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A plate of viruses: Viral metagenomics of supermarket chicken, pork and beef from Brazil

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ABSTRACT

A viral metagenomics study was conducted in beef, pork, and chicken sold in supermarkets from Southern Brazil. From chicken, six distinct gyroviruses (GyV) were detected, including GyV3 and GyV6, which for the first time were detected in samples from avian species, plus a novel smacovirus species and two highly divergent circular Rep-encoding ssDNA (CRESS-DNA) viruses. From pork, genomes of numerous anelloviruses, porcine parvovirus 5 (PPV5) and 6 (PPV6), two new genomoviruses and two new CRESS-DNA viruses were found. Finally, two new CRESS-DNA genomes were recovered from beef. Although none of these viruses have history of transmission to humans, the findings reported here reveal that such agents are inevitably consumed in diets that include these types of meat.

1. Introduction

Meat and meat products are the source of numerous bacteria (e.g. *Salmonella enterica*), protozoa (e.g. *Toxoplasma gondii*) and worms (e.g. *Trichinella spiralis*) that may cause significant illness in humans. Therefore, it has been rising the overall concern of viral transmission by food products, especially the ones causes by meat and meat-derived. Meats have been pointed in charge, once is well-known that food-source animals are themselves subject to virus infections that might led to transmission to other species along the food chain. A large number of viruses harbored by farm or wild animals can cause zoonotic infections such as noroviruses, rotaviruses and hepatitis E virus (Spahr et al., 2018; Villabruna et al., 2019). Furthermore, viral transmission between different animal species, including domestic and wild animals may also occur, facilitating viral adaptation and consequently enhancing the viral host range (Ma et al., 2009; Morse et al., 2012). Today, emerging viruses are a major concern, since devastating, occasionally life-threatening epidemics – e.g., COVID-19 and pandemic influenza – are caused by viruses which originally spilled over from animals to humans.

In 2014, an interesting study identified a series of viral genome sequences in beef, pork, and chicken purchased from stores in San Francisco, USA (Zhang et al., 2014b). Despite no association with disease, the innovative workflow lead to the detection of a large number of known and unknown viral genomes harbored by the meats sampled on the occasion. Bearing in mind that microorganism's evolution mimics the lines of a poem by Gonçalves Dias (The Song of Exile): the birds that chirp here are not the same as those who chirp there (…)" (Dias, 1846), we set up a metagenome-based study to examine the virome of meats sold in supermarkets in Porto Alegre, the capital of Rio Grande do Sul, the southernmost State in Brazil. The present study revealed that chicken, pork and beef samples harbor highly divergent CRESS-DNA viruses, including genomes that could not be classified at the family level. In chicken and pork samples, diverse anelloviruses (gyroviruses from chicken and Torque teno viruses from swine) were detected. In addition, in all meat samples analyzed, the vast majority of viral sequences detected were attributed to bacteriophages species hosted by *Acinetobacter* and *Pseudomonas*, common genera of bacteria isolated from meat samples.

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2. Material and methods

2.1. Chicken, pork and beef samples and virus enrichment

Ten samples of chicken (drummete), pork (chop) and beef (ground meat) were purchased in a supermarket in Porto Alegre City, Rio Grande do Sul, Brazil. All samples were from animals produced within the state and processed by local slaughterhouses. Tissue fragments (~1 cm^3) of each sample were aseptically excised and pooled. The three pools (chicken, pork and beef) were processed essentially as described previously (Cibulski et al., 2020). Briefly, pooled samples were macerated with sterile sand, resuspended in 10 vol of PBS (pH 7.2), followed by vigorous vortexing for 15 min. Subsequently, the suspensions were centrifuged at 3,000×*g* for 30 min at 4 °C and the supernatant filtered through a 0.45 μm membrane (Millipore) to remove bacteria and other particulate debris. The supernatants were centrifuged at 150,000×*g* on a 25% sucrose cushion for 4.5 h at 4 °C (in a Sorvall AH629 rotor), and the pellet containing viral particles was treated with a mixture of DNase, RNase A and benzonase to digest non-protected nucleic acids.

2.2. Viral nucleic acids isolation, enrichment and sequencing

Capsid-protected nucleic acids were extracted using a standard phenol-chloroform protocol (DNA) or TRIzol® (RNA). Viral DNA was enriched by multiple displacement amplification using phi29 DNA polymerase (da Silva et al., 2018). RNA was converted to cDNA using SuperScript III reverse transcriptase and random-amplificated (Clem et al., 2007). The DNA products resulting from enrichment protocols were purified using a Genomic DNA clean and concentrator (Zymo Research). DNA libraries were further prepared with 50 ng of purified DNA using a Nextera DNA sample preparation kit (Illumina) and sequenced using an Illumina MiSeq instrument (2x150 paired-end reads with the Illumina v2 reagent kit).

2.3. Viral metagenomic data assembly and sequence analyses

The quality of generated sequences was evaluated using FastQC tool (Andrews, 2010). Low-quality sequences were trimmed with the aid of CLC Workbench 12 software. The paired-end sequence reads were assembled into contigs with metaSPAdes and CLC Workbench v12. All assemblies were confirmed by mapping reads to contigs as described in Lima et al. (2019) using Geneious Software (version R9). The assembled contigs were examined in search for similarities to known sequences with BLASTx software. Sequences with E-values of 10^{-3} or less were classified as likely originating from a eukaryotic virus, bacteria, phages, eukaryote or unknown, based on the taxonomic origin of the sequence with the best E-value as described by Caesar et al. (2019).

