SWINE HEALTH INFORMATION CENTER
FINAL RESEARCH GRANT REPORT FORMAT

Project Title: Investigation of the clinical signs and lesions associated with PCV3
Project identification number: 18-201 SHIC
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Institution: University of Minnesota
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Industry Summary:

Porcine circovirus type 3 was discovered in 2016 in the US associated with cases of systemic disease and reproductive disorders. Multiple studies performed during the last two years have shown that this virus is widespread and has been around for decades. It can be found in multiple tissues and samples, in pigs with multiple clinical conditions and in healthy pigs. However, we are lacking precise information on the relevance of this virus and its potential to cause disease in pigs. The objective of this proposal was to mine the diagnostic data obtained by the MN VDL during the last 2 years to identify associations between the presence of PCV3 and its viral load and specific lesions and clinical conditions. PCV3 results, clinical signs and information on the lesions for each pig were retrieved from the MN VDL laboratory information management system, submission forms and diagnostic reports. PCV3 frequency in pigs with different clinical signs ranged from 12% to 27%. No significant associations were observed between clinical signs and the presence of PCV3. In PCV3-positive pigs, no clinical signs were significantly associated with having a higher load of PCV3. PCV3 frequency in pigs with different lesions ranged from 0% to 62%. The only lesion that had a significant association between its presence and PCV3 infection was heart vasculitis/perivascultis. In PCV3-positive pigs, higher viral loads were significantly associated with pigs with myocarditis, heart vasculitis/ perivasculitis, kidney vasculitis/perivasculitis and dermatitis. This study did not identify any significant associations with clinical signs. However, the presence of PCV3 in 20% of fetuses is remarkable. In addition, the samples with the highest PCV3 concentration in this study were from fetal tissues. Lesions of myocarditis and systemic vasculitis were associated with the presence or the amount of PCV3 in tissues. The lack of significant results for other lesions does not exclude the possibility of a real association and may be due to confounding factors or limited data. In summary, this study provides an objective view of the relationship between PCV3 and disease, based on a large dataset of diagnostic cases. PCV3 is a very common virus that circulates in healthy populations and can be detected in around 20% of the pigs submitted to the diagnostic laboratory. Therefore, it is important to differentiate when PCV3 plays a significant role and when it does not. The results from this study support previous studies that suggested that PCV3 may cause death in fetuses and myocarditis and systemic vasculitis in pigs.

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Keywords: PCV3, abortion, myocarditis, vasculitis, diagnostic laboratory, lesions, clinical signs

Scientific Abstract:

Porcine circovirus type 3 was discovered in 2016 in the US associated with cases of systemic disease and reproductive disorders. Multiple studies performed during the last two years have shown that this virus is widespread and has been around for decades. It can be found in multiple tissues and samples, in pigs with multiple clinical conditions and in healthy pigs. However, we are lacking precise information on the relevance of this virus and its potential to cause disease in pigs. The objective of this proposal was to mine the diagnostic data obtained by the MN VDL during the last 2 years to identify associations between the presence of PCV3 and its viral load

and specific lesions and clinical conditions. PCV3 results were retrieved using the MN VDL laboratory information management system (LIMS). Clinical signs and lesion information was retrieved from the LIMS, and/or from diagnostic reports and submission forms. Two by two tables were used to evaluate the association between PCV3 infection and clinical signs and lesions. For pigs that tested positive for PCV3, differences in Ct values were investigated using a Wilcoxon rank test. PCV3 frequency in pigs with different clinical signs ranged from 12% to 27%. No significant associations were observed between clinical signs and the presence of PCV3. In PCV3-positive pigs, the median Ct value ranged from 31 to 38. No clinical signs were significantly associated with having a lower Ct value. PCV3 frequency in pigs with different lesions ranged from 0% to 62%. The only lesion that had a significant association between its presence and PCV3 infection was heart vasculitis/perivascultis. In PCV3-positive pigs, the median Ct value ranged from 22 to 38. Lower Ct values were significantly associated with pigs with myocarditis, heart vasculitis/perivasculitis, kidney vasculitis/perivasculitis and dermatitis. This study did not identify any significant associations with clinical signs. However, the presence of PCV3 in 20% of fetuses is remarkable. In addition, the samples with the highest PCV3 concentration in this study were from fetal tissues. Lesions of myocarditis and systemic vasculitis were associated with the presence or the amount of PCV3 in tissues. The lack of significant results for other lesions does not exclude the possibility of a real association and may be due to confounding factors or limited data. The results from this study support previous studies that suggested that PCV3 may cause death in fetuses and myocarditis and systemic vasculitis in pigs.

