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Berl Münch Tierärztl Wochenschr 129, 437–443 (2016)
DOI 10.2376/0005-9366-16029

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ISSN 0005-9366

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Eingegangen: 07.03.2016
Angenommen: 03.05.2016

Online first: 25.05.2016
http://vetline.de/open-access/158/3216/

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Detection of Porcine Reproductive and Respiratory Syndrome Virus and Porcine Cirovirus Type 2 in blood and oral fluid collected with GenoTube swabs

Nachweis des Porzinen Reproduktiven und Respiratorischen Syndrom Virus und des Porzinen Circovirus Typ 2 in mittels GenoTube-Tupfern gewonnenem Blut und Speichel

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Summary

The earliest possible detection of pathogens is of utmost importance in limiting the spread of contagious diseases, such as Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Circovirus Type 2 (PCV-2). Alternative sample matrices must enable sensitive detection of these swine pathogens without the need for standard blood collection in pigs by venipuncture. Here, we investigate the potential use of GenoTube swabs for PCR detection of PRRSV and PCV-2 in individual blood and oral fluid swabs from pigs after experimental PRRSV infection. The results were compared with matched serum and pen-wise collected oral fluid. It was shown that PCV-2 detection in GenoTube blood swabs, oral fluid swabs and pen-wise oral fluid is at least as sensitive as in serum, while PRRSV detection is most sensitive in serum, followed by the blood swabs and oral fluids. A good correlation was observed between PCV-2 and PRRSV loads in serum and blood swabs, while there was little or no correlation between serum and oral fluids. A modified extraction protocol enabled further improvement of PRRSV RNA recovery from GenoTube swabs. Furthermore, we show that GenoTube swabs are suitable for long-term (up to 56 days) storage of specimens at room temperature without significant influence on PRRSV RNA stability. In conclusion, blood and oral fluid collected by GenoTube swabs are practical and reasonable sample matrices for PRRSV and PCV-2 detection by RTq-PCR and qPCR, respectively. However, it has to be kept in mind that viral loads in oral fluid do not accurately reflect those in serum.

Keywords: RT-qPCR, qPCR, pig, pen-wise, individual

Zusammenfassung

Eine möglichst frühzeitige Detektion von Pathogenen ist von größter Wichtigkeit für die Eindämmung der Ausbreitung von Infektionskrankheiten wie das Porzine Reproduktive und Respiratorische Syndrom Virus (PRRSV) und das Porzine Circovirus Typ 2 (PCV-2). Alternative Probenmaterialien müssen für den sensitiven Nachweis dieser Erreger geeignet sein und sollten ohne die herkömmliche und aufwendige Blutentnahme bei Schweinen auskommen. Hier untersuchen wir die mögliche Verwendung von GenoTube-Trockentupfern für den PCR-Nachweis von PRRSV und PCV-2 in individuellen Blut- und Speichelproben von Schweinen nach experimenteller PRRSV-Infektion. Die Ergebnisse wurden mit den korrespondierenden Serumproben und buchtenweise gewonnener Speichelflüssigkeit verglichen. Es zeigte sich, dass der PCV-2-Nachweis in Bluttupfern, Speicheltupfern und buchtenweiser Speichelflüssigkeit mindestens genauso sensitiv ist wie im Serum, während PRRSV am sensitivsten in Serum nachgewiesen wurde, gefolgt von Bluttupfern und Speichelflüssigkeit. Es bestand eine enge Korrelation zwischen den PCV-2- und PRRSV-Lasten in Serum und Bluttupfern, wohingegen die Korrelation zwischen Serum und Speichelflüssigkeit geringer oder nicht vorhanden war. Ein modifiziertes Extraktionsprotokoll führte zu zusätzlicher Verbesserung der PRRSV-RNA-Rückgewinnung aus den GenoTube-Tupfern. Weiterhin zeigen wir, dass GenoTube-Tupfer auch für die Langzeitlagerung (bis zu 56 Tage) bei Raumtemperatur geeignet sind, ohne dass es zu signifikanten

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Einflüssen auf die PRRSV-RNA-Stabilität kommt. Zusammenfassend kann gesagt werden, dass mit dem GenoTube-System gewonnene Blut- und Speicheltupfer eine praktische und sinnvolle Probenmatrix für den PRRSV- und PCV-2-Nachweis mittels RT-qPCR bzw. qPCR sind. Es sollte jedoch daran gedacht werden, dass die Viruslasten in der Speichelflüssigkeit nicht unbedingt jenen im Serum entsprechen.