Open reading frames (ORF) predictions and genome annotations of the complete near-full-length genomes were performed with the aid of Geneious software and NCBI ORF finder and later identified by BLASTx/BLASTn. Gene and protein comparisons were performed with BLASTn and BLASTp programmes. To identify the main protein motifs, Motif Scan (https://myhits.isb-sib.ch/cgi-bin/motif_scan) was applied; stem-loop structures were analyzed using Mfold (Zuker, 2003). Nucleotide and amino acid sequences were aligned using the MAFFT software (Katoh and Standley, 2013) and then visually inspected in Geneious. Rolling circle replication (RCR) and superfamily 3 helicase (SF3) motifs (Gorbalenya et al., 1990; Gorbalenya and Koonin, 1993; Ilyina and Koonin, 1992; Rosario et al., 2012) were analyzed from the sequence alignment using as reference *Cressdnaviricota* replication-associated proteins (Krupovic et al., 2020).

2.4. Construction of phylogenetic trees

Amino acid sequences representative of known viral families, as well as CRESS-DNA genomes, were obtained from GenBank (June 2020) and then aligned with the sequences identified in the present study using MAFFT software, which was optimized for accurate global alignment (option "G-INS-i") (Katoh and Standley, 2013) and further trimmed with trimAL (Capella-Gutiérrez et al., 2009). These alignments were used to generate maximum-likelihood phylogenetic trees using PhyML (Guindon et al., 2010) with best fit substitution models determined by Smart Model Selection (Lefort et al., 2017). Statistical significance analyses of tree topologies were performed with the approximate likelihood branch support test (aLRT) (Guindon and Gascuel, 2003). The GenBank accession numbers of the viral sequences used in the phylogenetic analyses are shown on phylogenetic trees.

3. Results and discussion

3.1. Viruses identified in chicken

Approximately 531,000 high-quality reads were generated by metagenomic sequencing. After assembly, a total of 2,370 contigs were generated; 50.3% were viral contigs. From these, 0.75% were assigned to eukaryotic viruses and 99.25% to bacteriophages. However, despite the relatively small number of contigs associated with viral genomes previously known to infect Eukarya, ~20% of the reads were mapped back to these contigs, indicating a high sequence coverage. A detailed taxonomic classification, including the numbers of reads for each Eukarya-related viral contig recovered is this study, is provided in Supplementary Table 1.

Nine Eukarya-related viral contigs were identified in this metagenome and, six contigs corresponded to gyrovirus sequences (Supplementary Table 1); in four of these, full genomes were obtained (chicken anemia virus, CAV; avian gyrovirus 2, AGV2; gyrovirus 3, GyV3; and gyrovirus 4, GyV4) (Fig. 1). The other two gyroviruses' contigs corresponded to a genome of gyrovirus 6 (GyV6) and to gyrovirus 3 (GyV3 376).

Gyroviruses are small naked DNA viruses with a negative sense, single-stranded circular DNA genome of about 2.3 kb classified in the family *Anelloviridae,* where it clusters close to Torque teno viruses. Based on information gathered from CAV (Welch et al., 2006), gyroviruses are highly resistant to chemical and heat inactivation, which gives them the ability to persist and spread in the environment. Chicken meat has been reported to carry numerous gyroviruses, including the highly prevalent CAV, the worldwide distributed, and recognized pathogen of chicken. Over the last decade, twelve new putative *Gyrovirus* species were described, most of these recovered from chickens. Nevertheless, several other gyroviruses have been reported in feces of humans and other mammals, suggesting possible dietary sources of contamination, such as consumption of gyrovirus-infected chicken meats.

Three of the four full genomes recovered in the present study, i.e. CAV chicken meat/Brazil, AGV2 chicken meat/Brazil and GyV4 chicken meat/Brazil (Fig. 1), are highly similar (>98%) to recently reported genomes from southern Brazil (Lima et al., 2019, 2017), indicating that the viruses circulating in chicken within that region are closely related (Fig. 1). The fourth complete genome recovered, GyV3, shares high nt identities with GyV3 genomes from human and cat feces (Phan et al., 2012; Zhang et al., 2014c). In addition, a VP1-coding genome fragment related to GyV3 was identified. This fragment shares low nt and aa identity with previously reported GyV3 isolates (~72% of nt and ~75% at amino acid level), and probably represents another genome variant, since the degree of VP1 identity between other GyV3 is greater than 70%. Another interesting remark is that, to date, GyV3 has only been identified in feces from mammalian hosts (human and cat). Therefore, it seems very likely that GyV3 may, in fact, be a virus of avian origin – here detected in chicken meat. The last gyrovirus contig identified in the chicken meat virome corresponds to a segment of a GyV6 genome. GyV6 was also described in human and cat feces (Gia Phan et al., 2013; Zhang et al., 2014c), both of which likely include chicken in their diets (although confirmatory evidence for consumption of chicken meats in

