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Use of Isolation and Antibody Detection for *Salmonella* Assessment

Marina Štukelj[1], Vojka Bole-Hribovšek[2],
Jasna Mićunović[2] and Zdravko Valenčak[1]
[1]*University of Ljubljana, Veterinary faculty*
Institute for the Health Care of Pigs, Ljubljana
[2]*University of Ljubljana, Veterinary faculty*
Institute for Microbiology and Parasitology, Ljubljana
Slovenia

1. Introduction

1.1 *Salmonella* in pigs

Salmonella infections of swine are of concern for two major reasons. The first is the clinical disease (salmonellosis) in swine that may result, and the second is that swine can be infected with a broad range of *Salmonella* serovars that can be a source of contamination of pork products. The genus *Salmonella* is morphologically and biochemically homogeneous group of Gram-negative, motile, non-spore-forming, facultative anaerobic bacilli with peritrichous flagella (Griffith et al., 2006). According to their biochemical characteristics it is divided in two species *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is further divided in six subspecies. Regarding their antigenic structure of somatic (O), flagellar (H) and capsular (Vi) antigens they are divided in serovars. Traditionally the serovars of *subspecies enterica*, which account for more than 99.5% of isolated *Salmonella* strains, have names, while all the others are named by their antigenic formula only (Grimont and Weill, 2007). Final differentiation within serovars is carried out by phage typing, plasmid profiling, restriction endonuclease analysis and resistance patterns. Serovars Typhimurium, Derby, Saintpaul, Infantis, Heidelberg, Typhisuis and Choleraesuis may all occur in pigs (Taylor, 2006).

The reservoir for *Salmonellae* is the intestinal tract of warm-blooded and cold-blooded animals. *Salmonellae* are hardy and ubiquitous bacteria that multiply at 7-47° C; survive freezing and desiccation well; and persist for weeks, months, or even years in suitable organic substrates. The bacteria are readily inactivated by heat and sunlight as well as by common phenolic, chlorine, and iodine disinfectants. Ability to survive in the environment, as well as prolonged carrier states in innumerable hosts ensures the widespread distribution of this genus worldwide (Griffith et al., 2006).

Pigs usually get infected through oral intake of the organism. After infection, animals can become carriers in the tonsils, the intestines and gut-associated lymphoid tissue (Wood et al., 1989; Fedorka-Cray et al., 2000). Most of the time, carriers are not excreting the bacteria

but under stressful conditions, re-shedding may occur. In this way, carriers are permanent potential source of infection for other animals and humans. Stress factors can occur during the fattening period, but also prior to slaughter, for instance during transport to the slaughterhouse or during the stay in the lairage (Seidler et al., 2001; Rostagno et al., 2010). Along the slaughter line, several steps can be critical for *Salmonella* contamination, removal of the pluck set and meat inspection procedures (De Busser et al. 2011). During these steps, the carcass can be contaminated with faeces and bacteria can be spread all over the carcass and to subsequent carcass.

After tracing the *Salmonella* data from the colon content isolated in the slaughterhouse back to the herd level, it was estimated that 40% of the herds were *Salmonella* positive at the moment of slaughter. A high level of herd contamination was also found in the Netherlands with 23% of the herds *Salmonella* positive sampled on the farm (van der Wolf et al., 1999) and in the UK with 63% positive farms (Davies et al., 1999). For interpretation of our data, it has to be kept in mind that the pigs with positive colon content and/or mesenteric lymph nodes in the slaughterhouse could have been infected on the farm and during transport or during the waiting period in the lairage before slaughtering. There are indeed indications that the contamination could already be detected in the faeces and the mesenteric lymph nodes as early as 3 h after infection (Fedorka-Cray et al., 1994). Especially the lairage and the high contamination level of the slaughterhouse environment are probably the major source for *Salmonella* infections prior to slaughter (Hurd et al., 2001; Swanenburg et al., 2001). Hurd et al. (2002) demonstrated that rapid infection during transport, and particularly during holding, is a major reason for increased *Salmonella* prevalence in swine: a sevenfold higher *Salmonella* isolation rate and twice as many different serovars were observed from pigs necropsied at the abattoir than from those necropsied on the farm.

There is currently an explosion of investigational activity related to issue of food safety, including *Salmonella* contamination of variety of foods. Salmonellosis is considered to be one of the most common food-borne illnesses in humans. There has been an increased public awareness of microbiological hazards of food and improved monitoring. Over the recent years, salmonellosis has been the second most commonly reported zoonoses in the European Union, accounting for 151,995 recorded human cases in 2007 (EFSA, 2009b) and 131,468 in 2008 (EFSA, 2010). Although *Salmonella* contamination of poultry and beef products exceeds that of pork, *Salmonella* control programs in swine will continue to be a primary focus of food safety initiatives. *Salmonella* reduction programs are becoming commonplace, with long-range goals to include the production and marketing of *Salmonella*-free pork products. Numerous dynamic programs are in place utilizing hazard analysis and critical control point (HACCP) principles (Griffith et al., 2006). Those programs, that have been in place for sufficient period of time, such as the Danish program, have significantly reduced the rate of *Salmonella* infection in pork products (Nielsen et al, 1995). Fortunately, most of the methods useful for pre-harvest *Salmonella* reduction in swine populations are related to sound management practices that also improve the overall health of swine operation.

Reduction of *Salmonella enterica subsp. enterica* (*Salmonella*) prevalence in the pig industry will be set as a target at the EU level and it is believed to significantly contribute to the protection of human health. The specific reduction target will be based upon the results of

a quantitative microbiological risk assessment on *Salmonella* in slaughter and breeder pigs as well as cost-benefit analyses, all conducted at the EU level. According to the Regulation EC-2160/20032, protection of human health from food-borne zoonotic agents is an issue of paramount importance. Farm-to-fork control programs will probably be needed to ensure a reduction of the prevalence of specified zoonoses and zoonotic agents. Moreover, Member States will have the responsibility to establish effective national control programs adjusted for the country-specific characteristics, including the disease burden and the financial implications for stakeholders. Results of the EU baseline survey on the prevalence of *Salmonella* in lymph nodes of slaughter pigs showed a wide range of prevalences in EU countries, from 0% to 29% infected pigs (EFSA, 2008). These findings suggest that country tailored surveillance-and-control strategies should be designed aiming to achieve the targets in a cost-effective way, assuring human-health protection (Baptista et al., 2010).

Bacteriological isolation methods are used to detect *Salmonella* positive pigs and to identify the *Salmonella* serovars, but because of the low sensitivity of bacteriological faecal or intestinal examination *Salmonella* positive pigs can be missed (Bager et al., 1991). Another method to screen pigs for *Salmonella* is detection of *Salmonella* serum antibodies. The *Salmonella* –LPS-ELISA (*Salmonella*-ELISA) has been developed in Denmark (Nielsen et al., 1995) and in The Netherlands (Van der Heijden et al., 1998). The setup of the *Salmonella*-ELISA is based on a mixture of lipopolysaccharides (LPS) from two *Salmonella* serovars and should theoretically detect 95% of *Salmonella* serovars (Baggesen et al., 1997). From field studies it became clear that the *Salmonella*-ELISA detects antibodies against serovars Typhimurium and Infantis more effectively than other *Salmonella* serovares (Basggsen et al., 1997). Experimental studies to investigate the feasibility of this method for other *Salmonella* serovars have not been carried out yet (Van Winsen et al., 2001).

Results from direct diagnostic methods (bacteriology) and indirect diagnostic methods (serology) cannot be compared easily. The actual shedding of *Salmonella* indicates true infection and transmission, whereas the positive serology indicates also silent transmission within the herd (Van Winsen et al., 2001). The two *Salmonella* ELISA´ s have been shown to be useful to screen herd or groups that are possibly infected with certain serovars but are of no use to judge individual animals (Nielsen et al., 1995; Van Winsen et al., 2001). The EU baseline study in fattening pigs showed that due to the diversity of tests and cut-off points, used by the 9 Member States (MSs) that chose to collect meat juice samples, no group level prevalence can be estimated. The sensitivity and specificity of these tests is not precisely known and in most MSs, some inconclusive results were reported. The sero-prevalence amongst these 9 MSs was estimated to have been from as low as 2.2% (lower boundary of 95% CI, classifying inconclusive results as negative) in Sweden to as high as 41.6% (upper boundary of 95% CI, classifying inconclusive results as positive) in Cyprus (EFSA, 2008). Community reference laboratory for *Salmonella* received from this study 60 meat juice samples per participating Member State and additionally tested them to evaluate possible comparison of results between member States. Four different ELISA kits were used by Member States and considerable discrepancies between Member States' results and the results of Community Reference Laboratory were found (Berk, 2008).

