Discussion: A host specific *Salmonella* Choleraesuis remains the prevalent serovar in Poland and some other EU Candidate Countries as well as in the US (Hoszowski A. and Wasyl D., 2002; Weide-Botjes, M. et al., 1996). *S*. Choleraesuis var. Kunzendorf is responsible for the majority of the clinical salmonellosis in swine including acute septicaemia and pneumonia in piglets and fattening pigs. It causes up to 80% of *Salmonella* infections and more than 90% cases of clinical disease in Poland. The aim of present study was to determine the genetic relatedness of recent *S*. Choleraesuis isolates. The tested strains belonged to two clonal lineages. One of them (II) was represented by single strain isolated in 2000 and it was not found in pigs any longer. The other line spread over three years within animal population. High similarity of macrorestriction profiles and ribotypes indicate the affinity of the strains (Weide-Botjes, M., Liebisch, B., Schwarz, S., and Watts, J. L., 1996) and suggest clonal spread of the pathogen among swine in Poland. Some shift of predominant macrorestriction profiles and ribotypes was noted. Sub-cluster (I') gathering mostly strains of Ch/X01 and Ch/X02 profiles predominated during first part of the three-year period. The second sub-cluster (I'') covered isolates showing mostly Ch/X03 profile was more often found in 2002. All isolates presenting R-2 and R-3 ribotypes were obtained in 2000. Either the macrorestriction profiles and the ribotypes revealed limited number of band differences that suggest that only a few genetic events took place during the spread of the clone (Tenover F.C. et al., 1995). The findings emphasise conservative character of *S*. Choleraesuis. Further long-time studies are needed to verify that thesis as well as to point out the possible crucial role of symptomless carriers or subclinical infected adult pigs for the disease spread among herds.

Conclusions: A low genetic divergence of *S*. Choleraesuis strains is concluded. It proves the clonal spread of *S*. Choleraesuis in Poland. PFGE and ribotyping proved to be useful and discriminative methods for *S*. Choleraesuis differentiation.

References:


Comparison of two commercial ELISA for the diagnosis of salmonellosis in swine

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Summary: Three hundred and sixty-one pig sera collected in farms of Catalonia were randomly selected from a serum bank. Samples were examined by using two commercial ELISA kits. Results were compared with the kappa value using WinEpiscope. Besides, sample/positive ratios (S/P) were
calculated. Comparison of results of both ELISA yielded a poor agreement (kappa 0.191), indicating that both ELISA did not measure the same. In addition, when raw optical densities were compared by means of a regression analysis, the results indicated a low correlation (r = 0.54). The results of this study clearly indicate that results of both kits are not interchangeable and that normalization of results by using S/P ratios did not serve to improve the agreement between tests. From our results, it is tempting to suggest that Salmotype detects a greater number of IgM positive pigs. The nature of these IgMs (salmonella-specific or not) is not known to us at this moment.

**Keywords:** Salmonella, kappa, Serology, Agreement, Farms

**Introduction:** Salmonellosis is one of the leading causes of human infectious enteritis worldwide. Poultry meat and eggs are thought to be the main source of human salmonellosis although several studies pointed out that pigs can have a considerable role in this sense. As a result, in several countries control programmes of pig salmonellosis are being implemented. Most of these programmes use ELISA as serologic diagnostic tool because of its reliability and simplicity of use. However, some discrepancies have been noted between different ELISA systems (Van de Heijden, et al., 2001). The purpose of the present study was to compare two ELISA kits routinely used in Europe for the diagnosis of swine salmonellosis.

**Material and methods:** Three hundred and eighty two pig sera collected in farms of Catalonia were randomly selected from a serum bank. This sample represented 194 fattening pigs and 167 sows. All animals come from farms of known Salmonella status (positive or negative) as determined by previous serological or bacteriological analysis. Samples were examined by using two commercial ELISA kits Salmonella-ab® (Svanova Biotech, Uppsala, Sweden) and Salmotype® (Labor Diagnostik, Leipzig, Germany). The ELISAs were performed according to the manufacturer directions and results were interpreted accordingly. Categorized results (positive versus negative) were compared with the Kappa value using WinEpiscope software. Besides, raw optical densities were used to calculate sample/positive ratios (S/P) following this formula: \( \frac{DO_{sample} - DO_{-ve\ control}}{DO_{+ve\ control} - DO_{-ve\ control}} \). These S/P ratios were used in a ROC analysis to determine the S/P value that better fitted the manufacturer’s criteria for classification of results. All statistical analysis were done using StatsDirect.