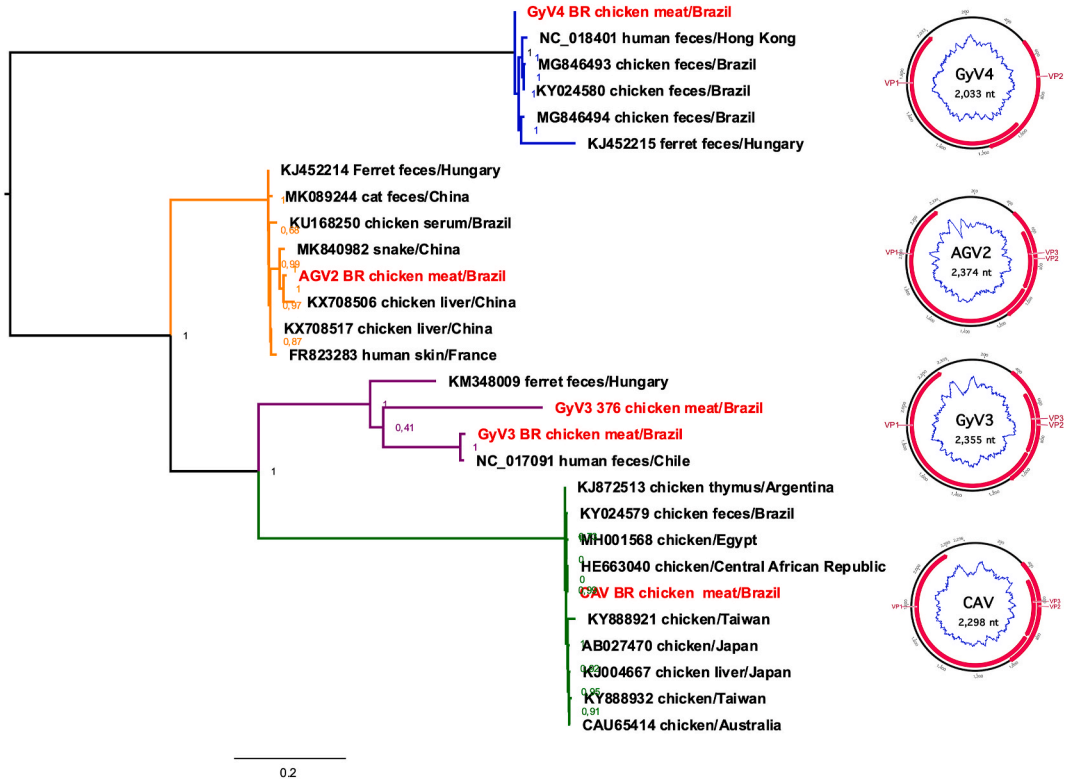

Fig. 1. Gyroviruses recovered from chicken meat. A VP1-coding region maximum likelihood phylogenetic tree was constructed using PhyML (Guindon et al., 2010) with best fit substitution models (GTR_G + I) determined by Smart Model Selection (Lefort et al., 2017). Statistical significance analyses of tree topologies were performed with the approximate likelihood branch support test (aLRT) (Guindon and Gascuel, 2003). In the right, the putative genomic organization of the assembled gyrovirus genomes. Sequences described in this study are marked in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the sampled populations were not available in those reports). Here, GyV6 is for the first time reported to be associated with chickens, once more bringing up the possibility of an avian origin for such virus.

Three contigs with the complete genome sequences assigned to the recently reported *Cressdnaviricota* phylum (Krupovic et al., 2020) were recovered from chicken virome. The first *Cressdnaviricota* contig evidenced clear characteristics from representatives of *Smacoviridae* family. Although also detected in invertebrates (Dayaram et al., 2015), smacoviruses have been reported mainly in samples of feces from humans and chickens. This is the case of 'Smaco 2466F' genome (2,466 nt) here reported, which is closely related to a smacovirus detected in feces from humans (human smacovirus 1) and chickens (chicken huchismacovirus 1) (Fig. 2). Due to high degree of nt and amino acid conservation and strong phylogenetic evidence, the smacoviruses recovered from chicken and human were classified in the same species within the *Huchismacovirus* genus (Varsani and Krupovic, 2018). Again, these findings might suggest that smacoviruses detected in human feces may also be of avian origin, as previously speculated for the gyroviruses above.

The last two genomes recovered were CRESS-DNA viruses that could not be allocated to any previously described viral family. CRESS 53F (5,532) exhibited four ORFs encoding Rep, Cap and two hypothetical proteins (with homologues in other CRESS-DNA viruses) (Fig. 3A). The *N*-terminus of Cap presented a R-rich region, whereas the Rep showed motifs implicated in RCR (Supplementary Table 2). In addition, between *cap* and *rep* genes, one stem-loop structure was putatively formed. At the apex of this secondary DNA structure, a conserved nonanucleotide sequence (TAATATTAC) was found. This large ssDNA virus was classified as type I genome (Rosario et al., 2012). Phylogenetically (Fig. 3B), CRESS 53F Rep forms a sister clade with CRESSV2 sequences (Kazlauskas et al., 2018) and was closely related to unclassified CRESS-DNA viruses detected in a chimpanzee (NC_030466) and in a meadow vole (*Microtus pennsylvanicus*; JF755404).

CRESS 77F (2,895 nt, 38.3% of GC content) showed a type II genome (Rosario et al., 2012), with *cap* and *rep* genes bidirectionally organized and separated by two untranslated regions (Fig. 3A). In the high GC-content (58%) portion of the untranslated region at the 5' terminal of the *cap* gene, the nonamer CAGTATTAC was located. The *Rep* displayed the RCR motifs I, II and III, as well as SF3 helicase motifs A, B and C. The coat protein was highly divergent, sharing <20% aa identity with a CRESS-DNA virus recovered from minnow tissue (MH617562). Phylogenetically (Fig. 3B), a clade within CRESSV1 was formed by CRESS 77F, CRESS-DNA sequences from rainbow trout (MH617109), red snapper (MH617681), CRESS 36B (from bovine meat, described in this study) and KX259434 (wastewater) (Kazlauskas et al., 2018).