Schlüsselwörter: RT-qPCR, qPCR, Schwein, gruppenweise, individuell

Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Circovirus Type 2 (PCV-2) are among the most significant viral pathogens of swine (Meng, 2012). PRRSV infection may lead to abortion or stillbirth in pregnant sows as well as to respiratory signs and high fever (Karniychuk and Nauwynck, 2013). PCV-2 may contribute to several potentially multifactorial disease complexes involving wasting, lung, enteric, kidney, reproductive or skin disease (Segales, 2012). Co-infection with PRRSV and PCV-2 may enhance disease severity, parallel detection of both viruses in the same sample is therefore of clinical significance. Apart from diagnosis of a clinically apparent disease, detection of clinically healthy virus carriers is of the utmost importance for pig trade, especially for farms with virus-negative status, or countries that have implemented control or eradication programmes. In live pigs, direct detection of both viruses is usually performed in blood serum, typically collected by venipuncture of the Vena cava cranialis. Alternative sample matrices, such as oral fluid or semen, have also been propagated (Kittawornrat et al., 2010). Oral fluid has the advantage of simple sample collection in groups of pigs and can be conducted without the need for a trained veterinarian and further has the potential to monitor groups of pigs over an extended period of time, without disturbing the animals. Semen is collected routinely from boars and would thus represent a sample matrix that is available in sufficient quantity without the need to disturb the animal any further. However, in comparison to serum, RT-qPCR from semen is also of lower diagnostic sensitivity (Gerber et al., 2013; Pepin et al., 2015), although the virus shedding in semen can sometimes last much longer than the viremia (Christopher-Hennings et al., 1995). Another alternative would be to collect drops of blood originating from a small puncture of superficial veins, i. e. with a scalpel or a needle, onto absorbent material, such as filter paper or swabs. While this procedure is probably not feasible for small pigs kept in groups, it would certainly simplify the sampling of large, individually kept pigs, such as boars. It was shown that PRRSV RNA can be detected by RT-qPCR in biological samples (serum, oral fluid, tissue) collected onto chemically treated filter paper cards (FTA-cards). However, the analytic sensitivity is about 100 times lower than in native sample material, thus potentially limiting the usefulness of FTA-cards for PRRSV diagnostic purposes (Linhares et al., 2012; Steinrigl et al., 2014). A commercially available swab system with an integrated active desiccant system was shown to efficiently preserve DNA (Garvin et al., 2013). Recently, a similar swab system adapted to veterinary purposes (GenoTube Livestock, Prionics, Schlieren, Switzerland), was successfully used for efficient detection of RNA and DNA viruses, i. e. African and Classical swine fever virus, in the same sample matrix (Petrov et al., 2014). However, the suitability of these swabs for detection of PRRSV and PCV-2 has not yet been tested.

The aim of the presented work was to determine whether GenoTube swabs represent efficient sampling tools for the diagnosis of PRRSV and PCV-2 infection. Therefore, PRRSV and PCV-2 detection in blood and individual oral fluid samples collected with GenoTube swabs were evaluated by comparison with matched serum and pen-wise oral fluid samples. Furthermore, the stability of PRRSV RNA in GenoTube swabs stored for up to 56 days at room temperature was evaluated, as well as the influence of different extraction regimens on PRRSV RNA recovery.

Material and Methods

Biological samples

Blood (via puncture of the Vena cava cranialis), pen-wise oral fluids and individual GenoTube (GT) oral fluid swabs were collected from pigs experimentally infected with a high pathogenic (HP) PRRSV type 2 field isolate (AGES/568-30FC/13, GenBank: KM588915) in the course of two different experiments. Experiment one included ten animals that were seven weeks of age at the time of challenge infection; they received 10^4 $TCID_{50}$ of AGES/568-30FC/13 by the intradermal route and the experiment was terminated at 14 days post infection. Experiment two included ten animals that were about ten weeks of age at the time of challenge infection; they received 2×10^5 $TCID_{50}$ of the same virus by the intranasal route and the experiment was terminated at 28 days post infection. In both experiments, experimental animals were obtained from a PRRSV-negative farm and were tested for freedom from PRRSV by both ELISA and RT-qPCR. In experiment one, animals were naturally PCV-2 infected, while animals involved in experiment two had been vaccinated against PCV-2 at the age of six weeks with Ingelvac Circoflex (1 ml i. m., Boehringer Ingelheim, Germany). Housing, animal care and experimental protocol of both trials have been approved by the local ethics committee (Agency of the Government in Lower Austria, Department of Agrarian Law).

