Porcine circovirus 3 (PCV-3) as a causal agent of disease in swine and a proposal of PCV-3 associated disease case definition

Viviane Saporiti1  |  Giovanni Franzo2  |  Marina Sibila1,3  |  Joaquim Segalés3,4,5

1 IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, Barcelona, Spain
2 Department of Animal Medicine, Production and Health (MAPS), University of Padua, Padua, Italy
3 OIE Collaborating Centre for the Research and Control of Emerging and Re-emerging Swine Diseases in Europe (IRTA-CReSA), Bellaterra, Barcelona, Spain
4 UAB, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, Barcelona, Spain
5 Departament de Sanitati Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Barcelona, Spain

Correspondence
Joaquim Segalés, OIE Collaborating Centre for the Research and Control of Emerging and Re-emerging Swine Diseases in Europe (IRTA-CReSA), 08193 Bellaterra, Barcelona, Spain.
Email: joaquim.segales@irta.cat

Authors Marina Sibila and Joaquim Segalés contributed equally.

1 INTRODUCTION

Porcine circovirus 3 (PCV-3) was discovered in the United States (2015) by next-generation sequencing (NGS) methods in swine affected by respiratory and neurological signs, cardiac and multisystemic inflammation, reproductive failure and a porcine dermatitis and nephropathy syndrome (PDNS)-like condition (Palinski et al., 2017; Phan et al., 2016). Since then, the virus has been detected worldwide in pigs displaying several clinical–pathological outcomes as well as in healthy animals. The objective of this review is to critically discuss the evidence existing so far regarding PCV-3 as a swine pathogen. In fact, a significant number of publications claim PCV-3 as a disease causal infectious agent, but very few of them have shown strong evidence of such potential causality. The most convincing proofs of disease association are those that demonstrate a clinical picture linked to multisystemic lymphoplasmacytic lymphohistiocytic perivascular inflammation and presence of viral nucleic acid within these lesions. Based on these evidence, individual case definitions for PCV-3-reproductive disease and PCV-3-systemic disease are proposed to standardize diagnostic criteria for PCV-3-associated diseases. However, the real frequency of these clinical–pathological conditions linked to the novel virus is unknown, and the most frequent outcome of PCV-3 infection is likely subclinical based on its worldwide distribution.

KEYWORDS
case definition, disease causality, porcine circovirus 3 (PCV-3), reproductive disease, systemic disease

Abstract

Porcine circovirus 3 (PCV-3) was discovered in 2015 using next-generation sequencing (NGS) methods. Since then, the virus has been detected worldwide in pigs displaying several clinical–pathological outcomes as well as in healthy animals. The objective of this review is to critically discuss the evidence existing so far regarding PCV-3 as a swine pathogen. In fact, a significant number of publications claim PCV-3 as a disease causal infectious agent, but very few of them have shown strong evidence of such potential causality. The most convincing proofs of disease association are those that demonstrate a clinical picture linked to multisystemic lymphoplasmacytic lymphohistiocytic perivascular inflammation and presence of viral nucleic acid within these lesions. Based on these evidence, individual case definitions for PCV-3-reproductive disease and PCV-3-systemic disease are proposed to standardize diagnostic criteria for PCV-3-associated diseases. However, the real frequency of these clinical–pathological conditions linked to the novel virus is unknown, and the most frequent outcome of PCV-3 infection is likely subclinical based on its worldwide distribution.
Experimental infections with PCV-3

PCV-3 in co-infection with other pathogens

Naturally occurring PCV-3 infection

There are several parallels between PCV-2 and PCV-3, since both are very similar from a molecular organization point of view (Franzo et al., 2018) and have been detected retrospectively many years before their first identification/report in potential association with disease (Jacobsen et al., 2009; Rodrigues et al., 2020). In turn, significant differences also apply, since PCV-2 evolves much more rapidly than PCV-3 (Franzo, Segalés, et al., 2018), a remarkably higher genomic variability has been detected in PCV-2 compared to PCV-3 (Franzo et al., 2020) and PCV-2 was discovered in the context of a new disease with epidemic proportions worldwide, while that has not been the case with PCV-3 (Opriessnig et al., 2020). Moreover, with regards the amino-acid (aa) similarity, PCV-3 is far distant from PCV-2, with an identity of Cap and Rep proteins around 26%–37% and 48%, respectively (Palinski et al., 2017; Phan et al., 2016).

Therefore, the objective of this review is to critically discuss the evidence existing so far regarding PCV-3 as a swine pathogen and to propose a disease case definition for these conditions that show a rather strong causal relationship.

2 | DISEASE ASSOCIATION

2.1 | Naturally occurring PCV-3 infection

The pathogenesis of PCV-3 infection is still a mystery. Several limitations account for such paucity of knowledge, including the very scarce availability of virus isolates (Mora-Díaz et al., 2020; Oh & Chae, 2020), the lack of serologically and virologically free pigs and the widespread nature of the virus, that make very difficult to get suitable tools, animals and conditions to perform experimental infections. Some laboratory reagents have been generated for intra-laboratory use only (Li et al., 2018; Zhang et al., 2019), but they have not been apparently validated by other research groups.

