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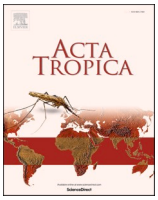

High detection frequency and genetic diversity of porcine circovirus 3 (PCV-3) in Namibian backyard farms and warthogs

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ABSTRACT

Since its first identification in 2015, porcine circovirus 3 (PCV-3) has been reported worldwide with a high frequency and in the presence of several clinical conditions, although its impact on pig health and productivity is still debated. Data on the presence of PCV-3 in Africa are, however, limited. A previous study performed on commercial pigs in Namibia failed to identify the pathogen. In the present study, the viral circulation in backyard farms, characterised by lower biosecurity measures and frequent animal exchange between farms, was assessed. The susceptibility of warthogs to PCV-3 infection and their potential epidemiological role were also evaluated. Tonsils from 77 pigs from backyard piggeries and 55 warthogs were collected in different regions of Namibia and tested by PCR. Positive samples were sequenced and compared to PCV-3 strains circulating globally. Forty-two out of 77 pigs (54.54 %) and 12 out of 55 warthogs (21.82 %) tested positive, demonstrating the presence of PCV-3 in the country and suggesting that the high biosecurity measures implemented in the commercial farms that previously tested negative for PCV-3 probably prevented viral introduction. The partial ORF2 gene was successfully sequenced in samples from 27 pigs and 6 warthogs. Genetically, the identified strains were part of 3 distinct groups which included both backyard pigs and warthogs from different regions of Namibia. There is also evidence for the occurrence of multiple introduction events most likely from Asian countries, either directly into Namibia or through other African countries. Considering the strict Namibian regulations on live animal importation, understanding the source of viral introduction is challenging, although semen importation or the habit of feeding backyard pigs with human food waste might have played a role. Pig exchanges between farms for breeding purposes or wildlife movements could also have been involved in PCV-3 dispersal within Namibia. Despite the significant advances in the field, further studies should be undertaken to properly understand PCV-3 epidemiology in Namibia and its impact on pig productivity and wildlife health.

1. Introduction

Porcine circovirus 3 (PCV-3) is a member of the family *Circoviridae*, genus *Circovirus,* identified for the first time in 2015 in the USA by metagenomic analysis of tissues collected from animals displaying porcine dermatitis and nephropathy syndrome (PDNS) and reproductive disorders (Palinski et al., 2017). It is characterised by a single-stranded circular genome of 2000 nucleotides encoding 2 major proteins. Open reading frame 1 (ORF1) encodes the Rep protein, involved in viral replication, while ORF2 codes for the Cap protein, the only constituent of the viral capsid (Klaumann et al., 2018). Like PCV-2, an ancient origin has also been estimated for PCV-3, followed by a prolonged, undetected circulation in the swine population, progressively increasing in tandem with the intensification of the global swine industry (Franzo et al.,

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2019b).

Interestingly, a much lower evolutionary rate has been estimated for PCV-3 compared to PCV-2, with only one PCV-3 genotype defined so far (Franzo et al., 2020, 2019b; Franzo and Segalés, 2023).

The ancient origin and undetected circulation of PCV-3 fit well with its worldwide distribution. Indeed, after its first detection it was rapidly identified around the world, including Asia (Fu et al., 2018; Ku et al., 2017; Zheng et al., 2017), Africa (Anahory et al., 2022; Chang'a et al., 2023; Luka et al., 2022), Europe (Faccini et al., 2017; Franzo et al., 2018; Stadejek et al., 2017) and South America (Assao et al., 2021; Rodrigues et al., 2020), in the presence of different clinical conditions, like PDNS (Palinski et al., 2017), reproductive disorders (Faccini et al., 2017; Zou et al., 2018), respiratory disease (Shen et al., 2018; Zhai et al., 2017), and myocarditis (Phan et al., 2016). However, as it is also commonly detected in asymptomatic subjects, its pathogenic relevance has been debated (Franzo et al., 2018b; Zheng et al., 2017). Although a formal case definition has currently been defined at least for systemic disease [PCV-3-systemic disease (PCV-3-SD)] and reproductive disorders [PCV-3-reproductive disease (PCV-3-RD)], the real frequency of these clinical–pathological conditions and their economic relevance are unknown (Saporiti et al., 2021).