Danish *Salmonella* scheme categorised pig farms in four levels from 0 to 3. Once a month, all herds were assigned to official *Salmonella* level (1, 2 or 3) according to the results from the

preceding 3 months. Level 1 included herds with low acceptable prevalence of *Salmonella*, Level 2 included herds with a moderate still acceptable prevalence of *Salmonella*, and Level 3 included herds with a high unacceptable prevalence (Alban et al., 2002). Farm category must be a result of several consequential serological testing (two or three) in different period (monthly or four times per year) which is for determination of "serological salmonella index" in monitoring schemes in EU members differently regulated. Number of samples from each farm is also important for estimation of seroprevalence for *Salmonellae*. In Danish *Salmonella* control program the sampling has been simplified into 60, 75 or 100 samples per herd per year depending on herd size after revision of their program in 2001. Also cut off for tested samples has been reduced from OD 40 % to OD 20 % which increases the number of seropositive samples approximately two times. Level 1 herds have an index of <40, Level 2 herds have an index between 40 and 70, and Level 3 herds have an index >70. A Level 0 category is currently being evaluated for herds in which the seroprevalence is 0 for 3 consecutive months. Three months results of the prevalence were weighed 0.2: 0.2: 0.6 where the immediate month is counting three times as much as the previous months. Producers are interested to be introduced in level 0 where herd is seronegative for *Salmonellae* in certain period (Alban et al. 2002; Benchop et al., 2008). Beginning in 2002, Germany initiated a voluntary *Salmonella* control program similar to the Danish one, and the United Kingdom introduced the Zoonoses Action Plan (ZAP) *Salmonella* monitoring program, also based on meat juice ELISA. The Netherlands and Belgium are considering similar programs (Nielsen, 2002). Presently, there is no national *Salmonella* monitoring program for pig producers in the United States or Canada. Sera collected as part of the National Animal Health Monitoring System (NAHMS) Swine 2000 Study being evaluated with the DME conducted at Iowa State University, Ames, Iowa (Turney, 2003). The Norwegian *Salmonella* surveillance and control programme (NSSCP) was launched in 1995 and has been approved by the EU (EFTA Surveillance Authority Decision No. 68/95/COL of 19 June 1995) as the background for accepting testing meat, meat products or live animals for *Salmonella* before it is allowed to enter Norway from EU member countries. The program covers activities directed towards both live animals (cattle, pig and poultry) and meat (cattle, pig, sheep and poultry) and is designed similarly to the Swedish and Finnish *Salmonella* control programmes (Hopp et al., 1999). The program includes systematic sampling in the breeding herds (BH) and random sampling of carcasses at the abattoirs in order to identify infected carcasses originating from BH, IH (integrated herds) and FH (finishing herds). The sample sizes have been calculated so that a prevalence of 5% in any breeding herd and 0.1% in the total population can be detected, assuming a diagnostic test sensitivity of 100% (Sandberg et al., 2002).

The control program was based on the assumption that there was an association between serological reaction and bacteriological *Salmonella* prevalence. This association has been described (Nielsen et al., 1995; Stege et al., 1997; Christensen et al., 1999; Sørsen et al., 2000). The general conclusion of these studies was that the serological test was effective mainly at herd-level and especially well suited to detect high prevalence herds. A central question is how to describe the association between serology and bacteriology, because the serological results from a herd may be interpreted differently (Alban et al. 2002).

In 2008 there were 43,124 breeding pigs and 432,011 fattening pigs in Slovenia, reared on 34,725 holdings. Pig production in 2010, which includes only pigs, slaughtered in slaughterhouses in Slovenia, was 241,332 for year 2010. Number of breeding pigs was 30,345

which were on 4,373 farms. From these farms there were 3,296 farms with five or less than five breeding sows. All these farms are one-site farms, which means, that all categories of pigs from breeding pigs till fatteners are located on one site. All pigs were raised indoor (Statistical office of the Republic of Slovenia, 2011).

Seroprevalence of *Salmonella* in Slovenia is low. Comparison of the seroprevalence between large and small farms shows that the number of positive breeding swine and fatteners are higher at the large farms than in small farms. The seroprevalence of fatteners from small farms was 0.1 and of breeding sows was 0.3. The seroprevalences of pigs from large farms were higher; the seroprevalence of fatteners was 0.3 and of breeding sows was 0.68 (Stukelj et al., 2004). In our Serology laboratory we tested annually 270 to 375 serum samples. Our tested farm could be classified into the level 1 according to revised Danish surveillance-and-control program for *Salmonella*. In our preliminary study we randomly selected 100 samples out of 375 tested in 2007 which would be the number of tested samples for that herd size according to Danish program. Seroprevalence to *Salmonellae* for year 2007 for mentioned farm was for all tested samples 12.8% for OD 40% and 24% for OD 20%. For randomly selected samples for the same year the prevalence was 7.5 % for OD 40% and 17% for OD 20%. We also compared results after testing with classification with weighted three months seroprevalence. Prevalence from all tested sera in the first three months in 2007 was 8% for OD 40% and 14% for OD 20%. In randomly selected samples for the same months prevalence was 7.5 % for OD 40% and 10% for OD 20%. Results from testing of all the samples and results for randomly selected samples show only differences in percentages but the classification level of the farm remains the same (Stukelj et al., 2009).

1.2 EU baseline studies of the prevalence of *Salmonella* in pigs

1.2.1 EU baseline study on the prevalence of *Salmonella* in slaughter pigs

To obtain an overview of the *Salmonella* prevalence in pigs in EU Member States (MSs) two baseline studies on the prevalence of *Salmonella* in slaughter and breeding pigs were conducted. The baseline study in slaughter pigs started on the 1st October 2006 and lasted till the 30th September 2007. Tested slaughter pigs were selected in slaughterhouses that together accounted for 80% of pigs slaughtered within each Member State (MS), which constituted the survey target population. Twenty-five EU MSs participated in the survey. Norway participated on a voluntary basis.

Slaughtered pigs with a live weight between 50 kg and 170 kg and their carcasses were randomly sampled in slaughterhouses representing at least 80% of MSs' total production of slaughtered pigs. The samples to take were stratified by the slaughterhouses' capacity (throughput) in the year 2005 and by the month. The day on which the samples were taken was also randomly chosen from all days of the month of sampling as was the slaughtered pig or its carcass from all scheduled pigs to slaughter on the selected slaughter day. From a selected slaughter pig at least 5 ileo-caecal lymph nodes weighing at least 15 grams were collected on a mandatory basis. The number of pigs to sample was 384 minimum and 2,400 maximum and was calculated for each MS. In addition, in order to assess the contamination of slaughter pig carcasses, 13 MSs (Austria, Belgium, Cyprus, Czech Republic, Denmark, France, Ireland, Latvia, Lithuania, Poland, Slovenia, Sweden and The United Kingdom) voluntarily sampled each at least 384 carcasses belonging to

the slaughtered pigs of which lymph nodes were taken. This additional sampling was done by swabbing the surface of the carcass in a standardized way, after evisceration and before chilling. Moreover, 9 MSs (Cyprus, Denmark, France, Ireland, Lithuania, Slovenia, Sweden, The Netherlands and The United Kingdom) voluntarily collected a muscle sample (to extract meat juice) or a blood sample from all pigs selected for lymph node sampling for antibody detection examination. Samples were taken by the competent authority in each MS or under its supervision.

The EU live pig population totalled 160 million heads in 2005. The largest population was in Germany, 17% of the EU live pig population. Seven MSs (Germany, Spain, Poland, France, Denmark, The Netherlands and Italy) accounted for 74% of the total EU population. Conversely, several MSs had very small live pig populations. The EU slaughtered pig population totalled 240 million heads in 2005. The largest population was in Germany, 20% of the EU slaughtered pig population. Eight aforementioned MSs plus Belgium, accounted for 81% of the total EU slaughtered pig population. Conversely, several MSs had very small slaughtered pig populations.