The blood serum was obtained by centrifugation of whole blood at 2400 g for 10 minutes at room temperature and stored at –20°C until nucleic acid extraction. In order to collect blood onto GT swabs, the swabs were quickly immersed into freshly from the Vena cava cranialis collected whole blood and then immediately re-inserted into the sheath tube. GT oral fluid swabs were collected by swabbing the buccal cavity of individual pigs. All GT

swabs were stored at room temperature until nucleic acid extraction. Pen-wise oral fluid was collected daily by exposing one cotton rope per barn (ten pigs) to the pigs for about 15 minutes. Thereafter, the ropes were centrifuged for 10 minutes at 2500 g in a 50-ml falcon tube with filter as described in Sattler et al. (2015). The resulting oral fluid was aliquoted and stored at –20°C until nucleic acid extraction.

In total, 103 GT blood swabs, 45 GT oral fluid swabs and 63 pen-wise oral fluid samples were obtained from both experiments. For all GT blood and oral fluid swabs, matched serum samples were available. For pen-wise oral fluid samples, matched serum samples from all pen-mates were available on days zero, three, seven, ten, 14 and 28 after challenge.

Nucleic acid extraction

Upon thawing, oral fluid samples were centrifuged at 1000 g for 10 minutes at 4°C and the resulting supernatants were further processed. Nucleic acids were extracted from 100 µl serum or oral fluid using the NucleoSpin® 96 Virus Core Kit (Macherey Nagel, Austria) on the Freedom Evo® 150 automated platform (Tecan, Austria). Just after addition of lysis buffer RAV1, 3 µl of XenoTM RNA Control (Life Technologies, Vienna, Austria) were added to each sample as extraction and inhibition control. Nucleic acids were eluted in 100 µl of elution buffer RE.

Nucleic acid was extracted from GT swabs by cutting a small piece (3–4 mm in diameter) of blood- or OF-soaked swab material from the tip of the swab with sterile scissors, immersing it in a mixture of 560 µl buffer AVL, 5.6 µl carrier RNA (QIAamp Viral RNA Mini kit, Qiagen, Germany) and 140 µl phosphate buffered saline (PBS) and vortexing it for 10 seconds. Afterwards, 3 µl of XenoTM RNA Control (Life Technologies, Austria) were added to each sample and extraction was further performed as suggested in the QIAamp Viral RNA Mini Handbook (QIAamp Viral RNA Mini kit, Qiagen, Germany). A set of duplicate GT blood swabs taken from two animals at all sampling time-points during the second animal experiment was extracted in a modified way: the whole swab was immersed in the same amounts of buffer AVL, carrier RNA and PBS as described above and all liquid from the soaked swab was collected by centrifugation at 3220 g for 10 minutes at room temperature. Thereafter, all further extraction steps were performed as described above.

RT-qPCR detection of PRRSV and qPCR detection of PCV-2

PRRSV RNA was detected and quantified in nucleic acids extracted from serum, GT swabs and oral fluid by a commercial reverse transcription quantitative PCR (RT-qPCR) assay (TaqMan® PRRSV Reagents and Controls, Life Technologies, Vienna, Austria), which features simultaneous, one-tube detection of North American (NA) and European (EU) genotype PRRSV, as well as of XenoTM RNA Control. RT-qPCR setup and thermoprofile was as recommended by the manufacturer. In each RT-qPCR run, a dilution series ranging from 8 x 10^7–8 copies of PRRSV NA RNA, as well as appropriate positive and negative controls were tested in parallel.

PCV-2 nucleic acid was detected and quantified in the same nucleic acid extracts in 25µl reaction mixes, consisting of 12.5 µl 2 x TaqMan Universal Master Mix (Life

TABLE 1: *Qualitative PCV-2 qPCR results in different sample materials of pigs naturally infected with PCV-2 and challenged with an HP PRRSV field strain*

		GT blood swabs		
		positive	**negative**	**total**
Serum	positive	24	0	24
	negative	5	2	7
	total	29	2	31
		GT oral fluid swabs		
		positive	**negative**	**total**
Serum	positive	32	0	32
	negative	13	0	13
	total	45	0	45
		pen-wise oral fluid		
		positive	**negative**	**total**
Serum (pen-wise)	positive	10	0	10
	negative	0	0	0
	total	10	0	10

