THE PREVALENCE OF \textit{SALMONELLA}, \textit{CAMPYLOBACTER} AND VTEC IN PIG FARMS

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\textbf{Abstract:} Four different pig farms were sampled for the prevalence of \textit{Salmonella}, \textit{Campylobacter} and VTEC. From a total of 215 rectal samples of individual pigs, 15 rectal samples taken from animals at the same farm were positive for \textit{Salmonella}. The \textit{Salmonella} status of the pigs at this farm differed from one age group to another. \textit{S. Typhimurium} was isolated from all the rectal samples and \textit{S. Typhimurium} and \textit{S. Schwarzengrund} were isolated from the environment. On two other farms \textit{Salmonella} was only present in the environment with \textit{S. London} and \textit{S. Typhimurium} as serotypes. With cut-off value \%OD$> 40$ in the ELISA we found a good correlation with the \textit{Salmonella} status of the farm. The presence of \textit{Campylobacter} was tested in 150 rectal swabs, 51 of these, spread over the four farms, turned out positive. All the strains were identified as \textit{Campylobacter coli} by a species-specific PCR. To determine if pigs are a reservoir of VTEC a total of 289 samples were screened for the presence of VTEC and 54 strains were isolated that each carried one virulence gene. Thirty-one strains carried the \textit{vt2e} variant of the \textit{vt2} gene, four strains harboured the \textit{hlyA} gene and 19 the \textit{eaeA} gene.

\textbf{Introduction:} Food safety including the absence of pathogens has become a very important aspect of meat quality. \textit{Salmonella} together with \textit{Campylobacter} are responsible for most of the bacterial human food borne infections. The last decade there has been a dramatic increase in the human cases of salmonellosis and campylobacteriosis. In 1999, 15774 infections with \textit{Salmonella} spp. were registered in Belgium (Belgian National Reference Centre for \textit{Salmonella} and \textit{Shigella}). For \textit{Campylobacter} the number of reported cases in the period 1987-1999 has more than doubled to 6514 cases in 1999. In Denmark, The Netherlands and Germany it is estimated that 15 to 20\% of all human cases of salmonellosis are associated with the consumption of pork (van Pelt \textit{et al.}, 2000; Steinbach and Kroell, 1999).

This study examines the prevalence of \textit{Salmonella}, \textit{Campylobacter} and VTEC in individual pigs on four swine farms.

\textbf{Materials and method:} In a first screening different age groups of pigs were tested for the presence of \textit{Salmonella}, \textit{Campylobacter} and VTEC. From each age
group two rectal samples and an overshoe sample of the floor of the pig house were taken to determine the pathogen status of the farm. In a second screening individual pigs of the same age group (20 to 30 pigs) were examined by taking a rectal swab (± 0.1g of faeces). For testing the environment of the farm an overshoe sample and a swab sample of the footwear of the farmer were taken. For serological examination blood samples were obtained from the same pigs that were already sampled rectally. Diaphragm meat juice samples of the individual pigs were taken at the slaughterhouse.

To measure the immunological response for *Salmonella* in swine meat juice and swine serum the German test kit Salmotype®-ELISA (Labor Diagnostik, Liepzig, Germany) was used following the suppliers instructions. The test result is presented as the percentage optical density of the sample, relative to the optical density of positive reference samples (%OD). As cut-off value both %OD >10 and %OD> 40 are presented.

**Results:** In total fifteen rectal samples (n=237) were positive for *Salmonella* Typhimurium O5+*, all from pigs taken at farm 1(Table 1). On that farm a big difference in prevalence between two age groups was noticed. In the environment of that farm S. Schwarzengrund was isolated from the feed trough and S. Typhimurium O5 and O5+ from an overshoe. In the other two positive farms only in the environment *Salmonella* was isolated. When we compared the bacteriological results with the serological findings (Table 1), we found a good correlation in farm 1 when a cut-off value higher than 40 was used. In the group with a high degree of *Salmonella* shedding (12/17 rectal samples were positive) 13 samples gave a %OD >40 (n=17) and all were positive when the %OD > 10 was used. In the low contaminated group (1 rectal sample was positive) only two samples were serologically positive with a %OD>40. At the *Salmonella* negative farm (farm 3) 7% of the pigs gave a %OD value higher than 40 (n=28) and with a cut-off value %OD higher than 10, 42% were positive. The correlation between the bacteriological and the serological data was even worse at farm 4 that was *Salmonella* negative in the rectal swab but positive in the environment, 46% of the samples gave a %OD higher than 40 and 75 % of the samples gave a %OD>10.