3.2. Viruses identified in pork

Approximately 465,000 high-quality reads were produced by metagenomic sequencing of pork sample. After assembly, a total of 2,922 contigs were generated; 55% of these were representative of viral sequences. From these, 1.4% were assigned to eukaryotic viruses, whilst the remaining sequences were representatives of bacteriophages. A detailed taxonomic classification, including the numbers of sequenced reads of each Eukarya-related viral contig recovered in this study, is provided in Supplementary Table 1.

In the same way that gyroviruses were found in large amounts in chicken meat, *Anelloviridae* contigs (Torque teno sus viruses, TTSuV) represented the majority of the contigs recovered from pork in this study. TTSuVs are ubiquitous entities, detected at high frequency in pigs and wild boars worldwide (Martínez et al., 2006; Ramos et al., 2018; Teixeira et al., 2013). These *Anelloviridae* genomes have been classified into two genera: *Iotatorquevirus* (TTSuV-1a and -1b) and

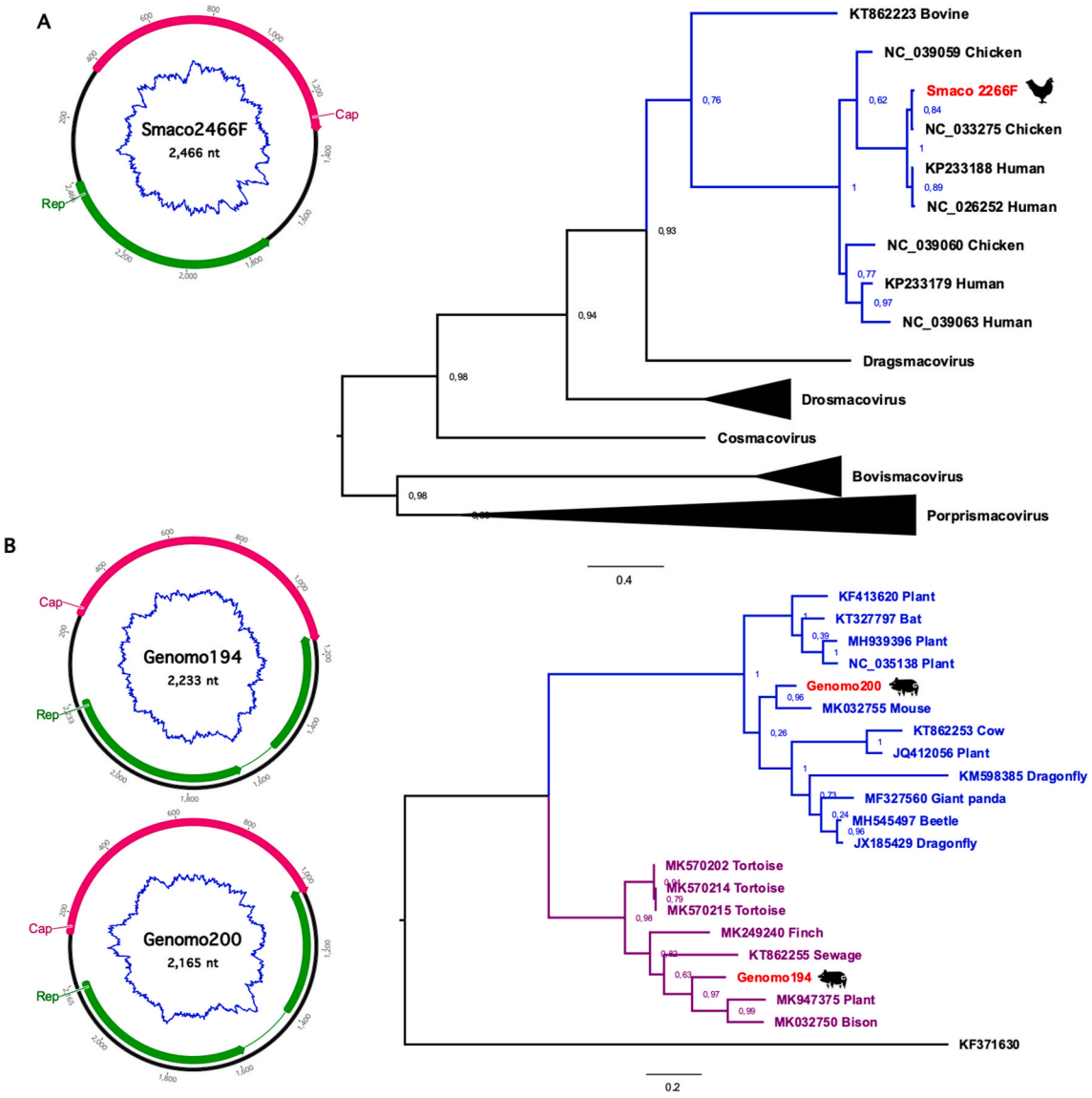

Fig. 2. Smacoviruses and genomoviruses genomes in chicken and pork meats. (A) Genomic organization and ML phylogenetic tree of smacoviruses. *Huchismacovirus* genus branch are highlighted in blue. (B) Genomic architecture of genomoviruses recovered from pork samples and phylogenetic assessment. *Gemycircularvirus* and *Gemykibivirus* genus are highlighted in blue and purple color. ML phylogenetic trees were constructed using PhyML (Guindon et al., 2010) with best fit substitution models (LG_G + I + F for both sequence sets) determined by Smart Model Selection (Lefort et al., 2017). Statistical significance analyses of tree topologies were performed with the approximate likelihood branch support test (aLRT) (Guindon and Gascuel, 2003). Sequences described in this study are marked in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Kappatorquevirus (TTSuV-k2a and -k2b). Interestingly, members of these genera display a remarkable genetic diversity. In this study, eleven contigs of TTSuVs (eight of which constitute complete genomes) were identified (Fig. 4A), including six contigs assigned to *Iotatorquevirus* TTSuV1a (with four complete genomes), yet with high genetic variability between genomes (76–96% of genomic identity). In addition, two *Iotatorquevirus* TTSuV1b also recovered here displayed 23% nt divergence between them. Phylogenetically, such iotatorqueviruses were close to TTSuVs previously detected in Southern Brazil (Fig. 4B) (Da Silva et al., 2020; Tochetto et al., 2020). Regarding kappatorqueviruses, one TTuSV-k2a genome (plus an additional partial genome) and one TTSuV-k2b genome were recovered. Phylogenetically, these genomes were closely related to kappatorqueviruses previously sequenced in South America (Fig. 4B) (Da Silva et al., 2020; Tochetto et al., 2020). In addition, it was noticed that most of the viral reads from pork virome are from TTSuVs, especially from kappatorqueviruses (Supplementary Table 1). Although many studies have attempted to link TTSuVs to disease in swine, their role in pathological conditions, if any, is still unclear, as TTSuVs have been detected in healthy and diseased pigs at high viral genome loads (Da Silva et al., 2020; Ramos et al., 2018; Rogers et al., 2017; Teixeira et al., 2015).

In addition to the well-knows anelloviruses, contigs related to *Parvoviridae* (genus *Copiparvovirus*) were also identified (Fig. 4C). These contigs had high nucleotide identities with porcine parvovirus 5 and porcine parvovirus 6 (PPV5 and PPV6) genomes. Whether these novel parvoviruses play some role in disease in pigs remains to be determined, since these have neither been isolated in cell culture nor evaluated in experimental infections (Miłek et al., 2019). To date, a few sequences of these viruses have been publicly released; all bearing a significant degree of conservation. Phylogenetically, the PPV5 genome fragment reported here clustered with sequences recovered in China and USA; whereas the PPV6 sequences reported here seem more closely related to sequences recovered from pigs in USA (Fig. 4D).