In consequence, only three experimental infections have been published in the literature to date, all of them using nursery aged pigs (4- to 8-week-old). Two of them were done by the same research group in the United States by means of a cell culture propagated virus (1 mL intranasal [IN] and 1 mL intramuscular [IM] of $6.6 \times 10^{10}$ genomic copies/mL) (Mora-Díaz et al., 2020) or tissue homogenate containing...
| Clinical signs/lesions                                                                 | Production phase | Tested samples                  | % (and proportion) of PCR positivity                                                                 | Disease animals | Healthy animals | Country | Reference |
|--------------------------------------------------------------------------------------|------------------|--------------------------------|-----------------------------------------------------------------------------------------------------|----------------|----------------|---------|-----------|
| Respiratory disease with dyspnea/diffuse moderate lymphohistiocytic interstitial pneumonia and acute bronchitis | Suckling/Nursery | Tissues'                        | 100.0% (3/3)**                                                                                           | NI             | USA            | Phan et al., 2016 |
| Respiratory disease                                                                 | NA               | Lung homogenate/oral fluid/nasal swab | 12.5% (34/271)                                                                                           | NI             | USA            | Palinski et al., 2017 |
| Severe respiratory disease                                                          | Nursery          | Sera                            | 63.7% (51/80)                                                                                           | 1.8% (4/216)   | China          | Zhai et al., 2017 |
| Mild respiratory disease                                                             | Nursery          | Sera                            | 13.1% (23/175)                                                                                           | 1.8% (4/216)   | China          | Shen et al., 2018 |
| Abdominal breathing/lung swelling and congestion                                    | Nursery          | Tissues/sera                    | NA***                                                                                                  | NI             | China          | Shen et al., 2018 |
| Respiratory disease/interstitial pneumonia, suppurative bronchopneumonia, pleuritis and fibrinous-necrotizing pneumonia | Nursery/growing  | Sera                            | 6.2% (8/129)                                                                                           | 6.6% (4/60)    | Spain          | Saporiti, Cruz et al., 2020 |
| Porcine respiratory disease complex related signs                                     | Growing          | Sera                            | 60.0% (15/25)                                                                                           | 28.0% (7/25)   | Thailand       | Kedkovid, Woonwong, Arunorat, Sirserewan, Sangpratum, Lumyai et al., 2018 |
| Porcine respiratory disease complex/bronchointerstitial pneumonia                    | Growing          | Lung and lymph node tissues      | 62.5% (5/8)                                                                                             | NI             | Thailand       | Kedkovid, Woonwong, Arunorat, Sirserewan, Sangpratum, Lumyai et al., 2018 |
| Respiratory distress/bronchointerstitial pneumonia and infiltrating lymphocytes      | Growing          | Tissues                         | 100.0% (2/2)                                                                                           | NI             | South Korea    | Kim, Park et al., 2018 |

NI: not included in the published manuscript; NA: non-available information in the published manuscript.
*Not specified.
**NGS results.
***The type of samples analyzed in control animals (feces) was different from the ones used in diseased pigs (lung tissues).
****Number of tested samples not included in the published manuscript.
Intestinal tissues/0.0%(0/42)*

Suckling Proposal of case definition for PCV-3 Qietal., 2019; China

of 106.53 TCID50/mL), with or without KLH/ICFA (Jiang et al.,2018).

by a Chinese research group, using a PCV-3 infectious clone (2 mL IN KLH/ICFA) (Temeeyasen et al., 2021). The third one was performed keyhole limpet hemocyanin mulsified in incomplete Freund’s adjuvant through the same routes and with the same doses), with or without IM of 1.04 × 1011 genomic copies/mL and re-inoculated after 7 days through the same routes and with the same doses), with or without keyhole limpet hemocyanin emulsified in incomplete Freund’s adjuvant (KLH/ICFA) (Temeeyasen et al., 2021). The third one was performed by a Chinese research group, using a PCV-3 infectious clone (2 mL IN of 106.52 TCID50/mL), with or without KLH/ICFA (Jiang et al., 2018). The group inoculated with KLH/ICFA also received an infectious booster after 4 days (Jiang et al., 2018).

The first two studies used caesarean-derived, colostrum-deprived piglets and no clinical signs upon inoculation occurred. However, mild-to-moderate lesions consisting of multisystemic inflammation and perivasculitis were observed, associated with a low to moderate amount of PCV-3 genome detected by ISH within the lesions (Mora-Díaz et al., 2020; Temeeyasen et al., 2021). These data mirror the limited number of studies on PCV-3 naturally infection cases (A; Arruda et al., 2020) and IgG in the other (Temeeyasen et al., 2021). The virus was detected in serum as soon as 3 days after inoculation until the end of the experiment at 42 DPC (Temeeyasen et al., 2021).

The Chinese study used specific pathogen-free animals inoculated with a PCV-3 infectious clone together or not with KLH/ICFA (Jiang et al., 2018). In contrast to previous studies, fever was observed in the challenged pigs, which showed anorexia, coughing, sneezing, diarrhea and respiratory signs; also, skin lesions consisting of multifocal papules were observed by 15 DPC until the end of the experiment (28 DPC). Although the authors indicated that PDNS was reproduced (Jiang et al., 2018), the reported kidney histopathological lesions were not compatible with systemic necrotizing vasculitis and fibrino-necrotizing glomerulonephritis, the well-known microscopic lesions of PDNS (Segalés, 2012). Therefore, based on current evidence, it is not possible to claim that PDNS has been reproduced by means of a PCV-3 experimental inoculation. Importantly, detection of this virus in tissues was attempted by immunohistochemistry, but published images (Jiang et al., 2018) are difficult to be interpreted.

TABLE 2 PCV-3 PCR detection in pigs suffering from enteric disorders

| Clinical signs/lesions | Production phase | Tested samples | % (and proportion) of PCR positivity | Country | Reference |
|-----------------------|------------------|----------------|-------------------------------------|---------|-----------|
| Diarrhea | Nursery | Fecal samples | 17.14% (6/35) | 2.86% (1/35) | China | Zhai et al., 2017 |
| Diarrhea/vomiting | Suckling | Intestinal tissues/ fecal samples | 10.4% (50/480) | 0.0% (0/42)† | China | Qi et al., 2019 |
| Digestive disorders/catarrhal enteritis with or without villi atrophy and fusion, and catarrhal colitis | Nursery/growing | Sera | 5.5% (7/126) | 6.6% (4/60) | Spain | Saporiti, Cruz, et al., 2020 |

*The type of samples analyzed in control animals (feces) was different from the ones used in diseased pigs (intestinal tissues).

high PCV-3 load (2 mL IN of 3.38 × 1012 genomic copies/mL and 2 mL IM of 1.04 × 1011 genomic copies/mL and re-inoculated after 7 days through the same routes and with the same doses), with or without keyhole limpet hemocyanin emulsified in incomplete Freund’s adjuvant (KLH/ICFA) (Temeeyasen et al., 2021). The third one was performed by a Chinese research group, using a PCV-3 infectious clone (2 mL IN of 106.52 TCID50/mL), with or without KLH/ICFA (Jiang et al., 2018). The group inoculated with KLH/ICFA also received an infectious booster after 4 days (Jiang et al., 2018).