From the 150 rectal swabs taken at the four farms 51 (34 %)were positive for *Campylobacter*. *Campylobacter* was isolated with about the same frequency in the rectal swabs of pigs from the different farms (between 30 and 40 %). Also in the environment *Campylobacter* was isolated on all farms. All the *Campylobacter* strains were identified as *Campylobacter coli*.

From a total of 177 individual rectal swabs 31.6 % reacts positively for the virulence gene in the multiplex EHEC-PCR. Twenty-four strains from the 56 positive virulence samples were isolated; six isolates contained additionally the heat stable enterotoxin gene *StTo*. From the rectal samples 11 eaeA positive strains, and 4 hlyA positive strains were isolated. No strain that carried a combination of virulence
genes (vt1 / vt2 + eaeA + hlyA) was isolated. E. coli serotype O157 was present in 30% of the rectal swabs. All 11 strains, which could be isolated, were sorbitol-positive on sorbitol MacConkey agar and did not possess any of the virulence genes tested in the multiplex PCR.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Rectal</th>
<th>Status of the farm</th>
<th>% OD &gt;40</th>
<th>% OD &gt;10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm 1</td>
<td>+ S. Typhimurium</td>
<td>+ S. Typhimurium O5* and O57 / S. Schwarzengrund</td>
<td>20/71*</td>
<td>47/71*</td>
</tr>
<tr>
<td>Farm 1 group 1</td>
<td>+ S. Typhimurium</td>
<td>+ S. Typhimurium O5*</td>
<td>13/17</td>
<td>17/17</td>
</tr>
<tr>
<td>Farm 1 group 2</td>
<td>+ S. Typhimurium</td>
<td>+ S. Typhimurium O5*</td>
<td>2/15*</td>
<td>10/15*</td>
</tr>
<tr>
<td>Farm 2</td>
<td>-</td>
<td>+ S. London</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Farm 3</td>
<td>-</td>
<td>-</td>
<td>2/28</td>
<td>12/28</td>
</tr>
<tr>
<td>Farm 4</td>
<td>-</td>
<td>+ S. Typhimurium</td>
<td>13/28</td>
<td>21/28</td>
</tr>
</tbody>
</table>

* The status of the farm is determined by presence or absence of Salmonella in the environment of the farm.
* ELISA tested on blood samples taken at the farm.

Table 1: The ELISA values of the individual pigs tested at the farm (blood sample) and at the slaughterhouse (meat juice sample).

**Discussion:** The overall prevalence of *Salmonella* in pigs, tested on the farm by taking rectal swabs was determined to 6%. This figure is much lower than the one found by serological examination of the same pigs (32% of these pigs had a %OD higher than 40 and 66% of the pigs had a %OD >10). This limited study on four farms does not allow drawing conclusions about the general prevalence of *Salmonella* in pigs during rearing. The serological data with a % OD >40 correlated remarkably better with the environmental status of the farm than with the status of the individual pigs. With % OD >10 no difference was seen between environmentally positive and negative farms. By taking an overshoe it was possible to isolate different serotypes, which indicates the efficiency of this type of sampling for monitoring the environmental *Salmonella* contamination on the farm.

Although 2 farms are environmentally contaminated with *Salmonella*, no strains were isolated from the pigs. This is in contrast to the *Campylobacter* results were in each environmentally positive farm also the rectal swabs were positive. It is possible that pigs on these farms were challenged with *Salmonella* before and were cured or became latent carriers afterwards. Another possibility is that hygienic barriers are more efficiently working towards *Salmonella* than towards *Campylobacter*.

No human pathogenetic VTEC strains were found among the pig isolates. Like for *Campylobacter* no real hygienic barrier is noticed between the farm environment and the pigs.
References