Microbial Pathogenesis 196 (2024) 106917

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Porcine astrovirus 3 RNA in the central nervous system of weaned pigs with neurologic disease and polioencephalomyelitis in Brazil

Carolina Yuka Yasumitsu [a], Alais Maria Dall Agnol [a,b], Ana Aparecida Correa Xavier [c], Flavia Helena Pereira Silva [c], Marco Aurélio Callegari [d], Ulisses de Pádua Pereira [e], Caio Abércio da Silva [f], Selwyn Arlington Headley [c], Alice Fernandes Alfieri [a,b], Amauri Alcindo Alfieri [a,b,*]

[a] *Laboratory of Animal Virology, Department of Preventive Veterinary Medicine, Universidade Estadual de Londrina, Londrina, Paraná, Brazil*
[b] *Multi-User Animal Health Laboratory, Molecular Biology Unit, Department of Preventive Veterinary Medicine, Universidade Estadual de Londrina, Paraná, Brazil*
[c] *Laboratory of Animal Pathology, Department of Preventive Veterinary Medicine, Universidade Estadual de Londrina, Paraná, Brazil*
[d] *Akei Animal Research, Fartura, São Paulo, Brazil*
[e] *Laboratory of Animal Bacteriology, Department of Preventive Veterinary Medicine, Universidade Estadual de Londrina, Londrina, Brazil*
[f] *Animal Science Department, Universidade Estadual de Londrina, Londrina, Brazil*

A R T I C L E I N F O

Keywords:
Swine
Weaned piglets
Neurological disease
Polioencephalomyelitis
Central nervous system
Mamastrovirus

A B S T R A C T

This report aims to describe the identification of porcine astrovirus 3 (PAstV3) RNA in the central nervous system (CNS) of weaned pigs with clinical signs of neurological disease associated with polioencephalomyelitis in southeastern Brazil. Three, 20 -35 days-old piglets that died after clinical manifestations of a neurological syndrome were submitted to *post-mortem* evaluations. Tissue samples were examined by histopathology, bacteriology, and molecular assays (RT-PCR, nested-PCR, RT-qPCR, and Sanger sequencing) to detect the primary infectious disease agents associated with neurological disease in pigs. The principal neuropathological alterations occurred in the grey matter of the spinal cord and brainstem resulting in nonsuppurative poliomyelitis and rhombencephalitis. PAstV3 RNA was detected in the CNS samples of all piglets with histopathological evidence of disease and was confirmed by nucleotide sequencing. Nucleic acids from pathogens commonly associated with neurological diseases in pigs, such as porcine teschovirus, porcine sapelovirus, porcine enterovirus G, atypical porcine pestivirus, senecavirus A, and encephalomyocarditis virus was not detected by molecular assays in the three piglets. This is the first report of PAstV3 in piglets with neurological disease and lesions consistent with polioencephalomyelitis in Brazil. This report highlights the importance of monitoring health events that could compromise pig farming productivity and animal welfare.

1. Introduction

Astroviruses (AstVs) are found worldwide and have been linked to both subclinical and clinical infections in birds [1], several mammalian species [2,3], including humans [4]. Furthermore, there is a high potential for interspecies transmission and recombination events between different astrovirus strains [5]. These characteristics emphasize the significance of these viruses for one health [6].

Porcine astrovirus (PAstV) belongs to the family *Astroviridae,* genus *Mamastrovirus,* and species *Mamastrovirus suis* [7]. The viral particles are spherical, not enveloped, and have a diameter of 28–30 nm. The viral genome is formed by single-stranded RNA with a size of 6.4–7.7 kb [8]. PAstV is an emerging virus of pigs with five lineages (PAstV1–PAstV5) [9], and infections can occur in asymptomatic or sick pigs [10,11].

Enteric diseases in pigs have often been associated with different PAstVs lineages, infections with a single lineage, associations between distinct lineages, or even with other etiological agents [10,12]. Neurological conditions have been more recently reported and attributed, especially to PAstV3 [13,14]. The initial descriptions of pigs with neurological manifestations associated with PAstV3 occurred between 2015 and 2016 in Hungary [14], followed by 2017 in the USA [13], and more recently (2020), in South Korea [unpublished data – Preprints

* Corresponding author. Laboratory of Animal Virology, Department of Preventive Veterinary Medicine, Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

E-mail address: alfieri@uel.br (A.A. Alfieri).

https://doi.org/10.1016/j.micpath.2024.106917
Received 20 May 2024; Received in revised form 2 July 2024; Accepted 3 September 2024
Available online 5 September 2024
0882-4010/© 2024 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

version; Park et al., 2023]. Furthermore, the virus has been identified in clinically healthy piglets and sows from pig farms with previous reports of PAstV3-associated neurological disease [15,16]. Since outbreaks of novel infectious disease agents can have devastating economic impacts on pig production, monitoring these infections is necessary to develop adequate control and prophylactic measures.

