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Comparing validation of four ELISA-systems for detection of *Salmonella* Derby- and *Salmonella* Infantis-infected pigs

Vergleichende Evaluierung von vier ELISA-Systemen zum Nachweis porciner Salmonella-Derby- und Salmonella-Infantis-Infektionen

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Summary

The objective of this study was the comparative evaluation of four indirect *Salmonella* ELISA tests at study time approved in Germany to detect *Salmonella* infection in pigs. Three tests are based on a LPS-antigen mix and directed against specific IgG antibodies. The fourth test is based on a purified *S.* Typhimurium whole-cell lysate antigen and discriminates between *Salmonella*-specific IgM-, IgA-, and IgG- antibodies.
In a longitudinal study, two groups of six weeks old hybrid piglets were orally infected with a porcine *S.* Infantis or *S.* Derby strain. Clinical and bacteriological parameters were monitored weekly during an observation period of 130 days after infection and serum samples were investigated in parallel with the respective ELISAs.
Apparently, the LPS-based ELISA systems used in this study failed to recognize *S.* Infantis-infected pigs although those animals shed the pathogen in high amounts throughout the study until day 81 post infection (p. i.). In contrast, the isotype-specific *Salmonella* Typhimurium whole-cell-lysate based ELISA was capable of detecting *Salmonella*-infected pigs from day ten p. i. at all tested serotypes and revealed the highest sensitivity in detection of *S.* Infantis-infected pigs. Furthermore, it became apparent that the often used surveillance cut-off value of 40 OD% is not appropriate for intra-vitam detection of *S.* Infantis- and *S.* Derby-infected pigs. In contrast, the cut-off values of the ELISAs given by the suppliers result in considerable higher detection rates.

Keywords: *Salmonella*, Derby, Infantis, pig, ELISA, validation

Zusammenfassung

Gegenstand dieser Studie war die vergleichende Evaluierung von vier zum Studienzeitpunkt in Deutschland für die serologische Diagnostik von *Salmonella*-Infektionen des Schweins zugelassenen ELISA-Testsystemen.
Zum Einsatz kamen drei LPS-basierte ELISA-Systeme zur Detektion von spezifischen IgG-Antikörpern sowie ein auf einem *S.*-Typhimurium-Vollzelllysat-Antigen-basierender ELISA, welcher zwischen *Salmonella*-spezifischen IgM-, IgA-, und IgG-Antikörpern unterscheiden kann.
Im Rahmen einer Langzeitstudie wurden zwei Gruppen von jeweils sechs Wochen alten Hybridschweinen mit Bakterien der *Salmonella*-Serovare *S.* Infantis und *S.* Derby oral infiziert. Nach der Infektion wurden über einen Zeitraum von 130 Tagen wöchentlich sowohl klinische als auch bakteriologische Parameter erfasst. Parallel wurden Blutproben mit den jeweiligen ELISA-Testsystemen unter-

sucht. Es zeigte sich, dass mit den in dieser Studie verwendeten LPS-basierten ELISA-Systemen keine mit *S.* Infantis infizierten Schweine erkannt werden konnten, obwohl die Tiere den Erreger bis zum Tag 81 p. i. in großen Mengen ausschieden. Dagegen war der *S.*-Typhimurium-Vollzelllysat-basierte ELISA zehn Tage p. i. in der Lage, die meisten dieser Tiere Serovar-unabhängig als Salmonellen-infiziert zu identifizieren und wies zudem die höchste Sensitivität bezüglich des Nachweises von *S.*-Infantis-infzierten Tieren auf.

Weiterhin erwies sich der in Deutschland angewandte Surveillance cut-off-Wert von 40 OD% als nicht geeignet für die Detektion von mit *S.* Infantis und *S.* Derby infizierten Schweinen. Im Gegensatz dazu führte die Anwendung der von den Hersteller-Firmen vorgeschlagenen cut-off-Werte der ELISA-Testsysteme zu einer deutlich höheren Test-Sensitivität.