The virus is commonly detected in *Suidae* species, domestic swine and wild boar with a comparable frequency, and occasionally in some non-Suidae species (Czyżewska-Dors et al., 2020; Franzo et al., 2019a; Jiang et al., 2019; Sun et al., 2019; Wang et al., 2019). Therefore, a relatively broad host spectrum can be suggested, similarly to what has been reported for PCV-2 (Zhai et al., 2019).

Despite its extensive geographical and host distribution, previous attempts to detect PCV-3 in Namibian commercial pig farms were unsuccessful (Molini et al., 2021b). This was an unexpected result and was in contrast with the ubiquitous nature of PCV-3 in other countries and the detection of other similar swine pathogens in Namibia like PCV-2 and porcine parvovirus (Molini et al., 2021a, 2023, 2022).

One explanation for the lack of PCV-3 detection could be the effectiveness of biosecurity measures implemented in commercial Namibian pig farms, primarily designed to control African Swine Fever outbreaks.

To test this hypothesis, a molecular epidemiology study was performed on Namibian backyard farms and warthog populations, where such strict prevention measures are not implemented.

2. Materials and methods

2.1. Sample collection and processing

The study included samples (tonsils) from 77 healthy slaughtered pigs (5–6 months of age, approximately 75–100 kg) collected between May 2022 and May 2023 in backyard piggeries located in three different regions of Namibia (Khomas, Hardap, and Erongo). All the backyard piggeries involved in the study presented a medium level of biosecurity and the number of animals ranged from 30 to 100. According to Namibian standards, such a biosecurity level implies limited access for people and vehicles (although without mandatory vehicle disinfection upon entry/exit from the farm), regular cleaning and disinfection of facilities, daily inspection of animals and reporting of any sick animals, and maintenance of a closed herd, but without ensuring that remount is sourced from disease-free providers. Farm compartmentalization and fences must also be present, although not designed to prevent contact with wild boars and other small animals.

In addition, the tonsils of 55 warthogs, collected after hunting between June 2019 and June 2023 and originating from four livestock farms in Khomas and Otjozondjupa regions, were also included in the study (Table 1). In Namibia, all livestock farms, typically covering an area of 10,000 hectares, can request permission from the Namibian government to annually cull a specified number of warthogs and other wild animals naturally present on the land and not farmed. The farms included in the study all had internal abattoirs where other livestock animals were slaughtered. During the hunting season, which runs from April to November, specific days were allocated for hunting and slaughtering warthogs. After the shooting, they were transported to the internal abattoir, where post-mortem inspection and slaughter took place.

Table 1
Summary of the samples included in the study and relative metadata.

Species	Site	District	Region	Date	PCV3 Tested/ positive
Sus scrofa	A	Windhoek	Khomas	May 2022	1/0
Sus scrofa	B	Windhoek	Khomas		6/4
Sus scrofa	C	Windhoek	Khomas		1/0
Sus scrofa	D	Mariental	Hardap		15/2
Sus scrofa	E	Windhoek	Khomas		7/2
Sus scrofa	F	Windhoek	Khomas	August 2022	5/0
Sus scrofa	G	Mariental	Hardap	February 2023	3/1
Sus scrofa	H	Windhoek	Khomas	May 2023	4/1
Sus scrofa	I	Walvis bay	Erongo		1/1
Sus scrofa	L	Mariental	Hardap		14/11
Sus scrofa	M	Mariental	Hardap		10/10
Sus scrofa	N	Mariental	Hardap		10/10
Phacochoerus africanus	O	Windhoek	Khomas	September 2019	11/1
Phacochoerus africanus	P	Windhoek	Khomas	June 2019 -June 2023	35/6
Phacochoerus africanus	Q	Windhoek	Khomas	June 2022	3/0
Phacochoerus africanus	R	Otjivarongo	Otjozondjupa	May 2022	6/4

All samples were collected during post-mortem inspection at the abattoir by veterinarians specialized in Veterinary Public Health. The tissue was removed from the carcasses using sterile disposable scalpels and metal forceps, which were carefully flamed with a portable Bunsen burner for each sample. During sampling, the veterinarian wore disposable and sterile gloves, a mask, and other protective clothing. All samples were then stored refrigerated in sterile and properly labeled airtight containers until delivery to the laboratory, and at −20 °C thereafter.

The tonsils were homogenized in 1 ml of sterile phosphate buffer saline (PBS) using a TissueLyser LT (Qiagen, Germany). Total genomic DNA was extracted from the homogenized samples using the High Pure Viral Nucleic Acid Kit (Hoffman-La Roche, Switzerland) with an elution volume of 100 µl, following the manufacturer's instructions.