In Brazil, only one description of PAstV was reported in fecal samples from healthy piglets, however, PAstV was not identified in the central nervous system (CNS) of piglets with neurological disease [17]. It must be highlighted that all previous descriptions of PAstV-associated neurological disease were observed in pigs from the Northern Hemisphere [13–15], and there are no reports of similar disease in the Southern Hemisphere. This report aims to describe the identification of PAstV3 RNA in the CNS of weaned pigs with clinical signs of neurological disease associated with polioencephalomyelitis in southeastern Brazil.

2. Materials and methods

2.1. Study location, clinical history, and sample collection

The outbreak occurred in 2023, at a multisite pig production farm with around 3,200 sows located in the state of São Paulo, Southeastern Brazil. The consulting veterinarian reported that, for over a year, weaned piglets at this farm have been showing neurological signs. The clinical manifestations included quadriplegia, opisthotonos, nystagmus, and occasional fever in suckling (from 20 days of age) and newly weaned piglets (up to 35 days old). It was noted that approximately 2 % of these piglets were affected, and the lethality rate was almost 100 %. Despite receiving broad-spectrum antimicrobials and anti-inflammatories the affected pigs showed no response to treatment.

In mid-April 2023, three weaned piglets, aged between 24 and 26 days, with similar neurological clinical signs but without receiving any supporting therapy, were found dead. All carcasses were submitted for *post-mortem* evaluation to determine the possible cause of the neurological syndrome within 4 h after death.

2.2. Pathological evaluation

Tissue samples of all major organs including the cerebrum, cerebellum, brainstem, cervical spinal cord, lungs, myocardium, liver, kidney, intestine, and spleen were collected. These samples were then fixed by immersion in 10 % buffered formalin solution, processed for routine histopathologic evaluation, and stained with the Hematoxylin and eosin (H&E) stain. Feces and duplicates of all organs (cerebrum, cerebellum, brainstem, cervical spinal cord, lungs, myocardium, liver, kidney, and spleen) collected were maintained at −80 °C until used in molecular assays designed to detect agents associated with neurological diseases of swine.

2.3. Bacterial analysis

Aseptic swabs were collected from the surface of the CNS of the three piglets during *post-mortem* evaluations, promptly plated on 5 % sheep blood agar, and then incubated at 37 °C for 24–48 h for bacterial culture and isolation.

2.4. Molecular detection of agents associated with neurological disease in swine

Suspensions (10 % w/v) were prepared from a 100 mg fragment of each organ collected during *post-mortem* evaluation and then mechanically disrupted using the TissueLyser LT (QIAGEN, Hilden, Germany). Fecal suspensions at 10–20 % (weight/volume) and disrupted tissue samples were homogenized in 0.01 M phosphate-buffered saline (PBS) at pH 7.2 and clarified by centrifugation at 1000×*g* for 5 min. Nucleic acid extraction was performed on 500 μL aliquots of the tissue/fecal suspensions that had been pre-treated with proteinase K and 1 % sodium dodecyl sulphate (Invitrogen™ Life Technologies, Carlsbad, CA, USA). The nucleic acid was extracted by a combination of phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidine isothiocyanate methods [18,19]. The extracted nucleic acids were eluted in 50 μL of UltraPure™ DEPC-treated water (Invitrogen™ Life Technologies, Carlsbad, CA, USA) and stored at −80 °C. Nuclease-free water was used as a negative control in all nucleic acid extractions and subsequent procedures.

Molecular assays were used to amplify the nucleic acids of the main infectious disease agents associated with neurological disease in pigs. The nucleic acids were tested using the semi-nested RT-PCR with Pan-Astrovirus primers [20]. The PAstV3 RNA presence in the tissue samples and Cq were determined using a TaqMan-based RT-qPCR assay. The RT-qPCR was carried out with SuperScript™ III Platinum™ One-Step Quantitative RT-PCR System (Invitrogen™ Life Technologies, Carlsbad, CA, USA) [16]. All reactions were performed in triplicate.

Additionally, other viral pathogens associated with the development of neurological diseases in swine were included in the analysis, such as porcine teschovirus (PTV), porcine sapelovirus (PSV), porcine enterovirus G (EV-G) [21,22], atypical porcine pestivirus (APPeV [23], Senecavirus A (SVA) [24,25], and encephalomyocarditis virus (EMCV) [26]. The specific gene targets, primers, amplicon size, and type of molecular assays used to identify neurological infectious disease pathogens are provided in Supplementary Table 1.

2.5. Sequencing and phylogenetic analysis

One amplicon from each piglet was chosen for nucleotide (nt) sequencing to confirm Pan-Astrovirus specificity. The amplicons were purified using a PureLink® Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen® Life Technologies, Carlsbad, CA, USA) and quantified using Qubit® Fluorometer (Invitrogen® Life Technologies, Eugene, OR, USA). Direct sequencing was performed using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®, Foster City, CA, USA) with the forward and reverse primers in a 3500 Genetic Analyzer sequencer (Applied Biosystems®).