TABLE 2: *Quantitative PCV-2 and PRRSV loads in different sample materials of pigs naturally infected with PCV-2 and challenged with an HP PRRSV field strain (only positive samples were calculated)*

	n	Viral load (copies/ml) median (1st; 3rd quartiles)	
PCV-2			**corresponding serum**
GenoTube blood swab	29	**9.2E+04** (2.4E+03; 2.1E+07)	**6.1E+05** (4.0E+03; 2.0E+07)
GenoTube oral fluid swab	32	**4.0E+05**[b] (1.1E+05; 1.0E+06)	**1.7E+06**[b] (4.0E+03; 3.3E+07)
Pen-wise oral fluid	10	**1.8E+07** (7.7E+06; 2.6E+07)	**2.5E+06** (1.9E+04; 1.7E+07)
PRRSV			**corresponding serum**
GenoTube blood swab	76	**2.9E+04**[c] (7.4E+03; 2.0E+05)	**7.3E+05**[c] (6.4E+04; 4.9E+06)
GenoTube oral fluid swab	31	**3.5E+03**[c] (2,3E+03; 1.2E+04)	**7.3E+05**[c] (1.3E+05; 7.3E+06)
Pen-wise oral fluid	9	**1.0E+04**[a] (3.9E+03; 2.5E+04)	**1.7E+06**[a] (1.7E+05; 4.2E+06)

a: significant difference P < 0.05

b: significant difference P < 0.01

c :significant difference P < 0.001

TABLE 3: *Qualitative PRRSV RT-qPCR results in different sample materials of pigs challenged with an HP PRRSV field strain*

		GT blood swabs		
		positive	**negative**	**total**
Serum	positive	76	17	93
	negative	0	10	10
	total	76	27	103
		GT oral fluid swabs		
		positive	**negative**	**total**
Serum	positive	31	14	45
	negative	0	0	0
	total	31	14	45
		pen-wise oral fluid		
		positive	**negative**	**total**
Serum (pen-wise)	positive	9	5	14
	negative	0	3	3
	total	9	8	17

Technologies, Vienna, Austria), 2.5 µl of sample, 200 nM of hydrolysis probe (Olvera et al., 2004) and each 900 nM of primers PCV2Fmod (5′-CCA GGA GGG CGT KBT GAC T-3′) and PCV2Rmod2 (5′-CGY TAC CGY TGG AGA AGG AA-3′). The primers are based on previously published ones (Olvera et al., 2004) that were modified to contain degenerate bases at variable positions of the PCV-2 genome within the primer binding sites. For absolute quantification, a standard dilution series ranging from 2.5 x 10^7–2.5 copies of PCV-2 plasmid DNA (determined by spectrophotometry) was included in each run. Proper positive and negative controls were included as well. The thermo-profile was as described elsewhere (Olvera et al., 2004).

Statistical analysis

The statistical analysis was done with the IBM SPSS statistics version 22. The qualitative test outcomes were classified into two-by-two contingency tables. The quantitative values were tested for normal distribution with the Kolmogorow-Smirnov test. Most parameters were not normally distributed. The quantitative values were compared by calculating regression curves and determining the goodness of fit (r^2). The rank correlation coefficient after Spearman was used to test for correlations for each comparative data set. Differences between the quantitative test outcomes were calculated with the Wilcoxon test. For comparisons of pen-wise oral fluids with serum, the median serum viral loads and the median GT oral fluid swab viral loads per pen were used. Differences with an error of possibilities $P < 0.05$ were considered significant.

Results

Agreement of PRRSV RT-qPCR and PCV-2 qPCR test outcomes between serum, GT swabs and pen-wise oral fluid

PCV-2 detection by qPCR, using GT blood swabs, GT oral fluid swabs or pen-wise oral fluid, was at least as sensitive as in serum (Tab. 1). Morever, more GT blood or GT oral fluid swabs were classified as PCV-2 positive than corresponding serum samples. An almost perfect correlation ($r = 0.96$) was observed between quantitative PCV-2 loads in GT blood swabs and serum, while correlation was less pronounced between serum and GT oral fluid swabs ($r = 0.50$) or pen-wise oral fluid ($r = 0.59$) (Fig. 1). Determination of the ratios of PCV-2 loads measured in GT blood swabs, GT oral fluid swabs or pen-wise oral fluid showed that quantitative results obtained from GT blood swabs and pen-wise oral fluids were highly similar to serum over the whole range of tested concentrations. PCV-2 loads determined from GT oral fluid swabs tended to be lower than corresponding values in serum towards higher PCV-2 serum concentrations (≥ 10^6 copies/ml), while the opposite was the case towards lower PCV-2 serum loads. This had a negative influence on the quality of fit of the regression curve (Fig. 1). Over all samples, PCV-2 loads in GT oral fluid swabs were significantly lower than in serum ($P < 0.01$) (Tab. 2). The PCV-2 load in pen-wise oral fluid samples was significantly lower than in the corresponding mean GT oral fluid swabs ($P < 0.05$).