DNA extracts were screened using a real-time PCR (qPCR) assay with specific primers and probe for PCV-3, as reported by Franzo et al. (2018a). Briefly, qPCR was performed on a C1000 Bio-Rad thermocycler (Bio-Rad Hercules, CA, USA) with PrecisionPLUS qPCR Mastermix (Genesig Primerdesign ltd, Camberley, UK). The cycling conditions were 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s, and 60 °C for 1 min. The fluorescence signal was acquired at the end of each cycle extension phase.

Positive samples with a Cq ≤ 35 were amplified using the primers: PCV3–1-F 5′ -TTACTTAGAGAACGGACTTGTAACG 3′ and PCV3–1-R 5′ AAATGAGACACAGAGCTATATTCAG 3′ (Ku et al., 2017) to obtain a fragment of 650 bp covering the ORF2 of PCV-3. The PCR reaction conditions were: 5 µL of extracted DNA in a total reaction volume of 20 µL containing a final concentration of 1.25 mM $MgCl_2$, 1X PCR buffer (Qiagen, Germany), 0.2 mM dNTPs, 10 pmol of each primer, and 2.5U of Taq DNA polymerase. The following thermal profile was implemented: initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and elongation at 72 °C for 60 s, followed by a final elongation at 72 °C for 5 min.

Amplicons of positive samples were purified using a Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced commercially by LGC Genomics (Berlin, Germany). The sequences were edited and

assembled using the Staden software package version 2.0.0b8. The sequences of the positive samples were submitted to the GenBank database.

2.2. Sequence analysis

Different datasets were created to investigate PCV-3 strain features and epidemiology:

(1) A dataset of Namibian sequences (625 bp) only
(2) A dataset of Namibian sequences plus the complete ORF2 reference sequences (645 bp) described in Franzo et al. (2023)
(3) A dataset based on dataset 2 plus all PCV-3 sequences available from African countries (i.e. Mozambique, Nigeria, Tanzania). Since only partial ORF2 sequences were available in most cases, a shorter genomic region (370 bp) was included in the present dataset.

In all instances sequences were aligned using MAFFT (Standley, 2013) and a phylogenetic tree was reconstructed using IQ-Tree (Nguyen et al., 2015), selecting the substitution model with the lowest Bayesian Information Criterion (BIC) calculated by jModelTest (Darriba et al., 2012). The branch support was assessed by performing 10,000 ultrafast bootstrap replicates.

3. Results

3.1. PCV-3 detection

Forty-two out of 77 pigs (54.54 %) and 12 out of 55 warthogs (21.82 %) (Table 1), from the 12 piggeries and the 4 farms involved in the study, tested positive for PCV-3 by qPCR. The Cq values of the samples ranged between 16.84 and 36.35. The partial ORF2 gene was successfully sequenced in 27 out of the 42 positive pigs and 6 out of the 12 positive warthogs.

3.2. Sequence analysis

Thirty-three partial ORF2 sequences (625 bp) were obtained (Acc. Numbers OR237449-OR237481) which could be classified in three main groups. The largest group included strains originating from both backyard pigs and warthogs (Fig. 1) and was part of a much larger clade (Clade A) including samples collected worldwide. All but 2 backyard pigs (collected in Khomas region) originated from Mariental, Hardap region. The 2 warthogs originated from the Khomas region. Clade B consisted of one backyard pig from Khomas and 4 warthogs from Otjozondjupa. The comparison with other strains included in dataset 2 highlighted the closest genetic relationship with Chinese strains sampled between 2014 and 2019 (Supplementary Fig. 1). However, using dataset 3, which was based on a shorter genetic segment and allowed the inclusion of other African strains, demonstrated a close relationship with strains from Nigeria (Fig. 2).