Sequence quality analyses and consensus sequences were obtained using Phred and CAP3 homepages, respectively (http://asparagin.cenargen.embrapa.br/phph/). Similarity searches were performed with nt sequences deposited in the GenBank database using the Basic Local Alignment Search Tool homepage (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple and pairwise alignments with strains available in GenBank were performed with MEGA software version 7.0.26 [27], and the nt sequence identity matrix was constructed using BioEdit software version 7.2.5 [28]. A phylogenetic tree based on nt sequences was obtained using the Neighbor-Joining method [29] with the Tamura-Nei method [30] using MEGA software version 7.0.26 [27]. The bootstrapping probabilities were calculated using 1000 replicates.

3. Results

3.1. Pathological and bacteriological findings

The most important gross alterations were restricted to the brain of all weaned piglets evaluated and the lungs of piglets #1 and 3. There was marked congestion of the meningeal vessels of the cerebrum of all piglets, while rib impressions were prominent at the lungs of piglets #1 and 3. The histological evaluations revealed similar patterns of neurological disease consistent with viral infections in the CNS of all piglets. These lesions were predominant at the grey matter of the brainstem and the cervical region of the spinal cord but mild at the cerebellum and rare at the cerebral cortex. The intensity and distribution of the neurological lesions were more severe at the brainstem and spinal cord, followed by the cerebellum, and lasty at the cerebral cortex. Lesions at the brainstem and spinal cord were similar in intensity and distribution and consisted

of acute, moderate, multifocal to coalescing, nonsuppurative rhombencephalitis and poliomyelitis, respectively, having perivascular cuffings formed by one to two layers of lymphocytes, foci of neuronal necrosis, neuronophagia, and glial nodes (Fig. 1A–F). Histological alteration identified at the cerebellum consisted essentially of several perivascular cuffs observed predominantly at the white matter (Fig. 1G). In contrast, the histopathological alterations at the cerebral cortex were sparse in all piglets and consisted of rare perivascular cuffings formed by one layer of inflammatory cells (Fig. 1H). Additional neuropathological alterations included mild cerebral congestion in all piglets and focal perivascular hemorrhage at the spinal cord of piglet #1.

Significant non-neurological pathological alterations were observed in the lungs of all piglets, characterized as moderate to severe, diffused, interstitial pneumonia. This was due to marked thickening of the alveolar walls caused by hyperplasia of type II pneumocytes with an influx of lymphocytes.

Bacteria were not isolated from the CNS swabs collected during the gross evaluation of these piglets.

3.2. Molecular detection of neurological agents and characterization of porcine astrovirus 3

PAstV RNA was amplified by the Pan-Astrovirus assay from the brainstem and spinal cord of all piglets, the cerebrum of two (# 1 and 2), as well as the cerebellum of piglet #1 with histopathological evidence of neurological disease. Furthermore, PAstV3 RNA was identified using specific RT-qPCR in most of the neuroanatomical regions that were positive with the consensus primers, except for the cerebrum of piglet #1 (Table 1). Additionally, PAstV3 RNA was only amplified from the myocardium of piglet #1. All other non-neurological organs evaluated, even the lungs with interstitial pneumonia, did not contain PAstV3 RNA. The nucleic acids of common agents (PTV, PSV, EV-G, SVA, APPeV, and

