*Real-time PCR assay*

**Sample selection for PCV2 DNA detection**

Out of the 42 samples from the unvaccinated against PCV2 pigs, a number of 17 samples from pigs seropositive to *T. gondii* and/ or *N. caninum* were selected for PCV2 DNA detection by real-time PCR (Table 1).

**Method**

All samples were subjected to nucleic acid extraction, using the QIAamp® cador® Pathogen Mini Kit (Qiagen, Hilden, Germany), under the manufacturer's recommendations. The extracts were subsequently tested for the presence of PCV2 genome, by applying the TaqMan probe-based real-time PCR method as previously described [57]. This method targets a 144 bp conserved region of the viral ORF1. The pair of primers used was PCV2-PT-rep6(F) and PCV2-PT-rep149(R) with sequences 5′-CAGCAAGAAGAATGGAAG-3′ and 5′-TTACCCTCCTCGCCAAC-3′, respectively. Reactions were performed on a LightCycler® 2.0 Instrument (Roche Life Science, Vilvoorde, Belgium) and fluorescence data were analyzed using the LightCycler® Software (version 4.1; Roche Life Science).

**Results**

Out of the 5 unvaccinated sows of T+ group, 4 (80%) were PCR positive to PCV2, while 7 (70%) of the 10 unvaccinated sows of N+ group were PCR positive to PCV2. The two animals seropositive to both pathogens were also PCR positive to PCV2 (Table 1).

**Table 1.** Number of seropositive sows against *T. gondii* and/or *N. caninum,* number of porcine circovirus 2 (PCV2) vaccinated and unvaccinated seropositive sows and number of PCV2 PCR positive and negative PCV2 unvaccinated sows.

| Seropositive sows | | PCV2 vaccination status in seropositive sows | | PCV2 PCR in unvaccinated sows | |
|---|---|---|---|---|---|
| | | + | - | + | - |
| *T. gondii* (T+) | 14 | 9 | 5 | 4 | 1 |
| *N. caninum* (N+) | 11 | 1 | 10 | 7 | 3 |
| Both | 2 | 0 | 2 | 2 | 0 |
| Total | 27 | 10 | 17 | 13 | 4 |

**Conclusion**

PCV2 circulation is evident in the majority of unvaccinated seropositive to *T. gondii* and/or *N. caninum* pigs.