**Results and Discussion:** Comparison of results of both ELISA yielded a poor agreement. Thus kappa value obtained for comparisons of results using manufacturer’s directions was 0.191 (confidence intervals at 95 %: 0.089 - 0.294), indicating that both ELISA did not measure the same (Table 1). In addition, when raw optical densities were compared by means of a regression analysis, the results indicated a low correlation (r = 0.546814, 95 % confidence interval (Fisher’s z transformed) = 0.470131 to 0.615304).

**Table 1. Comparison of results between Salmonella-ab and Salmotype ELISA kits**

<table>
<thead>
<tr>
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<th>Salmotype +</th>
<th>Salmotype -</th>
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<tbody>
<tr>
<td>Salmonella-ab +</td>
<td>118</td>
<td>62</td>
</tr>
<tr>
<td>Salmonella-ab -</td>
<td>84</td>
<td>97</td>
</tr>
</tbody>
</table>

Besides, sample/positive ratios (S/P) were calculated and comparison of results between both ELISAs using S/P ratios did not improve significantly the Kappa-value. To determine possible causes for this discrepancy, 10 randomly selected positive sera in Salmotype were heat inactivated (56 °C, 30 min) and re-analysed with this ELISA. All sera became negative after this treatment. In contrast, positive sera in Salmonella-ab were not affected by this treatment. When S/P ratios were used, ROC analysis showed that, in Salmonella-ab, a S/P cut-off ≥ 0.3389 agreed 100 % with the negative results obtained.
using the directions and 99.44 % with the positive results. Similarly, in Salmotype, a S/P cut-off ≥ 1.3776 agreed 99.37 % with the negatives using kit directions and 99.45 % with positives.

The results of this study clearly indicate that results of both kits are not interchangeable and that normalisation of results by using S/P ratios did not serve to improve the agreement between tests. From our results, it is tempting to suggest that Salmotype detects a greater number of IgM positive pigs. The nature of these IgMs (salmonella-specific or not) is not known to us at this moment.

Reference:

SALMONELLA SEROLOGY – WHICH SAMPLES SHOULD BE USED: COMPARISON OF MEATJUICE AND SERUM SAMPLES OF THE SAME PIGS

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Contamination of pork with Salmonella typhimurium is a potential source for fatal food born Salmonella-infections in humans. Screening programs are used in a number of countries to categorize pig farms into 3-4 Salmonella-risk-categories. A similar program will soon be implemented by the German government as well. A number of commercial ELISA-Testkits are registered in Germany for serum as well as meat juice samples. However little information is available regarding the question whether meat juice or serum samples of the same pigs will lead to the same results has not been investigated thoroughly.

Purpose
The investigation was performed to clarify if serum and meat juice samples from the same animal and taken at the same day would deliver comparable ELISA-results. Furthermore with a series of consecutive blood samplings on the same animals the time-effect on ELISA-results was to be investigated.

Methods
Random samples originated from two different slaughterhouses. Blood was taken immediately after the killing process and transported. Meat samples (1x1x1 cm) were taken from the???????? after evisceration of the carcasses. The meat was frozen and thawed in meatjuice-sampling tubes (Firma). A commercial ELISA test (Enterisol® Salmonellen-Diagnostikum, Boehringer Ingelheim Vetmedica GmbH), a mixed-ELISA based on the polysaccharide fraction of Salmonella-O-Antigen (1, 4, 5, 6, 7, 12) was used according to the test instructions. For the longitudinal study the same, randomly selected finisher pigs of one farm were sampled 3 times at different time points (jugular vein). All samples were tested with the same Testkitbatch at the same day.

Results
Slaughterhouse 1 (Graph 1)
PP-values of samples originating from slaughterhouse 1 showed a very good correlation between serum and meat juice (Graph 1). Not only were the qualitative ELISA-results all the same between the matching samples but also the quantitative results (PP-values) were almost identical in most of the cases.