Finally, strain 42 from Erongo was distantly related to other Namibian ones and was a close relative of a strain sampled in 2017 in USA

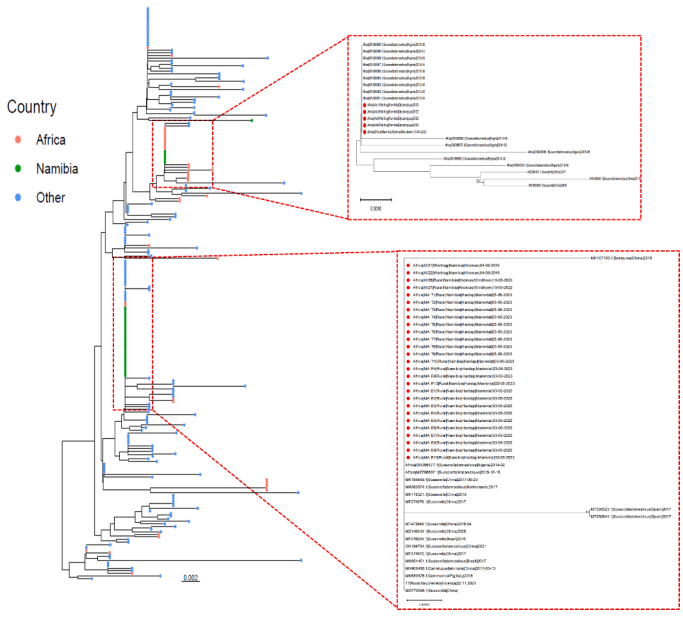

Fig. 2. Maximum likelihood phylogenetic tree reconstructed based on the partial ORF2 sequence of Namibian and international strains. The collection regions have been colour coded. The clades including the Namibian strain, marked with a red dot, have been magnified.

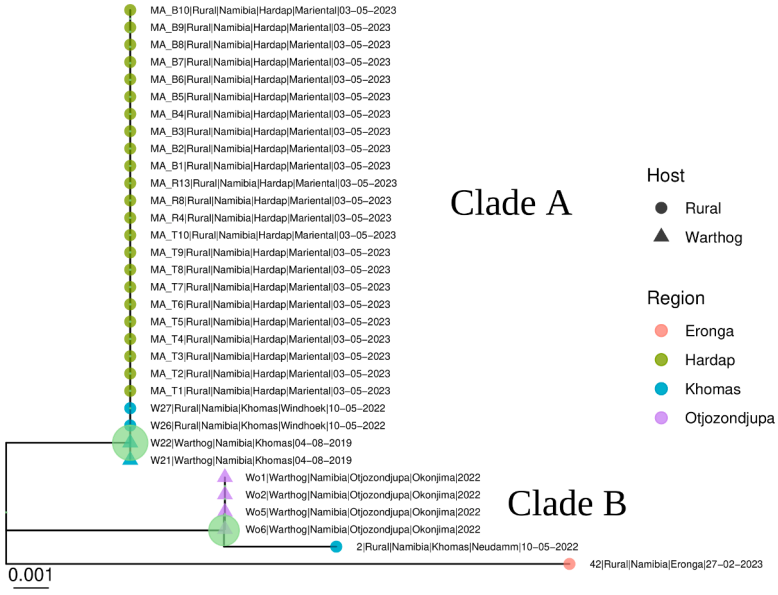

Fig. 1. Maximum likelihood phylogenetic tree reconstructed based on the partial ORF2 sequence of Namibian strains. The collection region and host have been coded with different colours and shapes.

(Fig. 1 and Supplementary Fig. 1).

4. Discussion

The observations from this study demonstrate the circulation of PCV-3 in Namibia in both backyard swine populations and wild animals. Since PCV-3 was not detected in a previous study involving intensive farms representative of the swine industry in Namibia, the biosecurity and management practices applied in these productive systems were probably effective enough to prevent viral introduction. Biosecurity shortcomings in small-scale, backyard farms most probably favor PCV-3 introduction, persistence, and spread over relatively long distances. The Namibian strains belonging to the two main clades (A and B) originated from the neighboring regions of Khomas, Hardap and Otjozondjupa. Viruses collected from Khomas belonged to both clades suggesting that this region, because of its central location, might be a point of virus exchange although further investigations are required to clarify this. Animal movements in Namibia, although discouraged, are relatively common and involve breeding stock particularly. Since February 2019, due to an outbreak of Foot-and-Mouth Disease (FMD), no live animals can be imported from South Africa. In addition, there are only two suppliers currently operating in Namibia which has resulted in limited competition and increased costs for breeders. Therefore, smaller farms have started exchanging pigs for breeding purposes. Such practices, which are currently forbidden in intensive farms, could explain the presence of PCV-3 in the backyard pig population and its dispersal over relatively long distances.