The first two studies used caesarean-derived, colostrum-deprived piglets and no clinical signs upon inoculation occurred. However, mild-to-moderate lesions consisting of multisystemic inflammation and perivasculitis were observed, associated with a low to moderate amount of PCV-3 genome detected by ISH within the lesions (Mora-Díaz et al., 2020; Temeeyasen et al., 2021). These data mirror the limited number of studies on PCV-3 naturally infection cases (A; Arruda et al., 2019; Phan et al., 2016; Saporiti et al., 2021), in which myocarditis and periarteritis were the dominant histological findings. Importantly, the experimental inoculation of the virus caused a detectable antibody response around 7–10 days post-challenge (DPC) in both studies, but with different profiles; IgM response dominated in one study (Mora-Díaz et al., 2019; Phan et al., 2016; Saporiti et al., 2021), in which myocarditis and periarteritis were the dominant histological findings. Importantly, the experimental inoculation of the virus caused a detectable antibody response around 7–10 days post-challenge (DPC) in both studies, but with different profiles; IgM response dominated in one study (Mora-Díaz et al., 2019) and IgG in the other (Temeeyasen et al., 2021). The virus was detected in serum as soon as 3 days after inoculation until the end of the experiment at 42 DPC (Temeeyasen et al., 2021).

The Chinese study used specific pathogen-free animals inoculated with a PCV-3 infectious clone together or not with KLH/ICFA (Jiang et al., 2018). In contrast to previous studies, fever was observed in the challenged pigs, which showed anorexia, coughing, sneezing, diarrhea and respiratory signs; also, skin lesions consisting of multifocal papules were observed by 15 DPC until the end of the experiment (28 DPC). Although the authors indicated that PDNS was reproduced (Jiang et al., 2018), the reported kidney histopathological lesions were not compatible with systemic necrotizing vasculitis and fibrino-necrotizing glomerulonephritis, the well-known microscopic lesions of PDNS (Segalés, 2012). Therefore, based on current evidence, it is not possible to claim that PDNS has been reproduced by means of a PCV-3 experimental inoculation. Importantly, detection of this virus in tissues was attempted by immunohistochemistry, but published images (Jiang et al., 2018) are difficult to be interpreted.

2.4 Proposal of case definition for PCV-3 associated diseases

The sole detection of an endemic virus in tissues or other biological samples is not enough to establish a causal association or to establish disease diagnoses (Arruda et al., 2019). Most studies so far published on PCV-3 detection in diseased animals have been based on molecular methods, with very few of them reporting macro- or microscopic lesions and even less using methods detecting the genome of the virus in the observed lesions (Arruda et al., 2019; Kim, Park, et al., 2018; Phan et al., 2016; Saporiti et al., 2021). Therefore, the latter studies associating the presence of the viral genome with the lesions provide the strongest evidence of this virus in association with pathological conditions.

The establishment of disease diagnosis criteria for widespread pathogens is not an easy task. A good example of such a scenario would be a relative of PCV-3, PCV-2. Three major criteria were proposed to establish the diagnosis of PCV-2-systemic disease (Segalés & Domingo, 1999; Sorden, 2000): (1) presence of compatible clinical signs, mainly wasting, (2) observation of moderate-to-severe histological lesions in lymphoid tissues (lymphocytic infiltration and histiocytic infiltration) and (3) detection of moderate to high amount of PCV-2 within such lesions. Such criteria were crucial to provide an ordered, concise and systematic approach for diagnosing a disease that was considered new by the end of the 1990s and early 2000s. Based on the general reluctance to accept that PCV-2 was truly pathogenic for swine at that time (Segalés et al., 2013), such demanding case definition guaranteed the necessary strictness and was found acceptable for most members of veterinary and scientific communities. The description of a novel PCV (PCV-3) almost 20 years after the report of PMWS was taken with more caution, and despite the lack of an associated severe and globally distributed disease, veterinarians and scientists have been more open-minded to accept its potential disease causality.
| Clinical signs/lesions | Production phase | Tested samples | % (and proportion) of PCR positivity | Disease animals | Healthy animals | Country | Reference |
|------------------------|-----------------|----------------|-------------------------------------|----------------|----------------|---------|-----------|
| **Reproductive failure** | Gestation       | Sera from sows  | 45.9% (39/85)                       | 21.9% (23/105) | China          | Zou et al., 2018 |
|                        |                 | Pool of tissues from aborted fetuses/Pool of tissues from stillborn piglets | 100.0% (2/2) | 100.0% (2/2) | Italy          | Faccini et al., 2017 |
|                        |                 | Tissues from mummified fetuses | 97.0% (270/276) | NI | Brazil | Dal Santo et al., 2020 |
| **Sow mortality and reproductive failure (aborted mummified fetuses)** |                | Sow tissues/fetal tissues | NA | NI | USA | Palinski et al., 2017 |
| **Sows delivering stillbirth piglets** |                | Pool of sera from sows | 100.0% (2/2) | 0.0% (0/2) | Brazil | Tochetto et al., 2018 |
|                        |                | Sera sows         | 67.4% (31/46) | 60.5% (26/43) | Brazil | Tochetto et al., 2020 |
| **Acute losses in neonatal piglets/increased rate of stillborn/sow mortality** |                | Stillborn/tissues/semen/sera | 34.7% (77/222) | NI | China | Ku et al., 2017 |
| **Reproductive losses/abortion and stillborn piglets** |                | Pool of tissues from aborted fetuses or stillborn piglets | 33.9% (18/53) | NI | Spain | Saporiti et al., 2021 |
| **Reproductive losses/abortion and stillborn piglets** |                | Sera from sows and thoracic/abdominal fluid from fetuses and spleen | 10% (sow sera) | 100% (fluid samples) | 70% (spleen) | Russia | Yuzhakov et al., 2018 |
| **Abortion/death of suckling piglets** | Gestation/suckling | Tissues from aborted fetuses/weak suckling piglets | 36.4% (8/22) | NI | South Korea | Kim, Nazki, et al., 2018 |
| **Acute loss of neonatal piglets** |                | Tissues from aborted fetuses/stillborn/weak-born piglets | 89.0% (49/55) | NI | Hungary | Deim et al., 2019 |
| **Reproductive failure/weak-born neonatal piglets/myocarditis/encephalitis** |                | Tissues from fetuses/suckling/weaning | 100.0% (25/25) | NI | USA | Arruda et al., 2019 |

