Presence of emerging Salmonella spp. serovars in pig farms: a risk to public health

Abstract

The protection of consumers from emerging Salmonella spp. serovars depends on surveillance and control of the pathogen in food producing animals. Among them, pigs are the second most important species for transmitting emerging Salmonella spp. serovars pathogenic to consumers. Thus, a microbiological investigation of pig salmonellosis was undertaken in Central Greece examining samples collected from multiple carcass sites, aiming at the identification of prevalent serovars and their carcass location. For this purpose 492 tissue samples and 378 environmental swab samples were cultured following ISO 6579:2002. Twelve different serovars were isolated from faecal and tissue samples and two from the environmental samples, representing a total of 79 isolates. Among the identified serovars were the emerging monophasic Typhimurium serovars, S. enterica subsp. enterica ser. 4,5, 12:i:- (16 isolates) and S. enterica subsp. enterica ser. 4,12:i:- (7 isolates), the monophasic S. enterica subsp. enterica ser. 6,7:i:- (6 isolates) and the rarer serovars of subspecies enterica subsp. diarizonae 61:k:1,5; and S. enterica subsp. houtenae 40:g:t:- (one isolate each). Of the remaining isolates, 37 were serovar Typhimurium, six (6) were serovars S. Bredeney (2 isolates), S. Agona, S. Derby, S. Infantis, S. Mendeleoides, and S. Cerro and four were untypeable. Although serovar Typhimurium was the most prevalent one (37 isolates), the other serovars could also be considered of emerging public health importance, due to pork contamination. Thus, the pig appears in Greece as a possible source of human salmonellosis caused not only by commonly reported serovars, but also of rarer ones.

Keywords: pig, salmonellosis, slaughterhouse, tissue

Introduction

Salmonella spp. are the commonest cause of foodborne enteric disease in man and the second most frequently reported zoonotic agent in the EU. A mean 10-20% of human infections across EU are attributed to the consumption of pork products, although in some members states this may reach 50%. Thus, the contribution of pork products to human infections appears high enough to make control of pig salmonellosis an objective of the pig industry. Effective control of pig salmonellosis depends on measures preventing the pathogen of entering a pig farm. Pigs usually get infected through the faecal-oral route: hence carrier animals (pigs or other species) should be prevented of entering the premises. After infection of the host, the pathogen initially colonizes the intestine and eventually the gut-associated lymphoid tissue and other organs of the digestive tract. From these tissues it is intermittently excreted into the gut and eventually the animal’s environment. In these events, important role play the lymph follicles (Peyer’s patches) of the ileum wall. Peyer’s patches are the main source of Salmonella serovars infecting other organs of the host, such as the lymph nodes and the gallbladder. Colonization of the mesenteric lymph nodes and gallbladder epithelium may result to the survival of various highly infective serovars, although the infected pigs usually remain asymptomatic. Pigs subclinically infected with Salmonella spp. are confirmed as carriers only after multisite carcass sampling and microbiological examination. This procedure is time consuming and cost ineffective, thus not routinely used. Hence, subclinically infected pigs, remaining largely unidentified, are a potential source of infection for other animals and humans. Furthermore, they are contributors to the development of an endemic ‘house flora’ of Salmonella within the slaughterhouse contaminating carcasses and eventually pork consumers. A strong correlation has been found between the proportion of animals with Salmonella positive faeces and contamination of carcasses. An estimated 70% of carriers are responsible of their own carcass contamination, while contaminated equipment, such as the carcass splitter, or the manual handling of carcasses are responsible for 15 to 30% of contamination. Contaminated slaughterhouse equipment is more important than handlers for carcass contamination, due to bacterial growth during the day inside or on the surface of equipment. Thus, by minimizing positive animals entering the slaughterhouse one minimizes the building up of an endemic “house flora” positively contributing to the protection of consumers. Therefore, the objectives of the present study were the identification of Salmonella serovars present in Greek swine farms, their carcass location and the discussion of the findings in relation to information reported from human cases occurring worldwide.

Material and methods

Herd information and sample collection

Data considered significant for the presence of Salmonella in pig farms of central Greece were collected through a number of questions answered by 15 farm owners agreeing to participate in the study. Sampling of finishers from this furrow-to-finish herds, having 20 to 500 sows each, was performed with regular visits to two slaughterhouses of the same area. Four hundred ninety two (492) tissue samples were collected from 123 randomly selected pigs. An estimated 5% of the fatteners (about 2500) slaughtered during the sampling period (September 2013 – March 2014) were microbiologically examined. Tissue samples collected after evisceration were from the pigs’ neck (site of carcass drainage). Environmental samples were collected at the manual handling of carcasses are responsible for 15 to 30% of contamination. Contaminated slaughterhouse equipment is more important than handlers for carcass contamination, due to bacterial growth during the day inside or on the surface of equipment. Thus, by minimizing positive animals entering the slaughterhouse one minimizes the building up of an endemic “house flora” positively contributing to the protection of consumers. Therefore, the objectives of the present study were the identification of Salmonella serovars present in Greek swine farms, their carcass location and the discussion of the findings in relation to information reported from human cases occurring worldwide.