Considering the lack of external biosecurity measures in backyard farms, a potential role of wild animals in the transmission of PCV-3, warthogs in particular, can also be speculated. In fact, a close genetic relatedness was observed between backyard and warthog strains from the Khomas and Otjozondjupa regions.

Although warthogs are territorial and do not undergo long-distance movements, overlaps of different animal groups do occur which could lead to the regional expansion of PCV-3. To be effective as a source of PCV-3 spread, however, prolonged viral persistence and circulation in these animals would have to be assumed. The detection of a group of PCV-3 strains in warthogs only (Fig. 1), is suggestive of a certain separation and independent evolution, which might support this hypothesis. Recent studies performed on wild boars suggested the epidemiological role of wild suids as a source of PCV-3 persistence, presence and potential evolution (Dei Giudici et al., 2020; Franzo et al., 2023, 2019a). However, further evidence will be needed to evaluate if such a scenario holds true for warthogs as well.

Identifying the initial sources of PCV-3 introduction in Namibia is also challenging. Clade A consists of strains identified worldwide, therefore the pathways of introduction into Namibia remain obscure. An Asian origin has been identified for other swine pathogens detected in Namibia and is supported by the close and growing political and economic relationships between African and Asian countries (Franzo et al., 2022b, 2022a).

An Asian origin, followed by independent evolution, is also supported by the members of Clade B which consist of African and Chinese strains only.

Nigerian sequences (Luka et al., 2022), identical or closely related to Namibian sequences were reported for both Clade A and B. The overall low genetic variability of PCV-3 variants makes it difficult to ascertain whether multiple introductions of the same strain occurred in Africa or whether a limited number of virus introductions occurred that was then followed by local dispersal. Since live animal importation from foreign countries is forbidden in Namibia, other sources might be hypothesized, including the purchase of contaminated semen by breeders or wild animal transboundary movements (Eddicks et al., 2022). However, the real impact of semen as an infection source is still unclear, and the huge geographical distance between Nigeria and Namibia makes the wild animal role unlikely. An alternative non-conflicting hypothesis might involve the common habit of feeding pigs with waste from hotels and restaurants (Penrith et al., 2013). This practice is a well-known risk of pathogen introduction into farms and, on a broader level, into countries through the importation of contaminated food. Although the presence of porcine circovirus in raw meat has been demonstrated (Opriessnig et al., 2009), further studies should be performed to verify the likelihood of this hypothesis.

The genetic separation of the strain identified in site I (i.e. Walvis Bay, Erongo) from other Namibian variants reflects the geographic distance of the other areas. This strain was identified in Erongo, which is separated from the other regions considered in this study by a vast desert constituting a clear barrier to wild animal movements, further reducing contact opportunities. In addition, it confirms the mainly local nature of pigs, fomites or other contaminated material movements.

5. Conclusion

Overall, the present study demonstrates the presence of PCV-3 in backyard farms and wild animals in Namibia, highlighting the role of these ecological niches in its epidemiology. However, further studies will be required for a clearer understanding of the epidemiological scenario and relevance of PCV-3 in Africa.

Data availability

All the obtained sequences have been submitted in GenBank under the Acc. Numbers OR237449-OR237481.

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Ethics statement

The study is exempt from ethics approval since all samples from domestic pigs and warthogs were obtained during routine clinical diagnostic abattoir activities. No sampling or experimental procedure was specifically designed for the study.

CRediT authorship contribution statement

Umberto Molini: Conceptualization, Data curation, Writing – original draft. **Lauren M. Coetzee:** Formal analysis. **Vernon Christians:** Formal analysis. **Maria Y. Hemberger:** Formal analysis. **Bernard Chiwome:** Formal analysis. **Maria Amukwaya:** Formal analysis. **Siegfried Khaiseb:** Formal analysis. **Giovanni Cattoli:** Funding acquisition. **William G. Dundon:** Writing – original draft, Supervision, Visualization. **Giovanni Franzo:** Conceptualization, Data curation, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2023.107085.

References

Anahory, I.V., Franzo, G., Settypalli, T.B.K., Mapaco, L.P., Achá, S.J., Molini, U., Cattoli, G., Lamien, C.E., Dundon, W.G., 2022. Identification of porcine circovirus-3 in Mozambique. Vet. Res. Commun. 46, 593–596. https://doi.org/10.1007/s11259-021-09858-4.