The cleaned validated dataset comprised data on 19,159 slaughter pigs. On the sample-level the dataset contained 18,663 samples of lymph nodes, 5,736 carcass swabs and 5,972 serological samples originating from 25, 13 and 9 MSs, respectively. The dataset also included data on 408 lymph node samples from Norway. For slaughter pigs and of lymph node samples some invalid lymph node test results were excluded. A total of 934 slaughterhouses in the EU and nine in Norway were sampled, varying from three in Cyprus and Luxembourg to up to 400 in Poland (EFSA, 2008).

Observed prevalence of slaughter pigs infected with *Salmonella* spp. in lymph nodes

It is important to note that the absence of any *Salmonella* from the tested samples does not imply that a MS is *Salmonella* - free, as firstly the detection method has a sensitivity of less than 100%, so false negative results are plausible. Secondly, the prevalence within the MS may be too low for even one positive animal to be detected with the sample size that was used. *Salmonella* spp. was found in 24 out of the 25 MSs providing data on lymph node samples of slaughter pigs. No lymph node tested positive in Finland, whereas one pig tested positive in Norway. The observed EU-level prevalence was 10.3% (95% CI: 9.2; 11.5). The unweighted prevalence (10.8%) was included in the CI 95%. Within MSs, the prevalence varied between 0.0% and 29.0%. Serovar Typhimurium was isolated in all the 24 MSs reporting positive results for *Salmonella* in lymph nodes. One pig tested positive in Norway. The observed EU-level prevalence was 4.7% (95% CI: 4.1; 5.3). The unweighted prevalence (4.2%) was included in the CI 95% CI. At the MS-level, the observed prevalence was highest in Luxembourg (16.1%). Serovar Derby was isolated in 20 MSs. No lymph node tested positive for Derby in Cyprus, Estonia, Finland, Lithuania, Sweden and in Norway. The observed EU-level prevalence was 2.1% (95% CI: 1.8; 2.6). The unweighted prevalence (1.8%) was included in the CI 95% CI. At the MS-level, the observed prevalence was highest in France (6.5%). Serovars of *Salmonella* other than Typhimurium and Derby were found in lymph nodes of slaughter pigs from 24 MSs. The observed EU-level prevalence was 5.0% (95% CI: 4.4; 5.7). The unweighted prevalence (5.6%) was included in the CI 95%. At the MS-level, the observed prevalence was highest in Greece (17.2%).

The EU prevalence of 10.3% can be interpreted as showing that one in ten pigs slaughtered in the EU was infected with *Salmonella* when slaughtered. This infection may have arisen on the farm of origin or at any time during transport to slaughter or lairage. About half of the MSs had a *Salmonella* prevalence in lymph nodes above the EU average, while the other half had prevalence below the EU mean. This was also the case for serovar Typhimurium, but less true for Derby and for serovars other than these latter two, for which fewer MSs had figures above the EU mean. It is noteworthy that although there was a large variation in the slaughter pig *Salmonella* prevalence, the serovar distribution was not remarkably varying between the MSs, because two specific *Salmonella* serovars, Typhimurium and Derby, accounted for a major part of the positive findings at the EU-level and for most *Salmonella*-positive MSs. All 24 *Salmonella*-positive MSs isolated *Salmonella* Typhimurium and 20 detected *Salmonella* Derby. These two serovars are common serovars found in *Salmonella* infection cases in humans, and are both amongst the ten most frequently reported serovars in humans (EFSA, 2008).

Observed prevalence of carcasses contaminated with *Salmonella* spp.

Salmonella spp. was found in 11 out of the 13 MSs providing data on surface swabs-sampling of carcasses. No carcass swabs tested positive in Slovenia and Sweden. The observed 13 MS-group level prevalence was 8.3% (95% CI: 6.3; 11.0). At the MS-level, the observed prevalence was highest in Ireland (20.0%). For this 13 MS-group the observed prevalence of slaughter pigs infected with *Salmonella* spp. in lymph nodes was estimated as 9.6% (95% CI: 8.2%; 11.1%). Thus, one in 12 pig carcasses produced in this group of 13 MSs was contaminated with *Salmonella*. This estimation cannot as such be extrapolated to the level of the EU, because this group of MSs may not be representative for all MSs. One group of participating MSs had a prevalence above the weighted average (Belgium, France, Ireland and the United Kingdom), and the other one below the average (Austria, Cyprus, Czech Republic, Denmark, Latvia, Lithuania, Poland). This was the case for *Salmonella* spp., for serovar Typhimurium, and to a lesser extend for Derby. It was not the case for serovars other than the two latter ones.

Serovar Typhimurium was isolated in 10 MSs reporting positive results for *Salmonella* in carcass swabs. No carcass swabs tested positive in Latvia, Slovenia and Sweden. The observed 13 group-level prevalence was 3.9% (95% CI: 2.8; 5.5). At the MS-level, the observed prevalence was highest in Ireland (11.7%). Serovar Derby was isolated in 10 MSs. No carcass swabs tested positive in Cyprus, Slovenia and Sweden. The observed 13 MSs group-level prevalence was 2.6% (95% CI: 1.7; 3.9). At the MS level, the observed prevalence was highest in France (5.9%). Serovars of *Salmonella* other than Typhimurium and Derby were found on carcass swabs from 11 MSs. No carcass swabs tested positive in Slovenia and Sweden. The observed 13 group level prevalence was 2.3% (95% CI: 1.6; 2.5). At the MS-level, the observed prevalence was highest in France (4.8%).

It is again noteworthy that although there was a large variation in the prevalence of *Salmonella* contaminated carcasses, the serovar distribution was not remarkably varying between these MSs, because two specific *Salmonella* serovars, Typhimurium and *D*erby, accounted for a major part of the positive findings at the EU-level and for most *Salmonella*-positive MSs. The contamination of the carcasses occurred in the slaughterhouse and may have been due to infection within the pigs or from the slaughterhouse environment. For this 13-MS group the carcass swab *Salmonella* spp. prevalence appears to be similar to the

lymph node prevalence. At the MS-level, the prevalence of contaminated carcass swabs tended to be similar or lower than the prevalence of slaughter pigs infected with *Salmonella* spp. in lymph nodes in 11 of the 13 MSs. Conversely, in two MSs (Belgium and Ireland) the prevalence of contaminated carcass swabs seemed higher than the prevalence of infected lymph nodes. However, sample size calculations have not been predicated for such comparison.

In this survey the carcass swab represents the closest sampled point to the exposure of the consumer, at the beginning of the food chain. Thus, since the imperative for control of *Salmonella* in pigs is the protection of public health, there is an argument that the carcass swab is the most appropriate measure of those utilised in this survey. Further, individual MSs might choose whether intervention at the farm, the slaughterhouse or some combined strategy afforded the best option for their particular circumstances (EFSA, 2008).

Observed prevalence of slaughter pigs with antibodies against *Salmonella*

Amongst the 9 participating MSs, two used the Salmotype Pig Screen® ELISA by Labor Diagnostik Leipzig, three MSs used the HerdCheck Swine *Salmonella*® ELISA by IDEXX, two MSs used an in house ELISA, one MS used the VetSign Porcine *Salmonella*® ELISA by Guildhay, and one MS used both the Salmotype Pig Screen® ELISA and the HerdCheck Swine *Salmonella*® ELISA. The NRLs used the cut-off of their choice. Eight MSs reported their results as relative optical densities (OD%) and one MS reported his results in S/P ratio (sample value related to positive control value). It was difficult to estimate the real seroprevalnece because of some inconclusive results, which could be counted as positive, negative or missing.

Seroprevalence (presence of *Salmonella* antibodies in meat juice or in sera) is a measure of the prior exposure of the pig to *Salmonella* infection. Due to the diversity of tests and cut-off points employed by the 9 MSs that chose to collect these samples, no group level prevalence can be estimated. The sensitivity and specificity of these tests is not precisely known and in most MSs, some inconclusive results were reported. The seroprevalence amongst these 9 MSs was estimated to have been as low as 2.2% (lower boundary of 95% CI, classifying inconclusive results as negative) in Sweden to as high as 41.6% (upper boundary of 95% CI, classifying inconclusive results as positive) in Cyprus.