NA: non-available information in the published manuscript; NI: not included in the published manuscript.
**TABLE 4** PCV-3 PCR detection in pigs suffering from neurological disorders

| Clinical signs/lesions                                      | Production phase | Tested samples          | % (and proportion) of PCR positivity | Disease animals | Healthy animals | Country | Reference                  |
|-------------------------------------------------------------|------------------|-------------------------|--------------------------------------|-----------------|-----------------|---------|----------------------------|
| Neurological signs                                          | Suckling         | Tissue pool             | 100.0% (1/1)*                         | NI              | USA             | Phan et al., 2016 |
| Congenital tremors                                         | Suckling         | Brain                   | 100.0% (7/7)                          | NI              | China           | Chen et al., 2017 |
| Congenital tremors, neurological signs in piglets after birth and multisystemic inflammation/non-suppurative encephalomyelitis | Suckling         | Brain, other tissues    | 100.0% (3/3)                          | NI              | UK              | Williamson et al., 2021 |
| Tremors, weak-born neonatal piglets/myocarditis, encephalitis, gliosis and lymphocytic perivascular cuffing | Suckling         | Brain, other tissues    | 100.0% (2/2)                          | NI              | USA             | Arruda et al., 2019 |

*NGS results.
NA: non-available information in the published manuscript; NI: not included in the published manuscript.

**TABLE 5** PCV-3 PCR detection in pigs suffering from other conditions not listed in previous tables

| Clinical signs/lesions                                      | Production phase | Tested samples | % (and proportion) of PCR positivity | Disease animals | Healthy animals | Country | Reference                  |
|-------------------------------------------------------------|------------------|----------------|--------------------------------------|-----------------|-----------------|---------|----------------------------|
| Myocarditis/periarteritis                                   | Suckling/nursery/fattening | Several tissues | 100.0% (3/3)*                         | NI              | USA             | Phan et al., 2016 |
| PDNS                                                        | NA               | Several tissues | 93.8% (45/48)                         | NI              | USA             | Palinski et al., 2017 |
| PDNS                                                        | Sows             | Pooled tissues   | NA*                                  | NI              | USA             | Palinski et al., 2017 |
| PDNS/acute deaths/myocarditis/arteritis/periarteritis       | Nursery          | Several tissues | 100.0% (11/11)                       | NI              | USA             | Arruda et al., 2019 |
| PDNS/systemic inflammation                                 | Nursery and fattening | Kidney and spleen | 40–50% (depending on tested tissue)   | NI              | Russia          | Yuzhakov et al., 2018 |
| Myocarditis/arteritis/periarteritis                         | Nursery          | Several tissues   | 100% (4/4)                            | 100% (2/2)**    | Portugal        | Alomar et al., 2021 |
| Arthrogryposis                                             | Stillborn piglets | Several tissues   | 100.0% (4/4)                          | NI              | UK              | Williamson et al., 2021 |

*NGS results. **Viral load was higher in sick animals; by in situ hybridization, only diseased animals were positive.
NA: non-available information in the published manuscript; NI: not included in the published manuscript.

In any case, and following the path paved by PCV-2 diagnostic approach (Segalés, 2012), the existence of PCV-3 associated disease (PCV-3-AD) diagnostic criteria would help in placing the novel virus into the general context of swine disorders. Therefore, based on existing information that provide clinical, pathological and virological assessments of PCV-3 infection cases (Arruda et al., 2019; Kim, Park, et al., 2018; Phan et al., 2016; Saporiti et al., 2021), the authors would like to propose two major disease outcomes related with PCV-3 infection: PCV-3-reproductive disease (PCV-3-RD) in sows and fetuses/neonatal piglets and PCV-3-systemic disease (PCV-3-SD) in pre- and post-weaning pigs (Table 8, Figure 1). The authors consider that PDNS, which has been linked with PCV-3 infection by some studies (Jiang et al., 2018; Palinski et al., 2017; Yuzhakov et al., 2018), does not fulfill so far specific criteria demonstrating a putative etiological association with PCV-3 based on clinical, pathological, virological and epidemiological facts.

