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Use of RT-PCR Ct values to assess trends in the magnitude of PRRS virus viremia over time

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Introduction and Objective

Real-time polymerase chain reaction (RT-PCR) is the standard diagnostic tool to monitor and evaluate the progress of PRRS virus (PRRSv) control programs. RT-PCR cycle threshold (Ct) values have shown a strong correlation to the amount of a pathogen's genetic material. RT-PCR Ct values may be a useful quantitative indicator of infection level in veterinary diagnostics.[2] Polson reported a correlation between PRRSv quantitative PCR (qRT-PCR) and PRRSv non-quantitative PCR (rtRT-PCR) Ct values.[3] The ease of interpretation and lower cost of rtRT-PCR vs qRT-PCR point to Ct values as a potentially useful tool in monitoring PRRSv control programs. The objective of this study was to evaluate the use of rtRT-PCR Ct testing as a relative quantitative measurement tool for PRRSv viremia as part of a PRRSv control program in a large production system.

Materials and Methods

The study was conducted at a large-scale, PRRS positive and unstable, production system located in the US. In July, 2008, a PRRS modified live virus (MLV) pig vaccination program was implemented in pigs upon weaning (2 ml, I.M.). By February 2009, all growing pigs in the system had been vaccinated. Late nursery and mid-finishing pigs (n=120) were blood sampled monthly. Hospital pens in both production phases were targeted for sampling. The individual sera were pooled 5:1 within production phase and tested by PRRS rtRT-PCR. Reported Ct values reflect the number of PCR amplification cycles that are required to exceed a defined threshold. Therefore a higher reported value (#cycles) reflects a lower viral load initially present in the sample. A Ct value for every PRRSv rtRT-PCR positive pool was determined and summarized over two month intervals: Jul-Aug08 (n=41), Sep-Oct08 (n=69), Dec08–Jan09 (n=77), Feb–Mar09 (n=62) and Apr-May09 (n=63). The Ct values for each period were analyzed within and across production phases.

Results

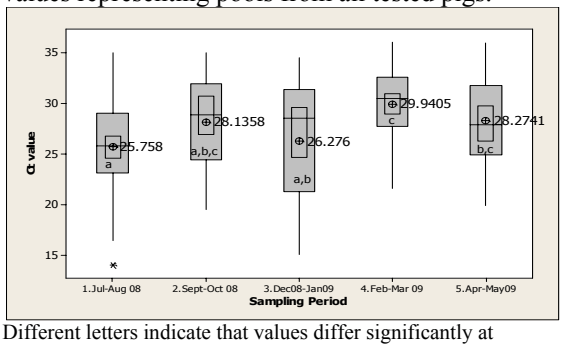
Both wild type and MLV PCR positives were included in nursery pig data analyses. Nursery Ct values rose over time (data not shown), consistent with decreasing viral load. All PCR positives detected in finishers were wild type virus. When comparing finishing pigs Ct values during and after full system vaccination , Ct values rose significantly indicating a decrease in viral load subsequent to vaccination ($P \leq 0.05$, Table 1). Across production phases (all PCR positives tested), Ct values rose over time consistent with decreasing PRRS viral load across the collective populations (Figure 1).

Table 1. PRRS rtRT-PCR Ct values in pooled finishing pig sera (Wild Type positive only).

Period	rtRT-PCR Ct value: mean (SD)
During implementation - system wide vaccination (Jul08-Jan09) n=60	29.5 (4.310)[a]
After full implementation - system wide vaccination (Feb-May09) n=33	32.31 (2.740)[b]

Different letters indicate that values differ significantly at $P<0.05$ (Mann-Whitney test).

Figure 1. BoxPlot & CI 95% PRRS rtRT-PCR Ct values representing pools from all tested pigs.



Different letters indicate that values differ significantly at $P \leq 0.05$ (Tukey HSD test).