Two genomes of unrelated genomoviruses were detected in pork

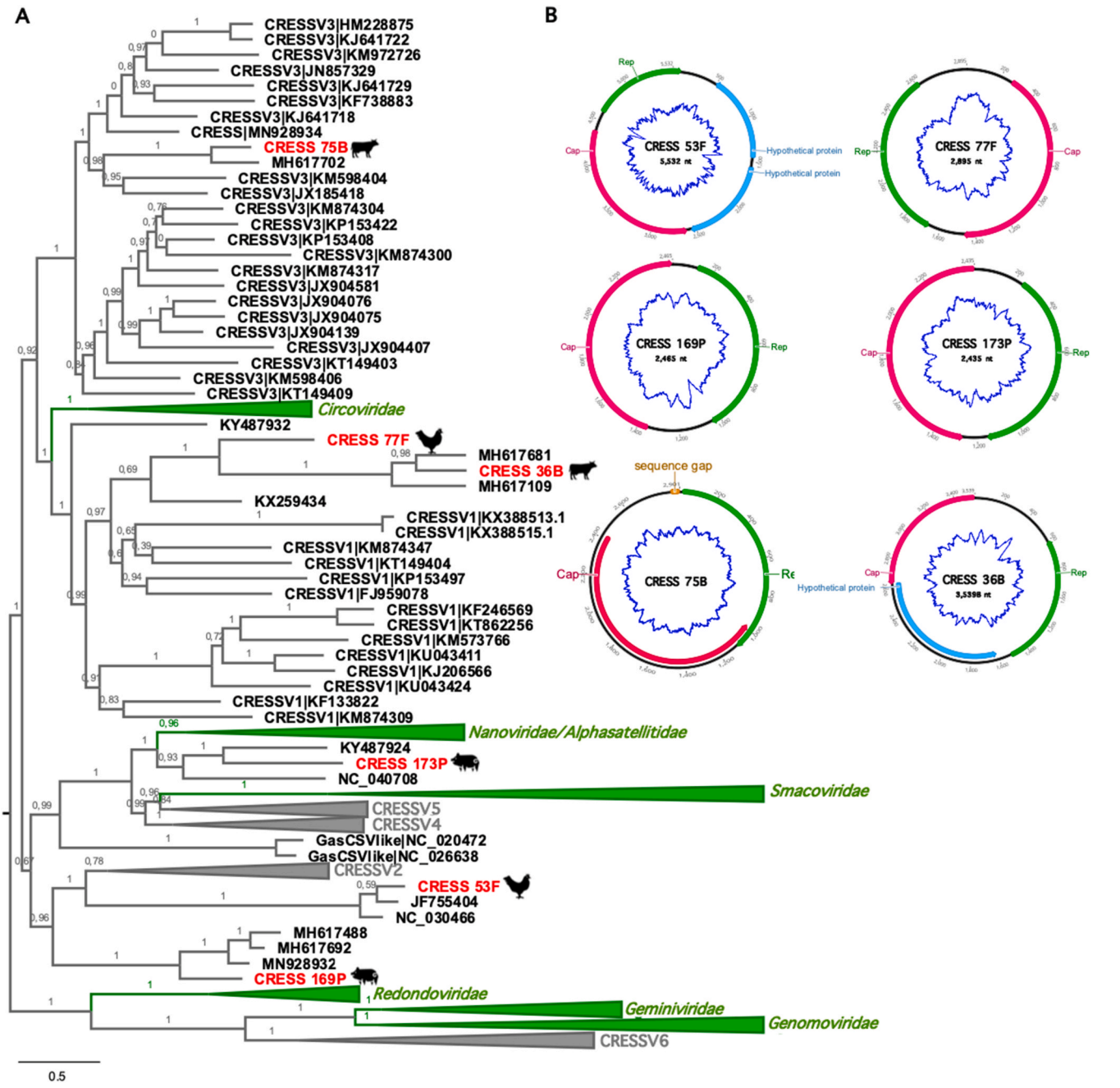

Fig. 3. Unclassified CRESS-DNA viruses recovered from chicken, pork and beef meat. (A) ML phylogenetic tree of unclassified *Cressdnaviricota* genomes discovered in this study. ML phylogenetic trees were constructed using PhyML (Guindon et al., 2010) with best fit substitution models (rtRev_G + F) determined by Smart Model Selection (Lefort et al., 2017). Statistical significance analyses of tree topologies were performed with the approximate likelihood branch support test (aLRT) (Guindon and Gascuel, 2003). Sequences described in this study are marked in red. (B) Genomic architecture of new CRESS-DNA viruses recovered from chicken, pork and beef samples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

virome. Genomoviruses are closely related to geminiviruses and have often been detected in metagenomic studies in samples from mammals, birds, plants and environmental samples. The genomes reported here, Genomo194 and Genomo200, exhibited the classical *Genomoviridae* architecture (Fig. 2B) (Krupovic et al., 2016), with the replication-associated protein containing the motifs involved in RCR (Supplementary Table 2). Phylogenetically (Fig. 2B), Genomo194 clustered with sequences previously detected in plants (MK947375) and bison and were assigned to the *Gemykibivirus* genus. The second genomovirus, Genomo200, could be assigned to the *Gemycircularvirus genus,* clustering along with a genomovirus detected in mouse (MK032755). The species demarcation criterion in *Genomoviridae* is genome identity >78% (that define a same species) (Varsani and Krupovic, 2017). Thus, Genomo194 (diverges >25% with your closest counterpart) was tentatively classified as a novel species to which the name "porcine associated gemykibivirus 1" (PAGkV-1) is suggested. Likewise, Genomo200 seems to be another new species within the *Gemycircularvirus* genus (diverges >30% with your closest counterpart), to which the name "porcine associated gemycircularvirus 3" (PAGcV-3) is here proposed.

Two unclassified *Cressdnaviricota* genomes were also identified in pork samples: CRESS 169P (2,465 nt, type V genome) and CRESS 173P (2,435 nt, type I genome) (Fig. 3A) with the nonanucleotide motif (NANTATTAC) located at untranslated region. As other *Cressdnaviricota*,