Schlüsselwörter: *Salmonella*, Derby, Infantis, Schwein, ELISA, Validierung

Introduction

Salmonellosis is one of the most important enteric infections in man and in livestock. Various serovars of *Salmonella enterica* can cause clinical inapparent infections or disease which most often becomes manifest as a self-limiting gastroenteritis or systemic diseases. Besides *Salmonella* (*S.*) Typhimurium, *S.* Derby and *S.* Infantis are the most important cause of porcine *Salmonella* infections in the EU (EFSA, 2008). Although pigs usually do not develop clinical salmonellosis, they may become shedders and carriers resulting in a substantial disease-causing potential for both other pigs from the herd and humans via contaminated foods. The consumption of *Salmonella* contaminated pork and pork products was estimated to be responsible for about 20% of all registered cases of human salmonellosis in Germany (Steinbach and Kroell, 1999).

Salmonella infections can be diagnosed directly in the piggery or at the slaughterhouse by isolating salmonellae with various established cultural methods or by serodiagnosis using lipopolysaccharide-based ELISA systems (Nielsen et al., 1995; Mousing et al., 1997). In different countries (e. g. Denmark, Germany) these serological results are used to classify pig herds in one of three categories. Category 3 has the highest prevalence of *Salmonella* antibodies, defined as at least 40% of the pigs examined being seropositive. Category 2 herds have a moderate number of antibody-positive pigs, whereas herds of category 1 have up to 20% antibody-positive pigs. Based on the result of categorization, different measures for *Salmonella* reduction on herd level have to be implemented. For the efficient surveillance and control of *Salmonella* in pig herds, reliable ELISA test systems are of major importance to identify swine herds which were exposed to *Salmonella* organisms. However, problems may arise from the different configuration of ELISA systems and the resulting discrepancy of test results. An international ring trial on ELISAs used for *Salmonella* antibody detection in swine revealed large differences regarding the sensitivity of the tests (van der Heijden, 2001).

The objective of this study was to compare four indirect ELISA tests regarding detection sensitivity in pigs after long-term infection with *S.* Derby and *S.* Infantis. First results of the in parallel performed experimental infection of pigs with *S.* Typhimurium have been already published (Szabo et al., 2008). This study presents the results of the comparing evaluation of the ELISA systems after infection of pigs with the two important *S. enterica* serovars Infantis and Derby.

Material and Methods

Bacterial strains and culture conditions

The *Salmonella enterica* strains used for the comparing investigation were the following: a *S.* Infantis strain (571/03) isolated from a pig showing peritonitis and endocarditis, and a *S.* Derby strain (531/05) originating from a pig with enteritis. The strains were kindly provided by the National Reference Laboratory for *Salmonella* infections at the Federal Institute for Risk Assessment (BfR), Berlin, Germany.

All *Salmonella* strains were cultured in Luria Bertani (LB) broth to mid-logarithmic growth phase in an orbital incubator at 37°C and subsequently pelleted and washed once in PBS by centrifugation (2000 x g for 10 min). Bacteria were then counted using a counting chamber and adjusted in ice-cold phosphate buffered saline (PBS; pH 7.2) to a final cell count of 2×10^8 cfu/ml.

Animals and experimental design

16 six-weeks-old hybrid piglets (landrace x pietrain) stemming from a herd with no history of *Salmonella* infection and tested negative for *Salmonella* by faeces culture and ELISA (Salmotype® Pig STM-WCE, Labor Diagnostik Leipzig, Germany) were divided into two groups. The first group (n = 7) received *S.* Infantis and the second group (n = 9) was infected with *S.* Derby.

Both groups were housed in air-conditioned stables facilitated for biosafety 2 level. Food and water were provided ad libitum. The pigs of the two infection-groups were kept and handled separately. All animal experiments were performed according to the German law of animal welfare (permit no. TVV 7/05 by the Regierungspräsidium Leipzig).