3 DISCUSSION

Traditionally, swine veterinarians have dealt with overt diseases, with the main task of counteracting them and improving the profitability of farms. Several decades ago, the most important diseases affecting pigs were considered mostly ‘unifactorial’, in which the unique presence...
**TABLE 6** PCV-3 PCR detection in healthy pigs

| Clinical signs/lesions | Production phase | Tested samples | % (and proportion) of PCR positivity | Country | Reference |
|------------------------|------------------|----------------|--------------------------------------|---------|-----------|
| Asymptomatic           | Weaning/growing/ | Oral fluids    | 43.4% (142/327)                       | South Korea | Kwon et al., 2017 |
|                        | finishing        |                |                                      |         |           |
| Asymptomatic           | Sows/fetuses     | Tissues        | 59.5% (132/222)                      | China   | Zheng et al., 2017 |
| Asymptomatic           | Sows (in lactation) | Sera    | 47.3% (18/38)                        | Thailand | Kedkovid, Woonwong, Arunorat, Sirisereewan, Sangpratum, Kesdangsakonwut, et al., 2018 |
| Asymptomatic           | Sows             | Sera           | 15.7% (19/121)                       | Spain   | Saporiti, Martorell, et al., 2020 |
| Asymptomatic           | Sows and fetuses | Tissues (brain and lung) | 33.7% (86/255)                     | Denmark | Franzo, Legnardi, et al., 2018 |
| Asymptomatic           | Different production phases | Tissues and sera | 56.4% (44/78)         | Italy   |           |
| Asymptomatic           | Different production phases | Tissues, sera and nasal swabs | 37.4% (37/99) | Spain   |           |
| Asymptomatic           | Different production phases | Pool of sera    | 15.0% (14/94)                       | Spain   |           |
| Asymptomatic           | Non-available    | Lymph node tissues | NA                             | Sweden  | Ye et al., 2018 |
| Asymptomatic           | Growing          | Tissues, serum and nasal swabs | 5.9% (5/90)                      | Poland  | Stadejek et al., 2017 |
| Asymptomatic           | Nursery/finishing | Sera           | 10% (7/73)                          | Spain   | Klaumann, Franco, et al., 2018 |
| Asymptomatic           | Nursery/finishing | Sera           | 6.4% (7/110)                        | Spain   | Saporiti, Huerta, et al., 2020 |
| Asymptomatic           | Nursery/finishing | Sera           | 13.0% (13/100)                      | Belgium |           |
| Asymptomatic           | Nursery/finishing | Sera           | 10.4% (7/67)                        | France  |           |
| Asymptomatic           | Nursery/finishing | Sera           | 6.3% (5/80)                         | Germany |           |
| Asymptomatic           | Nursery/finishing | Sera           | 4.5% (3/67)                         | Italy   |           |
| Asymptomatic           | Nursery/finishing | Sera           | 6.3% (5/80)                         | Denmark |           |
| Asymptomatic           | Nursery/finishing | Sera           | 14.0% (7/50)                        | The Netherlands |           |
| Asymptomatic           | Nursery/finishing | Sera           | 4.0% (2/50)                         | Ireland |           |
| Asymptomatic           | Nursery/finishing | Sera           | 15.0% (3/20)                        | Sweden  |           |

Of the infectious agent was sufficient to cause disease or production losses. However, the current worldwide swine disease scenario is dominated by disorders that are considered of ‘multifactorial’ nature, since the mere presence of the agent is not sufficient to induce the disease (Segalés, 2013). Moreover, most of the new swine pathogens discovered in the last 20 years are infectious agents that (1) had been circulating in pigs for extended periods but remained undetected until recently or (2) infectious agents that had newly emerged in swine because of host species jump and further evolution (Fournié et al., 2015). Detection of these novel pathogens has been driven by advances in diagnostic methods such as broad-range PCR and NGS methods (Blomström, 2011), as well as increased surveillance efforts and particular research interests (Fournié et al., 2015). PCV-3 is an excellent example of a virus discovered through NGS that has been circulating for an extended period before its first detection (Rodrigues et al., 2020) and for which the surveillance efforts, mainly linked to research, have remarkably increased in the last 5 years (Opriessnig et al., 2020).

In contrast with PCV-2, PCV-3 was not discovered because of the emergence/identification of a new disease with severe impact on swine production, but as an extra-diagnostic effort on cases with different clinical outcomes and lack of etiologic diagnosis (Palinski et al., 2017; Phan et al., 2016). This starting point prompted the search for PCV-3 employing molecular methods, which was soon demonstrated to be a widespread virus in the swine population (Klaumann, Correa-Fiz et al., 2018). Unlike PCV-2, isolation of PCV-3 in cell culture was unsuccessful (Facciniet al., 2017; Palinski et al., 2017) until recently (Mora-Díaz et al., 2020; Oh & Chae, 2020), and the availability of virus isolates
TABLE 7  List of pathogens found concomitantly with the presence of PCV-3 in domestic swine