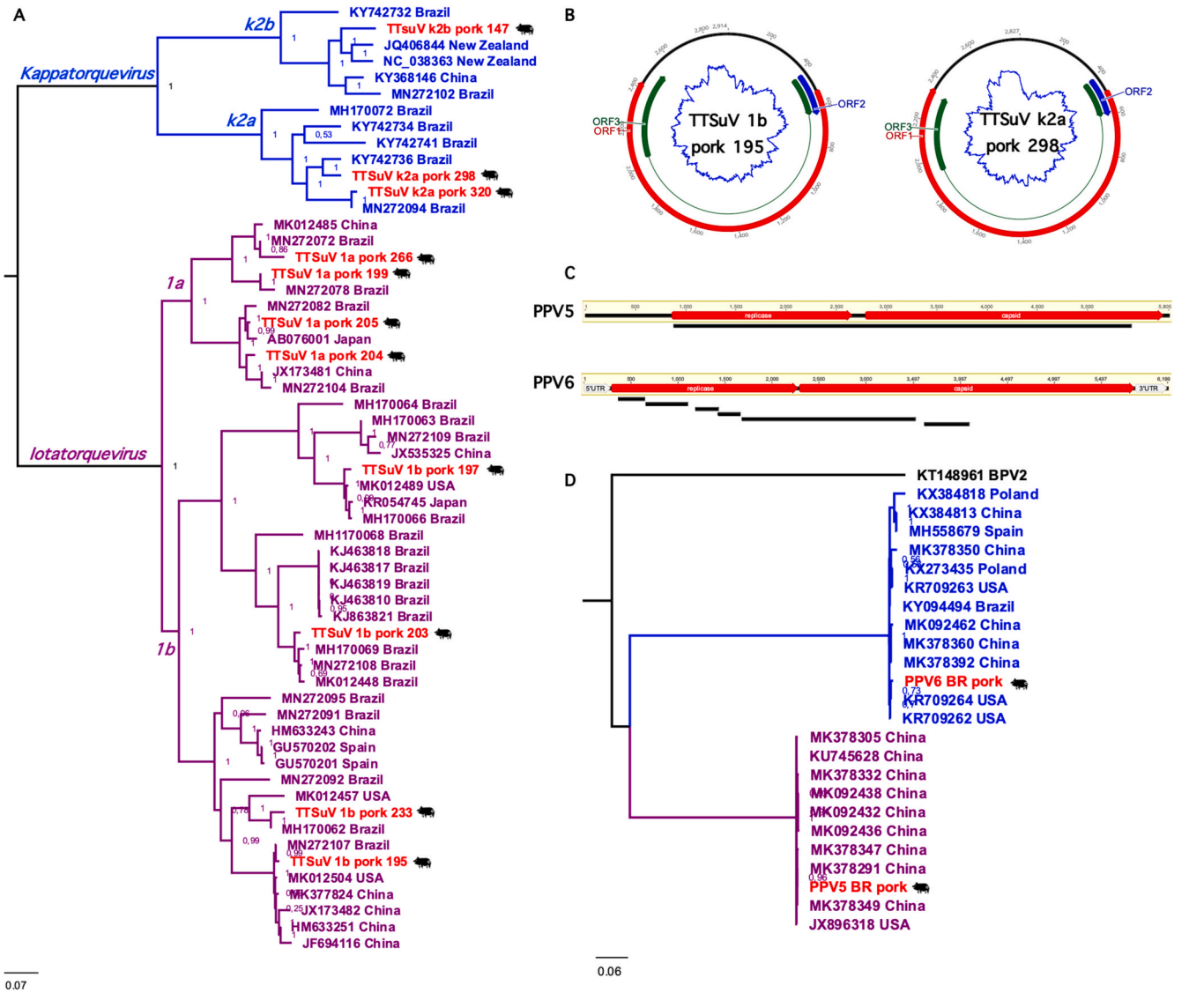

Fig. 4. Porcine anellovirus and parvovirus genomes in pork samples. (A) ML phylogenetic tree (based in VP1 coding sequence) of Torque teno sus viruses. (B) Genomic organization of iotatorqueviruses (TTSuV 1b pork 195) and kappatorqueviruses (TTSuV k2a pork 298). (C) Contigs from PPV5 and PPV6. (D) Capsid-coding ML phylogenetic tree of PPV5 and PPV. BPV2 is used as outgroup. ML phylogenetic trees were constructed using PhyML (Guindon et al., 2010) with best fit substitution models (LG_G + I + F for both sequence sets) determined by Smart Model Selection (Lefort et al., 2017). Statistical significance analyses of tree topologies were performed with the approximate likelihood branch support test (aLRT) (Guindon and Gascuel, 2003). Sequences described in this study are marked in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the Rep of these CRESS-DNA viruses possesses the classical motifs involved in RCR. Phylogenetically, CRESS 169P was distantly related to CRESSV2 replication-associated proteins (Kazlauskas et al., 2018), and formed a cluster with sequences of CRESS-DNA genomes recovered from mouse tissues and from a small passerine bird from temperate Asia, *Phoenicurus auroreus* (MN928932).

CRESS 173P, formed a sister clade with replication-associated proteins from *Nanoviridae* and *Alphasatellitidae*, and was closely related to unclassified CRESS-DNA viruses recovered from *Ilex paraguariensis* (NC_040708) and wastewater (KY487924). *Nanoviridae* is a family of plant-infecting viruses with genomes composed by multiple segments of circular ssDNA. Despite the similarity with nanoviruses Rep, when Cap sequence is examined, no similarities to nanovirus coat proteins were found. CRESS-DNA viruses evolved on several independent occasions, and recombination seems to be an important drive of the evolution of these viruses (Kazlauskas et al., 2019; Martin et al., 2011). Coat proteins are key proteins involved in entry in host cells by interaction with cell receptors. So, since it is a virus that apparently infects mammals, a different capsid was expected.

3.3. Viruses identified in beef