After one week of adaptation, the pigs were infected orally with the respective *Salmonella* strain. Feed was withdrawn twelve hours before infection. Pigs were sedated with 1 mg/kg i. m. Azaperon and fixed in a hammock in ventral position. Fifty millilitres of bacterial suspension (1×10^{10} cfu/ pig of *S.* Infantis and *S.* Derby) were administered via a gastric tube (Roesler et al., 2004).

Subsequent to infection, clinical signs including general demeanour, respiration, coughing, vomiting, ingestion, diarrhoea and rectal body temperatures of each pig were monitored daily after infection. Body weight was

measured every third day for each pig. The pigs were euthanized by bleeding under anaesthesia at day 123 (*S.* Infantis) and day 130 post infection (p. i.). (*S.* Derby). Samples from the quadriceps muscle, liver, spleen, lung, tonsils, ileum, jejunum, caecum, colon, mandibular lymph node, lung lymph node, jejunal lymph node, ileocolic lymph node, colic lymph node and bile fluid were collected for qualitative bacteriology.

Bacteriological examinations

After infection, the number of pigs shedding salmonellae was determined weekly until slaughtering. Faecal samples were cultured and examined quantitatively and qualitatively according to ISO 6579:2007. Tissue and faecal samples were analysed as described before (Roesler et al., 2004, Brumme et al., 2007). Colonies suspected as *Salmonella* spp. were further serotyped by agglutination using polyclonal antisera (SIFIN, Germany).

Serological examinations

Swine sera (collected twice a week until day 32, subsequently at weekly intervals) and meat juice samples (taken at the end of the experiment from the diaphragm muscle) were analysed for the presence of antibodies against *Salmonella* according to manufacturer's instructions using following kits: (A) Salmotype® PigScreen (LDL, Germany), (B) Enterisol® Salmonellen-Diagnostikum (Boehringer Ingelheim, Germany), which is in the meantime in Germany not anymore available on the market, (C) HerdChek® Swine *Salmonella* Antibody (IDEXX Laboratories, Germany), (D) Salmotype® Pig STM-WCE (LDL, Germany). Tests A, B and C are able to detect IgGantibodies against *Salmonella* spp. in porcine serum, plasma, and meat juice samples and the results are given as optical density (OD) or OD%, respectively. The Salmotype® Pig STM-WCE Test Kit (D) is designed to discriminate between *Salmonella* Typhimurium-specific IgM-, IgA-, and IgG-antibodies in the same matrices, but the results are calculated by the reference standard method and are indicated as ELISA units per ml (EU/ml).

Cut off values for positive diagnosis were: 20 OD% for test A, 40 OD% test B, 10 OD% test C and for test D 34 EU for IgA, 58 EU for IgG and 66 EU for IgM.

Statistical analyses

The results of the serological investigation (OD% values) and the bacteriological quantitative examination were calculated and plotted as notch boxes. The median (internal horizontal line), upper and lower quartiles (the upper and lower horizontal margins of the boxes), the 95% confidence limits (the oblique margins of the boxes) and the extreme values are shown. Significant differences were tested by Mann-Whitney U test. A *p*-value of ≤ 0.05 was considered significant.

For calculation of sensitivities of the different ELISA test systems all tested experimentally infected pigs were voted to be "*Salmonella*-infected" at the respective time point. To calculate test sensitivities for different stages of the infection, the mean of the calculated test sensitivities of the ELISA systems for the individual time points were used. For test system D the samples were voted to be serological "*Salmonella*-positive" if the results were higher than the cut off value of one of the three tested immunoglobulin isotypes.

Results

Clinical signs

All pigs infected with *S.* Derby showed mild clinical symptoms of salmonellosis, such as disturbed demeanour, fever or diarrhoea for about one week whereas *S.* Infantis-infected pigs had a more intensive diarrhoea in that period.

