Research Square

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First description of Protoparvovirus in surface water and human Adenovirus in green leafy vegetable, Environmental Education Center - Southern Brazil

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Abstract

This study analyses the presence of coliforms, *Mastadenovirus* (AdV) and *Canine protoparvovirus* (CPV) in an Environmental Education Center (EEC), which includes a city kennel in Southern Brazil, in October 2017 and February 2018. In both collections were analyzed surface water at AdV (human and canine species), CPV, total (TC) and fecal coliforms (FC) presence; lettuce at AdV; and dogs stool at canine AdV (CAV) and CPV. In total, 67 samples were analyzed: 33 dog stool samples; 10 points of surface water and 24 fractions (root, stem and leaves) originated of eight lettuce. Coliforms and viral analyses were performed by Colilert and PCR assays, respectively. Amplicon was sequenced, and species/types of virus were characterized. TC and FC were detected in all surface water in at least one of the collections. Occurrence of viruses was of the 34.3% (23/67) in all samples. HAdV-C was observed in 13% [2/23] (water sample); HAdV-E in 8,7% (2/23) – water and lettuce; CAV-1 in 13% (3/23) – two stool samples and one water sample; and CPV-2a in 56.5% (13/23) – dog stools. *Raccoon* CPV-like and AdV (not characterized) were detected in one surface water sample and one dog stool sample, respectively. As far as we know, this is the first molecular characterization of the presence of one wildlife animal *Protoparvovirus* in water and AdV in lettuce reported in Brazil. Detection of HAdV-E in lettuce confirmed the role of vegetables as a potential vehicle of human food-borne viral diseases. Nonetheless, more studies are needed about *Raccoon* CPV-like to elucidate the potential transmission and threat of this virus in wildlife and interspecies (wild and domestic).

Introduction

Environmental monitoring through water is important to improve human, crops and animal health. However, water has been deprived of its characteristics by the lack of water resources management and sanitation. This issue happens due to sewage disposal in springs and soil, which causes waterborne diseases in human and animal species [4].

Microbiological contamination can be strategically used in basin monitoring and management. Coliforms found in stools, soil and vegetables are classified as total (TC) and thermotolerant or fecal coliforms (FC). TC is present in the soil and FC is solely of fecal origin (warm-blooded species), which *Escherichia coli* (*E. coli*) is the major example of the latter [10].

Viruses, the main pathogens of enteric diseases, are also the key of tracking environmental monitoring. Their presence in water can cause gastroenteritis in humans and animals. Owing to their resistance and easy infiltration in the soil, viruses can reach the groundwater sources, spreading in the environment and overcoming negative impacts on public health [34]. Besides, the water used for irrigation can generate food contamination, mainly in raw vegetables [26]. In Sicily, a study with 70 vegetable samples (including Swiss chard, chicory, parsley, celery, escaroles, endive and lettuce) detected: 2.9% of Norovirus (NoV), 2.9% of Enterovirus (EV) and 1.4% of Hepatitis E virus (HEV) [33]. In Argentina, a research with green leafy crops (lettuce, spinach, chicory and arugula) detected NoV, Rotavirus (RV), human Astrovirus e EV [32]. In Brazil, just one study has been found, which identified RV - A (RVA), *Human mastadenovirus* (HAdV),

Hepatitis A virus (HAV) and NoV in vegetables (cucumber, spring onions, lettuce and arugula). RVA has been detected in arugula, cucumber and spring onions; and HAdV just in cucumber species [13].

Adenoviruses (AdV), besides EV, HEV, HAV, NoV and RV, are commonly enrolled at environmental contamination, however AdV has been usually used as reference of bioindicators in environmental matrices [39]. They can be eliminated in large quantities by infected hosts secretions and excretions – human and animal origin. Nevertheless, viruses' markers are not yet a requirement in water quality standards in Brazil [31].