Approximately 523,000 high-quality reads were obtained at metagenomic sequencing. After assembly, a total of 1,739 contigs were generated; 51.6% were viral contigs. From these, 0.5% were assigned to eukaryotic viruses, whereas remaining contigs corresponded to bacteriophage sequences. A detailed taxonomic classification, including numbers of sequenced reads of each Eukarya-related viral contig recovered in this study, is provided in Supplementary Table 1.

Four contigs of CRESS-DNA viruses were identified in the beef virome (Fig. 3A). CRESS 36B (3,539 nt) is a full genome sequence of a highly distinct CRESS-DNA virus. The coding region of Rep and Cap proteins are in the same strand (negative), what places such genome as a type VI virus (Rosario et al., 2012). It possesses a conserved nonamer (TATTATTAT) at the top of a stem-loop structure; also, the replication-associated protein contains the RCR motif III and SF3

helicase motifs, implicated in genome replication (Supplementary Table 2). Interestingly, CRESS 36B replication-associated protein lack the RCR motif I and II (or possess highly divergent motifs). Between *cap* and *rep* ORFs, a putative ORF coding for an as yet undescribed protein was found. Such ORF, has been previously detected in some CRESS-DNA viruses (based in BLASTp analysis), in which it seems to code for a conserved peptide of unknown function. Contig CRESS 75B (2,851 nt) contains the complete *cap* and *rep* coding regions; following the *cap* coding region, at the apex of a hairpin structure, the nonamer TATTATTAC was found. This genome is classified as type I virus, since its *rep* and *cap* are encoded in opposite directions (Rosario et al., 2012) (Fig. 3A); RCR and SF3 motifs were found in replication-associated protein; Cap protein, differentially of CRESS 36B, contains at *N*-terminus a R-rich region. The two other viral sequences detected here were small contigs with identity to replication-associated protein (CRESS 852B contig) and capsid proteins (CRESS 1094B contig). These share low aa identity (58% in Rep and 31.9% in Cap) with an unclassified CRESS-DNA virus recovered from the genital tract of cattle in China (MH782428).