Faecal shedding and colonisation

Salmonellae of the respective serotypes were isolated from faeces of all pigs infected. Starting from about 100% shedding the experimentally infected pigs remained intermittent shedders until slaughter. The shedding rate at slaughtering decreased to 57% for the *S.* Infantis-infected group and to 33% for the *S.* Derby-infected group, respectively. After slaughtering the respective *Salmonella* strains could be re-isolated from lymphatic tissues from all *S.* Infantis-infected pigs (100%) and from the majority of the pigs infected with *S.* Derby (89%).
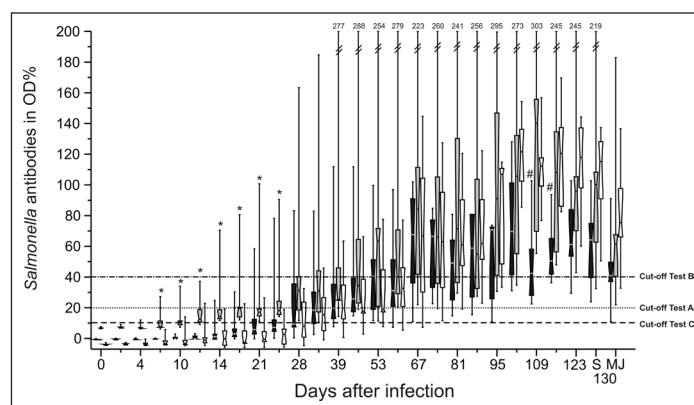

FIGURE 1: *Salmonella Derby antibodies (in OD%) detected by ELISA tests A (black), B (gray), and C (white) in pigs (n = 9) in serum (S) tested ante-mortem and meat juice (MJ) tested postmortem. * significant (p ≤ 0.05) higher OD%-values compared to the two other used tests. #, significant (p ≤ 0.05) lower OD%-values compared to the two other used tests.*
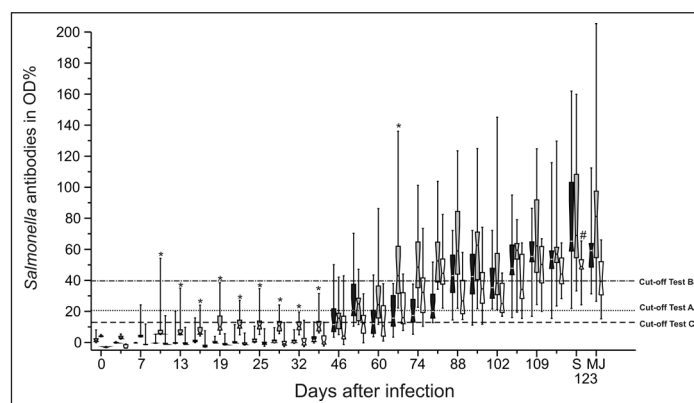

FIGURE 2: *Salmonella Infantis antibodies (in OD%) detected by ELISA tests A (black), B (gray), and C (white) in pigs (n = 7) in serum (S) tested ante-mortem and meat juice (MJ) tested postmortem. * significant(p ≤ 0.05) higher OD%-values compared to the two other used tests. #, significant (p ≤ 0.05) lower OD%-values compared to the two other used tests.*

Serological results

Results for *S.* Derby and *S.* Infantis antibodies (in OD%) detected ante-mortem by ELISA tests A, B, and C are presented in Figures 1 and 2 (for comparison with *S.* Typhimurium antibodies detected in pigs see also Szabo et al., 2008). The summary for sensitivities of the four ELISA-systems used in pigs for serological detection of the two *Salmonella* serovars is shown in Table 1. The sensitivities are summarised in three intervals (0–33, 39–95, 102–130d p. i.) with respect to the test cut-offs recommended by the manufactures (test A, 20 OD%; test B, 40 OD%; test C, 10 OD%) and the surveillance cut-off (40 OD%, not done for test system D, as there is no surveillance cut-off).

Concerning detection of *Salmonella* infection in fattening pigs, ELISA systems A, B and C varied with regard to sensitivity. The highest sensitivity of all tested LPS-based ELISA systems was observed in the third interval (102–123d p. i.), whereas only test C could detect all animals as seropositive from day 102 p. i. until slaughter.