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the day of sampling carcasses. In each sampling day three sampling rounds were performed: one before slaughterering and two during carcass processing. The samples collected were from the hands and knives of two cleaners of the dirty zone and the carcass splitter. The knife blades were swabbed from the tip to base twice on both sides, as was also the blade from the splitter. Each swab sample of the gloves was collected by swabbing the whole palm surface of both hands. In total, 378 environmental samples were collected.

Isolation and serotyping of *Salmonella* spp.

The *Salmonella* ISO 6579-2002, Annex D for food and animal feeding stuffs was strictly followed for the isolation of *Salmonella* serovars. The selected “choice laboratory medium” were *Salmonella-Shigella* agar (SS Merck- Germany) and Brilliant Green agar (BG) (CM329; Oxoid, England). Thus, three selective media were included in the isolation procedure. Suspect colonies on these media were subcultured on Columbia blood agar (Oxoid, England). They were examined with Gram stain and if they were Gram negative rods, they were tested for oxidase production and the utilization of Triple Sugar Iron Agar (Merek- Germany). Two biochemical systems, API 20E (BioMerieux, France) and the MicrogenTM GnA+B-ID (Microgen Bioproducts Ltd, UK) suitable for Gram (-) bacteria were used for assigning isolates to species. The isolates identified as *Salmonella spp.* were further tested, with a polyvalent slide agglutination test (Remel Europe Ltd; Dartford, England) detecting O- and H-antigens. If they were positive, they were sent to the Greek National Reference Laboratory (GNRL) for specific serotyping.

**Results**

Prevalence of *Salmonella* and herd conditions

Only 3 (20%) of the 15 herds examined were culture negative in all types of tissue samples collected. They were small herds having about 40 sows each and not reporting a recent entrance of replacement animals. Their owners did not report prophylactic use of antimicrobials in feeding stuffs, but they reported whitewashing between batches in addition to common cleaning and disinfection procedures. Herds above 100 animals. Their owners did not report prophylactic use of antimicrobials for common cleaning and disinfection procedures. Herds above 100 animals. Their owners did not report prophylactic use of antimicrobials for the prophylactic purposes had slaughtered animals infected in at least one carcass site. One of these farms used also acidifiers in weaners’ feed, as a preventing measure for restricted carcass site sampling or changes due to live pigs arriving in Greek herds from a variety of community sources and spreading to pork consumers across EU. This is, perhaps, the reason that although the most prevalent serovar was Typhimurium (Table 1), recognized in EU as one of the serovars frequently associated with human and pig salmonellosis, an equivalent number was emerging serovars not reported previously from Greek swine farms.

Among the emerging serovars were monophasic variants of *S. Typhimurium, S. enterica* ser. 4,12:i:- and *S. enterica* ser. 4,12:i:-, found here to be the second most prevalent serovars (Table 1). These variants have recently increased in prevalence among consumers, replacing gradually other important serovars associated to swine and pork products. Interestingly, these variants were not isolated from feaces in the present study due, perhaps, to their adaptation to the tissue of other digestive tract organs, such as the gallbladder and lymph nodes. At such organs, they remain dormant until stressful factors cause their multiplication and subsequent gut and environmental contamination, putting at risk other animals and eventually humans. Such adaptation could also be responsible for the increased reporting of these variants from animals only recently, coinciding with carcass multisite sampling rather than just feaces and environmental sampling. The increased frequency of their isolation from human cases in different countries is, perhaps, a result of this adaptation. Hence, consumers are, perhaps, infected through consumption of undercooked meat harboring the pathogen in lymphatic tissue, rather than meat contaminated during its processing e.g. at slaughtering. This was evident from the positive neck muscle tissue deriving only from animals having at least one more positive site.