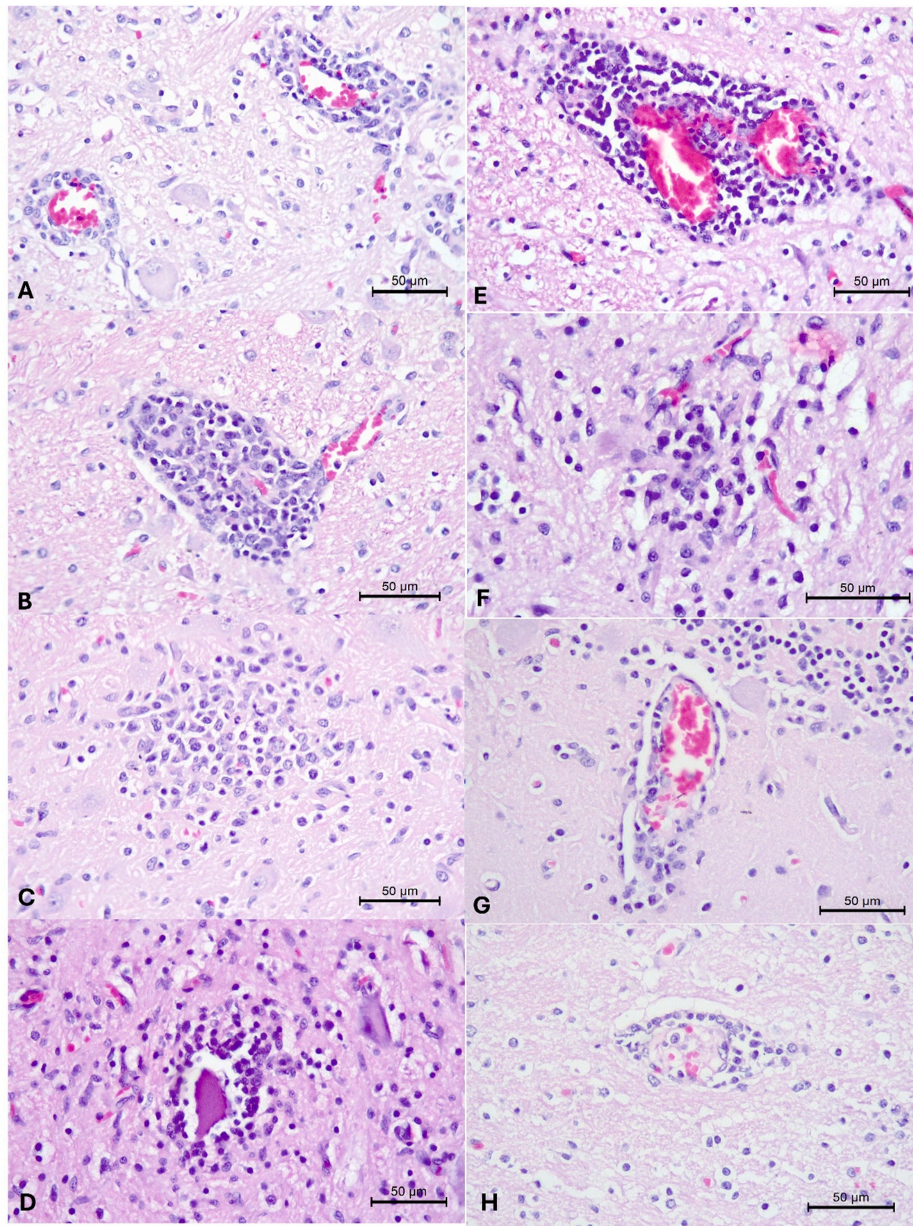

Fig. 1. Principal histopathological findings were observed at the brainstem (A–C), spinal cord (D–H), cerebellum (G), and cerebrum (H) of piglets infected with porcine astrovirus 3. Observe the lymphocytic perivascular cuffings (A–B), neuronal necrosis, and glial node (C) at the brainstem. There is severe neuronophagia (D), perivascular cuffing (E), and a glial node (F) at the cervical spinal cord. Observe perivascular cuffings formed by one layer of inflammatory cells at the cerebellum (G) and cerebrum (H). Hematoxylin and eosin stain. Bars: A-H, 50 µm.

Table 1
Detection of porcine astrovirus by semi-nested RT-PCR (Pan-Astrovirus) and RT-qPCR (PAstV3) assay in biological samples of weaned piglets with histopathological confirmation of neurological disease.

Piglet #	Organs	sn-RT-PCR	RT-qPCR – Cq
#1	Brainstem	+ve	30.42
	Cerebrum	+ve	ND
	Cerebellum	+ve	30.15
	Spinal cord	+ve	24.55
	Myocardium	+ve	30.51
	Feces	ND	ND
#2	Brainstem	+ve	24.67
	Cerebrum	+ve	26.56
	Cerebellum	ND	ND
	Spinal cord	+ve	26.49
	Myocardium	ND	ND
	Feces	+ve	ND
#3	Brainstem	+ve	31.19
	Cerebrum	ND	ND
	Cerebellum	ND	ND
	Spinal cord	+ve	32.51
	Myocardium	ND	ND
	Feces	ND	ND

Legend: sn-RT-PCR (semi-nested RT-PCR): Pan-Astrovirus; RT-qPCR: Porcine Astrovirus 3; Cq: quantification cycle; +ve: positive; ND: Not detected.

EMCV) associated with the development of neurological diseases in swine were not detected by their respective molecular assays in the three piglets.

The quantification cycles (Cq) ranged from 24.55 to 32.51, piglet #1 had the lowest Cq (24.55) in the spinal cord, while piglet #3 had the highest Cq (32.51) in the spinal cord. In the other tissues evaluated (non-CNS tissues), the RNA of PAstV3 was only identified in the myocardium of piglet #1 (Cq 30.51). In the lung, liver, and spleen it was not detected PAstV3 RNA in any of the piglets. Only the fecal sample from piglet #2 was positive by the conventional assay (Pan-Astrovirus). However, in the specific RT-qPCR for PAstV3, it was not possible to identify the presence of RNA.

Sequencing of the amplicons generated by the Pan-Astrovirus assay confirmed the presence of PAstV RNA in the tissue samples. The three nt sequences from the partial RdRp of PAstV were named BRA/UEL/SP-3156/23, BRA/UEL/SP-3157/23, and BRA/UEL/SP-3158/23 (GenBank accession numbers PP475135 to PP475137, respectively). Phylogenetic analyses revealed that the three sequences of PAstV identified in this study clustered with members of the PAstV3 group (Fig. 2). Furthermore, the PAstV strains herein identified show 87.3–90.6 % nt sequence identity with other PAstV3 strains previously identified in pigs with neurological disease, while with the other porcine astroviruses (PAstV1-2; and PAstV4-5) the nt identity was <60.9 %. The comparative analysis between the PAstV field strains identified in this study showed a range of 99.6–100 % nt identity to each other.

