

Lack of detection of *Porcine circovirus 3* (PCV-3) in formalin-fixed, paraffin-embedded tissues from porcine abortions in Switzerland

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Fehlender Nachweis von *porzinem Circovirus 3* (PCV-3) in Formalin fixierten und in Paraffin eingebetteten Geweben aus Schweineaborten in der Schweiz

Das porcine Circovirus 3 (PCV-3) wurde in den letzten Jahren mit verschiedenen Schweinekrankheiten in Verbindung gebracht, darunter auch mit Reproduktionsstörungen. Das mögliche Vorkommen von PCV-3 bei Aborten aus Schweizer Schweinebeständen wurde bisher nicht untersucht. Das Hauptziel der vorliegenden retrospektiven Studie war es, fetale und/oder plazentare Gewebe aller Schweineaborte, welche in den letzten 10 Jahren an unser Institut geschickt worden sind, auf das Vorhandensein von PCV-3 zu untersuchen. Zwölf der 53 untersuchten Fälle zeigten leichte histopathologische Veränderungen, wie sie zuvor bei PCV-3-positiven Fällen beschrieben wurden. Allerdings konnte in keinem der Fälle PCV-3-Genmaterial in den untersuchten Formalin fixierten und in Paraffin eingebetteten Geweben nachgewiesen werden. Vergleichbar zu anderen Studien konnte nur in einem Drittel der Fälle eine Abortursache festgestellt werden. Die Untersuchungen lassen vermuten, dass PCV-3 im letzten Jahrzehnt nicht an den bei uns untersuchten Schweineaborten beteiligt war.

Schlüsselwörter: Histopathologie, In-situ-Hybridisierung, PCV-3, Fortpflanzungsstörung, Schwein

Abstract

The novel *Porcine circovirus 3* (PCV-3) has been associated in the past years to different porcine diseases, including reproductive failure. The potential occurrence of PCV-3 in abortions from Swiss pig herds has not been investigated so far. Thus, we conducted a retrospective study on pig aborted cases submitted to our laboratory in the University of Bern during the last 10 years with the main aim of investigating the possible presence of PCV-3 in foetal and/or placental tissue. Twelve out of the 53 studied cases showed mild histopathological changes as previously described in PCV-3 positive cases. However, in none of the cases, PCV-3 genetic material could be detected in the examined formalin-fixed, paraffin-embedded tissues. In only one third of the cases, a cause for the abortion was found, which is similar to other studies. Our survey suggests that PCV-3 was not involved in the porcine abortion cases submitted over the last decade at our institution in Switzerland.

Keywords: Histopathology, in situ hybridization, PCV-3, reproductive failure, swine

Abortion in pigs remain a significant global threat for the swine industry, and many infectious agents are described as causative. However, determining those is often difficult, even when applying a broad spectrum of laboratorial tests, and abortion aetiology remains often unidentified.¹ This considerably compromises our possibility to reduce abortions in pigs and thus improve animal welfare and reduce economic losses.

A few years ago, *Porcine circovirus 3* (PCV-3), a presumably novel circovirus, was identified in swine affected by reproductive failure, respiratory and neurological signs, multisystemic inflammation and a porcine dermatitis and nephropathy syndrome (PDNS)-like condition.^{14,16} Periarthritis, interstitial pneumonia and myocarditis have been associated with the presence of PCV-3.^{6,21} However, an unambiguous proof of pathogenicity could not be demonstrated yet, as the virus is also frequently found in healthy animals and experimental studies have failed to reproduce overt clinical disease.^{12,20} Nevertheless, a recent experimental infection in pregnant gilts resulted in transplacental infection, histological lesions in piglets mimicking those of natural occurring disease and lesser body weight in piglets with vascular lesions at weaning age, without causing any abortion.⁵

Although a worldwide distribution of PCV-3 in pig herds has been shown,^{2,7–9,11–13,21,22,24} there are no peer-reviewed reports indicating its possible presence in Switzerland so far.¹² Nevertheless, PCV-3 has recently been detected in Switzerland as single case in three postweaning pigs, which showed systemic lymphoplasmacytic and histiocytic perivascular lesions (<https://dzntg.ch/news/pcv-3>). Interestingly, the Swiss domestic pig population has the particularity of being free [i.e. *African swine fever virus* (ASFV), *Porcine reproductive and respiratory syndrome virus* (PRRSV), *Suid herpesvirus 1* (PRV)] or almost free (*Mycoplasma hyopneumoniae*) from several pathogens, some of which are present in other neighbouring European countries.²³