Monophasic *S. Typhimurium* or “*S. Typhimurium*-like” variants are currently in the fourth place among important serovars reported from man, in the second reported from live pigs and in the third reported from pig meat. They have also been isolated from a number of other animal species, such as poultry, cattle, turtles and food products, such as dried pork sausages. They show very high molecular relatedness to the ‘classic’ *S. Typhimurium*, a reason for possible misclassifications without studies of molecular relatedness.
Thus, molecular relatedness, in respect to pathogenicity genes, helps the avoidance of misclassifications, but it also changes the epidemiological importance of newly emerging serovars from animals and consumers. In Greece, S. enterica serovar 1,4,[5],12:i:- was firstly recorded from humans in 2007, accounting for only 0.3% of human cases of salmonellosis; its rate of isolation increased sharply thereafter, ranking third in frequency since 2009. In an EFSA report for the EU-wide baseline survey on the prevalence of Salmonella in slaughter pigs, S. serovar 1,4,[5],12:i:- was among the ‘top five’ serovars in Greece accounting for the 2.7% of the isolates. In addition, rarer serovars and subspecies, such as S. enterica 6,7:k:- and S. enterica subsp. diarizonae 61:k:1,5 were also identified in the present investigation. The source of subspecies diarizonae infecting man is thought to be reptiles kept as pets. This subspecies is considered host adapted to reptiles, colonizing their nasal mucosa. Its isolation from pigs could indicate an emerging serovar for pork consumers and the same could be the cases with subspecies S. enterica subsp. houtenae, which, like the previous one, has traditionally been considered a subspecies infecting exotic reptiles, and humans only when coming in contact with these animal species. Such hosts pass in the environment their strains infecting food producing animals and eventually exposing consumers. Thus, physicians treating human salmonellosis could rather reflect the sampling site. Feecal samples are traditionally the samples used for confirming a positive clinical or subclinical case. However, as it is evident (Table 1), from the 52 isolates only 11 (21.1%) derived from feaces. As previously reported, the best sites for confirming infection are the mucosal of the ileum and the mesenteric lymph nodes and this is possible only during carcass processing. In the present study 35 of the 52 isolates derived from the ileum (18 isolates) and mesenteric lymph nodes (17 isolates). In addition, due to that only nine animals were positive to more than one site; concurrent multiple-site carcass sampling should be considered as giving reliable results. Such a sampling protocol could also give information on serovars possibly co-existing in the same host. This is rarely reported either because it rarely happens or the serotyping procedure is limited, due to costs, to only phenotypically different isolates. However, in one instance of the present investigation two different serovars were identified from different carcass sampling sites of the same animal: serovar 6,7:k:- from the lymph nodes and serovar infantis from feaces. Unfortunately, such multiple site isolations are impossible from human salmonellosis, thus it’s unknown if one or more serovars cause an observed clinical case.

Furthermore, colonization of lymphatic tissue increases the risk to meat consumers, if cooking or handling does not eliminate the pathogen. This increased risk from lymphatic tissue colonization rather than carcass contamination during handling was evident in the present investigation through the examined meat and environmental samples. Six (6) of the muscle neck samples (site of carcass drainage) were positive to Salmonella and of them two came from a carcass that had Salmonella in their feaces, although all derived from animals with another positive site. As for the environmental samples considered evidence of possible slaughterhouse carcass contamination, the present investigation does not confirm other studies. Specifically, Salmonella was repeatedly isolated from the non-disposable gloves of only one of the skinners. Thus, hygiene measures were largely effective in the examined facilities, with the exemption of this worker’s gloves. This type of gloves should be changed between handlings and meticulously cleaned. The serovars identified were Typhimurium and S. enterica subsp. enterica ser 4,5,12:i-, both potential consumer pathogens, if passed to carcasses. Although the worker was not handling carcasses, he was part of the “house flora”, which could contaminate carcasses, if HACCP procedures fail due to workers’ negligence.

### Table 1 Salmonella serovars found at five different bacteriological sampling sites of finishers

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Feaces No</th>
<th>Ileum No</th>
<th>Lymph nodes No</th>
<th>Pig meat No</th>
<th>Total isolates per serovar</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>S. enterica subsp. enterica ser 4,5,12:i-</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>S. enterica subsp. enterica ser 4,12:i-</td>
<td>-</td>
<td>2</td>
<td>5</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>S. Bredeney</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>S. Agona</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>S. Derby</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>S. enterica subsp. enterica 6,7:k:-</td>
<td>2</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>S. Infantis</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

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Table Continued

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Feaces No</th>
<th>Ileum No</th>
<th>Lymph nodes No</th>
<th>Pig meat No</th>
<th>Total isolates per serovar</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Melangridis</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>S. Cerro</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>S. enterica subsp. diarizonae 61:k:1,5</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>S. enterica subsp. houtenae 40:2:-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Rough</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>18</td>
<td>17</td>
<td>6</td>
<td>52</td>
</tr>
</tbody>
</table>

Conclusion
From the above one concludes that the pig, thus pork meat, is a potential source of, not only serovars accepted as commonly pathogenic to consumers, but also of other rarer serovars and subspecies. These, colonizing the lymphatics of subclinically infected pigs, could eventually infect multiple consumers, if meat cooking and handling methods fail to protect them. In addition, the present evidence showed that strict HACCP control effectively controls the building up of “house flora” having microbes pathogenic to consumers.

Acknowledgements
None.

Conflict of interest
The author declares that they do not have a commercial or other association that might pose a conflict of interest.

References
2. EFSA, European Food Safety Authority. EFSA assesses risk of Salmonella from pig meat. 2010.