4. Discussion

The neurological manifestations described in these pigs were previously associated with infections by PAstV3 [13,31]. Furthermore, the neuropathological findings of rhombencephalitis and poliomyelitis herein diagnosed have been described in pigs experimentally [31] and spontaneously [13,32] infected with PAstV3. These initial histopathological findings of viral-induced lesions in the cervical spinal cord and brainstem of these weaned piglets contrasted previously diagnosed encephalitic diseases of pigs in Brazil, which prompted a suggestive diagnosis of astrovirus-related infections [33].

The presence of PAstV3 was confirmed by amplifying and quantifying the RNA from neuroanatomical regions with histopathological evidence of viral disease. This demonstrated that the virus was associated with the development of these lesions. Moreover, the non-detection of the RNA of important neurological disease pathogens of swine, including PTV, PSV, EV-G, SVA, APPeV, and EMCV, with the non-isolation of bacteria suggests that these agents were not associated with the neurological manifestations observed. The results from this investigation differ from the previous description of PAstV in piglets from Brazil. That study investigated the etiology of neurological manifestations in piglets, during which PAstV was not detected within the CNS of affected animals but within fecal samples of healthy pigs [17]. Accordingly, the findings herein reported may represent the first identification of PAstV3 in association with neurological disease in pigs from Brazil and Latin America.

During the investigation, we observed neuropathological changes in weaned piglets, primarily in the grey matter of the spinal cord and brainstem, with significantly reduced histological patterns of viral-induced infections in the cerebellum and cerebrum. Experimentally induced and naturally occurring PAstV3 infections in pigs and other mammalian species showed similar selective tropism for specific neuroanatomical regions of the brain [13,31,32,34,35]. These findings suggest that the brainstem and cervical spinal cord must be collected and evaluated whenever neurologic astrovirus-related infections are suspected in mammalian species. Only spinal cord sections were evaluated in this study.

During this study, RNA of an astrovirus was detected in the fecal sample of piglet #2 using the Pan-Astrovirus assay but without corresponding quantification with the specific PAstV3 assay. These findings may suggest the possible identification of a distinct enteric lineage of PAstV, resulting in the non-detection by the RT-qPCR assay. Additionally, it is important to note that the Pan-Astrovirus molecular assay uses a semi-nested PCR [20]. However, the nested PCR assays have greater sensitivity when compared to quantitative PCR assays, including assays designed for AstV and PAstV3 [14]. Therefore, samples with low viral loads may have negative results in the RT-qPCR assay. The detection of the virus in feces is not a common finding in all infected animals. Even in experimental pigs infected with PAstV3, it was not possible to recover the virus in the feces of all animals [31].

The RNA of PAstV3 was identified in the spinal cord and brainstem of all pigs, whereas detection in the brain (piglet #2) and cerebellum (piglet #1) varied depending on the piglet. Regarding the detection of PAstV3 in non-neurological organs, the viral RNA was not detected in most non-neurological organs evaluated, except in the myocardium of piglet #1, which presented a Cq similar to that identified in the cerebellum and brainstem. However, the Cq detected in the cervical spinal cord was lower, suggesting a higher viral load. Similar findings were described in experimentally infected piglets, where PAstV3 was identified only in the neurological organs [31]. Our findings contrast with a study conducted in Hungary involving naturally infected pigs. In that study, PAstV-3 was detected in various tissues, including lymph nodes, tonsils, liver, lungs, and myocardium of symptomatic animals. However, the viral load in these tissues was lower than that found in neurological organs [14]. Therefore, tracking the presence of the virus in organs from the nervous system is necessary for more accurate future diagnoses.

The phylogenetic analysis of the nt fragment of the RdRp gene revealed five clades of PAstV. The sequences from each piglet originating in this study were grouped into the PAstV3 clade, along with the neurotropic strains identified in the USA and Hungary. The RdRp gene is conserved, but the nt sequence analysis showed that the field strains identified in this study have low similarity to other PastV3 strains associated with neurological disease (87.3–90.6 %) and even lower similarity to strains from non-neurological PAstV (<60.9 %). This indicates a high genetic variability between these viral types.

The limitations of this study are concentrated on the small number of animals used and the absence of complementary assays to demonstrate the agent within the observed lesions. The use of immunohistochemistry (IHC) and/or *in situ* hybridization assays would have contributed greatly to the intralesional detection of viral antigens and proteins, respectively, thereby confirming infection. Accordingly, an international