In the light of these considerations, we conducted a retrospective study including all swine abortions that, within the last decade, were submitted to the (ITPA) at the University of Bern, Switzerland. The aim of the study was to determine a potential association of PCV-3 within our collection of pig abortion cases. In addition, we assessed the outputs of the diagnostic work in the studied cases and compared it to previous studies.

Between 2012–2022, 228 aborted piglets or formalin fixed foetal organs from n=53 sows (cases) as well as maternal placenta (n=49) and blood (n=21), were submitted to the ITPA at the University of Bern, Switzerland. Organs examined for histopathology included lung, liver, heart, brain, spleen, kidney, intestine, umbilical cord, lymph nodes and maternal placenta. Lung and placenta were available in 49 cases (92%), further organs to a lesser degree (Sup. Table 1). Tissues were

fixed by immersion in 4% neutral buffered formaldehyde, embedded in paraffin, cut at 4 µm and stained with haematoxylin and eosin (H&E). In addition, samples of placenta, lung, liver, and the ligated stomach were collected and submitted for general bacteriology at the Institute for Veterinary Bacteriology at the Vetsuisse faculty of the University of Bern. Moreover, a broad molecular panel was employed to investigate the involvement of well-known bacterial and viral abortigenic infectious agents including *Brucella* spp. [modified Ziehl-Neelsen staining (Stamp) foetal tissues; serology, blood from sow for *B. suis* (Rose-Bengal-Test)], *Chlamydia* spp. (PCR, foetal organs), *Leptospira* spp. (real-time PCR- (rtPCR-TR), Stomach and placenta; microagglutination test, blood from sow), ASFV (serology, foetal body fluid or blood), *Classical swine fever virus* (serology, foetal body fluid or blood), *Porcine circovirus 2* (IHC or qPCR, FFPE from several foetal organs), *Porcine parvovirus 1* (rtPCR or antibody ELISA, foetal organs or foetal body fluid respectively), PRRSV (serology, foetal body fluid or blood) and PRV rtPCR, foetal organs). Examinations were conducted at the respective institutes for bacteriology and virology of the Vetsuisse faculties in Bern and Zurich (Switzerland). All of the above-mentioned tests were carried out as part of the diagnostic procedure at the time of the submission.

In order to assess the potential presence of PCV-3 in all 53 cases, in addition of reviewing the postmortem reports, we reassessed the HE slides focussing on the reported hallmarks of PCV-3 infection: myocarditis, arteritis/periarthritis and interstitial pneumonia. Subsequently, the presence of PCV-3 was investigated by using *in situ* hybridization (ISH) to detect its genome using the RNAscope® technology (Advanced Cell Diagnostics, Newark, CA, USA) as previously described.²¹ Briefly, an additional 4 µm cut was obtained from each FFPE tissue block containing the above mentioned lesions and was deparaffinized. Endogenous peroxidase was blocked, and protease and target retrieval treatments were performed. Then, subsequent incubations with a PCV-3 probe targeting *rep* gene sequence (catalog no. 491021) and six amplifiers were performed between washes with wash buffer solution, and, finally, signal was detected using red solution. Specificity of labelling was further confirmed using a negative probe (catalog no. 310043). This ISH was chosen given its high sensitivity, which is only slightly lower than the sensitivity of a quantitative PCR for PCV-3 in FFPE tissues.⁶

Histological evidence of interstitial pneumonia (n=7), myocarditis (n=1) and arteritis/periarthritis (n=12) in several parenchymatous organs and the placenta (n=3) were found in aborted piglets tissues coming from a total of 12 cases of abortion (Table 1). All affected litters were derived from different farms. The histological lesions were, however, of mild intensity in all cases. The organs displaying these lesions had been processed in 28 formalin-fixed, paraffin-embedded blocks, which were investigated using the PCV-3

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ISH for the purpose of this study. All the examined tissues gave negative results for PCV-3 ISH.