Conclusions

This is the first field report using Ct values as a relative quantitative indicator of viral loads as part of a PRRSv control project and therefore, the results should be interpreted with prudence. Ct values rose over time subsequent to implementation of a MLV PRRS vaccination program suggesting that vaccination decreased viral load on a system-wide basis. However, further correlation of RT-PCR Ct values with quantitative PCR values and PRRS strain type are needed.[3]

References.

1. Cikos S et al 2007. *BMC Molecular Biology* 8:113.
2. http://www.wvdl.wisc.edu/PDF/WVDL.Info.PCR_Ct_Values.pdf
3. Polson D et al 2010. 21st IPVS Proccedings. P.169, Pp 475.

Control and elimination of a porcine reproductive and respiratory syndrome virus field isolate from a continuous flow, single-site nursery-finisher unit using Ingelvac® PRRS MLV vaccination

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Introduction and Objectives

Porcine reproductive and respiratory syndrome (PRRS) Area Regional Control (ARC) projects have developed throughout the United States in the past year. The establishment of individual herd plans is a major component of these ARC projects. Herd plans for continuous flow growing pig sites are one of the more challenging to develop and implement.

A plan to control and subsequently eliminate an endemic PRRS virus isolate; (1-18-4 RFLP cut pattern), that infected a multi-age group growing pig herd was developed by the practicing veterinarian and then implemented by the farm employees. The objective of this study was first, to control the circulation and shedding of the endemic PRRS virus isolate and to subsequently eliminate it from the farm.

Materials and Methods

The site houses three groups of 2800 pigs per group with nine-to-ten weeks of age difference between each group. The site is comprised of two nursery buildings with four and six nursery rooms in each building. There are two sets of finisher buildings with four buildings each dedicated to the grower and finisher age groups. Each set of finisher buildings has a dedicated hallway; however, exhaust air is shared between the buildings on the site. During the study period, pigs from the oldest group were agressively marketed two weeks (day -14 to 0) prior to the first vaccination (day 0). On day 0, all pigs from the three groups housed in the farm (3-4, 12-13 and 21-22 weeks of age) were vaccinated intramuscularly with a full dose (2ml) of Ingelvac® PRRS MLV. The pigs to be marketed within the next 21 days (~200 head) were not vaccinated. Twenty-one days later (day 21), the same pigs were vaccinated with a second full dose of Ingelvac® PRRS MLV excluding pigs marketed (~200 head) or pigs to be marketed within 21 days (~1,000 head). Between day 42 and 52 a new group of PRRS virus negative weaned pigs from a PRRS positive stable farm undergoing an elimination process; (Standard Herd Classification II-B)*, was entered into the study farm. Each of the pigs in this group was vaccinated with a single full dose of Ingelvac® PRRS MLV. Between day 125 and 135 another PRRS virus negative wean group was entered into the nursery but was not vaccinated. Prior to and throughout the control and elimination process, internal biosecurity was increased. The protocol indicated the use of dedicated boots and clothes for each age group, no supply sharing between groups was allowed without previous disinfection and Synergize was used to disinfect the facility, equipment and supplies.

Results

A random subset of pigs (n=10) from the group placed between day 125 to 135 (PRRS virus negative and not vaccinated) were bled on day 184 (9 weeks post placement) and sera was tested for the presence of PRRS virus antibodies by ELISA Herd Check PRRS 2XR (IDEXX Laboratories Westbrook, ME). All serum samples were negative.

Discussion and Conclusions

The goal to control and eliminate the endemic PRRS virus isolate from the farm was achieved. Double-mass vaccination with Ingelvac® PRRS MLV of pigs already on the site, vaccination of the first incoming group after mass vaccination, and a short herd closure period between groups decreased shedding, and limited horizontal transmission. The negative PRRS virus pigs entered between day 125 to 135 remained negative to PRRSv antibodies, indicating no exposure to either field or modified live vaccine virus and therefore successful implementation of the protocol. The farm will continue monitoring PRRSv status and respecting internal and external biosecurity. Custom herd plans, such as this, are necessary in area regional control projects.

*Holtkamp, D., Polson, D., et.al., *JSHAP,* 2011.