Real-time PCR diagnostic package for diagnosis of porcine respiratory disease.

Jorsal, Sven Erik Lind; Hjulsager, Charlotte Kristiane; Kokotovic, Branko; Larsen, Lars Erik

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

National Veterinary Institute

Technical University of Denmark

Real-time PCR diagnostic package for diagnosis of porcine respiratory disease

Sven Erik Jorsal, Charlotte K. Hjulsager, Branko Kokotovic, Lars E. Larsen

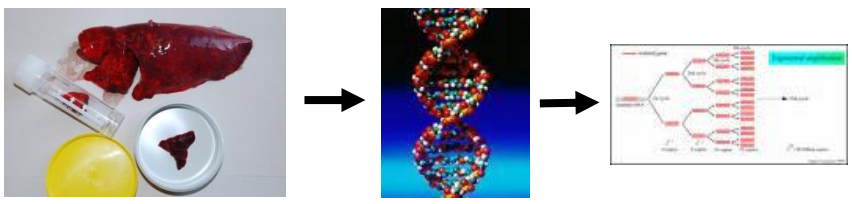
National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark

Introduction

Several viruses, mycoplasmas and bacteria are involved in respiratory disease in pigs. Most of them can be detected by PCR, but conventional bacteriological examination is preferable for most bacterial infections, because of the broad spectrum of potential species and the need for detection of antimicrobial resistance. A PCR diagnostic package for detection of the common respiratory pathogens swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhyo) was developed at the National Veterinary Institute (NVI). Here we present a summary of the results of samples tested since the launch in 2007.

Materials and Methods

Lung tissue samples were submitted to NVI from veterinary practitioners as 3x3x3 cm pieces of lung tissue from 3 individual pigs with respiratory disease. RNA was extracted with RNeasy Mini Kit (QIAGEN) and DNA was extracted with QIAamp DNA Mini Kit (QIAGEN). RNA was used for test of SIV and PRRSV, and DNA was used in the Mhyo and PCV2 tests. Testing was performed with separate real-time PCR assays for Mhyo, SIV, PCV2 and PRRSV, respectively. SIV and Mhyo results were reported as detected / not detected. PCV2 results were quantitatively expressed as copies of PCV2 pr. 500 ng DNA extracted. PCV2 copies >10^7 was defined as massive load. Detection of PRRSV Type1/Type2 (EU/US type) was an option in the package, but only chosen in relatively few submissions.



Samples from 3 separate lungs → **DNA/RNA extraction** → **Real-time PCR**

Influenza:	**detected / not detected**
M. hyopneum.:	**detected / not detected**
PCV2:	**level of PCV2 copies pr. 500 ng DNA**
Optional PRRSV:	**type1/type2 detected / not detected**

Results

In the period from late 2007 until 31 December 2013, NVI received 1085 submissions with 2797 samples of lung tissue. The number of submissions with at least one sample positive for SIV was 345 (32%) and for Mhyo 231 (21%). For PCV2, the number of submissions with >10^7copies of PCV2 per 500 ng extracted DNA was 131 (12%). The annual prevalence of positive samples varied during the period for SIV (23-52%) and PCV2 (6-15%), but was quite stable for M. hyo (19-23%). Detection of PRRSV was optional and only requested in 84 out of the 1085 submissions. PRRSV Type 1 was detected in 2 submissions and PRRSV type 2 in 13 submissions.

Table 1.

Number of submissions, samples and positive submissions.

	Submissions	Samples	SIV positive	Mhyo pos.	PCV2 >10^7
2007[1]	31	77	8 (26)	6 (19)	4 (13)
2008	224	463	57 (23)	52 (23)	33 (15)
2009	198	482	55 (28)	46 (23)	28 (14)
2010	176	465	60 (34)	38 (22)	24 (14)
2011	144	400	32 (22)	30 (21)	9 (6)
2012	122	368	63 (52)	22 (18)	11 (9)
2013	190	542	70 (37)	37 (20)	22 (12)
07-13	1085	2797	345(32)	231(21)	131(12)

1: Fourth quarter in 2007

Discussion

SIV was the pathogen most often detected, often in combination with other pathogens which may indicate that swine influenza is often involved in respiratory disease in Danish pig herds. The proportion of SIV positive submissions increased simultaneously with launch of the diagnostic package, from around 20 % positives before to around 30 % positives after launch, probably due to an increased number of samples per submission/herd.

In Denmark ca. 3000 herds are registered in the SPF System. However, around 2/3 of the SPF herds are infected with Mhyo and around 1/2 of the herds with PRRSV. Hence, the PCR diagnostic package is still a relevant diagnostic tool for many Danish pig herds.

The diagnostic predictive value of PCV2 detection in lung tissue has not been documented and cannot replace standard PCV2 diagnostic tests for PCVD/PMWS. However, detection of massive PCV2 load in lungs implies that supplementary tests for the PCV2 significance is relevant.

Poster no. 120