

in 2009. *S. Enteritidis* strains of phage type PT8 (17.30; $n = 14$) were isolated only in herd III in 2008 from the faecal samples of the weaners and the environmental samples from the weaning sections. *S. Infantis* strains (7.40; $n = 6$) were isolated in 2009 in herd I from the faecal samples of the sows, and in herd II from the faecal samples of the sows and weaners, and from the environmental swabs from the weaning sections.

The comparison of seroprevalence in the sows and slaughtered fattening pigs in the latently infected herds during 2008 and 2009 is presented in Figure 1. In 2008, seroprevalence in the sows from herd I was found to be 54.30, however, no *Salmonella* antibodies were found in the fattening pigs. Seroprevalence in the sows of herd II was 47.80 and 8.70 in the fattening pigs. The corresponding figures in herd III were 23.90 and 19.60. High *Salmonella* seroprevalence was recorded in the sows from all three herds in 2009, reaching 67.40, 56.50 and 80.40, respectively. These findings corresponded with the increased seroprevalence values in the slaughtered fattening pigs, which were 19.50, 21.70, and 21.7%, respectively.

DISCUSSION

In 9 EU member states, the prevalence of slaughtered pigs with *Salmonella* antibodies ranged from 3.50 to 33.30 (ANONYMOUS 2008). NOWAK *et al.* (2007) reported seroprevalence of 70, at a cut-off level of OD ≥ 400 , in meat juice samples taken at slaughterhouses in Germany. While these results appear to be similar to those in the present study, where 8.70 of finishing pigs were found to be seropositive, it is not possible to compare directly the results due to different sample types, different ELISA tests, and different cut-off values used in the respective studies.

Our study found low or very low seroprevalence (0 to 200; OD0 ≥ 20) in 14/15 herds; interestingly, these included all 3 herds which were previously known to be latently infected with *Salmonella*. High seroprevalence of 73.90 was found only in one herd with previously unknown *Salmonella* status. Similarly, most herds of fattening pigs in Germany were serologically negative (810) or showed low seroprevalence (130). Few herds were in higher seroprevalence categories (30 of herds with seroprevalence 200 to 400; 30 of herds with seroprevalence > 400) (NOWAK *et al.* 2007).

A meat juice antibody ELISA test has been used in Germany to classify pig herds according to the *Salmonella* risk in the *Salmonella* control programme under the QS-System (Quality and Safety). The herds with middle and high seroprevalence (category II and III) are considered to be *Salmonella* positive. The pigs from such risk herds are usually slaughtered only at the end of the slaughtering day. A thorough cleaning and disinfection follows. The measures to reduce *Salmonella* status have been implemented in such herds; their efficiency has been monitored serologically. The results of our cross sectional study support the implementation of serological ELISA testing of *Salmonella* in herds of fattening pigs into the control programmes in the Czech Republic similarly to the QS-System in Germany.

The results of our longitudinal study revealed the carriers among the sows which may contribute to *Salmonella* persistence in the latently infected farrow-to-finish swine herds. According to the baseline survey on *Salmonella* in breeding pigs in the EU in 2008, it was found that 33.30 of the production holdings had positive results of culture for *Salmonella*. In the Czech Republic it was 15.50 (ANONYMOUS 2009). Breeding pigs, predominantly sows, may be an important source of dissemination of *Salmonella* throughout the pig production chain. After farrowing, faecal shedding of *Salmonella* increases in sows, contaminating pens and the environment which creates potential sources of infection for piglets (NOLLET *et al.* 2005). A direct relationship has been confirmed between breeder pig herd *Salmonella* prevalence in faeces (above 100) and findings of the same serotypes in ileocaecal lymph nodes and swabs from carcasses of fattening pigs at slaughter. For these reasons, *Salmonella* control in the infected breeder herds is essential for the reduction of *Salmonella* transmission into fattening pigs and for the prevention of pork contamination, thus ensuring food safety (ANONYMOUS 2010).

In our study, fairly high values of seroprevalence, ranging from 23.90 to 83.40, were found in the sows after farrowing in all three herds monitored over two years, while *Salmonella* findings ranged between 1.80 and 24.50, and 0 to 25.00 in the faecal and environmental samples, respectively. This analysis of *Salmonella* status in the latently infected farrow-to-finish herds corresponds with the findings of other authors (NOLLET *et al.* 2005; FARZAN *et al.* 2007; RAJIC *et al.* 2007) who demonstrated a low correlation between seropreva-

lence of specific *Salmonella* antibodies and their isolation from swine faeces. The presence of high titre of specific antibodies does not always signify infection, but is indicative of previous exposure. Our findings suggest an association between high seroprevalence in sows and the occurrence not only of *S. Typhimurium* but also of *S. Derby* and *S. Infantis* serotypes in weaners, similar to the data reported by KRANKER *et al.* (2001).

With the weaned piglets, *Salmonella* findings from faeces ranged from 6.3% to 48.0 and those from the environmental samples were in the range of 0 to 90. The sows were in all three herds the suspect source of infection with the prevalent serotype *S. Derby* for the piglets and stable environment, followed by *S. Typhimurium* phage type DT104 and *S. Infantis*. The serotype *S. Enteritidis* phage type PT8, which was isolated from faeces of the weaners in the rearing section of herd III, was not isolated from the sows. Feedstuff or stable environments contaminated by mice droppings could be the source of *S. Enteritidis* infection for the weaned piglets; however, this aspect was not examined in this study. The environmental samples collected in the farrowing and weaning sections were reliable indicators of *Salmonella* contamination in the latently infected swine herds monitored. In accordance with other authors (LURETTE *et al.* 2008; WILKINS *et al.* 2010), we found an increased *Salmonella* incidence in piglet weaning. We suppose that the cause of this enhanced *Salmonella* spreading in the weaning can be the results of the mixing of litters from the culture positive and culture negative sows and increased susceptibility of weaners to the infection.

The comparison of seroprevalence in the sows and slaughtered fattening pigs from the herds latently infected with *Salmonella* offered different results during long-term monitoring. While in the sows from herd I and II seroprevalence was high, in the fattening pigs it was zero or low in 2008. The corresponding figures in herd III were 23.90 and 19.60. The relationship between the high *Salmonella* seroprevalence in the sows and increased rate of the seropositive slaughtered fattening pigs in 2009 thus suggests an epidemiological link in the latently infected herds. This variation in serological findings may be potentially explained by the season, changes in hygiene level, management, and other risk factors in the herds which might have affected the course of *Salmonella* infection and seroconversion in fattening pigs (BELOEIL *et al.* 2007).

CONCLUSION

Serological surveillance of *Salmonella* infections in the herds of fattening pigs allows the detection of high risk herds and assessment of the efficiency of the control measures implemented into the primary production, which are the preconditions for the reduction of pig carcass contamination. The occurrence of carrier sows in seropositive farrow-to-finish swine herds may constitute a long-lasting but substantial risk for *Salmonella* transmission to piglets in the farrowing and weaning sections and further to pig fattening including the environmental contamination. For these reasons, it is essential that effective *Salmonella* control measures should be taken first at the sow level.

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Received for publication July 28, 2011

Accepted after corrections October 18, 2011

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