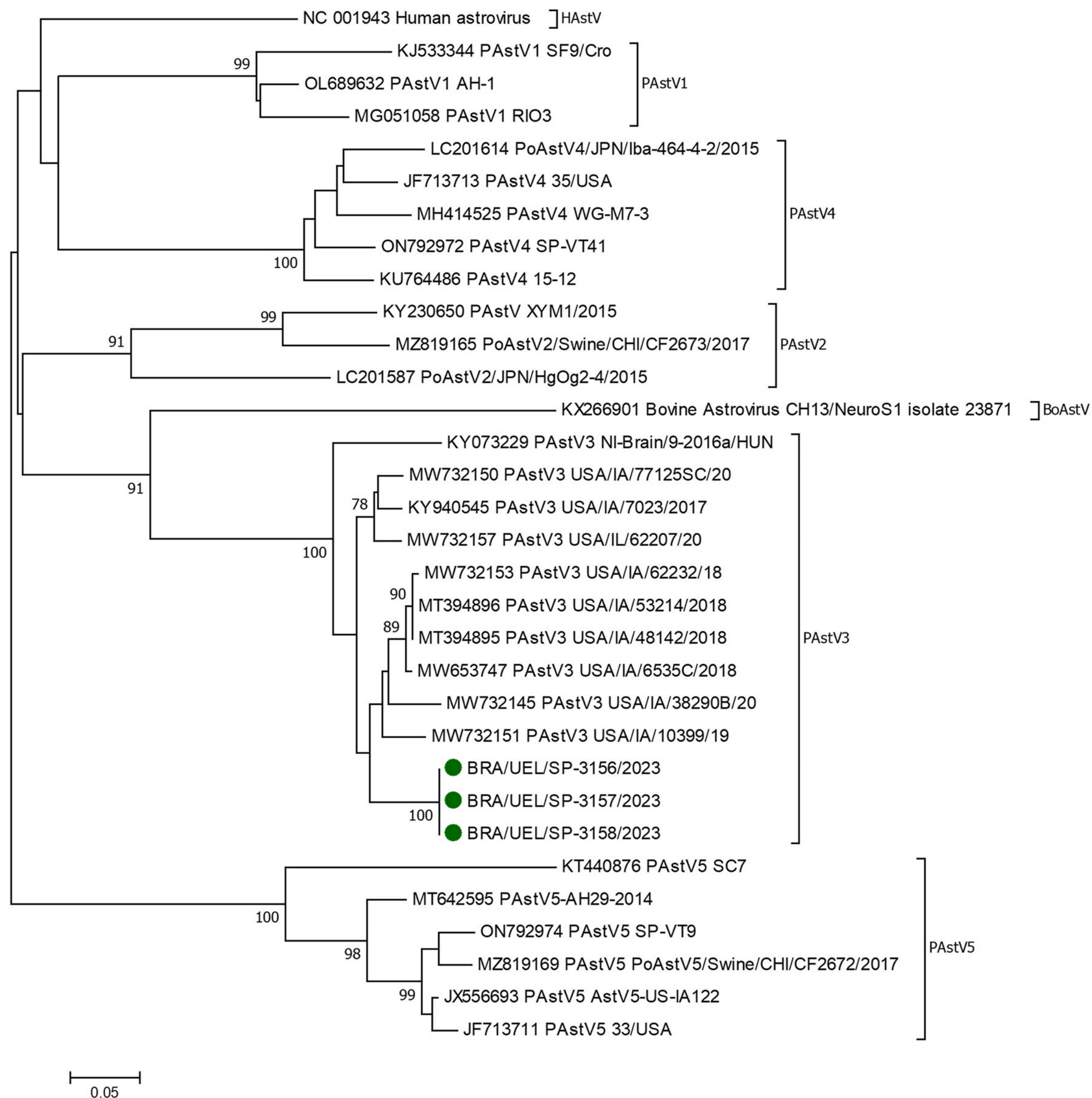

Fig. 2. The evolutionary history was inferred using the Neighbor-Joining method of partial (269 nt) RNA-dependent RNA polymerase (RdRp) of porcine astrovirus (PAstV). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Tamura-Nei method [3] and are in the units of the number of base substitutions per site. The analysis involved 30 nucleotide sequences of PAstV, one bovine astrovirus, and another human astrovirus. Evolutionary analyses were conducted in MEGA7. The PAstV3 strains identified in this study are highlighted with green-filled circles.

collaboration is being elaborated to detect viral particles either by IHC or *in situ* hybridization. Additionally, a retrospective study is being implemented to revise all histopathological diagnosis of porcine neurological disease over the last 10 years in an attempt to identify additional cases of PAstV3 in pigs.

5. Conclusion

In this report, we describe the first molecular detection of PAstV3 in weaned piglets with neurological disease and lesions consistent with polioencephalomyelitis in Brazil.

Data accessibility

Nucleotide sequences for PAstV3 data from this study are deposited in GenBank (accession numbers PP475135 to PP475137).

Ethics approval

This study was submitted to the Ethics Committee on Animal Experiments of the Universidade Estadual de Londrina (CEUA/UEL) and approved under identification number 11363.2015.16. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

CRediT authorship contribution statement

Carolina Yuka Yasumitsu: Writing – original draft, Software, Methodology. **Alais Maria Dall Agnol:** Writing – original draft, Methodology, Formal analysis. **Ana Aparecida Correa Xavier:** Methodology. **Flavia Helena Pereira Silva:** Methodology. **Marco Aurélio Callegari:** Methodology. **Ulisses de Pádua Pereira:** Writing – review & editing, Supervision. **Caio Abércio da Silva:** Writing – review & editing, Conceptualization. **Selwyn Arlington Headley:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation.

