Comparative examination and validation of ELISA test systems for Salmonella diagnosis of slaughtering pigs

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Introduction
Infections with Salmonella enterica are one of the most important sources of human gastroenteritis. The consumption of contaminated pork products was found to be associated with 20% of human salmonellosis in Germany, whereas S. Typhimurium, especially phagetype DT 104, is the most frequently isolated Salmonella serotype from pork (Steinbach and Kroell, 1999). Salmonella infection can be directly diagnosed in the abattoir or at farm level by serodiagnosis using anti-LPS ELISA (Nielsen et al., 1995, Mousling et al., 1997). Serological results are used to classify swine herds in three categories for assessing the hygienic status of farm in regard to Salmonella infection in pigs. In this context, reliable ELISA test systems are required for the categorisation of swine herds.

The object of our study was to monitor antibody response and faecal shedding in sixteen weaned pigs experimentally infected with Salmonella Typhimurium DT 104. For evaluation of serological results, four ELISA tests approved in Germany were used. Three tests were directed at IgG isotype and one test discriminated between antibody classes IgA, IgM and IgG.

Material and methods

Swine and experimental design
Sixteen 6 weeks old Salmonella-free piglets were orally exposed to S. Typhimurium DT 104 and followed by clinical examination, blood and faecal sampling until day 130 post inoculation (p.i.). Faecal samples of pigs were tested negative for Salmonella at day of arrival. A porcine isolate of multiresistant Salmonella Typhimurium strain (BB 440) obtained from a herd of swine with acute salmonellosis was used for infection of pigs. The strain was additionally provided with a nalidixic acid resistance in the National Reference Laboratory for Salmonella. Each pig was infected with 4.4x10⁶ cfu DT 104 by intragastric application using a nasal stomach tube. Prior infection animals were sedated with 1.0 – 2.0 mg/kg azaperon, i.m. Animals were fed with a commercial antibiotic-free feed and provided water ad libitum. Feed was withdrawn 20 h before infection.

Blood samples were taken from the cranial vena cava on the first and second day p.i., until 30 days p.i. twice a week and then at weekly intervals until slaughter. Prevalence of Salmonella in faeces was determined daily within the first eleven days p.i., until 30 days p.i. in intervals of 3 days and then once a week until slaughter at day 130 p.i.

Bacteriological examination
Faecal samples of approximately 10 g were inoculated in 1 % Buffered Peptone Water (1.12535, Merck) (1:10), homogenised using a Stomacher 400 (Seward, London, UK) for 2 min at high speed and incubated at 37°C for 24h. Afterwards 0.1 ml was inoculated on MSRV (modified semisolid Rappaport-Vassiliadis, CM 900100, SR161E, Oxoid) agar plates and incubated at 42°C for 24h.

Serological examination
Blood samples were coagulated for 20 h at 4°C and centrifuged for 10 min at 3500 g. Serum was collected and stored at −20°C until analysis. Swine sera were analysed for the presence of antibodies against Salmonella according to producers instructions using following kits:
Salmotype® Pig Screen (LDL Leipzig, Germany),
- HerdChek® Swine Salmonella Antibody (IDEXX Laboratories, Wörrstadt, Germany),
- Enterisol® Salmonellen-Diagnostikum (Boehringer Ingelheim, Germany)
- Salmotype® Pig STM-WCE (LDL Leipzig, Germany)

Results

Clinical findings

During the first week p.i., 31% of pigs suffered from semi-liquid diarrhoea for 1-2 days. Only one pig showed anorexia, vomiting and diarrhoea until the 6th week p.i., thereafter it became convalescent. 69% of pigs had elevated body temperature up to 41.6°C within 24h to 48h p.i. that became normal within 9 days p.i. Weight increased continuously, at day 130 p.i. (age 24.5 weeks) the average weight was 121 kg per pig.

Faecal shedding of S. Typhimurium

S. Typhimurium was isolated from faeces of all 16 infected pigs after one day p.i. All animals excreted Salmonella in faeces until day 16 p.i. Thereafter shedding was intermittent until slaughter except one animal which remained Salmonella-negative (Figure 1).

Serological results

Figure 1 shows the results for percentage of Salmonella-positive animals over the study period for ELISA tests 1-3. In ELISA test 1, individual seroconversion in pigs was observed between day 13 and 67 p.i., whereas the majority of pigs (88%) was positive for Salmonella antibodies between day 28 and 47 p.i. Apart from one pig which became negative for Salmonella at day 130 p.i., all animals remained seropositive. ELISA test 2 detected seroconversion among pigs between day 16 and 47 p.i. However, the majority of pigs (94%) seroconverted between day 22 and 39 p.i. Afterwards, all pigs were tested positive for Salmonella antibodies until slaughter except one pig which became seronegative at day 88 p.i. By use of ELISA test 3, three pigs seroconverted from day 13 p.i. whereas the latest was positive for Salmonella antibodies from day 39 p.i. All animals remained seropositive until the end of experiment.

