O 24 Discrimination of Vaccinated and infected Pigs by *Salmonella*-specific IGa antibodies

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Summary: Isotype switching from IgM to IgG or IgA leads to generation of high affinity antibodies during the immune response. This phenomenon can be applied for differentiation of early and late infection stages. The purpose of this study was to evaluate the applicability of a newly developed Ig-isotype specific *Salmonella* antibody ELISA for discrimination between vaccinated and infected pigs.
Using this novel ELISA we were able to detect different patterns of *Salmonella*-specific IgM, IgG and IgA antibodies following immunization with a *S.* Typhimurium live vaccine or after experimental infection with a nalidixic-acid resistant wild-type strain of *S.* Typhimurium. Interestingly, *Salmonella*-specific IgA antibodies represented an excellent tool for the recognition of fresh infection in vaccinated pigs. Under SPF conditions, we were able to discriminate between naive, vaccinated, experimentally infected nonvaccinated, and experimentally infected vaccinated animals. However, the highest specific IgA levels were detected in challenged vaccinated pigs. Preliminary results from field trials support the findings from experiments using SPF animals.

Keywords: Serology, Diagnostics, ELISA, Ig class, Vaccination

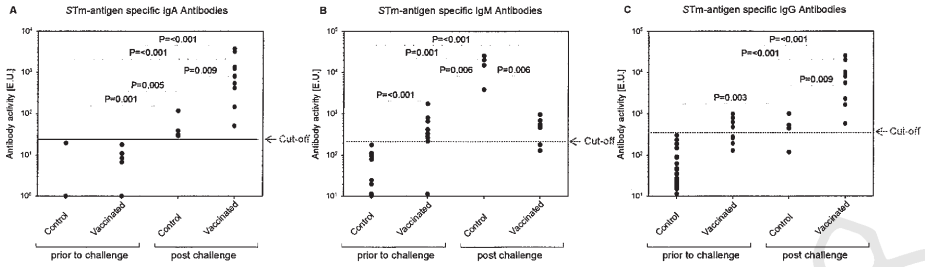
Introduction: Due to intensified stockbreeding the risk of *Salmonella* transmitted to man by consumption of animal food products increased. Vaccination with live attenuated vaccines represents an effective tool for reduction of *Salmonella* burden of pig herds. Nevertheless, farmer often refuse live vaccines being afraid to be unable to distinguish between antibody formation following the vaccination or infection with wild strains. Unfortunately, there exists no reliable assay to discriminate vaccinated from infected pigs so far which could overcome this dilemma. Therefore, we attempted to apply a novel isotype-specific ELISA system for discrimination between infected pigs and pigs immunized with a *S.* Typhimurium live vaccine.

Materials and Methods: The ELISA developed for this purpose uses antigens from whole cellular extract of *S.* Typhimurium (SALMOTYPE® Pig WCE-ELISA, Labor Diagnostik Leipzig, Leipzig, Germany). Antigen-bound IgM, IgG, and IgA antibodies were detected in swine serum samples by isotype-specific peroxidase-conjugated secondary antibodies. Sera derived from *S.* Typhimurium vaccination/challenge experiments using SPF pigs (n=4-20) and two different field trials were analysed for evaluation of the test. In order to minimize interassay variances and to normalize the measured signals any individual serum was $\log_2$ diluted over four steps and compared to a defined reference standard serum which was equally diluted (Butler, 1991). For each Ig class (IgM, IgA, IgG) an especially calibrated reference standard serum was used. OD raw data were calculated into ELISA units (E.U.) by using a new software developed for this purpose (SALMOSoft™, Labor Diagnostik Leipzig). The calculated E.U. were plotted statistical significances were calculated by using the SigmaStat™ software (SPSS Science, Erkrath, Germany).

Results: Using the novel Ig isotype-specific ELISA we were able to detect different patterns of *Salmonella*-specific IgM, IgG and IgA antibodies following oral immunization with a *S.* Typhimurium live vaccine (SALMOPORC® IDT, Rosslau, Germany) or experimental infection with a nalidixic-acid resistant wild-type

strain of *S.* Typhimurium DT104 (958/96). Interestingly, *Salmonella*-specific IgA antibodies represented an excellent tool for the recognition of fresh infection in vaccinated pigs but to a lower extent also in nonvaccinated control animals. Thus, under SPF conditions, we were able to differentiate between naive, vaccinated, experimentally infected nonvaccinated, and experimentally infected vaccinated pigs. However, the highest specific IgA levels were detected in challenged vaccinated pigs (Fig. 1A). Although the IgM values were found to be very high at day 7 post infection in nonimmunized pigs, which recommends IgM as an indicator of very early infection in nonvaccinated pigs, IgM is not a reliable parameter for *Salmonella* infections in vaccinated pigs since there was an overlapping region with the IgM levels of healthy, nonimmunized controls animals (Fig. 1B). A similar result was observed in terms of IgG. But in contrast to IgM, *Salmonella*-specific IgG was an appropriate indicator of the secondary immune response induced by an infection in immunized pigs similar to IgA, but did not clearly identify the vaccine induced primary immune response since the half of the values overlapped with those of control animals (Fig. 1C). In general, the background IgG level was higher than the background levels of IgM and IgA. Therefore, IgA revealed to be the most appropriate parameter for our purpose. Preliminary results from field trials confirm the findings from experiments using SPF animals. Taken together, these data provide a novel approach for the identification of *Salmonella*-infected pigs and the discrimination from pigs immunized with *Salmonella* live attenuated vaccines.

Figure 1: Recognition of *S.*-Typhimurium infected SPF pigs by detection of *Salmonella*-antigen specific IgM, IgG, and IgA antibodies. IgA was most appropriate for discrimination of infected animals. Statistical significances were calculated by using the Mann-Whitney rank sum test.



Discussion and Conclusion: The evaluation of *Salmonella* incidence in pig herds or even individual animals, particularly in breeding facilities, by serological tests has been a matter of discussion for many years. However, the high sensitivity represents an important advantage of serological compared to bacteriological diagnostic methods. A disadvantage of many serological tests is their low specificity which depends mainly on the nature of the antigen used. As demonstrated in this study the use of whole cellular extract antigen in combination with the detection of certain Ig isotypes allows the recognition of *Salmonella* infection in pigs with relatively high precision. This novel test offers a hopeful tool not only for highly reliable evaluation of *Salmonella* incidence in pig herds or the infection and/or immune status of individual animals but also to promote the acceptance of immunization of pig herds using live vaccines under diagnostic control.

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