Alice Fernandes Alfieri: Writing – review & editing, Supervision. **Amauri Alcindo Alfieri:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

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.

Acknowledgements

The authors thank the following Brazilian Institutes for their financial support: the National Council of Technological and Scientific Development (CNPq), the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES), the Financing of Studies and Projects (FINEP), and the Araucaria Foundation (FAP/PR). Carolina Y. Yasumitsu, Ana A. C. Xavier, and Flavia H. P. Silva are recipients of CAPES studentships. Ulisses P. Pereira, Selwyn A. Headley, Alice F. Alfieri, and Amauri A. Alfieri are recipients of CNPq fellowships.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2024.106917.

References

References

[1] L. Li, M. Sun, Y. Zhang, M. Liao, A review of the emerging poultry visceral gout disease linked to avian astrovirus infection, Int. J. Mol. Sci. 23 (2022).
[2] D. Comeau, M.T. Spinato, D. Ojkic, R.A. Foster, J.L. Caswell, Bovine astrovirus and its role in lymphocytic encephalitis in cattle in Ontario, Canada, 1988-2019, J. Vet. Diagn. Invest. (2024) 10406387241237192.
[3] K. Kuczera, A. Orłowska, M. Smreczak, M. Frant, P. Trębas, J. Rola, Prevalence of astroviruses in different animal species in Poland, Viruses 16 (2024).
[4] P.S. Porto, A. Rivera, R. Moonrinta, C.E. Wobus, Entry and egress of human astroviruses, Adv. Virus Res. 117 (2023) 81–119.
[5] N. Wohlgemuth, R. Honce, S. Schultz-Cherry, Astrovirus evolution and emergence, Infect. Genet. Evol. 69 (2019) 30–37.
[6] V. Cortez, V.A. Meliopoulos, E.A. Karlsson, V. Hargest, C. Johnson, S. Schultz-Cherry, Astrovirus biology and pathogenesis, Annu Rev Virol 4 (2017) 327–348.
[7] ICTV, International committee on taxonomy of viruses, Virus Taxonomy: 2023 Release (2024). Available online: https://ictv.global/taxonomy. (Accessed 1 July 2024).
[8] ICTV, International committee on taxonomy of viruses, Chapter Version: ICTV Ninth Report; 2009 Taxonomy Release (2009). https://ictv.global/taxonomy. (Accessed 30 April 2024).
[9] C.T. Xiao, L.G. Giménez-Lirola, P.F. Gerber, Y.H. Jiang, P.G. Halbur, T. Opriessnig, Identification and characterization of novel porcine astroviruses (PAstVs) with high prevalence and frequent co-infection of individual pigs with multiple PAstV types, J. Gen. Virol. 94 (2013) 570–582.
[10] R. Kour, P. Kumar, N. Jindal, S. Kumari Minhas, R. Kumar, A.K. Gupta, A. Malik, Molecular detection and characterization reveals circulation of multiple genotypes of porcine astrovirus in Haryana, India, Arch. Virol. 166 (2021) 2847–2852.
[11] S. Salamunova, A. Jackova, R. Mandelik, J. Novotny, M. Vlasakova, S. Vilcek, Molecular detection of enteric viruses and the genetic characterization of porcine astroviruses and sapoviruses in domestic pigs from Slovakian farms, BMC Vet. Res. 14 (2018) 313.
[12] Vaishali, R. Gupta, M. Kumar, N. Bansal, Vivek, P. Kumar, P. Kumar, N. Jindal, Coinfection of porcine astrovirus and other porcine viruses in diarrheic pigs in Haryana, India, Arch. Virol. 168 (2023) 246.
[13] B. Arruda, P. Arruda, M. Hensch, Q. Chen, Y. Zheng, C. Yang, I.R.H. Gatto, F. M. Ferreyra, P. Gauger, K. Schwartz, L. Bradner, K. Harmon, B. Hause, G. Li, Porcine astrovirus type 3 in central nervous system of swine with polioencephalomyelitis, Emerg. Infect. Dis. 23 (2017) 2097–2100.
[14] Á. Boros, M. Albert, P. Pankovics, H. Bíró, P.A. Pesavento, T.G. Phan, E. Delwart, G. Reuter, Outbreaks of neuroinvasive astrovirus associated with encephalomyelitis, weakness, and paralysis among weaned pigs, Hungary, Emerg. Infect. Dis. 23 (2017) 1982–1993.
[15] G. Rawal, F.M. Ferreyra, N.R. Macedo, L.K. Bradner, K.M. Harmon, G. Allison, D.C. L. Linhares, B.L. Arruda, Ecology of porcine astrovirus type 3 in a herd with associated neurologic disease, Viruses 12 (2020).