The future value of testing of serological samples probably lies in their application within a MS for surveillance purposes and identification of positive herds, since these tests are relatively cheap, sample collection is straightforward and can be done by a slaughterhouse technician and in the case of meat samples, can be frozen for transport and batch testing. However, it should be recalled that these samples are poor predictors of the *Salmonella* status of the individual pig or carcass. This was further underpinned by the survey concordance-discordance results, at the MS-level, between the test for *Salmonella* spp. using lymph nodes and meat juice and sera samples. These analyses results revealed no to low agreement (EFSA, 2008).

Frequency distribution of *Salmonella* serovars in lymph nodes and carcass swabs

The serotyping of *Salmonella* isolates was mandatory according to the technical specifications of the survey. At least one isolate from each positive sample was to be typed

according to the Kaufmann-White Scheme. Results from any sample where the serovar information was not available for any isolate were excluded from the final dataset. In total there were 2,600 *Salmonella*-positive lymph node samples. Two different *Salmonella* serovars were isolated from three *Salmonella*-positive lymph nodes. Eighty-seven different serovars were isolated from the lymph nodes of slaughter pigs across the EU. Serovars Typhimurium and Derby were highly predominant. Serovar Typhimurium was the most frequently reported serovar from the slaughter pigs' lymph nodes in EU and Norway, isolated in 40.0% of the *Salmonella* positive slaughter pigs, and reported by all (24) MSs having found *Salmonella* positive slaughter pigs and by Norway. The next common reported serovar was Derby, isolated from 14.6% of the positive slaughter pigs. Serovar Derby was also the second serovar most commonly isolated in terms of number of reporting MSs (20). Serovars Rissen and monophasic 4,[5],12:i:- were the third and the fourth most frequently recovered serovars, with an isolation rate in lymph nodes of 5.8% and 4.9%, respectively. Serovar Rissen was isolated in five MSs and *S.* 4,[5],12:i:- in eight MSs. Serovar Enteritidis was the fifth most common reported serovar and recovered in 19 MSs, in particular in Cyprus, Estonia, Poland and Slovenia where it was the most frequently isolated serovar in lymph nodes.

There were a total of 387 carcasses testing positive for *Salmonella* by surface swab-sampling in the 13 MSs. Thirty different serovars were isolated on the surface of the slaughter pig carcasses. Serovar Typhimurium was the most frequently recovered serovar from the surface of the slaughter pig carcasses in EU, representing 49.4% of the *Salmonella* positive carcasses. The second most frequent serovar was Derby (24.3% of the positive carcasses). The three next most frequent serovars were Infantis, Bredeney, and Brandenburg (3.4%, 2.1% and 1.8% of the positive carcasses, respectively). Serovar Typhimurium was the dominant serovar in 10 MSs. In Austria and in Poland, serovar Derby was isolated as frequently as Typhimurium.

A greater diversity of *Salmonella* serovars were isolated from lymph nodes than from carcass swabs, although there were five serovars that were only isolated from carcass swabs. Firstly, carcass swabs were collected from fewer MSs and secondly, the overall prevalence of *Salmonella* positive swabs was lower than that of lymph node samples within those MSs that tested both. The number of bacteria that may be collected from a carcass is also likely to be lower than the number found in the lymph node of an infected pig except in case of extreme contamination. Finally, the presence of *Salmonella* on a carcass swab may reflect post-slaughter contamination with serovars that exist in the slaughterhouse environment as well as infection originating from within the slaughtered pigs.

Serovar Typhimurium was isolated in all of the 24 MSs that found *Salmonella* in lymph node samples and in Norway. It was the most frequently isolated serovar in all MSs except Bulgaria (Derby), Cyprus (Enteritidis), Estonia (Enteritidis), Italy (Derby), Latvia (Brandenburg), Poland (Enteritidis), Slovenia (Enteritidis) and Slovakia (Derby). In six of these 8 MSs, serovar Typhimurium was the second most common serovar to be isolated whilst in Bulgaria, serovar Infantis was the second most prevalent serovar and in Latvia, where Derby came second. Serovar Typhimurium has long been recognised in many European countries as a common serovar amongst pigs although it has a wide host range and has also been isolated from domesticated mammals and poultry species. Overall, *S.* Typhimurium accounted for 40% of the serovars isolated in the survey.

In 18 of 24 MSs that isolated *Salmonella* from lymph nodes, serovar Derby was amongst the top three serovars to be isolated. In Spain and Portugal, serovar Derby was ranked fourth whilst it was not detected in Cyprus, Estonia, Lithuania or Sweden. It is widely recognised as a common serovar in pigs although it does occur in other livestock species. It accounted for 14.6% of the S*almonella* isolated in this survey.

A wide range of other serovars were also detected, many in very low numbers. Serovar Enteritidis, which is usually associated with poultry, was found in 19 MSs and from 4.9% of all lymph node samples. It was as noted above, the most common isolate in Cyprus, Estonia, Poland, and Slovenia and the second most frequent isolate from Austria, Czech Republic, and Hungary. Serovar Enteritidis is the most frequent cause of human salmonellosis in the EU.

It can further be mentioned that *S.* Typhimurium and *S.* Derby were the most frequent serovars both in lymph nodes and on the surface of carcasses, suggesting that the serovars that exist in the slaughterhouse environment come mainly from the infected pigs that are slaughtered there. Overall, this survey demonstrates a wide variation in the distribution of *Salmonella* serovars in slaughter pigs and the presence of two dominant serovar in this species (EFSA, 2008).

Interpretation of the results from each of the three used survey tests

Salmonella infection results from ingestion or occasionally inhalation of viable bacteria. In pigs, infection within the intestinal tract may be followed by invasion of the cells of the gut and thence, infection is established in the intestinal lymph nodes. It is possible for pigs to ingest material containing *Salmonella* and for this to be in passive transit through the gut without actively establishing infection. Infected pigs may become carriers and excrete *Salmonella* in their faeces intermittently. Therefore, the presence of *Salmonella* within the lymph node is incontrovertible evidence that a pig is infected, as it is very unlikely that *Salmonella* can be isolated from lymph nodes of uninfected pigs and false positive results are rare. However, the test sensitivity is not 100% and there may therefore be false negative results. *Salmonella* excretion by carrier pigs is thought to be provoked by stress and may occur as the pigs are loaded and transported to the slaughterhouse. It is possible for pigs to become infected and for that infection to be transferred to the intestinal lymph nodes in a matter of hours. Therefore, a positive lymph node result may reflect infection on the farm of origin or during transport or lairage. The longer the duration of the transport and lairage phases, the more contaminated the environment during those phases, and the more stressful the conditions that are experienced, the greater the risk of infection occurring after departure from the farm.

Presence of *Salmonella* on carcass swabs reflects the surface contamination of the carcass. Although this may occur during transport or in the lairage, normal slaughterhouse practices including passing pigs through a scald tank and singeing to remove bristles act to reduce *Salmonella* contamination. Presence of *Salmonella* infection in the pig need not result in carcass contamination unless e.g. there is faecal leakage from the anus or the gut is accidentally nicked during processing. *Salmonella* may also survive in slaughterhouse environments, especially in equipment that is difficult to clean thoroughly. Poor hygiene in a slaughterhouse or amongst staff may also result in contamination of carcasses and one

contaminated carcass may touch others, resulting in cross-contamination. Thus, the prevalence of positive carcass swabs is a product of the risk of infection within a pig, the risk that the infection is released to the exterior and the risk of cross-contamination from other carcasses or the slaughterhouse environment. It is predictable that presence of *Salmonella* in the gut is not completely associated with carcass contamination. It is also important to consider that the presence of *Salmonella* infection in the intestinal lymph nodes, which are removed from the carcass and are not consumed, may only represent a limited public health threat whilst a contaminated carcass is likely to be a greater risk to public health as the carcass is the start of the food chain.

Salmonella infection stimulates an immune response and circulating antibodies can be detected in blood, serum or meat juice. As antibodies persist beyond the time of infection, unsurprisingly a positive serological result is a poor indicator of current infection. Infection during transport to a slaughterhouse or in lairage does not result in a seropositive reaction, as there is insufficient time for a detectable immune response to occur before death. However, the prevalence of seropositive pigs does give a good estimate of the lifetime exposure to *Salmonella*. Therefore, it may be a valuable tool for surveillance of *Salmonella* infection on farms as part of a control programme (EFSA, 2008).