Assao, V.S., Santos, M.R., Pereira, C.E.R., Vannucci, F., Silva-Júnior, A., 2021. Porcine circovirus 3 in North and South America: epidemiology and genetic diversity. Transbound. Emerg. Dis. 68, 2949–2956. https://doi.org/10.1111/tbed.14238.

Chang'a, J.S., Bitanyi, S.S., Kamigwe, A., Magidanga, B., Guo, S., Makoroma, P., Francis, G., Jumbe, J., Jeremiah, M., Nyakilinga, D., Mwasha, M., Msangi, K., Cattoli, G., Franzo, G., Molini, U., Dundon, W.G., 2023. Molecular characterization of porcine circovirus-2 and-3 in pigs in Tanzania. J. Vet. Med. Sci. 85, 691–694. https://doi.org/10.1292/jvms.23-0031.

Czyżewska-Dors, E., Núñez, J.I., Saporiti, V., Huerta, E., Riutord, C., Cabezón, O., Segalés, J., Sibila, M., 2020. Detection of porcine circovirus 3 in wildlife species in Spain. Pathogens 9, 341. https://doi.org/10.3390/pathogens9050341.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. Nat. Methods. https://doi.org/10.1038/nmeth.2109.

Dei Giudici, S., Franzoni, G., Bonelli, P., Angioi, P.P., Zinellu, S., Deriu, V., Carta, T., Sechi, A.M., Salis, F., Balzano, F., Oggiano, A., 2020. Genetic characterization of porcine circovirus 3 strains circulating in sardinian pigs and wild boars. Pathogens 9. https://doi.org/10.3390/PATHOGENS9050344.

Eddicks, M., Müller, M., Fux, R., Ritzmann, M., Stadler, J., 2022. Detection of porcine circovirus type 3 DNA in serum and semen samples of boars from a German boar stud. Vet. J. 279, 105784 https://doi.org/10.1016/j.tvjl.2021.105784.

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. Transbound. Emerg. Dis. 64, 1661–1664. https://doi.org/10.1111/tbed.12714.

Franzo, G., De Villiers, L., De Villiers, M., Ravandi, A., Gyani, K., Van Zyl, L., Coetzee, L. M., Khaiseb, S., Molini, U., 2022a. Molecular epidemiology of canine parvovirus in Namibia: introduction pathways and local persistence. Prev. Vet. Med. 209 https://doi.org/10.1016/j.prevetmed.2022.105780.

Franzo, G., Delwart, E., Fux, R., Hause, B., Su, S., Zhou, J.Y., Segalés, J., 2020. Genotyping porcine circovirus 3 (PCV-3) Nowadays: does it make sense? Viruses 12. https://doi.org/10.3390/v12030265.

Franzo, G., Grassi, L., Tucciarone, C.M., Drigo, M., Martini, M., Pasotto, D., Mondin, A., Menandro, M.L., 2019a. A wild circulation: high presence of porcine circovirus 3 in different mammalian wild hosts and ticks. Transbound. Emerg. Dis. 66, 1548–1557. https://doi.org/10.1111/tbed.13180.

Franzo, G., He, W., Correa-Fiz, F., Li, G., Legnardi, M., Su, S., Segalés, J., 2019b. A shift in porcine circovirus 3 (PCV-3) history paradigm: phylodynamic analyses reveal an ancient origin and prolonged undetected circulation in the worldwide swine population. Adv. Sci. 6 https://doi.org/10.1002/advs.201901004.

Franzo, G., Legnardi, M., Berto, G., Dal Maso, M., Genna, V., Menandro, M.L., Poletto, F., Cecchinato, M., Drigo, M., Tucciarone, C.M., 2023. Wilder than intense: higher frequency, variability and viral flows of Porcine circovirus 3 (PCV-3) in wild boars and rural farms compared to intensive ones in Northern Italy. Front. Microbiol. 14, 1234393.

Franzo, G., Legnardi, M., Centelleghe, C., Tucciarone, C.M., Cecchinato, M., Cortey, M., Segalés, J., Drigo, M., Franzo, G., Legnardi, M., Centelleghe, C., Tucciarone, CM., Cecchinato, M., Cortey, M., Segalés, J., Drigo, M., 2018a. Development and validation of direct PCR and quantitative PCR assays for the rapid, sensitive, and economical detection of porcine circovirus 3. J. Vet. Diagn. Investig. 30, 538–544. https://doi.org/10.1177/1040638718770495.