| Pathogen                                      | Country     | % (and proportion) of PCR positivity for PCV-3 | Reference                                                                 |
|-----------------------------------------------|-------------|------------------------------------------------|---------------------------------------------------------------------------|
| PCV-2                                         | China       | 15.8% (35/222)                                  | Ku et al., 2017                                                           |
|                                               | South Korea | 28.3% (13/46)                                   | Kim et al., 2017                                                          |
|                                               | Thailand    | 20.0% (1/5)                                     | Kedkovid, Woonwong, Arunorat, Sirisereewan, Sangpratum, Lumyai, et al., 2018 |
|                                               | Poland      | 4.8% (8/166)                                    | Wozniak et al., 2019                                                     |
|                                               | USA         | 5.4% (115/2125)                                 | Wang, Noll et al., 2019                                                  |
|                                               | European countries | 2.6% (16/624)                                        | Saporiti, Huerta et al., 2020                                           |
|                                               | Brazil      | 78.3% (216/276)                                 | Dal Santo et al., 2020                                                  |
|                                               | Colombia    | 24.0% (12/50)                                   | Vargas-Bermúdez et al., 2021                                         |
|                                               | Spain       | 1.9% (1/53)                                     | Saporiti et al., 2021                                                  |
| **Porcine reproductive and respiratory syndrome virus (PRRSV)** | Thailand    | 20.0% (1/5)                                     | Kedkovid, Woonwong, Arunorat, Sirisereewan, Sangpratum, Lumyai, et al., 2018 |
|                                               | South Korea | 100.0% (2/2)                                    | Kim, Park et al., 2018                                                  |
|                                               | China       | 0.6% (1/159)                                    | Chen et al., 2019                                                       |
|                                               | Spain       | 3.8% (2/53)                                     | Saporiti et al., 2021                                                  |
| **Porcine parvovirus (PPV)**                  | China       | 20.0% (8/40)                                    | Zhao et al., 2018                                                       |
|                                               | Brazil      | 58.7% (162/276)                                 | Dal Santo et al., 2020                                                  |
| **Classical swine fever virus (CSFV)**        | China       | 90.0% (9/10)                                   | Sun et al., 2018                                                        |
|                                               | Brazil      | 43.8% (115/2125)                               | Wang, Noll et al., 2019                                                  |
| **Porcine epidemic diarrhea virus (PEDV)**    | China       | NA                                              | Chen et al., 2017                                                       |
| **Atypical porcine pestivirus (APPV)**        | China       | NA                                              | Chen et al., 2017                                                       |
|                                               | UK          | 42.8% (3/7)                                    | Williamson et al., 2021                                                |
| **Porcine kobuvirus (PKV)**                   | China       | NA                                              | Chen et al., 2017                                                       |
| **Porcine pseudorabies virus (PRV)**          | China       | NA                                              | Chen et al., 2017                                                       |
|                                               | UK          | 5.0% (2/40)                                    | Zhao et al., 2018                                                        |
| **Porcine sapelovirus (PSV)**                 | China       | NA                                              | Chen et al., 2017                                                       |
| **Porcine bocavirus (PBoV)**                  | China       | NA                                              | Chen et al., 2017                                                       |
| **Torque teno sus virus (TTSuV1 and 2)**      | China       | 50% (66/132)                                   | Zheng et al., 2018                                                      |
| **Streptococcus spp**                         | USA         | NA                                              | Phan et al., 2016                                                       |
|                                               | Thailand    | 20.0% (1/5)                                    | Kedkovid, Woonwong, Arunorat, Sirisereewan, Sangpratum, Lumyai, et al., 2018 |
|                                               | South Korea | 100.0% (2/2)                                   | Kim, Park, et al., 2018                                                 |
|                                               | South Korea | 100.0% (2/2)                                   | Kim, Park et al., 2018                                                 |

(Continues)
Table 7 (Continued)

| Pathogen                        | Country     | % (and proportion) of PCR positivity for PCV-3 | Reference                  |
|---------------------------------|-------------|-----------------------------------------------|-----------------------------|
| *Glaeserella parasuis*          | USA         | NA                                            | Phan et al., 2016           |
| *Mycoplasma hyorhinis*          | USA         | NA                                            | Phan et al., 2016           |
| *Mycoplasma hyopneumoniae*      | South Korea | 100.0% (2/2)                                  | Kim, Park, et al., 2018     |
| *Pasteurella multocida*         | Thailand    | NA                                            | Kedkovid, Woonwong, Arunorat, Sirisereewan, Sangpratum, Lumyai, et al., 2018 |
| *Leptospira spp*                | Brazil      | 9.4% (26/276)                                 | Dal Santo et al., 2020      |

NA: non-available information in the published manuscript.

Table 8  Proposed diagnostic criteria for the individual case definition of PCV-3 associated diseases (PCV-3-AD)

| PCV-3-AD proposed name (acronym) | Main clinical sign                                                                 | Individual diagnostic criteria                                                                 |
|----------------------------------|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| PCV-3-reproductive disease (PCV-3-RD) | Late abortion, malformations, mummified fetuses, stillborn fetuses, weak-born piglets | 1. Late reproductive problems and higher perinatal mortality  
2. Multisystemic lymphoplasmacytic to lymphohistiocytic perivascular inflammation  
3. Moderate to high amount of PCV-3 genome in damaged tissues |
| PCV-3-systemic disease (PCV-3-SD)  | Wasting, weight loss, ill thrift or poor-doers, neurological signs                  | 1. Weight loss, rough hair, neurological signs  
2. Multisystemic lymphoplasmacytic to lymphohistiocytic perivascular inflammation  
3. Moderate to high amount of PCV-3 genome in damaged tissues |

or other reagents is extremely restricted still today. Therefore, the progress made on PCV-3 pathogenesis knowledge, immunity and diagnostic technique development is still very limited.

Nevertheless, evidence of PCV-3 involvement in certain pathological conditions is expanding. The presence of a particular infectious agent within certain histopathological lesions of animals showing overt disease, when consistently detected, is probably the strongest evidence of potential disease causality. In such regards, a laboratory technique such as ISH has ultimately allowed detecting PCV-3 nucleic acid within lesions of diseased animals. More specifically, the viral genome has been detected at moderate/high amounts in fetuses and stillborn/weak-born piglets from cases of reproductive disorders as well as in pre- and post-weaning pigs with wasting, sudden death or neurological signs showing multisystemic inflammatory infiltrates, mainly at perivascular level (Arruda et al., 2019; Kim, Park, et al., 2018; Phan et al., 2016; Saporiti et al., 2021; Williamson et al., 2021). Therefore, the existing combination of clinical, pathological and virological data provides a potential diagnostic framework for PCV-3-AD case definition.