The sensitivity among pigs infected with *S.* Derby was 10–20% and 70–85% in the first and second interval, respectively. In contrast, only test system C was able to detect seroconversion in some pigs infected with *S.* Infantis from 0–33d p. i (mean sensitivity 2%). The sensitivity of all tests in both, the second and third interval, was also significant lower (47–80% and 83–100%, respectively) than in the *S.* Derby group.

Figures 3 and 4 show the results for *Salmonella* serum antibody classes IgM, IgA, IgG (in EU) detected with ELISA test D in serum samples of pigs infected with
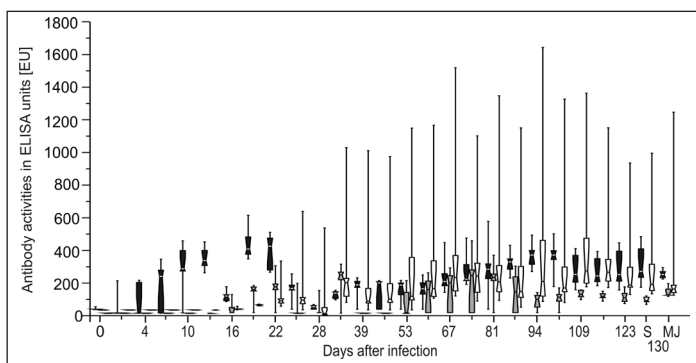
S. Derby and *S.* Infantis, respectively (see also for *S.* Typhimurium in Szabo et al. 2008). When applied to serum samples from pigs infected with *S.* Infantis this isotype-specific *Salmonella* Typhimurium whole-cell-lysate based ELISA was more sensitive than the other assays (Tab. 1). Furthermore, the ELISA was able to firstly detect all *Salmonella*-infected pigs from day ten after infection (Fig. 3 and 4).

Apparently, individual ELISA units of the WCE ELISA varied within all antibody classes especially for anti-*Salmonella* IgG. Out of *S.* Infantis- and Derby-infected animals, 42% and 67% were already detected as seropositive at seven and 13 days p. i., respectively, which was due to the early seroconversion of IgM for all serovars. IgA and IgG classes, however, seroconverted later in the second intervals (39–95d p. i.). All *S.* Derby-infected pigs remained positive for all three immunoglobulin classes until slaughter. Unlike to the *S.* Derby-infected animals, not all animals of the *S.* Infantis-infected group were tested seropositive in the last interval (102–130d. p .i) and at slaughter, respectively. From day 94 p. i., one animal was tested as seronegative and at slaughter two animals had antibody levels over the cut off values for positive diagnosis.

Using the in Germany used surveillance cut-off of 40 OD% for test A and C, sensitivity decreased in many cases significantly, due to lower cut-off values (test A: 20 OD%; test C: 10 OD%) recommended by the manufacturers. For instance, in the *S.* Derby-infected group, the sensitivity of test C decreased significantly from 85% to 44% in the second and from 100% to 95% in the third interval. In the *S.* Infantis group, the sensitivity decline was even more evident for all tests: for example for test C it decreased from 80% to 25% in the second and from 100% to 60% in the third interval, respectively. The recommended cut-off of test B is equivalent to the surveillance cut-off.

Results of the evaluation of meat juice and serum samples taken at slaughter are shown in Figures 1 to 4 and in Table 1. Concerning the OD% values, there were no significant differences between blood serum and meat juice for all *Salmonella* serovars. Using the surveillance cut-off of 40 OD% for test A and C, sensitivity became up to 34% lower (test A) for *S.* Derby and 14% lower (test C) for *S.* Infantis.

Test system D detected all blood sera and meat juice samples taken at slaughter as seropositive for *S.* Derby, whereas 14% of the *S.* Infantis blood sera were detected as seronegative. Analysing the meat juice samples with test D, all pigs were detected as seropositive.