Franzo, G., Legnardi, M., Hjulsager, C.K., Klaumann, F., Larsen, L.E., Segales, 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. Transbound. Emerg. Dis. 65, 602–606. https://doi.org/10.1111/tbed.12836.

Franzo, G., Legnardi, M., Tucciarone, C.M., Drigo, M., Klaumann, F., Sohrmann, M., Segalés, J., 2018b. Porcine circovirus type 3: a threat to the pig industry? Vet. Rec. 182, 83. https://doi.org/10.1136/vr.k91.

Franzo, G., Segalés, J., 2023. Porcine circovirus 3 (PCV-3) variability: is it in the virus or in the classification criteria? Virol. J. 20, 6–8. https://doi.org/10.1186/s12985-023-01984-6.

Franzo, G., Settypalli, T.B.K., Agusi, E.R., Meseko, C., Minoungou, G., Ouoba, B.L., Habibata, Z.L., Wade, A., de Barros, J.L., Tshilenge, C.G., Gelaye, E., Yami, M., Gizaw, D., Chibssa, T.R., Anahory, I.V., Mapaco, L.P., Achá, S.J., Ijomanta, J., Jambol, A.R., Adedeji, A.J., Luka, P.D., Shamaki, D., Diop, M., Bakhoum, M.T., Lo, M.M., Chang'a, J.S., Magidanga, B., Mayenga, C., Ziba, M.W., Dautu, G., Masembe, C., Achenbach, J., Molini, U., Cattoli, G., Lamien, C.E., Dundon, W.G., 2022b. Porcine circovirus-2 in Africa: identification of continent-specific clusters and evidence of independent viral introductions from Europe, North America and Asia. Transbound. Emerg. Dis. 69, e1142–e1152. https://doi.org/10.1111/tbed.14400.

Fu, X., Fang, B., Ma, J., Liu, Y., Bu, D., Zhou, P., Wang, H., Jia, K., Zhang, G., 2018. Insights into the epidemic characteristics and evolutionary history of the novel porcine circovirus type 3 in southern China. Transbound. Emerg. Dis. 65, e296–e303. https://doi.org/10.1111/tbed.12752.

Jiang, S., Zhou, N., Li, Y., An, J., Chang, T., 2019. Detection and sequencing of porcine circovirus 3 in commercially sourced laboratory mice. Vet. Med. Sci. 5, 176–181. https://doi.org/10.1002/vms3.144.

Klaumann, F., Correa-Fiz, F., Franzo, 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. Front. Vet. Sci. 5, 1–13. https://doi.org/10.3389/fvets.2018.00315.

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. Transbound. Emerg. Dis. 64, 703–708. https://doi.org/10.1111/tbed.12638.

Luka, P.D., Adedeji, A.J., Jambol, A.R., Ifende, I.V., Luka, H.G., Choji, N.D., Weka, R., Settypalli, T.B.K., Achenbach, J.E., Cattoli, G., Lamien, C.E., Molini, U., Franzo, G., Dundon, W.G., 2022. Coinfections of African swine fever virus, porcine circovirus 2 and 3, and porcine parvovirus 1 in swine in Nigeria. Arch. Virol. 167, 2715–2722. https://doi.org/10.1007/s00705-022-05593-6.

Molini, U., Coetzee, L.M., Hemberger, M.Y., Khaiseb, S., Cattoli, G., Dundon, W.G., 2023. Evidence indicating transmission of porcine parvovirus 1 between warthogs and domestic pigs in Namibia. Vet. Res. Commun. 47, 981–985. https://doi.org/10.1007/s11259-022-10038-1.

Molini, U., Franzo, G., Gous, L., Moller, S., Hemberger, Y.M., Chiwome, B., Marruchella, G., Khaiseb, S., Cattoli, G., Dundon, W.G., 2021a. Three different genotypes of porcine circovirus 2 (PCV-2) identified in pigs and warthogs in Namibia. Arch. Virol. 166, 1723–1728. https://doi.org/10.1007/s00705-021-05035-9.

Molini, U., Franzo, G., Settypalli, T.B.K., Hemberger, M.Y., Khaiseb, S., Cattoli, G., Dundon, W.G., Lamien, C.E., 2022. Viral co-infections of warthogs in Namibia with African swine fever virus and porcine parvovirus 1. Animals 12. https://doi.org/10.3390/ani12131697.