In contrast to PCV-2, PRRSV detection sensitivity was lower than in serum for all three tested alternative specimens (GT blood swabs, GT oral fluid swabs and pen-wise oral fluid) (Tab. 3). False negative PRRSV test results from GT blood swabs were obtained in 52% of the samples with corresponding PRRSV serum loads below 9 x 10^4 copies/ml and in 100% of samples with corresponding PRRSV serum loads below 3 x 10^3 copies/ml. False negative results were observed only very early (on day one) or late (on day 14) after experimental infection and were never observed when the corresponding PRRSV serum load was above 9 x 10^4 copies/ml. PRRSV loads obtained with GT blood and GT oral fluid swabs were mostly correlating well with serum ($r = 0.88$ and $r = 0.59$) (Fig. 2), although the PRRSV load in GT blood and in GT oral fluid swabs was significantly lower than in serum ($P < 0.001$) (Tab. 2). PRRSV loads in pen-wise

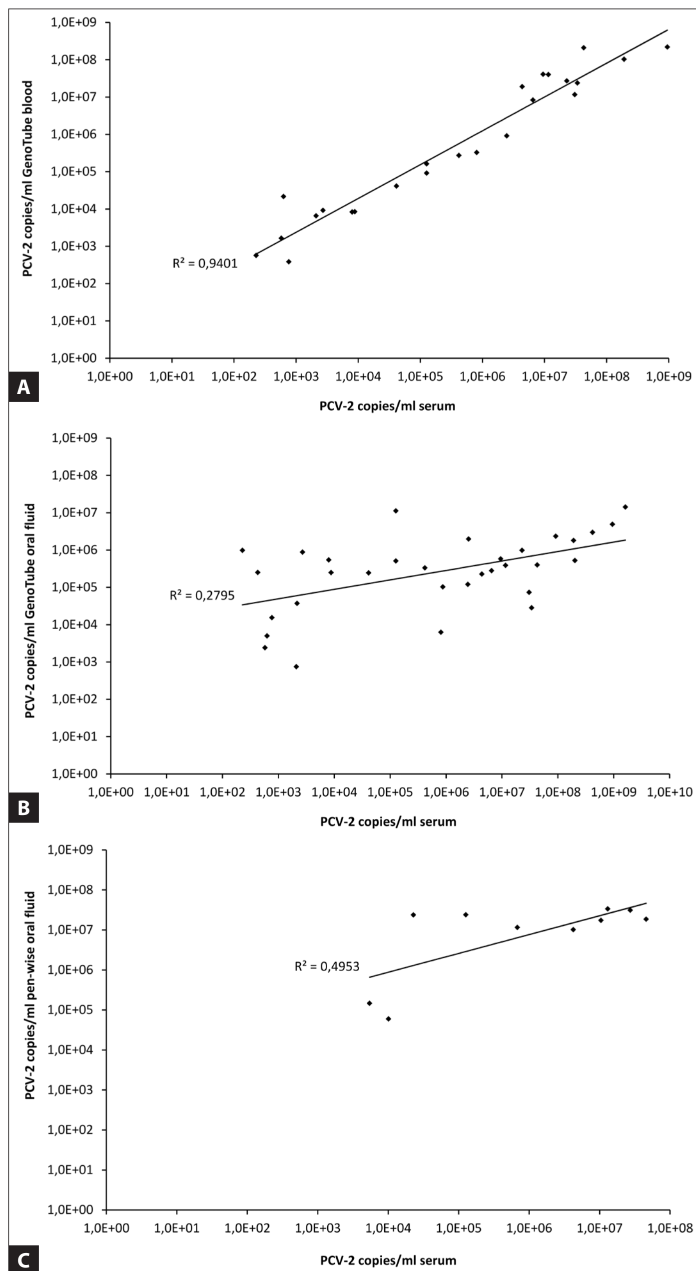

FIGURE 1: *Scatterplot indicating the correlation between PCV-2 load in serum and A) GenoTube blood swabs, B) GenoTube oral fluid swabs and C) pen-wise oral fluid in pigs naturally infected with PCV-2 and challenged with an HP PRRSV field strain.*