FIGURE 3: *Anti-Salmonella Derby antibody activities of immunoglobulin classes M (black), A (gray), and G (white) detected with ELISA test D in S. Derby infected pigs (n = 9).*
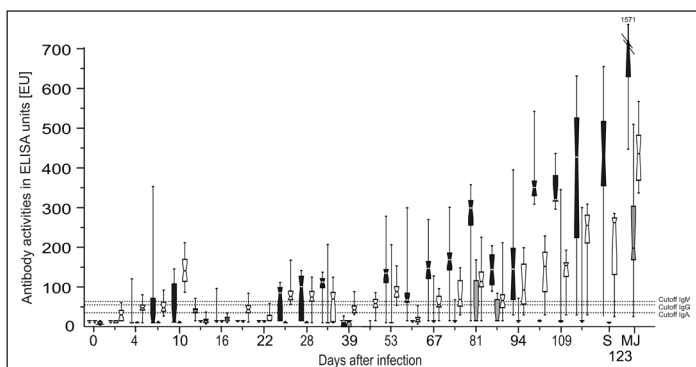

FIGURE 4: *Anti-Salmonella Infantis antibody activities of immunoglobulin classes M (black), A (gray), and G (white) detected with ELISA test D in S. Infantis infected pigs (n = 7).*

Discussion

In a comparative study we examined for the first time different ELISA systems for the detection of humoral antibodies in pigs experimentally infected with the *Salmonella* serovars Derby, Infantis, and Typhimurium (Szabo et al. 2008). Previous challenge studies in pigs were mainly performed with *S.* Typhimurium since *S.* Typhimurium is the most frequently isolated serovar in pigs in the European Union (EFSA, 2008) and because of its high zoonotic potential for humans (EFSA, 2007).

S. Typhimurium is found to be more invasive and associated with a higher level of antibodies in pigs compared to other serovars of *S. enterica* (Stege et al., 2000).

TABLE 1: *Sensitivity of ELISA tests A, B, C, and D depending on manufacturer cut-off values (A = 20 OD%, B = 40 OD%, C = 10 OD%) and the surveillance cut-off value (40 OD%), regarding to infection with S. Derby and S. Infantis. S, serum; MJ, meat juice. For sensitivities of test systems in different stages of the infection, the mean of the calculated test sensitivities of the ELISA systems for the individual time points were calculated. The given sensitivities are rounded*

Salmonella serotype used for experimental infection	Test	Sensitivity [%]									
		Test cut-off					40% OD cut-off				
		Day 0–33	Day 39–95	Day 102–123	At slaughter		Day 0–33	Day 39–95	Day 102–123	At slaughter	
					S	MJ				S	MJ
S. Infantis	A	0	59	91	86	86	0	23	86	86	86
	B	0	48	83	86	86	0	48	83	86	86
	C	2	80	100	100	100	0	25	60	86	43
	D	42	75	94	86	100	-	-	-	-	-
S. Derby	A	10	70	98	100	78	6	44	70	67	56
	B	8	53	93	89	78	8	53	93	89	78
	C	15	85	100	100	100	0	44	95	100	89
	D	67	98	100	100	100	-	-	-	-	-

Very little is known about pathogenesis of *S.* Derby and *S.* Infantis in pigs. They are generally considered as low virulent and less invasive than *S.* Typhimurium, although these assumptions are based on observations in single animals, sometimes infected by non-natural infection routes (Rycroft, 2000; Karasova et al., 2009). However, in recent years, beside *S.* Typhimurium the serovars *S.* Derby and *S.* Infantis have been frequently isolated from pigs in the EU and, therefore, become increasingly important. So, *S.* Derby is with 24% the most frequently isolated serovar in pig production stables in the EU, and also *S.* Infantis could be very often isolated (5%) from pigs (EFSA, 2008). Furthermore, besides *S.* Typhimurium and *S.* Enteritidis these two other *S. enterica* serovars are also assigned to have a high impact for human health.

Our investigations could not confirm a lack of invasiveness and low virulence of *S.* Infantis since there were also strong clinical findings and high colonisation rates of lymphoid tissues in the *S.* Infantis-infected group. This may be related to a higher virulence of the *S.* Infantis strain used in our study as this clinical isolate was originally obtained from a swine with pericarditis.