[16] G. Rawal, F.M. Ferreyra, N.R. Macedo, L.K. Bradner, K.M. Harmon, A. Mueller, G. Allison, D.C.L. Linhares, B.L. Arruda, Detection and cellular tropism of porcine astrovirus type 3 on breeding farms, Viruses 11 (2019).
[17] M.E. Hammerschmitt, P.R. de Almeida, B.S. de Cecco, M.P. Lorenzett, C. I. Schwertz, R.A.S. da Cruz, R.A. Caprioli, D.T. Schuh, M. Demoliner, A.K.A. Eisen, F.R. Spilki, S.P. Pavarini, D. Driemeier, Swine polioencephalomyelitis in Brazil: identification of teschovirus A, sapelovirus A, and enterovirus G in a farm from southern Brazil, Braz. J. Microbiol. 52 (2021) 1617–1622.
[18] A.A. Alfieri, M.E. Parazzi, E. Takiuchi, K.C. Médici, A.F. Alfieri, Frequency of group A rotavirus in diarrhoeic calves in Brazilian cattle herds, 1998-2002, Trop. Anim. Health Prod. 38 (2006) 521–526.
[19] R. Boom, C.J. Sol, M.M. Salimans, C.L. Jansen, P.M. Wertheim-van Dillen, J. van der Noordaa, Rapid and simple method for purification of nucleic acids, J. Clin. Microbiol. 28 (1990) 495–503.
[20] D.K.W. Chu, L.L.M. Poon, Y. Guan, J.S.M. Peiris, Novel astroviruses in insectivorous bats, J. Virol. 82 (2008) 9107–9114.
[21] D.G. Donin, R.d.A. Leme, A.F. Alfieri, G.C. Alberton, A.A. Alfieri, First report of *Porcine teschovirus* (PTV), *Porcine sapelovirus* (PSV) and *Enterovirus G* (EV-G) in pig herds of Brazil, Trop. Anim. Health Prod. 46 (2014) 523–528.
[22] A. Krumbholz, R. Wurm, O. Scheck, E. Birch-Hirschfeld, R. Egerer, A. Henke, P. Wutzler, R. Zell, Detection of porcine teschoviruses and enteroviruses by LightCycler real-time PCR, J Virol Methods 113 (2003) 51–63.
[23] F. Possatti, S.A. Headley, R.A. Leme, A.M. Dall Agnol, E. Zotti, T.E.S. de Oliveira, A. F. Alfieri, A.A. Alfieri, Viruses associated with congenital tremor and high lethality in piglets, Transbound Emerg Dis 65 (2018) 331–337.
[24] C. Feronato, R.A. Leme, J.A. Diniz, A.M.D. Agnol, A.F. Alfieri, A.A. Alfieri, Development and evaluation of a nested-PCR assay for Senecavirus A diagnosis, Trop. Anim. Health Prod. 50 (2018) 337–344.
[25] R.A. Leme, E. Zotti, B.K. Alcântara, M.V. Oliveira, L.A. Freitas, A.F. Alfieri, A. A. Alfieri, Senecavirus A: an emerging vesicular infection in Brazilian pig herds, Transbound Emerg Dis 62 (2015) 603–611.
[26] H. Vanderhallen, F. Koenen, Rapid diagnosis of encephalomyocarditis virus infections in pigs using a reverse transcription-polymerase chain reaction, J Virol Methods 66 (1997) 83–89.
[27] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (2016) 1870–1874.
[28] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, in: Nucleic Acids Symposium Series, 1999, pp. 95–98.
[29] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406–425.
[30] K. Tamura, M. Nei, Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, Mol. Biol. Evol. 10 (1993) 512–526.
[31] F.M. Ferreyra, P.E.H. Arruda, L.K. Bradner, K.M. Harmon, M. Zhang, L. Giménez-Lirola, B.L. Arruda, Experimental porcine astrovirus type 3-associated polioencephalomyelitis in swine, Vet. Pathol. 58 (2021) 1064–1074.
[32] F.S.M. Ferreyra, L.K. Bradner, E.R. Burrough, V.L. Cooper, R.J. Derscheid, P. C. Gauger, K.M. Harmon, D. Madson, P.E. Piñeyro, K.J. Schwartz, G.W. Stevenson, M.A. Zeller, B.L. Arruda, Polioencephalomyelitis in domestic swine associated with porcine astrovirus type 3, Vet. Pathol. 57 (2020) 82–89.
[33] A.A.C. Xavier, A.M. Dall Agnol, C.A. Da Silva, F.H.P. Silva, M.M. Zanim, A.A. A, S. A. Headley, Outbreak of swine polioencephalomyelitis associated with possible infection by porcine astrovirus, in: 7° Congresso Brasileiro de Patologia Veterinária (CBPV), João Pessoa-PB, 2023.
[34] G. Reuter, P. Pankovics, Á. Boros, Nonsuppurative (aseptic) meningoencephalomyelitis associated with neurovirulent astrovirus infections in humans and animals, Clin. Microbiol. Rev. 31 (2018).
[35] N. Wildi, T. Seuberlich, Neurotropic astroviruses in animals, Viruses 13 (2021).