Molini, U., Marruchella, G., Matheus, F., Hemberger, Y.M., Chiwome, B., Khaiseb, S., Cattoli, G., Franzo, G., 2021b. Molecular investigation of porcine circovirus type 3 infection in pigs in Namibia. Pathogens 10. https://doi.org/10.3390/pathogens10050585.

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274. https://doi.org/10.1093/molbev/msu300.

Opriessnig, T., Patterson, A.R., Meng, X.J., Halbur, P.G., 2009. Porcine circovirus type 2 in muscle and bone marrow is infectious and transmissible to naïve pigs by oral consumption. Vet. Microbiol. 133, 54–64. https://doi.org/10.1016/j.vetmic.2008.06.018.

Palinski, R., Piñeyro, P., Shang, P., Yuan, F., Guo, R., Fang, Y., Byers, E., Hause, BM., 2017. A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. J Virol 91, e01879–e01916. https://doi.org/10.1128/JVI.01879-16.

Penrith, M.L., Vosloo, W., Jori, F., Bastos, A.D.S., 2013. African swine fever virus eradication in Africa. Virus Res. 173, 228–246. https://doi.org/10.1016/j.virusres.2012.10.011.

Phan, T.G., Giannitti, F., Rossow, S., Marthaler, D., Knutson, T.P., Li, L., Deng, X., Resende, T., Vannucci, F., Delwart, E., 2016. Detection of a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. Virol. J. 13, 1–8. https://doi.org/10.1186/s12985-016-0642-z.

Rodrigues, I.L.F., Cruz, A.C.M., Souza, A.E., Knackfuss, F.B., Costa, C.H.C., Silveira, R.L., Castro, T.X., 2020. Retrospective study of porcine circovirus 3 (PCV3) in swine tissue from Brazil (1967–2018). Braz. J. Microbiol. 51 (3), 1391–1397. https://doi.org/10.1007/S42770-020-00281-6, 202051.

Saporiti, V., Franzo, 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.

Shen, H., Liu, X., Zhang, P., Wang, L., Liu, Y., Zhang, L., Liang, P., Song, C., 2018. Genome characterization of a porcine circovirus type 3 in South China. Transbound. Emerg. Dis. 65, 264–266. https://doi.org/10.1111/tbed.12639.

Stadejek, T., Woźniak, A., Miłek, D., Biernacka, K., 2017. First detection of porcine circovirus type 3 on commercial pig farms in Poland. Transbound. Emerg. Dis. 64, 1350–1353. https://doi.org/10.1111/tbed.12672.

Standley, K., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability.(outlines version 7). Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010 [doi].

Sun, W., Wang, W., Xin, J., Cao, L., Zhuang, X., Zhang, C., Zhu, Y., Zhang, H., Qin, Y., Du, Q., Han, Z., Lu, H., Zheng, M., Jin, N., 2019. An epidemiological investigation of porcine circovirus 3 infection in dogs in the Guangxi Province from 2015 to 2017, China. Virus Res. 270, 197663 https://doi.org/10.1016/j.virusres.2019.197663.

Wang, W., Sun, W., Cao, L., Zheng, M., Zhu, Y., Li, W., Liu, C., Zhuang, X., Xing, J., Lu, H., Luo, T., Jin, N., 2019. An epidemiological investigation of porcine circovirus 3 infection in cattle in Shandong province, China. BMC Vet. Res. 15, 1–8. https://doi.org/10.1186/s12917-019-1793-0.

Zhai, S.L., Lu, S.S., Wei, W.K., Lv, D.H., Wen, X.H., Zhai, Q., Chen, Q.L., Sun, Y.W., Xi, Y., 2019. Reservoirs of porcine circoviruses: a mini review. Front. Vet. Sci. 6, 319. https://doi.org/10.3389/fvets.2019.00319.

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. Virol. J. 14, 222. https://doi.org/10.1186/s12985-017-0892-4.

Zheng, S., Wu, X., Zhang, L., Xin, C., Liu, Y., Shi, J., Peng, Z., Xu, S., Fu, F., Yu, J., Sun, W., Xu, S., Li, J., Wang, J., 2017. The occurrence of porcine circovirus 3 without clinical infection signs in Shandong Province. Transbound. Emerg. Dis. 64, 1337–1341. https://doi.org/10.1111/tbed.12667.

Zou, Y., Zhang, N., Zhang, J., 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. Arch. Virol. 1–7. https://doi.org/10.1007/s00705-018-3914-7.