Conclusions

The main conclusions made by reporting team were:

- The survey provides valuable data for risk managers on the prevalence and distribution of *Salmonella* in EU MSs, and results are suitable to be used for setting targets for the reduction of the frequency of the *Salmonella* infection in slaughter pigs in the EU.
- Three tests were used in the survey: bacteriological tests of lymph nodes and of carcass swabs and a test for antibodies. *Salmonella* prevalence in lymph nodes reflects the infection of the pigs at the level of the primary production (i.e. on the farm and during subsequent transport and lairage). *Salmonella* contamination of the carcass may derive from the infection within the pig or from the slaughterhouse environment, whereas the presence of antibodies reflects past exposure of the pigs to *Salmonella*.
- The observed prevalence of slaughter pigs infected with *Salmonella* spp. varied widely amongst MSs.
- A large variety of serovars of *Salmonella* were isolated from ileo-caecal lymph nodes of slaughter pigs in the EU.
- A more limited range of serovars was identified on the surface of carcasses.
- With regard to seroprevalence, the observed estimates in slaughter pigs varied among the 9 participating MSs. However, these seroprevalence estimates are not directly comparable because of different tests and different thresholds used within participating MSs. No prevalence was therefore estimated at the MS-group level. Credible estimate of prevalence amongst these MSs varied from as low as 2% to as high as 42% (EFSA, 2008).

1.2.2 EU baseline study on the prevalence of *Salmonella* in holdings with breeding pigs

European Union Baseline survey on the prevalence of *Salmonella* in holdings with breeding pigs was carried out at farm level to determine the prevalence of *Salmonella* in pig breeding

holdings. The herds were randomly selected from holdings constituting at least 80% of the breeding pig population in a Member State.

Sampling took place between January 2008 and December 2008. A total of 1,609 holdings housing and selling mainly breeding pigs (sows or boars of at least six months of age kept for breeding purposes) (breeding holdings) and 3,508 holdings housing breeding pigs and selling mainly pigs for fattening or slaughter (production holdings) from 24 European Union Member States, plus Norway and Switzerland were included in the survey. In each selected breeding and production holding, fresh voided pooled faecal samples were collected from 10 randomly chosen pens, yards or groups of breeding pigs over six months of age, representing the different stages of production of the breeding herd (maiden gilts, pregnant pigs, farrowing and lactating pigs, pigs in the service area, or mixed). The pooled samples from each holding were tested for the presence of *Salmonella* and the isolates were serotyped.

The overall European Union prevalence of *Salmonella*-positive holdings with breeding pigs was 31.8% and all but one participating Member State detected *Salmonella* in at least one holding. Twenty of the 24 Member States isolated *Salmonella* in breeding holdings and at European Union level 28.7% of the holdings were estimated to be positive for *Salmonella*. This prevalence varied from 0% to 64.0% among the Member States. The estimated European Union prevalence of breeding holdings positive to serovar Typhimurium and to serovar Derby was 7.8% and 8.9%, respectively. Twenty-one of the 24 Member States isolated *Salmonella* in production holdings and at the European Union level 33.3% of the production holdings were estimated to be positive for *Salmonella*. This prevalence varied from 0% to 55.7% among the Member States. The estimated European Union prevalence of production holdings positive for serovars Typhimurium and Derby was 6.6% and 9.0%, respectively. For the two non-Member States, Switzerland detected *Salmonella* in both breeding and production holdings while Norway did not detect any *Salmonella* in its surveyed holdings.

The number of different *Salmonella* serovars isolated in breeding holdings and production holdings across the European Union was 54 and 88, respectively. Serovar Derby was the most frequently isolated serovar in both breeding and production holdings, detected in 29.6% and 28.5% of the *Salmonella*-positive holdings, respectively. The next most commonly isolated serovar was serovar Typhimurium accounting for 25.4% and 20.1% of *Salmonella*-positive breeding holdings and production holdings, respectively. These serovars were also commonly found in the EU-wide baseline survey of fattening pigs at slaughter in 2006-2007. The next most frequently reported serovars were serovars London, Infantis and Rissen both in breeding and production holdings and each accounted for approximately 7% of the positive holdings, in each type of holding. Also *Salmonella* isolates with the incomplete antigenic formula 4,[5],12:i:-, which are likely to be related to the recent emergence of monophasic serovar Typhimurium, were reported by several Member States.

Salmonella infection in breeding pigs may be transmitted to slaughter pigs through trade and movement of live animals and contamination of holding, transport, lairage and slaughter facilities. This may lead to *Salmonella*-contamination of pig meat and consequently to human disease. Further studies in surveillance and control methods for *Salmonella* in breeding pigs

as well as in the public health importance of consumption of meat from culled breeding pigs are recommended. Also investigations on the epidemiology of monophasic serovar Typhimurium would be welcome. The results of this survey provide valuable information for the assessment of the impact of *Salmonella* transmission originating from holdings with breeding pigs as a source of *Salmonella* in the food chain. These baseline prevalence figures may be used for the setting of targets for the reduction of *Salmonella* in breeding pigs, to follow trends and to evaluate the impact of control programmes (EFSA, 2009a).

1.3 Objectives of our investigation

The objectives of our investigations were to obtain an overview on *Salmonella* prevalence in pigs in Slovenia, which was part of EU Baseline study on the prevalence of *Salmonella* in slaughter pigs in 2008. Within this study Slovenia was one of the 9 countries that voluntarily included also detection of antibodies against *Salmonella* in meat juice. To assess the suitability of antibody detection for *Salmonella* we had previously monitored one of our big holdings already in 2007 (Stukelj et al., 2009).

2. Baseline study on the prevalence of *Salmonella* in slaughter pigs in Slovenia

2.1 Materials and methods

2.1.1 Pigs and holdings

In the EU baseline study on the prevalence of *Salmonella* in slaughter pigs 440 pigs from 178 holdings in Slovenia were tested. Almost a half of the pigs (212 or 48% of all tested) originated from small holdings (163 or 92% of all tested), which were represented in this study by only one to three pigs. For *Salmonella* isolation intestinal lymph nodes (minimum 15 grams) and carcass surface swabs were collected at slaughter and a piece of either diaphragm or neck muscles were collected for detection of antibodies in meat juice. Samples were sampled by official veterinarians and proceeded to Veterinary Faculty, National Reference Laboratory for *Salmonella*.

2.1.2 Detection of *Salmonella*

Isolation and identification were performed according to ISO/FDIS 6579, Annex D: 2007. We used Buffered peptone water (Biolife) for pre-enrichment, enrichment on Modified semisolid Rappaport-Vasilliadis agar (MSRV, Biocar) and plating on Xylose-lisine-desoxicholat agar (XLD, Biolife) and Rambach agar (Merck). *Salmonella* suspicious colonies were identified biochemically either by API 20 E (Biomérieux) or Crystal Enteric/nonfermenter ID kit (BBL). Serovars were identified by slide agglutination with STATENS SERUM INSTITUT *Salmonella* antisera according to White-Kauffman-Le Minor scheme (Grimont and Weill, 2007).

2.1.3 Antibody detection

The diaphragm samples were stored in plastic bags in freezer at -18° C. Before testing with ELISA, bags were taken from the freezer and the diaphragm samples were thawed, the

angles of the plastic bags were cut and the meat juices from each bag were poured over to the micro tubes. The samples were prepared for further testing.

The Swine Salmonella Antibody IDEXX ELISA allows rapid screening for the presence of antibodies to three *Salmonella enterica* serogroups indicating swine herds' exposure to the bacteria. The assay is designed to detect antibodies to *Salmonella* in swine serum, plasma and meat juice. LPS antigen (serogroups O:4 (B), O:7 (C1) and O:9 (D1)) is coated on 96-well plates.

The presence or absence of antibody to *Salmonella* in the sample was determined by relating the absorbance value at 650 nm of the unknown to the positive control mean by calculating the sample to positive (S/P) ratio. In many countries and/or laboratories the results are calculated in OD% referring to a set of standard sera, defined according to the Danish Mix-ELISA system. To obtain a result comparable to this OD% scale, a correlation factor has been experimentally determined. The S/P value was divided by this factor to give an approximate OD% value. Samples with OD% equal or grater than 40% (S/P = 1. 0) were considered positive in general screening, and samples with OD% equal or grater than 20% (S/P = 0. 5) were considered positive in more stringent screening.