Introduction:

Porcine circovirus type 3 was discovered in 2016 in the US associated with cases of systemic disease and reproductive disorders (Palinski et al. 2016, Phan et al. 2016). Multiple studies performed during the last two years have shown that this virus is widespread and has been around for decades (it was detected in samples from 1993 in Sweden). At the University of Minnesota Veterinary Diagnostic Lab (MN VDL) we have been monitoring for the presence of PCV3 on a routine basis during the last two years. We learned that PCV3 is widespread in the U.S. It can be found in multiple tissues and samples, in pigs with multiple clinical conditions and in healthy pigs. We now have a very good understanding of the frequency of the virus in the country and worldwide. However, we are lacking precise information on the relevance of this virus and its potential to cause disease in pigs. Therefore, this study investigates the relevance of PCV3 by mining the information accumulated from diagnostic cases.

Objectives:

The general objective of this proposal is to study the relevance of PCV3.
The specific objective is: to mine the diagnostic data obtained by the MN VDL during the last 2 years to identify associations between the presence of PCV3 and its viral load and specific lesions and clinical conditions.

Materials & Methods:

Veterinary diagnostic submissions from the University of Minnesota Veterinary Diagnostic Laboratory (MN VDL) were used for this study. All porcine submissions received from the United States that had at least one PCV3 PCR test between Feb 2016 and Jan 2018 were retrieved using the UMN VDL laboratory information management system (LIMS). Each individual laboratory submission was considered a single case regardless of number of pig samples submitted. For the evaluation of the association with clinical signs, only cases that had tissues available and thus were considered to be representative of clinical cases, were selected. Clinical signs information was retrieved directly from the LMIS, and/or from the diagnostic reports and submission forms. PCV3 positivity rate for each clinical sign was calculated in Microsoft Excel. Two by two tables were used to evaluate the association between PCV3 infection and clinical signs in Epi info™, and the odds ratio and p values of Fisher Exact test were recorded.
For the investigation of the association of PCV3 with histological lesions, lesion information was extracted from case diagnostic reports, and categorized into different organs and lesion

types. For each or the lesions, each pig was assigned one of three possible outcomes: lesion present, lesion absent or tissue not available. The 2x2 table was used to illustrate and present measures of association between PCV3 infection and lesion presence, and the odds ratio and p value were recorded. For pigs that tested positive for PCV3, the PCR CT data was used to investigate differences in the amount of virus between pigs with and without each lesion, using a Wilcoxon nonparametric rank test.

Results:

The records from a total of 730 cases containing 2,177 samples were extracted from LIMS. The cases were collected from 474 farms in 21 states of the United States including Colorado, Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Missouri, Montana, Nebraska, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, South Dakota, Tennessee, Texas, Wisconsin, and Wyoming.

From those, 1,323 pigs had tissues and therefore were included in the analysis of association of PCV3 with clinical signs and lesions. The results of the associations with clinical signs are summarized in table 1. PCV3 frequency in pigs with different clinical signs ranged from 12% in pigs with CNS signs to 27% in pigs with lameness or weight loss. No significant associations were observed between clinical signs and the presence of PCV3. In PCV3-positive pigs, the median Ct value ranged from 31 in pigs with respiratory disease to 38 in pigs with CNS signs. No clinical signs were significantly associated with having a lower Ct value. There were significant associations between having a higher Ct value and pigs affected with CNS signs or weight loss.