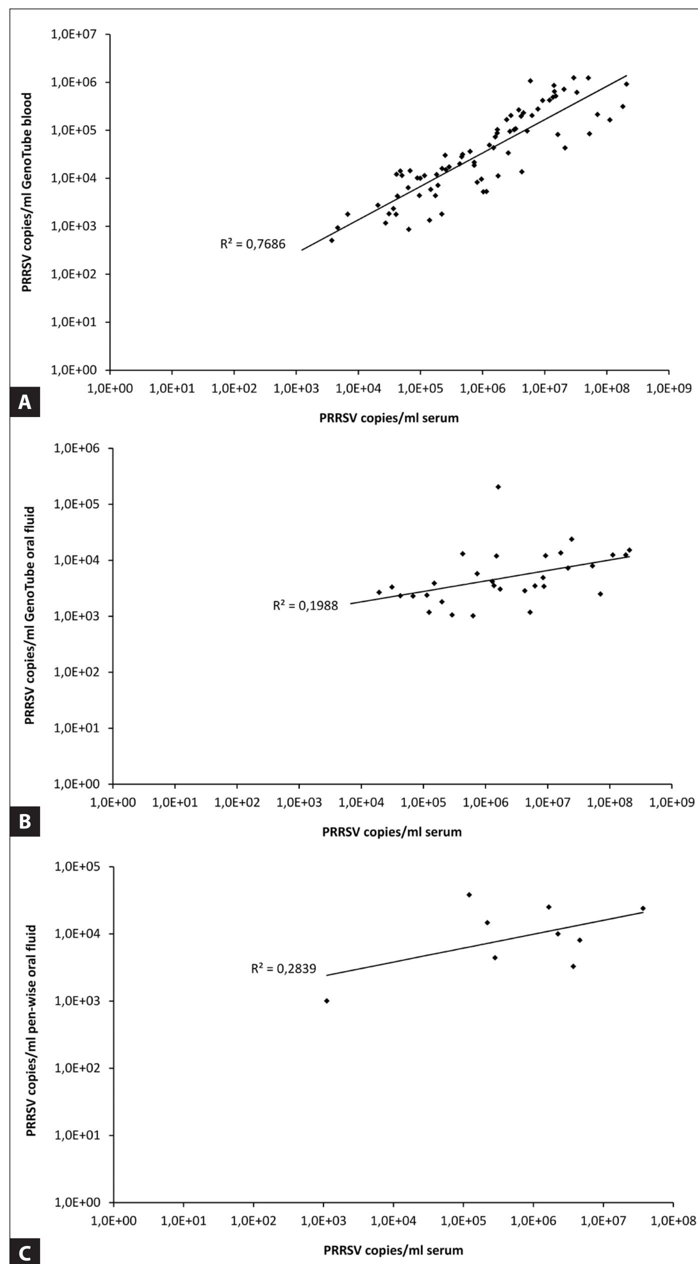

FIGURE 2: *Scatterplot indicating the correlation between PRRSV load in serum and A) GenoTube blood swabs, B) GenoTube oral fluid swabs and C) pen-wise oral fluid in pigs challenged with an HP PRRSV field strain.*

oral fluids showed no correlation with values obtained in serum (Fig. 2). A significantly higher PRRSV load was observed in serum than in pen-wise oral fluid ($P < 0.05$). No significant differences were seen between PRRSV loads in GT oral fluid swabs and pen-wise oral fluid.

Comparison of different extraction regimes on PRRSV RT-qPCR test outcome

In order to test if the observed differences in PRRSV load obtained from serum and GT blood swabs could be improved, a second nucleic acid extraction protocol was tested in which the whole blood soaked swab material was immersed in buffer as opposed to taking only a small piece of swab material from the tip of the swab (see Material and Methods section). Comparison of PRRSV RT-qPCR results on samples collected from two different animals from day three through day 21 after challenge showed an improvement of PRRSV RNA recovery by applying the modified protocol: while median PRRSV load difference between serum and GT-blood swabs extracted with the tip-cutting protocol was at 16-fold, application of the alternative extraction regimen to the same samples reduced this difference to fourfold (Fig. 3). After extraction with the modified protocol, the PRRSV load was significantly higher than with the tip-cutting protocol ($P < 0.05$), but still significantly lower than in the corresponding serum samples ($P < 0.05$). This improvement also impacted qualitative test outcomes, as a sample from one animal collected on day 21 after challenge (PRRSV serum load: 8.2×10^3 copies/ml) tested PRRSV RT-qPCR negative by the tip-cutting extraction protocol, but positive when extraction was performed following the alternative protocol.