Serological results of our study and the in parallel performed study of Szabo et al. (2008) revealed that specific antibodies could be detected earlier in sera of pigs infected with *S.* Typhimurium than in pigs infected with *S.* Infantis and *S.* Derby.

The *S.* Typhimurium-infected group showed continuously the highest serum IgG titre, especially in the last third of the experiment. This is probably due to the high rate of invasion in the lymphoid organs which causes a constant stimulation of B lymphocytes and thus the production of immunoglobulins. Also, Baptista et al. (2009) showed that *S.* Typhimurium-infected herds have a lower probability of changing from high to lower antibody levels compared to *S.* Derby-infected herds. In agreement with this observation, antibody titres of *S.* Typhimurium-infected pigs remain detectable for longer periods (Nielsen et al., 1995; Szabo et al., 2008). A possible higher amount of main antigens from *S.* Typhimurium compared with antigens from *S.* Choleraesuis in the LPS mixture used as antigen for the ELISA systems could be a possible reason for that observation. As demonstrated, the LPS-based ELISA systems failed especially to detect antibodies in *S.* Infantis-infected pigs in the early stage of infection. This may pose a problem for the monitoring where false-negative findings will result in a lower seroprevalence and finally in a wrong categorisationof the farm.

In the present longitudinal study on detection of *Salmonella* infection in fattening pigs, ELISA tests A, B and C varied with regard to sensitivity. The ability of these tests to identify *Salmonella*-positive pigs mainly depends on the respective cut-off value recommended by the suppliers for the individual test. Apparently, test C revealed the highest sensitivity due to the lowest cut-off. However, ELISAs with high sensitivity often lack in specificity what could not be investigated in this study because of the enormous costs of a long-time keeping of *Salmonella* free pigs under biosafety conditions. But, this might be a problem, especially if the true prevalence is low, because this will reduce the positive predictive value of the test. Overall, evaluation and setting of cut-offs should consider sensitivity and specificity. Therefore, categorising swine herds based on serological results according to status I, II and III, should base on ELISA-systems which perform comparable sensitivity and specificity. Otherwise, the level for the categorised farm may differ depending on the ELISA test used for serological examination of pigs.

If ELISA results are evaluated according to a higher cut-off as demonstrated for tests A and C using a cut-off of 40 OD% (which is used for categorization of herds according to the German National Regulation on Reduction of *Salmonella* in Slaughtering Pigs, "Schweine-Salmonellen-Verordnung"), test sensitivity is significantly lower than using the cut-off recommended by the test producer. This may lead to misdetection of *Salmonella*-positive pigs particularly animals infected with *S.* Infantis or *S.* Derby.

The findings of the present study confirm the problem of a "diagnostic window" in the early stage of infection for all three serovars. It was already shown that the peak of *Salmonella* excretion in faeces is followed by an immune response not earlier than two weeks p. i. (Nielsen et al., 1995). This corresponds to our results of serological examination using ELISA tests A, B, C and the bacteriological findings where the majority of pigs did not develop anti-*Salmonella* IgG as early as three weeks p. i. despite of high *Salmonella* excretion in faeces.

During the later, clinically inapparent stage of infection which covers the main part of life span of a fattening pig, a higher rate of animals was detected as seropositive by the use of all three LPS ELISA tests compared to a

lower rate of pigs shedding *Salmonella* in faeces during that period. Considering the probability to find *Salmonella*-positive animals in a farm, antibody detection by ELISA will surpass the bacteriological examination of faeces. However, our study results also show that a conclusion from a seropositive result to the status of *Salmonella* infection (early vs. late stage) is not possible. Therefore, ELISA systems detecting *Salmonella*-specific IgG antibodies represent valuable screening tests on herd level, but they are not considered a suitable tool to test individual pigs (Nollet et al., 2005).