In summary, compiled data on PCV-3 knowledge so far points it out as a virus with pathogenic potential, implying the need to standardize diagnostic criteria for at least reproductive and pre-/post-weaning disorders. Such proposal is independent of the frequency, geographic distribution or economic impact of PCV-3-AD, which are rather unknown at present. While the PCV-3-SD in pre- and post-weaning pigs has been scarcely diagnosed at a global level to date (Arruda et al., 2019; Williamson et al., 2021), PCV-3-RD (Arruda et al., 2019; Saporiti et al., 2021; Williamson et al., 2021) seems to occur more often. So far, however, the most frequent presentation of this viral infection is likely subclinical, and its potential health and economic impact on the swine industry worldwide is to be determined.

Acknowledgements
The authors thank the funding by E-RTA2017-00007-00-00 INIA Project from the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (Spanish Government) and the CERCA Programme/Generalitat de Catalunya.

Conflict of Interest
None of the contributing authors has any conflict of interest.

Data Availability Statement
Data used in this review is available in the different journals referenced.
FIGURE 1  Proposed diagnostic criteria for the individual case definition of PCV-3-associated reproductive (A, B, C) and systemic disease (D, E, F). PCV-3-reproductive disease: (A) stillborn piglet from a litter with a late reproductive problem characterized by increased percentage of stillborn and weak-born piglets, (B) mild-to-moderate mononuclear inflammatory infiltrates in the arterial wall of the fetal spleen and (C) moderate to high amount of PCV-3 nucleic acid in the damaged arterial area. PCV-3-systemic disease: (D) clinical picture of a pig showing wasting, (E) moderate-to-severe non-suppurative arteritis in the heart and (F) high amount of PCV-3 genome in the damaged artery

ETHICAL STATEMENT
Non-applicable; this study did not include sample collection or questionnaires from animals or humans.

REFERENCES
Alomar, J., Saporiti, V., Pérez, M., Gonçalves, D., Sibila, M., & Segalés, J. (2021). Porcine circovirus 3 associated wasting in postweaning pigs. Unpublished manuscript.
Arruda, B., Piñeyro, P., Derscheid, R., Hause, B., Byers, E., Dion, K., Long, D., Sievers, C., Tangen, J., Williams, T., & Schwartz, K. (2019). PCV3-associated disease in the United States swine herd. Emerging Microbes and Infections, 8(1), 684–698. https://doi.org/10.1080/22221751.2019.1613176
Saporiti et al.

Jiang, S., Zhou, N., Li, Y., An, J., & Chang, T. (2019). Detection and genomic sequencing of porcine circovirus 3 in neonatal pigs with congenital tremors in South China. Transboundary and Emerging Diseases, 64(6), 1650–1654. https://doi.org/10.1111/tbed.12702

Chen, N., Huang, Y., Ye, M., Li, S., Xiao, Y., Cui, B., & Zhu, J. (2019). Co-infection status of classic swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circoviruses (PCV2 and PCV3) in eight regions of China from 2016 to 2018. Infection, Genetics and Evolution, 68(November 2018), 127–135. https://doi.org/10.1016/j.meegid.2018.12.011

Czyżewska-Dors, E., Núñez, J. I., Saporiti, V., Huerta, E., Rütord, C., Cabezón, O., Segalés, J., & Sibila, M. (2020). Detection of porcine circovirus 3 in wildlife species in Spain. Pathogens, 9(5), 7–12. https://doi.org/10.3390/pathogens9050341

Dal Santo, A. C., Cezarro, K. C., Bennemann, P. E., Machado, S. A., & Martins, M. (2020). Full-genome sequences of porcine circovirus 3 (PCV3) and high prevalence in mumified fetuses from commercial farms in Brazil. Microbial Pathogenesis, 141, 104027. https://doi.org/10.1016/j.micpath.2020.104027

Deim, Z., Dencso, L., Erdélyi, I., Valappil, S. K., Varga, C., Pósa, A., Makrai, L., & Rákhely, G. (2019). Porcine circovirus type 3 detection in a Hungarian pig farm experiencing reproductive failures. Veterinary Record, 185(3), 84. https://doi.org/10.1136/vr.104784

Faccini, S., Barbieri, I., Gilioli, A., Sala, G., Gibelli, L. R., Moreno, A., Sacchi, C., Rosignoli, C., Franzini, G., & Nigrelli, A. (2017). Detection and genetic characterization of porcine circovirus type 3 in Italy. Transboundary and Emerging Diseases, 64(6), 1661–1664. https://doi.org/10.1111/tbed.12714

Fournié, G., Kearsley-Fleet, L., Otte, J., & Pfeiffer, D. U. (2015). Spatiotemporal trends in the discovery of new swine infectious agents. Veterinary Research, 46(1), 1–9. https://doi.org/10.1186/s13567-015-0226-8

Franzo, G., Legnardi, M., Hjulsager, C. K., Klaumann, F., Larsen, L. E., Segalés, J., & Drigo, M. (2018). Full-genome sequencing of porcine circovirus 3 field strains from Denmark, Italy and Spain demonstrates a high within-Europe genetic heterogeneity. Transboundary and Emerging Diseases, 65(3), 602–606. https://doi.org/10.1111/tbed.12836

Franzo, G., Delwart, E., Fux, R., Hause, B., Su, S., Zhou, J. Y., & Segalés, J. (2020). Concurrent infections are important by infection with porcine circovirus type 3. Veterinary Medicine and Science, 5(2), 176–181. https://doi.org/10.1002/vms3.144

Kedkovid, R., Woonwong, Y., Anunorat, J., Sirisereewan, C., Sangpratum, N., Kedkovid, R., Woonwong, Y., Anunorat, J., Sirisereewan, C., Sangpratum, N., Lumyai, M., Kedkovid, R., Semark, S., Tantummarak, P., Asavachees, P., Jitlimanee, S., & Thanawongnuwech, R. (2018). Porcine circovirus type 3 (PCV3) shedding in sow colostrum. Veterinary Microbiology, 220(April), 12–17. https://doi.org/10.1016/j.vetmic.2018.04.032