Influence of extended storage of GT blood swabs at room temperature on PRRSV load determination

To assess the potential influence of extended storage of GT swabs on the stability of PRRSV RNA, replicate GT blood swabs collected from two different pigs were stored at room temperature for one, three, seven, 14, 28 and 56 days prior to nucleic acid extraction and PRRSV RNA quantification. The GT blood samples had a PRRSV load of 1.5×10^5 and 2.4×10^5 copies/ml (corresponding to Cq values of 31.5 and 30.8) when nucleic acid was extracted on the day following collection. There was no difference in qualitative test outcome in GT blood swabs subjected to extended room temperature storage, i. e. both samples still tested positive when extracted after 56 days of storage. During the first seven days of storage, there was also no drop in PRRSV load. From 14 days of storage onwards, the PRRSV load dropped slightly (Fig. 4). The maximal difference in PRRSV load observed between samples from the same animal during the course of the experiment was 16-fold, indicating that extended storage of GT blood swabs at room temperature does not have a major impact on PRRSV RNA stability.

Discussion

In order to facilitate animal-friendly and cost-effective repeated sampling for the monitoring of infectious diseases like PRRSV and PCV-2, alternative diagnostic specimens, which do not require standard venipuncture have been propagated (Kittawornrat et al., 2010). Collection of suitable biological samples especially from boars for diagnostic purposes is of utmost importance for early detection of PRRSV introduction into boar studs, in order to limit the consequences arising from distribution of PRRSV contaminated semen (Nathues et al., 2016). A number of studies have been published regarding the potential use of pen-wise and individual oral fluids, semen, FTA-cards and blood swabs, with partly contradictory results (Inoue et al., 2007; Prickett et al., 2008a; Linhares et al., 2012; Kittawornrat et al., 2014; Decorte et al., 2015; Pepin et al., 2015). However, the training of the boars to chew on cotton ropes or the overnight application of substances like apple juice on the ropes, like it was described in a study of Kittawornrat et al. (2010) will not be accepted by the practitioners and farmers of boar studs for routine diagnostics. GT swabs are a commercial swab system with a

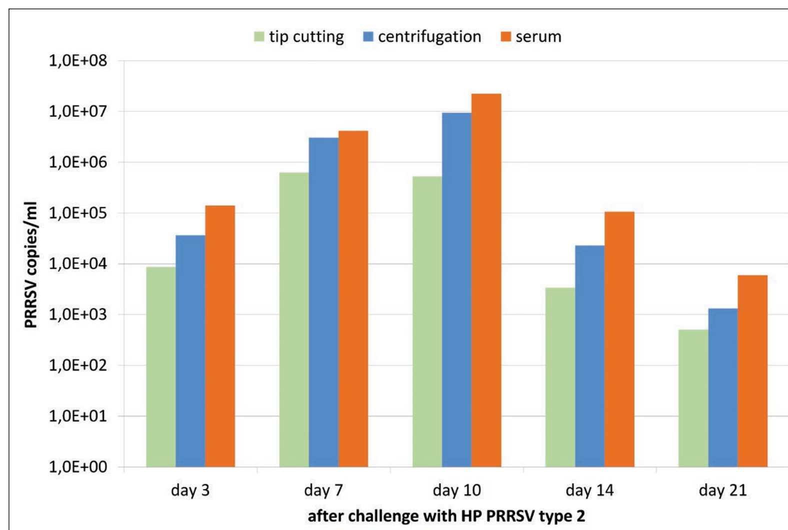

FIGURE 3: *PRRSV load in GenoTube blood swabs after nucleic acid extraction with two different protocols in comparison to the serum in two pigs challenged with an HP PRRSV field strain (mean).*