2.2 Results

2.2.1 Prevalence of *Salmonella* in pigs and holdings

All the carcass swabs tested negative for *Salmonella*. From lymph nodes of 28 pigs (6.36%) from 18 holdings (10.11%) we isolated *Salmonella enterica* subsp. *enterica*, belonging to 13 serovars, including four of five serovars of public health importance: Enteritidis (7 pigs from 6 holdings), Typhimurium (3 pigs from 3 holdings), Virchow (2 pigs from 2 holdings) and Infantis (1 pig from 1 holding). All the serovars belonged to sero-groups O:4 (B), O:7 (C1) and O:9 (D1), which are covered by IDEXX ELISA, used for antibody detection. From some bigger holdings, represented by 11 to 65 pigs, we isolated two to four different serovars.

For antibody detection we used two criteria: OD 40% and OD 20%. In IDEXX ELISA at OD 20% 91 (20.68%) pigs from 45 (25.28%) holdings tested positive. At OD 40% 48 (10.91%) pigs from 25 (14.04%) holdings, tested positive. This means that 52.75% of pigs positive at OD 20% from 55.56% serologically positive holdings reacted with high antibody titres.

Of 178 holdings in 102 (57.30%) holdings, represented by 165 (37.50%) pigs, all pigs tested negative in both tests. Another 165 pigs (37.50%) originating from 16 positive holdings (either by culture or ELISA) tested negative in both tests. All together 110 (25.00%) pigs from 50 (28.09%) holdings tested positive either by culture or ELISA and 9 (8.18%) of these 110 pigs from 6 holdings (12.00%) tested positive in both tests. Of 18 holdings positive by culture, 13 (72.22% of positives) were represented by 122 pigs (27.73% of all pigs). Of these 122 pigs 67 (54.92%) tested positive either by culture or ELISA or both and 55 (45.08%) tested negative both by culture and ELISA. Of 5 (27.78%) holdings with pigs testing positive only by culture and represented by 9 pigs (2.05% of all pigs), 6 pigs were positive by culture and 3 negative. Percentage of positive pigs (either by culture or ELISA) within holdings varied considerably. In the holdings represented by at least 10 pigs the range of ELISA positive pigs at OD 20% was from 5.00% to 53.33% with the average 22.91% and at OD 40% it was from 0.00% to 53.33% with the average of 15.46%. The results of culture and ELISA positive pigs and holdings are presented in Figure 1 and 2 respectively.

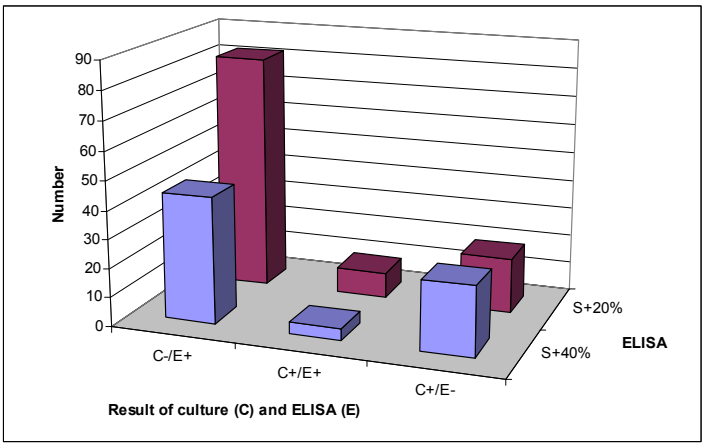

Fig. 1. Results of culture and ELISA positive pigs (N = 110).

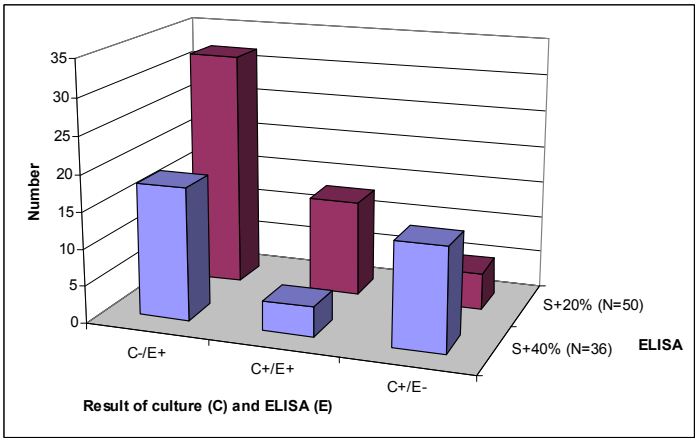

Fig. 2. Results of culture and ELISA positive holdings (N = 50).

2.2.2 Suitability of diaphragm and neck muscle meat juice

We also compared the results of ELISA of meat juice from diaphragm and neck muscles. For 9 pigs we did not have the data on the sampling site, so the results of altogether 439 pigs were processed. Meat juices of 304 (70.53%) pigs were from diaphragm and 127 (29.47%) from neck muscles. From diaphragm 71 (23.36%) meat juices were positive and from neck muscles 17 (13.39%). In the chi-square test the difference was statistically significant ($t = 4.88$, $P < 0.05$). Since different holdings were represented by different number of pigs, we compared also holdings from which only samples of diaphragm muscles, only neck muscles or both were tested. We had data for 175 holdings, of which 132 (75.43%) were represented only by diaphragm meat juices, 34 (19.43%) by neck muscle juices and 9 (5.14%) by both meat juices. Of the holdings with only diaphragm meat juices 35 (26.52%) had at least one positive pig. Of the holdings with only neck muscles juice 7 (20.59%) had at least one

positive pig. Of the holdings with both juices 5 (55.56%) had at least one positive pig. Since the number of holdings with both juices was low and the proportion of pigs with either of juices varied greatly within holdings, we compared only holdings with one type of juice. In the chi-square test the difference was not significant (t = 0.24, P <0.05). The comparison of diaphragm and neck muscles' ELISA are presented also in Figure 3.

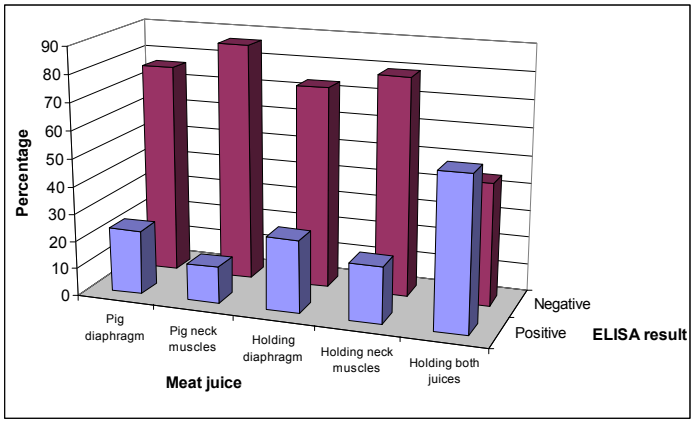

Fig. 3. Comparison of pig and holding meat juices' results.

2.2.3 Comparison of ELISA and culture

We found no correlation between culture and ELISA results. In the chi-square test the difference between them was statistically highly significant (t = 39.523, P< 0.005). We present the results of culture and ELISA in the holdings represented with at least 10 pigs in the Figure 4.

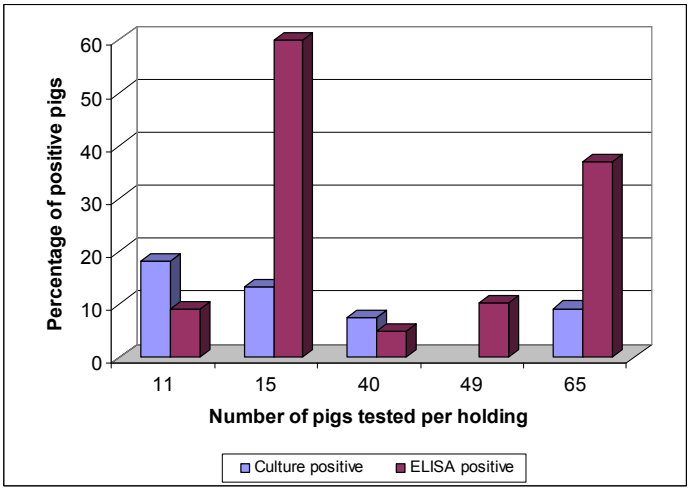

Fig. 4. Comparison of ELISA and culture.