Results for ELISA test 4 are presented in Figure 2 as mean ELISA units for the different antibody classes IgG, IgM and IgA over the entire study period. Apparently, individual ELISA units varied within all antibody classes especially for anti-Salmonella-IgG. Almost 56% and 94% of infected pigs were already detected 7 and 13 days p.i., respectively by dominant IgM antibodies. Seroconversion of IgA and IgG was observed later whereas majority of animals yielded a positive result from day 22 (75%) and 25 (69%) p.i., respectively. All 16 pigs remained positive for all three immunoglobulin isotypes until day 130 p.i. except one pig which became negative for IgA at day 88 p.i. and another one which did not develop any anti-Salmonella-IgA.

Discussion

In the present study, all 16 animals excreted Salmonella within two weeks p.i., thereafter shedding rate declined and remained intermittent. Similar results were obtained in a study of experimental infections by Nielsen et al. (1995) where 80% of pigs excreted Salmonella Typhimurium during the first week p.i., thereafter excretion decreased and intermittent excretion could be observed. Although primary infection of pigs was induced by experimental inoculation of Salmonella via stomach tube, a spontaneous re-infection of animals due to Salmonella contaminated faeces even during the intermittent shedding stage is most likely.
According to results of serological examination by use of ELISA tests 1-3 and bacteriological findings, the majority of pigs developed no anti-Salmonella IgG within the third week in spite of high Salmonella excretion in faeces. It is known that the peak of Salmonella excretion in faeces is followed by an immune response after 1-2 weeks because it takes time to develop a detectable serological response (Nielsen et al. 1995). Own results confirm the problem of “diagnostic window” in the early stage of infection that may cause false-negative results during serological testing.

In the longitudinal study on detection of Salmonella infection in fattening pigs, ELISA tests 1-3 varied in regard to sensitivity. Dependent on the test applied, at least 50% of infected animals were tested positive for Salmonella antibodies from day 22 (test 3), day 25 (test 2), and 39 p.i. (test 1), respectively. The highest sensitivity was observed 39, 47, and 67 days p.i. (with test 3, 2, and 1, respectively) when all infected animals were serologically tested positive. This observation may be explained by the fact that the sensitivity to detect Salmonella antibodies mainly depends on the respective cut-off recommended for the specific test. Apparently, test 3 revealed the highest sensitivity due to the lowest cut-off. However, ELISA tests with a higher sensitivity likely lack in specificity what was not investigated in this study.

During the chronic stage of infection which covers the main part of life span of a fattening pig, animals showed a higher rate of seropositive reactors by use of all three ELISA tests compared to a lower rate of pigs shedding Salmonella in faeces. Considering the probability to find Salmonella-positive animals in a farm, antibody detection by ELISA will surpass the bacteriological examination of faeces. However, a conclusion from a seropositive result to the status of Salmonella infection (acute vs. chronic stage) is not possible. Therefore, ELISA tests directed at IgG are used as screening tests on herd level, but they are not suitable for individual pig testing confirming results from other studies (Nollet et al., 2005).

Meanwhile, novel ELISA systems have been developed, which besides IgG, additionally detect antibody classes IgM and IgA in order to distinguish between an early and older infection on individual level (Lehmann, 2004, Ehlers et al., 2006). Own results obtained with ELISA test 4 confirm the usefulness to detect pigs in the early stage of infection better than ELISA tests 1-3. The higher sensitivity of ELISA test 4 is mainly due to the ability to detect antibodies of the early class IgM and finally, contributes to avoid false-negative results. However, this test is more labour intensive, needs a special software for evaluating results and should be considered from the financial point of view. Further investigations on ELISA test 4 are planned on distinction between an early and older infection with special regard to identification of Salmonella shedding pigs.

References


Figure 1: Rate of seropositive pigs (%) detected by ELISA tests 1-3 compared with shedding rate in faeces of pigs (16) infected with S. Typhimurium (day 1 to 130 p.i.)

Figure 2: Results for ELISA test 4 (E.U./ml) for antibody classes IgM, IgA, IgG compared with shedding rate in faeces of pigs (16) infected with S. Typhimurium (day 1 to 130)