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Establishment and application of a cell culture assay to quantify neutralizing antibodies against Clostridium perfringens type C β-toxin in pigs

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Key words
Clostridium perfringens type C, beta toxin, cell culture assay, pigs, colostrum, serum

Aim of the study
This study was performed to establish, validate and apply a cell culture assay to detect and quantify neutralizing antibodies against *Clostridium perfringens* type C beta toxin in serum, colostrum and milk of sows and piglets

Material and methods
Two different trials were conducted, a vaccination and a field trial. In both trials gilts were vaccinated five and two weeks prior to farrowing and subsequently received booster vaccinations once before every farrowing.
Vaccination trial: group 1 consisted of 10 vaccinated gilts and their piglets; group 2 consisted of 10 non-vaccinated gilts and their piglets. Vaccine: Toxoid of *C. perfringens* type C (IDT, Dessau, Germany). Blood and colostrum samples of all sows and 2 piglets per sow were collected at different time points.
Field trial: Three vaccinating farms and one none vaccinating farm participated. Sows were immunized using commercially available vaccines. On the vaccinating farms blood and colostrum samples of 9 gilts and 36 multiparous sows and 2 piglets per sow were collected. On the non-vaccinating farm blood and colostrum samples of 2 gilts and 14 multiparous sows as well as 2 piglets per sow were collected.
Cell culture assay: 100 ng of recombinant beta-toxin were incubated with a serial dilution of sera or colostrum for 1 h. Primary porcine endothelial cells, grown on 96-well plates, were incubated with this mixture for 24 hours at 37°C. Cell viability was determined using a redox dye and colorimetric quantification.

Results and significance
During the first part of our study, we successfully established a cell culture assay, allowing specific and quantifiable detection of neutralizing antibodies against the *C. perfringens* beta-toxin. In the vaccination trial the initial immunization with generated a mean colostral antibody titer high enough to protect the piglets from the first litter on. Antibodies were readily detected in serum of all piglets. Between the initial immunization and the booster vaccination during the second pregnancy the mean antibody titer of the sows decreased, but significantly increased after the booster vaccination. The mean colostral antibody titer was significantly higher at the second farrowing compared to the first. Additionally, over the period of four weeks post partum, the piglets of the second litter had a significantly higher mean antibody titer than the piglets of the first litter.
In the field trial there was a significant difference in the antibody response between the vaccinated gilts and multiparous sows and their piglets. No antibody titers were detected in the serum of gilts after the first immunization. After the second immunization low antibody titers in serum and colostrum were detected in only five out of the nine gilts. In 1/3 of the piglets of gilts no serum antibody titers could be detected. The remaining 2/3 showed low serum antibody titers.
In contrast, multiparous sows showed significantly increased antibody titers after the booster vaccination. The

serum antibody titers after the booster vaccination highly correlated with the antibody titers in the colostrum and in piglet serum. The mean antibody titer in piglet sera of multiparous sows was three times higher than in piglets of gilts.

In conclusion we successfully established a cell culture based bioassay which detects neutralizing anti-beta-toxin antibodies in serum and colostrum of pigs. Moreover, our results clearly show that repeated and continuous vaccination of sows with commercially available *C. perfringens* type C vaccines ensures passive immunity of piglets. The currently recommended and applied vaccination schemes however do not induce sufficient antibody responses to beta-toxin in all gilts, which could lead to insufficient passive immunity in piglets of gilts. Therefore piglets of gilts still have a risk of succumbing to necrotizing enteritis caused by *C. perfringens* type C, even under correctly performed vaccination programs. This could be avoided by an adaptation of the vaccination scheme with more frequent initial vaccinations of gilts before the first farrowing. We propose to investigate this in a short follow up study, as this could lead to new recommendations on the vaccination intervals in pig herds.

Publications, posters and presentations

Richard, O. K. (2017), Entwicklung eines Zellkulturassays zum Nachweis von neutralisierenden Beta-Toxin Antikörpern. Oral presentation: 23.01.2017, IDT Biologika GmbH, Business Unit Animal Health, Research and Development, Am Pharmapark, Dessau, Germany

Richard, O. K. (2017), Establishment and application of a cell culture assay to quantify neutralizing antibodies against *Clostridium perfringens* type C β-toxin in pigs. Poster presentation: Infectious Diseases of Swine: Clinico-pathological Approaches and Introduction of Novel Diagnostic Methods, 25-27 January 2017 in Bern

Richard O. K. (2017), Establishment and application of a cell culture assay to quantify neutralizing antibodies against *Clostridium perfringens* type C β-toxin in pigs. Poster presentation: European symposium of porcine health management (ESPHM), 03.-05. May 2017 in Prague, Czech Republic

Richard, O.K.; Grahofer, A.; Nathues, H.; Springer, S.; Vidondo, B.; Posthaus, H. Establishment and application of a cell culture assay to quantify neutralizing antibodies against *Clostridium perfringens* type C β-toxin in pigs. (*To be submitted to international peer reviewed journal. For submission to a journal we will have to wait for final vaccine approval at the IDT Biologica, which is anticipated by July 2017. Once accepted this manuscript will serve as inaugural Dissertation at the Vetsuisse Faculty.)*

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