2.2.4 Sampling for *Salmonella* reduction program

What happens if we do not have enough results over the whole year is presented in the figures 5, 6 and 7. In the figure 5 we present the results of holding A. The sampling covers all the twelve month, but the number of samples is too small, so the results are not reliable enough. We isolated three different serovars of *Salmonella*. The first was serovar Virchow in December and the next was Derby in January. In January and February high levels of antibodies were detected. Till August when serovar Coeln was isolated, there were no isolates. Its effect on seroconversion can not be estimated due to low number of samples.

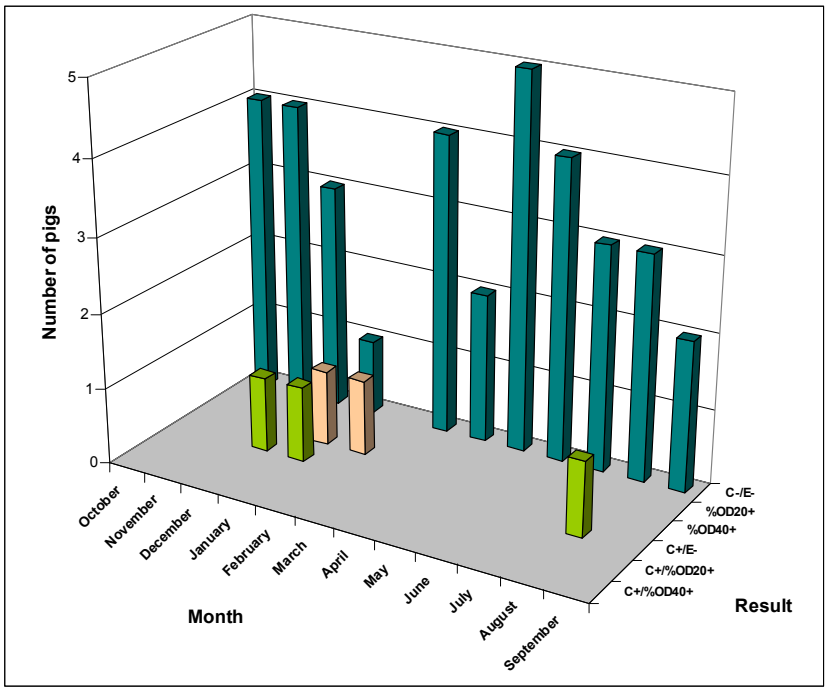

Fig. 5. Results of holding A over the year (N = 40 pigs tested; C = culture, E = ELISA).

In the holdings B and C there was no sampling in the second half of the study (spring and summer). In the holding B only 7 months were covered, and in the holding C only 6 months. In the holding B we found only some seroconversion, but no positive culture for *Salmonella* (Figure 6).

In the holding C we found seroconversion and culture positive pigs, but we didn't have an overview over the whole year. In October it seems that seroconversion remained from previous infection. In October we isolated serovar Choleraesuis var. Decatur, in November serovar Heidelberg and in December serovar Enteritidis. In February we isolated serovar Infantis and in March Enteritidis. From April to September, when the rate of *Salmonella* infection in humans is usually the highest, there were no samples. Some seroconversion was detected in October, which increased till January when the highest number of pigs positive at %OD 40 was detected. (Figure 7).

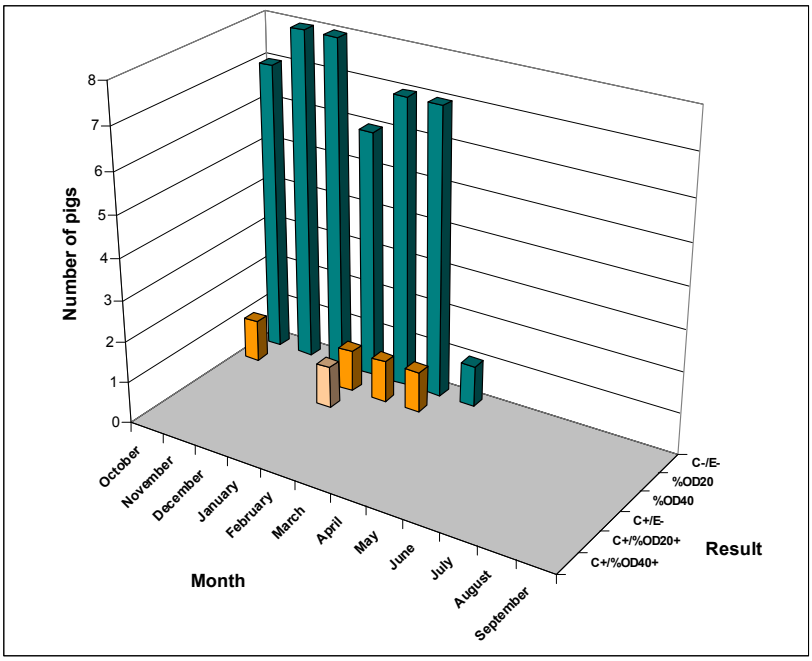

Fig. 6. Results of holding B over the year (N = 49 pigs tested; C = culture, E = ELISA).

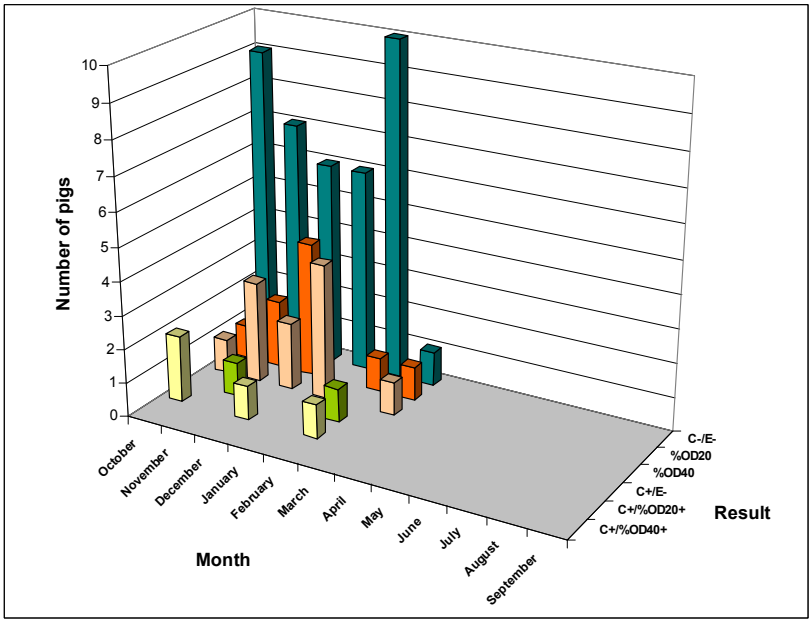

Fig. 7. Results of holding C over the year (N = 65 pigs tested; C = culture, E = ELISA).

2.3 Discussion

Current surveillance of *Salmonella* in pigs involves intensive and expensive scheme for herd classification. Isolation of *Salmonella* from lymph nodes is believed to reflect long-term exposure at herd level, but might indicate infection during transport and lairage. Bacteriological techniques for *Salmonella* detection are reported to have very high specificity (up to 100%) but sensitivity is low (Funk et al., 2000). In the EU Interlaboratory comparison study food II (2007), organized by CRL-*Salmonella*, the sensitivity of use of MSRV and XLD media depended on serovar and number of colony forming units (cfu) in samples. It ranged from 54.3% for 10 cfu of serovar Enteritidis to 100.0% for 50 cfu of serovar Typhimurium (Kuijpers et al., 2008). Besides, *Salmonella* are usually localized focally, so thorough homogenisation of samples is important. In faecal and dust samples competitive microflora might also lower the recovery of *Salmonella*.

ELISA detects specific antibodies against *Salmonella* and therefore it indicates past or resent exposure and different serological stages. According to producers manual the IDEXX Swine *Salmonella* Ab ELISA test specificity is 99.4%. Among *Salmonella* negative herds, some might be misclassified due to small number of pigs tested per holding - in 126 (70.79%) holdings in our Baseline study only one. More herds would be expected to be positive, if more samples had been collected. To improve herd test sensitivity, more samplings of herds would have been desirable (Baptista et al., 2010). CRL-*Salmonella* organized comparability of different ELISAs on the detection of *Salmonella* spp. antibodies in meat juice and serum. Ten national reference laboratories participated, using four different ELISA kits. The kits were designed to detect antibodies against *Salmonella* serogroups O:4 (B) and O:7 (C1) and one of them also against O:9 (D1). Laboratories used different OD% cut-off values from 15% to 40%. The comparison of results between laboratories was difficult. In nine of ten NRLs the results were significantly different from the results of CRL (Berk et al., 2008). Similar results were observed by Vico who compared three commercial enzyme-linked immunosorbent assays for meat juice samples. When these three kits were used in the same herd, the results deffered substantially. Thus caution is advised if it is decided to use these assays for herd health classivication in *Salmonella* control programs (Vico et al., 2010).