Phylogenetically, CRESS 36B (Fig. 3B) was related to viruses detected in red snapper fishes (CRESS virus sp. isolate ctcd80, MH617681) and clustered with CRESSV1 sequences (Kazlauskas et al., 2018). Interestingly, some regions of CRESS 36B genome are quite similar to CRESS ctcd80, sharing 52.5% of global nt identity. CRESS 75B clustered with CRESSV3 sequences (Fig. 3B) (Kazlauskas et al., 2018) and was close related to an unclassified CRESS-DNA virus detected in haddock tissues (CRESS virus sp. isolate ctcd114, MH617702). CRESS 75B and CRESS ctcd114 shared similar genomic architecture, with remarkable synteny.

It is interesting to point that only DNA viral sequences were identified in beef chicken and pork viromes. This may be due to limited sampling of tissues from presumably healthy animals. Furthermore, RNA viruses seems to predominate fecal material of farm animals (Sachsenröder et al., 2014; Shan et al., 2011; Zhang et al., 2014a). Additionally, it is necessary to consider the lability of RNA, since the meat evaluated here was stored at 4 °C and submitted to a complex workflow to viral metagenomics study. In order to clarify the presence (or absence) of RNA viruses in chicken, pork and samples, total RNA was isolated from individual tissues, retrotranscribed and used as template to broadly range PCRs able to detect astroviruses (Chu et al., 2008), coronaviruses (Lima et al., 2013), kobuviruses (Reuter et al., 2009) and hepatitis E virus (Jothikumar et al., 2006) – common RNA viruses with high frequency of detection in Brazilian farm animals. All samples were negative in these assays, reinforcing the viral metagenomic data.

3.4. Bacteriophages in chicken, pork and beef

Bacteriophages or phages are the most abundant organisms in the biosphere and a ubiquitous feature of prokaryotic existence (Clokie et al., 2011). Not surprisingly, in virome studies, the phage fraction is the most abundant one (Džunková et al., 2015; Perez Sepulveda et al., 2016).

Here, in all types of meat, bacteriophages were the dominant viruses, corresponding to 99% of the obtained contigs. Sequences from *Caudovirales* order (tailed bacteriophages with dsDNA genome) were the most abundant in the three metagenomes, comprehending >95% of the contigs. Viruses from *Siphoviridae*, *Podoviridae* and *Myoviridae* families represented the majority of the sequences recovered (Supplementary Figure 1). Regarding phage host, from chicken, phages from *Pseudomonas*, *Acinetobacter*, *Bacillus* and Enterobacteriaceae species were most frequent; from pork, phages associated to *Pseudomonas* and *Acinetobacter* and Enterobacteriaceae; from beef, *Acinetobacter, Pseudomonas* and *Staphylococcus* were the most frequent.

Meat is among the most perishable foods and a favorable environment for the replication of microorganisms because of its high concentrations of nutrients and high water activity (Odeyemi et al., 2020). During slaughterhouse processing, meats may be contaminated by the skin, intestinal content, personnel, and processing equipment. The microbial load varies from 10^2 and 10^4 cfu/cm^2 (Russo et al., 2006). The commonest microbial genera isolated from meat samples are *Acinetobacter*, *Pseudomonas*, *Brochothrix*, *Flavobacterium*, *Psychrobacter*, *Moraxella*, *Staphylococcus*, *Micrococcus*, lactic acid bacteria, and Enterobacteriaceae (Doulgeraki et al., 2012). Then, it is not surprising that most of the phages found in this study are hosted by bacteria that are often found in meat samples.

4. Concluding remarks

Viral metagenomics techniques shed to light a myriad of viral entities that change the current mindset about viral quantity and diversity in all forms of life, in all Earth's environments (Koonin et al., 2020; Mokili et al., 2012; Simmonds et al., 2017). Therefore, the breathed air, the drinking water and the food are full of viruses. In this study, the main objectives were first, to evaluate which viruses are present in the most consumed animal protein sources in Brazil and second, to verify if any of these viruses could pose risks to human health. Not surprisingly, a large number of known viruses, as well as a vast number of genome sequences of new viral entities were recovered from chicken, pork and beef samples. It was a pleasant satisfaction concerning the second main objective, since no viruses associated with human diseases was found. Moreover, even analyzing a small number of samples, we bring to light several viral genomes, highlighting the still high and unexplored viral diversity in farm animals.

Compared with the viral composition of US meat samples (Zhang et al., 2014b), we found certain similarities, such as the presence of well-known and ubiquitous anelloviruses and parvoviruses in pork, and gyroviruses in chicken samples. In addition, CRESS-DNA viruses were found in the three analyzed viromes. However, they were not phylogenetically related – showing that the viruses found in Brazilian meat samples are not the same as those reported in North American samples. Moreover, Brazilian meat samples seem to have a greater diversity of viral species, particularly in chicken and pork. Finally, it shows that despite the high rates of globalization in agribusiness – which can favor the spread of different viruses, including the potentially pathogenic ones –, the viral community present in these species from different geographic locations is significantly different. In addition to virological data, this study highlights valuable viral metagenomic techniques that can be used in routine applications of metagenomic sequencing in diagnostic context, facilitating viral detection and offering huge potential for tracing viruses in (foodborne) outbreaks.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virol.2020.09.005.

Author contributions section

Samuel Cibulski, Diane Alves de Lima, Helton Fernandes dos Santos, Thais Fumaco Teixeira and Fabiana Quoos Mayer performed

experiments, analyzed data, and wrote manuscript. Paulo Michel Roehe and Caroline Tochetto, analyzed data and wrote manuscript. Samuel Cibulski, Thais Fumaco Teixeira and Paulo Michel Roehe designed the study. All authors read and approved the final version of the manuscript.

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