The results of the associations with histopathological lesions are summarized in table 2. Very infrequent lesions (observed in less than 10 pigs) were excluded from the analysis. PCV3 frequency in pigs with different lesions ranged from 0% in pigs with ear necrosis or epidermitis to 62% in pigs with heart vasculitis/perivasculitis. The only lesion that had a significant association between its presence and PCV3 infection was heart vasculitis/perivascultis. For three lesions, there was an association between the absence of the lesion and PCV3 infection: bronchopneumonia, atrophic enteritis and epidermitis. In PCV3-positive pigs, the median Ct value ranged from 22 in pigs with heart vasculitis/perivasculitis to 38 in pigs with purulent arthritis. Lower Ct values were significantly associated with pigs with myocarditis, heart vasculitis/perivasculitis, kidney vasculitis/perivasculitis and dermatitis.

Discussion:

In this study, we analyzed two years of laboratory results to investigate the relationship of PCV3 infection with clinical signs and lesions. PCV3 was detected in pigs with all categories of clinical signs and no significant associations were observed. However, the presence of PCV3 in 20% of fetuses is remarkable. Fetuses tend to remain free of viral infections during a healthy pregnancy. Even in cases of viral abortion due to other viruses, it is not always easy to detect the virus in all of the fetuses. In addition, the samples with the highest PCV3 concentration in this study were from fetal tissues. Therefore, these findings suggest that PCV3 may be responsible for fetal death, as has been proposed before (Palinski et al. 2016).

Lesions of myocarditis and systemic vasculitis were associated with the presence or the amount of PCV3 in tissues. This supports the findings reported by Phan et al (2016). The lack of significant results for other lesions does not exclude the possibility of a real association and may be due to confounding factors or limited data.

In summary, this study provides an objective view of the relationship between PCV3 and disease, based on a large dataset of diagnostic cases. PCV3 is a very common virus that circulates in healthy populations and can be detected in around 20% of the pigs submitted to the diagnostic laboratory. Therefore, it is important to differentiate when PCV3 plays a significant role and when it does not. The results from this study support previous studies that suggested that PCV3 may cause death in fetuses and myocarditis and systemic vasculitis in pigs.

Table1:

Clinical Signs	*PCV3 frequency (PCV3+/total, (%))*	*Odds Ratio (Lower-Upper)*	*P-value*	*Median Ct (min- max)*	*P-value*
Respiratory	163/746 (21)	0.93 (0.67 – 1.28)	0.6785	31.6 (15.2 – 39.8)	0.4279
GI	78/383 (20)	0.84 (0.62-1.14)	0.2798	33.0 (15.2 – 39.8)	0.8546
Weight loss	24/87 (27)	1.37 (0.84-2.25)	0.2253	37.5 (25.5 -39.7)	0.0006
Lameness	20/72 (27)	1.38 (0.8-2.36)	0.2417	32.1 (24.3 - 39.7)	0.9261
Sudden death	17/71 (23)	1.11 (0.63 – 1.95)	0.7673	35.3 (15.2 – 40.0)	0.3468
CNS	7/49 (12)	0.48 (0.22-1.08)	0.0966	38.6 (30.0 – 39.7)	0.0170
Others	21/83 (25)	1.20 (0.72-2.02)	0.4921	31.1 (19.3 -37.4)	0.2176
Abortion	57/284 (20)	NA	NA	33.3 (10.1 – 39.7)	NA

Table 2:

Lesion	*Lesion frequency (lesion presence/pigs evaluated)*	*PCV3 frequency (PCV3+/total, (%))*	*Odds Ratio (95% CI)*	*P-value*	*Median Ct (min- max)*	*P-value*
Serositis/polyserotis	88/1022	21/88 (23)	1.11 (0.66-1.85)	0.6884	29 (15.2-40)	0.9513
Bronchopneumonia	392/1013	74/392 (18)	0.72 (0.53-0.99)	0.0439	30 (16.6-39.8)	0.5813
Interstitial pneumonia	302/1013	61/302 (20)	0.84 (0.61-1.18)	0.3230	28.6(15.2-39.8)	0.0607
Pleuritis	196/1013	36/196 (18)	0.75 (0.50-1.11)	0.1801	31.8 (20.5-39.7)	0.4267
Peribronchitis/peribronchiolitis	75/1013	14/75 (18)	0.79 (0.43-1.44)	0.5634	28.7 (24.3-39.4)	0.1650
Bronchiolitis/bronchitis	73/1013	18/73 (24)	1.16 (0.67-2.02)	0.6608	30.6 (16.4-39.6)	0.6642
Lung congestion and edema	73/1013	19/73 (26)	1.25 (0.73-2.16)	0.4644	32.2 (23.2-40)	0.9062
Septic pneumonia	27/1013	7/27 (25)	1.23 (0.51-2.95)	0.6402	25.9 (15.2-39.7)	0.5573
Fibriosuppurative/necrotizing pleuropneumonia	18/1013	7/18 (38)	2.27 (0.87-5.92)	0.0919	35.7 (28.3-39.4)	0.2221
Liver/hepatitis	78/866	16/78 (20)	0.93 (0.52-1.66)	0.8860	30.6 (20.7-38.5)	0.1724
Liver hypoxia lesion	27/866	8/27 (29)	1.55 (0.67-3.60)	0.4275	32.4 (25.6-38.6)	0.9255
Splentitis	44/832	10/44 (22)	1.02 (0.49-2.10)	1.0000	33.3 (20.7-39)	0.8172
Spleen lymphoid depletion	27/832	7/27 (25)	1.22 (0.51-2.92)	0.6423	29.7 (20.5-39.4)	0.7353
Epicarditis	132/819	24/132 (18)	0.78 (0.48-1.25)	0.3556	29 (20.5-39.7)	0.9897
Myocarditis	85/819	22/85 (25)	1.30 (0.78-2.19)	0.3301	25.2 (16.4 -39.4)	0.0007
Endocarditis	19/819	2/19 (10)	0.42 (0.10-1.84)	0.3952	31.5 (25.6-37.4)	0.9945
Heart vasculitis and perivasculitis	16/819	10/16 (62)	6.35 (2.27-17.72)	0.0004	22.1(18.5-32.9)	<0.0001
Heart necrosis and hemorrhage	11/819	3/11 (21)	0.99 (0.27-3.58)	1.0000	35.4 (30.1-38)	0.4636
Kidney glomerular and tubular lesion	31/747	8/31 (25)	1.26 (0.55,2.87)	0.6562	27.7 (23.2-39.7)	0.1587
Kidney/vasculitis and perivasculitis	14/747	6/14 (42)	2.75 (0.94-8.05)	0.0929	23.9 (21.7-28.4)	0.0041
Kidney/interstitial nephritis	12/747	1/12 (8)	0.32 (0.04-2.51)	0.4791	NA	NA
Atrophic enteritis	117/672	16/117 (13)	0.53 (0.30-0.94)	0.0257	35.3 (23-39.7)	0.3873
Suppurative/necrotic enteritis	68/672	14/68 (20)	0.95 (0.51-1.77)	1.0000	30.1 (20.7-37.7)	0.3416
Colitis	67/554	14/67 (20)	1.04 (0.55-1.94)	0.8728	31 (23.3-39.4)	0.7079
Lymphadenitis	48/443	8/48 (16)	0.61 (0.28-1.36)	0.2817	30.1 (20.5-39)	0.5260
Lymph node depletion	14/443	3/14 (21)	0.87 (0.24-3.19)	1.0000	29.7 (20.5-39.5)	0.9616
Supprative mengitis/encephalitis	23/185	1/23 (4)	0.15 (0.02-1.14)	0.0517	NA	NA
Non-suppurative mengitis/encephalitis	21/185	4/21 (19)	0.87 (0.27-2.74)	1.0000	30.2 (25.9-36.1)	0.5628
Skeletal muscle/necrosis	16/84	4/16 (25)	0.70 (0.20-2.41)	0.7655	27.7 (26.2-36.7)	0.2831
Epidermitis	12/53	0/12 (0)	0 (-1-1)	0.0476	NA	NA
Dermatitis	12/53	4/12 (33)	2.06 (0.49-8.60)	0.4337	27.2 (25.5-32.4)	0.0216
Non-purulent arthritis	13/50	5/13 (38)	2.68 (0.67-10.73)	0.2557	26.2 (22.9-36.3)	0.2020
Purulent arthritis	11/50	3/11 (27)	1.25 (0.27-5.73)	1.0000	38.6 (22.9-39.3)	0.4818
External ear necrosis	15/26	0/15 (0)	NA	1.0000	NA	NA