In our study, the seroprevalence at cut-off 20 OD% was in pigs 20.7% and in holdings 25.3%. The seroprevalence for cut-off 40 OD% in pigs was 10.9% and in the holdings 14%. Results from the bacteriological testing showed 6.4% positive pigs from 10.1% positive holdings. The results of statistical analysis showed the poor correlations between serology and bacteriology in pigs and in holdings.

In the instructions for sampling in the baseline study both meat juices from neck muscles and diaphragm were treated as equivalent. Our results also show that at the holding level there were no significant differences, although at pig level the difference seemed significant. We attribute this to the difference of seroprevalence between holdings and the differences in the numbers of pigs tested per single holding.

EU Member States approach the problem of reduction of *Salmonella* prevalence in pigs with different reduction programs. They categorize holdings in categories regarding seroprevalence. Danish *Salmonella* surveillance and control program in slaughter pigs was introduced in 1995 and started with cut-off for positive serology result 40 OD%. In August 2001 a new assignment was introduced which among others included reduction from cut-

off 40 OD% to 20 OD% in the interpretation of the individual meat juice sample results (Alban et al., 2002). German *Salmonella* surveillance and control program in slaughter pigs classified herds in three categories: I (0-20%), II (20-40%), III (<40%) by their percentage of yearly positive samples, which was re-calculated quarterly. The number of participating herds increased continuously since the start of the monitoring program, with regional differences in the degree of participation. In the forth quarter of 2008, 81.9% of the herds were allocated to category I, 14.0% to category II and 4.0% to category III. However, the prevalence of *Salmonella* tended to decrease in herds that participated over of long period (Merle et al., 2011). In Slovenia only one holding sent samples monthly, so it was used in our preliminary study. Seroprevalence to *Salmonellae* for year 2007 for the mentioned holding was for all tested samples 12.8% for OD 40% and 24% for OD 20%. For randomly selected samples for the same year the prevalence was 7.5 % for OD 40% and 17% for OD20 % (Stukelj et al., 2009). In the baseline study in 2008 this holding was represented by 9 pigs, sampled in four months over summer and only one pig tested positive at OD 20%. The example of the three holdings (A, B, C) from the baseline study clearly shows the necessity of monthly testing of relevant number of pigs. In Danish *Salmonella* surveillance and control program herds with annual kill less than 100 pigs were excluded; they were considered insignificant, because of pigs from such herds only constituted around 1% of the total number of pigs slaughtered at the time of study. Also relatively more animals would need to be sampled to estimate the prevalence in these herds with an acceptable precision. The minimal number of tested pigs was 60 per year (Alban et al., 2001). In the baseline study 168 holdings (94.4% of all tested) in Slovenia were represented only by 1-5 pigs. Also in Slovenia such a program for monthly testing with relevant number of pigs would be appropriate but adapted to the high percentage of small herds. Sandberg et al. reported that the unit for testing should be the herd rather than the individual animal. The sampling should focus on the larger herds that supply most of the meat in the market and on the herds that distribute sows and piglets to other herds and can thus contribute to the spread of *Salmonella* among herds (Sandberg et al., 2002).

Sørensen et al. reported that they found no linear association between the proportion of positive lymph nodes and herd serology. In general, the highest proportion of positive pigs was observed for finishers originating from herds with seroprevalences varying from 61-70% (Sørensen et. al., 2004). CRL-*Salmonella* came to the same conclusion that there is no correlation between antibody levels and detection of *Salmonella* from lymph-nodes and carcass swabs (Berk et al., 2008). Österberg et al. reported that the seroconversion and excreting of *Salmonella* were serovar and dose-dependent. Pigs inoculated with levels of 10^6 and 10^9 cfu of serovar Derby produced specific antibodies, while pigs inoculated with 10^3 cfu of Derby or serovar Cubana produced no detectable antibody levels (Österberg et al., 2009). Within our study we compared individual pigs instead of serological statuses of the farm to *Salmonella*. The *Salmonella* prevalence in lymph nodes in individual pig is irrespective of herd serology. The presence of *Salmonella* in the lymph nodes may be caused by an infection so early in the pig's life that the serological response is no longer there, but bacteria has remained in the lymph nodes, or the pig has been infected very recently (Sørensen et. al., 2004). We, too, had cases, where pigs were culture positive and serologically negative. The *Salmonella* bacteria probably related to infection in the herd, but this infection may have occurred several weeks or months prior to slaughter. If the aim is to monitor the *Salmonella* prevalence in the herd, than herd serology is better indication than in

caecal lymph nodes. Additionally, the presence of *Salmonella* in intestinal lymph nodes has a negligible impact on food safety as they are neither cut nor eaten. Usually leaking of intestinal content is more likely and more dangerous cause of carcass contamination with *Salmonella* and other enteric pathogens, so the technology and the way of handling pigs and their carcasses in slaughterhouse is very important. The results of De Busser et al. indicate that the lairage area is primary source of *Salmonella* in slaughter pigs and the carcass contamination originates from the environment rather than from the pig (inner contamination) itself (De Busser et al., 2011). Despite this, some countries used analyses of caecal lymph nodes to measure the *Salmonella* prevalence in pigs and herds.

The strong correlation between bacteriological findings and herd serology indicates that despite the fact that most *Salmonella* infections are silent in pigs, they nevertheless undergo an infectious process that results in immune response. The question is how *Salmonella* should be measured? Bacteriological measures as well as the measure of antibodies are strongly correlated. Therefore, four bacteriologically tested sites (carcass surface, pharix, lymph nodes, caecal content) and herd serology can in principle be used. The results of the study conducted by Sørensen et. al. demonstrated a strong association between herd serology measured by use of Danish mix - ELISA and the presence of *Salmonella* in caecal - contents, or carcass surface, and in pharynges, but not in caecal lymph nodes. This applies to Danish conditions where the transport time and duration of lairage is short (Sørensen et. al., 2004). The transport time and duration of lairage is short also in Slovenia, so similar measures would be indicated. In this study we tested by culture only lymph nodes and compared the results with serology. The results were not comparable, which was also expected from other studies (Sørensen et. al., 2004). This can also be expected in the case that *Salmonella* remains only in intestine and is occasionally excreted in environment.

3. Conclusion

For food safety assurance both approaches can be valuable. Antibody detection is an indicator of possible previous or on-going *Salmonella* infection in a herd, while *Salmonella* detection by culture in faeces, lymph nodes or animal environment indicates possible threat of food contamination, especially with serovars of public health importance. To obtain a reliable overview of the *Salmonella* prevalence in individual holdings regular monthly testing of relevant number of pigs is mandatory. Hygienic measures during pig production in holdings and in food production in slaughterhouses and food production plants are the key to reduction of *Salmonella* problem in humans.

4. Acknowledgment

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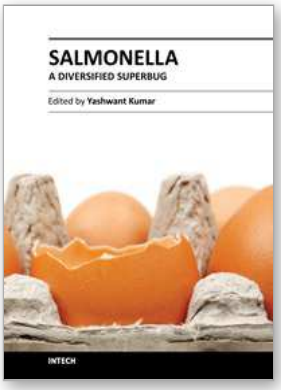

Salmonella - A Diversified Superbug
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Salmonella is an extremely diversified genus, infecting a range of hosts, and comprised of two species: enterica and bongori. This group is made up of 2579 serovars, making it versatile and fascinating for researchers drawing their attention towards different properties of this microorganism. Salmonella related diseases are a major problem in developed and developing countries resulting in economic losses, as well as problems of zoonoses and food borne illness. Moreover, the emergence of an ever increasing problem of antimicrobial resistance in salmonella makes it prudent to unveil different mechanisms involved. This book is the outcome of a collaboration between various researchers from all over the world. The recent advancements in the field of salmonella research are compiled and presented.

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Phone: +86-21-62489820
Fax: +86-21-62489821