A summary of the detected infectious agents is provided in Table 2. In 18 out of the 53 cases (34%), the cause of abortion could be conclusively determined. *Streptococcus* sp. and *E. coli*, considered as non-contagious bacterial causes for porcine abortion, were the most commonly isolated agents (n=11, 21%), as in some previous studies.^{1,3,10,18} Detected concomitant histological changes were necro-suppurative placentitis (n=5), suppurative bronchopneumonia (n=4), lymphoplasmacytic periarteritis (n=4), interstitial pneumonia (n=2), and/or perihepatitis (n=2), consistent with a bacteria induced abortion. PPV1 infection was noted in 5 of the 53 cases (9%). This percentage is slightly higher than the PPV1 prevalence reported in the most recent Swiss study (3%),¹⁰ but much lower than in older Swiss studies, where it ranged from 15 to 48%.^{3,4,17,25} Nevertheless, further studies performed in the USA, Europe, and India found this primary abortigenic virus in varying percentages of the examined cases: 2,4%¹, 3,6%¹⁸ and 22,2%,¹⁵ respectively. In two cases (3,7%), pathogenic *Leptospira* sp. were detected by means of rtPCR in the placenta and stomach content, and it was interpreted as the cause

of the abortion.¹ Lastly, 2 cases (3,7%) displayed no histological changes, but bacteriological examination yielded high yields of pure *E. coli* and *Staphylococcus aureus*, respectively, which led to the assumptions that these usually secondary pathogens have caused the abortion. No additional abortigenic agents were identified. Nevertheless, in six cases (11%) there was histologically a prominent inflammation (necro suppurative placentitis (n=4); suppurative bronchopneumonia (n=1), vasculitis (n=1)), suggesting a possible infectious abortion cause, but no infectious agents were detectable in these cases. This phenomenon has been reported in other studies, accounting for varying percentages of examined cases ranging from 3 to 12%.^{1,3,10} The overall success rate of finding an infectious cause for abortion cases in our study was 34%, within the range of previous studies,^{1,3,4,10,17,18,25} showing our relative inability to clarify porcine abortions. Non-infectious causes for porcine abortion were not recorded, whose detection, however, require clinical investigations and other ancillary tests. Detailed postmortem diagnostic available data (gross lesions, weight, crown-rump-length, histological diagnoses and ancillary testing) is provided in the Supplementary Table 1.

Table 1: Summary of histological lesions potentially indicative for a PCV-3 infection. All lesions were of mild intensity. The findings came from 12 cases originated from different farms, with multiple organs affected in some of them, which are therefore listed more than once.

Type of lesion	Affected foetal organ	Case No.
Peri-/arteritis	Kidney	3, 31, 39
	Spleen	9, 15, 17
	Liver	3, 9, 23
	Placenta	9, 14, 39
	Heart	15, 17
	Lung	14
Endo-/Myocarditis	Heart	17
Interstitial pneumonia	Lung	2, 14, 15, 26, 37, 39, 46

Table 2: Summary of detected infectious agents in the studied aborted cases. Performed tests included general bacteriology, serology, PCR and immunohistochemistry on foetal organs, blood from the sow or foetal body cavity fluid, depending on the studied pathogen and available sample. In four cases, more than one infectious agent was detected. Percentages are referred to total number of cases (n=53).

Aetiology	Infectious agent	Number of positive cases (%)
Viral	<i>Porcine parvovirus 1</i> (PPV1)	4 (7,5%)
Viral & bacterial	<i>E. coli</i> & PPV1	1 (1,9%)
Bacterial	<i>Streptococcus</i> (<i>Sc.</i>) spp.	3 (5,7%)
	<i>E. coli</i>	3 (5,7%)
	<i>Staphylococcus aureus</i>	3 (5,7%)
	<i>Leptospira</i> spp.	1 (1,9%)
	<i>Sc. spp.</i> & <i>Leptospira</i> spp.	1 (1,9%)
	<i>E. coli</i> & <i>Sc. spp.</i>	1 (1,9%)
	<i>Sc. sp.</i> & <i>T. abortusuis</i>	1 (1,9%)