Meanwhile, ELISA systems have been developed which detect antibody classes IgM and IgA in addition to IgG to distinguish between an early and late stage of infection at an individual level (Lehmann, 2004; Ehlers et al., 2006). Our results confirmed that ELISA test D detects pigs infected with *S.* Typhimurium, *S.* Infantis, and *S.* Derby in the early stage of infection significantly (p < 0.05) more effectively than ELISA tests A, B and C. This higher sensitivity of ELISA test D is mainly due to the ability to detect antibodies of the early immunoglobulin class M. Furthermore, our results showed a remarkable persistence of high IgM activity in the *S.* Infantis-infected group until the end of the investigation. This can be explained by continuous re-infection with the challenge strain via the faecal-oral route which thereby causes a permanent stimulation of lymphocytes of the GALT (gut-associated lymphatic tissue) and the related humoral immune response. However, it is still open why test D detects antibodies against *S.* Infantis so effective as this ELISA system is based on whole cell lysate from *S.* Typhimurium only, whereas the tests A, B, and C are coated with LPS from *S.* Typhimurium and *S.* Choleraesuis to detect also infections by serovars of group C.

With respect to test sensitivity, the LPS-based tests revealed significant lower sensitivities especially in pigs experimentally infected with *S.* Infantis and *S.* Derby. Thus, the majority of animals infected with *S.* Infantis or *S.* Derby were detected with these tests as seronegative until day 39 after infection. The *Salmonella* Typhimurium whole-cell-lysate based ELISA, however, was able to recognise most of these animals as *Salmonella*-positive at initial phase of infection. In this regard, the specificity of test D, but also of the other three compared ELISA systems is of special interest and has to be elucidated in future. The *Salmonella* Typhimurium whole-cell-lysate based ELISA offers a clear advantage regarding test sensitivity, however, this test is more laborious, requires special software for evaluating results and should be considered from the financial point of view. This might be the reason, why the test is only available (since spring 2010 in Germany) on request for scientific purposes.

The comparison of the test sensitivities with regard to the matrices (meat juice and blood serum) showed a tendency to lower sensitivities when analysing meat juice. Wilhelm et al. (2007) observed a non-linear correlation between antibody detection in serum and meat juice in particular if *Salmonella* prevalence in pig production units is high. There are reports that serum/meat juice ratios may widely diverge due to several factors such as stress and the state of hydration of pigs (Davies et al., 2003). In contrast, Steinbach et al. (2003) reported a positive correlation between the antibody level of meat juice and serum. In our study, test C showed significant

lower sensitivities of 43% for *S.* Infantis and 11% for *S.* Derby by testing meat juice compared to sera using the surveillance cut-off (40%). These findings suggest that blood sera are more suitable for a serological diagnosis of *Salmonella* antibodies in pigs than meat juice. This is unfortunately contrary to the obvious benefits of collecting and testing of meat juice samples, which can be handled more cost-effectively and, since it is collected after death, avoids stressful treatment of the pigs. Furthermore, the use of the recommended cut-off scientifically determined by the manufacturers for routine diagnosis of *Salmonella* may result in a considerably increase of sensitivity regardless of *Salmonella* serovars. This has to be considered especially when monitoring breeding herds and slaughter pigs used for the winning of raw goods.

Conclusion

The in parallel performed study of Szabo et al. (2008) showed that all tested ELISA systems are able to detect *S.* Typhimurium infection in pigs. Both sample matrices, blood sera and meat juice, are suitable for antibody detection. However, the test sensitivity mainly depends on the respective cut-off used for the specific test.

Our findings indicate that the currently used LPS-ELISA systems have diagnostic uncertainties particularly in detecting porcine *S.* Infantis and *S.* Derby infection when combined with the cut-off value of 40 OD%. Therefore, future intra-vitam *Salmonella* control measures should use the cut-off value of 20 OD%. But, even by use of the 20 or 10 OD% cut-off value the currently used LPS-ELISAs identify *Salmonella*-infected pigs about 30 days later than the *Salmonella* Typhimurium whole-cell-antigen based isotype-specific ELISA.

Acknowledgement

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