Kim, H. R., Park, Y. R., Lim, D. R., Park, M. J., Park, J. Y., Kim, S. H., Lee, K. K., Lyoo, Y. S., & Park, C. K. (2017). Multiplex real-time polymerase chain reaction for the differential detection of porcine circovirus 2 and 3. Journal of Virological Methods, 250, 11–16. https://doi.org/10.1016/j.jviromet.2017.09.021

Klaumann, F., Franco, G., Sohrmann, M., Correa-Fiz, F., Drigo, M., Núñez, J. I., Sibila, M., & Segalés, J. (2018). Retrospective detection of porcine circovirus 3 (PCV-3) in pig serum samples from Spain. Transboundary and Emerging Diseases, 65(5), 1290–1296. https://doi.org/10.1111/tbed.12876

Klaumann, F., Correa-Fiz, F., Franco, G., Sibila, M., Núñez, J. I., & Segalés, J. (2018). Current knowledge on porcine circovirus 3 (PCV-3): A novel virus with a yet unknown impact on the swine industry. Frontiers in Veterinary Science, 5, 315. https://doi.org/10.3389/fvets.2018.00315

Klaumann, F., Correa-Fiz, F., Sibila, M., Núñez, J. I., & Segalés, J. (2019). Infection dynamics of porcine circovirus type 3 in longitudinally sampled pigs from four Spanish farms. Veterinary Record, 184(20), 619. https://doi.org/10.1136/vr.105219

Ku, X., Chen, F., Li, P., Wang, Y., Yu, X., Fan, S., Qian, P., Wu, M., & He, Q. (2017). Identification and genetic characterization of porcine circovirus type 3 in China. Transboundary and Emerging Diseases, 64(3), 703–708. https://doi.org/10.1111/tbed.12638

Kwon, T., Yoo, S. J., Park, C. K., & Lyoo, Y. S. (2017). Prevalence of novel porcine circovirus 3 in Korean pig populations. Veterinary Microbiology, 207, 178–180. https://doi.org/10.1016/j.vetmic.2017.06.013

Li, X., Bai, Y., Zhang, H., Zheng, D., Wang, T., Wang, Y., Deng, J., Sun, Z., & Tian, K. (2018). Production of a monoclonal antibody against porcine circovirus type 3 cap protein. Journal of Virological Methods, 261, 10–13. https://doi.org/10.1016/j.jviromet.2018.07.014

Mora-Díaz, J., Piñeyro, P., Shen, H., Schwartz, K., Vannucci, F., Li, G., Arruda, B., & Giménez-Lirola, L. (2020). Isolation of PCV3 from perinatal and reproductive cases of PCV3-associated disease and in vivo characterization of PCV3 replication in CD/CD growing pigs.Viruses, 12(2), 219. https://doi.org/10.3390/v12020219

Oh, T., & Chae, C. (2020). First isolation and genetic characterization of porcine circovirus type 3 using primary porcine kidney cells. Veterinary Microbiology, 241, 108576. https://doi.org/10.1016/j.vetmic.2020.108576

Oppriessnig, T., & Halbur, P. G. (2012). Concurrent infections are important for expression of porcine circovirus associated disease. Virus Research, 164(1–2), 20–32. https://doi.org/10.1016/j.virusres.2011.09.014

Oppriessnig, T., Karuppannan, A. K., Castro, A. M. M. G., & Xiao, C. T. (2020). Porcine circoviruses: Current status, knowledge gaps and
Zhai, S. L., Zhou, X., Zhang, H., Hause, B. M., Lin, T., Liu, R., Chen, Q. L., Wei, W. K., Lv, D. H., Wen, X. H., Li, F., & Wang, D. (2017). Comparative epidemiology of porcine circovirus type 3 in pigs with different clinical presentations. Virology Journal, 14(1), 222. https://doi.org/10.1186/s12985-017-0892-4

Zhang, S., Wang, D., Jiang, Y., Li, Z., Zou, Y., Li, M., Yu, H., Huang, K., Yang, Y., & Wang, N. (2019). Development and application of a baculovirus-expressed capsid protein-based indirect ELISA for detection of porcine circovirus 3 IgG antibodies. BMC Veterinary Research, 15(1), 79. https://doi.org/10.1186/s12917-019-1810-3

Zhao, D., Wang, X., Gao, Q., Huan, C., Wang, W., Gao, S., & Liu, X. (2018). Retrospective survey and phylogenetic analysis of porcine circovirus type 3 in Jiangsu province, China, 2008 to 2017. Archives of Virology, 163(9), 2531–2538. https://doi.org/10.1007/s00705-018-3870-2

Zheng, S., Shi, J., Wu, X., Peng, Z., Xin, C., Zhang, L., Liu, Y., Gao, M., Xu, S., Han, H., Yu, J., Sun, W., Cong, X., Li, J., & Wang, J. (2018). Presence of Torque teno sus virus 1 and 2 in porcine circovirus 3-positive pigs. Transboundary and Emerging Diseases, 65(2), 327–330. https://doi.org/10.1111/tbed.12792

Zou, Y., Zhang, N., Zhang, S., Jiang, Y., Wang, D., Tan, Q., Yang, Y., & Wang, N. (2018). Molecular detection and sequence analysis of porcine circovirus type 3 in sow sera from farms with prolonged histories of reproductive problems in Hunan, China. Archives of Virology, 163(10), 2841–2847. https://doi.org/10.1007/s00705-018-3914-7

How to cite this article: Saporiti, V., Franco, G., Sibila, M., & Segalés, J. (2021). Porcine circovirus 3 (PCV-3) as a causal agent of disease in swine and a proposal of PCV-3 associated disease case definition. Transbound. Emerg. Dis., 68, 2936–2948. https://doi.org/10.1111/tbed.14204