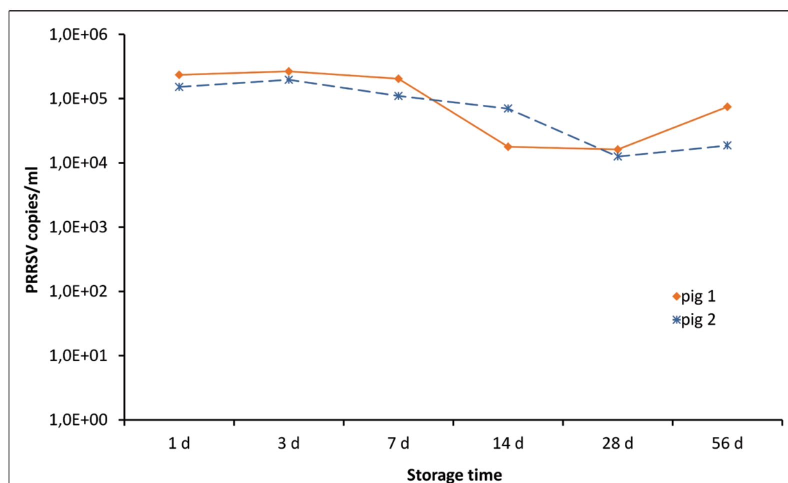

FIGURE 4: *PRRSV load in GenoTube blood swabs after storage at room temperature for different durations, obtained from two pigs challenged with an HP PRRSV field strain.*

special self-drying medium that was shown to be suited for PCR-diagnostics of African and Classical swine fever virus (Petrov et al., 2014) and for the detection of PRRSV antibodies in oral fluid (Sattler et al., 2015). To our knowledge, this is the first study determining the suitability of GT swabs for PCR detection of PRRSV and PCV-2. The presented results suggest that GT blood swabs can be used for both PRRSV and PCV-2 PCR detection, with little (for PRRSV) or no (for PCV-2) impact on test sensitivity as compared to serum. In fact, PCV-2 detection was more sensitive with GT blood swabs than with serum. Regarding comparability of quantitative results, PRRSV loads in GT blood swabs were consistently lower than corresponding serum loads, while PCV-2 loads were almost identical between both matrices. Other blood swab systems like the one used by Pepin et al. (2015) resulted in a lower sensitivity and a considerably lower PRRSV load than in serum and in oral fluid. We did not perform a puncture of small superficial veins in our study to collect blood for the swab system, because we preferred a direct comparison between serum and GT blood swab from the same blood sample. Furthermore, the collection from superficial veins would be suitable rather for individually kept large pigs like boars during semen collection.

In our study, we used the tip-cutting protocol for extraction of the GT swabs as it was successfully established by Petrov et al. (2014). However, PRRSV RNA recovery from GT blood swabs could be improved by using an alternative extraction protocol with centrifugation of the swabs that can easily be standardized. A potential disadvantage of this extraction protocol is that one GT swab can only be used for one extraction. Long-term storage at room temperature had no significant influence on test sensitivity and PRRSV load in GT blood swabs. This is in accordance with the conclusions of Garvin et al. (2013) that self-drying swabs similar to the GT swab can be stored at room temperature for several weeks without loss of sensitivity.

GT oral fluid swabs and pen-wise oral fluids were found to be less sensitive for detection of PRRSV, but not for PCV-2 infection, most likely due to comparatively low PRRSV loads or rapid degradation of PRRSV in oral fluid. The inhibition measured by Chittik et al. (2011) was not observed in our study and in a former study (Steinrigl et al., 2014). For both, PRRSV and PCV-2, the correlation between serum viral loads and viral loads measured in GT oral fluid swabs and pen-wise oral fluids was low. A lower PRRSV load in oral fluid than in serum was found in other studies as well, thus limiting sensitivity in animals with a low viral load or in groups of pigs with a low proportion of virus shedders (Prickett et al., 2008b; Steinrigl et al., 2014; Pepin et al., 2015). The usage of oral fluid stabilizers was studied and discussed (Decorte et al., 2013) and would be applicable for native oral fluid samples but not for the GT oral fluid swabs.

It can be concluded that both GT blood and GT oral fluid swabs can be used for the diagnosis of PCV-2 without a significant reduction of sensitivity. This can be used for instance in the genetic characterisation of the PCV-2 present in the respective farm as it was suggested by Prickett et al. (2008b). The sensitivity of PRRSV detection as well as the viral load in GT blood swabs and more so in GT oral fluid swabs and pen-wise oral fluid is reduced compared to the sensitivity in serum. This should be considered in the interpretation of the results of laboratory investigations.

Acknowledgement

The excellent technical assistance provided by the teams of the Department for Molecular Biology and Virology and Electron Microscopy (AGES Institute for Veterinary Disease Control Mödling, Austria) is acknowledged. We thank Jutta Pikalo and Eveline Wodak for the valuable help in animal care and sampling. This project was supported financially by the company Sanphar.

Conflict of interest

The authors declare that there are no protected financial, professional or other interests in a product or a company that could affect the contents or opinions stated in this publication.

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