The role of PCV-3 as cause of reproductive disease has not been fully elucidated. Whereas some studies found it worldwide associated to aborted foetuses in a very broad range ranging from 2 to 100% of the studied cases,^{2,7-9,11,21,22,24} others detected PCV-3 DNA in clinically healthy animals.^{7,19} In some of the first, it was the sole detected putative pathogen, raising the question whether PCV-3 could be a novel abortigenic pathogen.^{2,8,9,11} In addition, a recent PCV-3 experimental infection in pregnant gilts did not reproduce abortions but demonstrated transplacental infection and reduced body weights in piglets with vascular lesions at weaning age. In the current study, we were not able to retrospectively detect PCV-3 in the examined FFPE tissues. In a previous study, it was shown that the PCV-3 ISH and a qPCR in FFPE yielded similar results, with ISH being unable to detect positivity on some cases with very low viral loads.⁶ Therefore, we cannot totally rule out that PCV-3 may have been present in some of the cases investigated in this study. Ideally, further studies should be performed using fresh or frozen tissue, which was unfortunately not available for this study. Indeed, during the process of publication of this article, a single case of systemic PCV-3 infection was detected in Switzerland in piglets, which confirmed the expected presence of PCV-3 this country.

In summary, our survey suggests that PCV-3 was not involved in the porcine abortion cases submitted over the last decade at our institution in Switzerland. Only about one third of the abortions were found to be related with an infectious cause, those being mostly bacteria or PPV1. To investigate further the impact of PCV-3 in Switzerland, larger studies involving pigs suffering from several conditions across the entire country are needed.

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Absence de détection du *Circovirus porcin 3* (PCV-3) dans les tissus fixés au formol et inclus en paraffine provenant d'avortements de porcs en Suisse

Le nouveau *Circovirus porcin 3* (PCV-3) a été associé ces dernières années à différentes maladies porcines, y compris des troubles de la reproduction. La présence potentielle du PCV-3 dans les avortements de porcs en Suisse n'a pas été étudiée jusqu'à présent. Nous avons donc mené une étude rétrospective sur les cas d'avortements de porcs soumis à notre laboratoire de l'Université de Berne au cours des 10 dernières années, dans le but principal d'étudier la présence éventuelle du PCV-3 dans les tissus fœtaux et/ou placentaires. Douze des 53 cas étudiés présentaient des changements histopathologiques légers, tels que décrits précédemment dans les cas positifs au PCV-3. Cependant, dans aucun des cas, le matériel génétique du PCV-3 n'a pu être détecté dans les tissus examinés fixés au formol et inclus en paraffine. Dans un tiers des cas seulement, une cause d'avortement a été trouvée, ce qui est similaire à d'autres études. Notre étude suggère que le PCV-3 n'a pas été impliqué dans les cas d'avortements porcins soumis au cours de la dernière décennie dans notre institution en Suisse.

Mots clés: Histopathologie, hybridation in situ, PCV-3, troubles de la reproduction, porcs

Assenza di rilevamento del *Porcine circovirus 3* (PCV-3) nei tessuti fissati in formalina e incorporate in paraffina provenienti da aborti di suini in Svizzera

Il nuovo *Porcine circovirus 3* (PCV-3) è stato associato negli ultimi anni a diverse malattie suine, tra cui le disfunzioni riproduttive. La potenziale presenza di PCV-3 negli aborti di suini provenienti da mandrie svizzere non è stata finora indagata. Abbiamo quindi condotto uno studio retrospettivo con l'obiettivo di esaminare i tessuti fetali e/o placentari degli aborti di suini presentati all'Università di Berna negli ultimi 10 anni per indagare la possibile presenza di PCV-3. Dodici dei 53 casi esaminati hanno mostrato lievi alterazioni istopatologiche come descritto precedentemente nei casi positivi al PCV-3. Tuttavia, in nessuno dei casi è stato possibile rilevare materiale genetico di PCV-3 nei tessuti fissati in formalina e incorporati in paraffina esaminati. Solo in un terzo dei casi è stata trovata una causa per l'aborto, il che è simile ad altri studi. Il nostro sondaggio suggerisce che PCV-3 non è stato coinvolto nei casi di aborto suino presentati nell'ultimo decennio in Svizzera.

Parole chiave: istopatologia, ibridazione in situ, PCV-3, disfunzioni riproduttive, suini