AdV is a non-enveloped virus, double-stranded DNA belonging to *Adenoviridae* family, divided in five genera. HAdV belongs to *mastadenovirus* genus and it is classified into seven species (from A to G) [19, 46]. HAdV can infect a range of tissues, and the intestinal and respiratory tract are the most impacted [41]. HAdV-C, B and E are responsible for respiratory diseases, but they can be eliminated by stools from carriers hosts [36]. Although HAdV-F (gastrointestinal) is chosen by tracking environmental sources, HAdV-C and E are commonly founded [6]*. Mastadenovirus* can also infect animal species, as dogs. *Canine mastadenovirus* (CAV) is an example [5], subdivided in type 1 and 2. CAV-1 induces a systemic disease, causing hepatitis; CAV-2 replicates in the respiratory epithelium and, when co-infected with *Bordetella* sp. and *canine parainfluenza* virus, can evolve to kennel cough [9]. The transmission may occur through direct or indirect contact or fomites (water and food) and interspecies [21]

However, the presence of the *parvovirus* family in environmental matrices is not commonly investigated. Just a few studies seeking parvovirus genome from animal and/or human species in the environment have been reported [7]. *Human bocavirus* (HBoV) has been detected in effluent samples in the United States. In Brazil, DNA of HBoV was detected in surface water in the metropolitan region of Rio Grande do Sul (RS) State, Southern Brazil [25]. In Spain, *chicken parvovirus* (ChPV) and *turkey parvovirus* (TuPV) were found after mapping of fecal contamination of avian origin [12]; which has not yet been reported in Brazil.

Parvoviruses are small non-enveloped viruses with a single strand DNA genome that may infect a wide range of hosts (mammals, birds and reptiles), including the order *Carnivora* [22]. *Carnivore protoparvovirus 1* comprises the most important *parvoviruses* that infect carnivores, such as CPV, *feline panleukopenia* virus and *mink enteritis* virus [40]. CPV belongs to the genus *Protoparvovirus*, subfamily *Parvovirinae* and *Parvoviridae* family. CPV is classified into type 1 and 2 (CAV-1 and CAV-2). CAV-1 can cause myocarditis in neonatal pups, in which hemorrhagic gastroenteritis (HGE) is the most common syndrome. CPV-2 is responsible for severe gastroenteritis. Some genetic alterations have occurred, such as CPV-2a, CPV-2b subtypes and CPV-2c, more recently [8, 17]. The contamination of the different types of CPV usually occurs through oronasal exposure, stools and contaminated environments. Insects, rodents, people and fomites can spread this virus, which can infect vaccinated an unvaccinated dog [17, 42].

This study aims to perform a microbiological survey in the Environmental Education Center (EEC), a countryside area located in Southern Brazil, metropolitan region of RS. EEC have some native forest

remnants, wildlife and weirs. Since 2015, EEC has been shared with the city animal shelter. The focus of this research is a) to analyze the presence of FC, TC, AdVs (human and canine species) and CPV in different sources of EEC surface water; b) to investigate the presence of AdV in lettuce irrigated by water sources available in EEC; and c) to investigate the elimination of CAV and CPV in the kennel through stool samples.

Material And Methods

Area of study and survey samples

The study was carried out on the EEC (coordinates 29°44'27.1"S and 51°03'06.2"W) and in the city animal shelter (coordinates 29°44'20.06"S and 51°03'03.4"W) [Fig. 1]. EEC receives groups that do ecological tours and perform some workshops. The kennel receives abandoned dogs and cats in need of veterinary care, including vaccination and castration.

This study was performed in mid October, 2017 and late February, 2018. Both considered surface water, vegetables and dog stool samples.

Water samples were collected in one stream, three weirs and in one animal shelter septic tank, being the latter close to the kennel (see Fig. 1). The samples were named P1 – stream (29°44'29.2"S and 51°03'09.5"W), P2 – weir (29°44'23.5"S and 51°03.06.7"W), P3 – weir (29°44'23.1"S and 51°03.06.3"W), P4 – weir (29°44'23.4"S and 51°03'05.7"W) and P5 – septic tank (29°44'21.0"S and 51°03'04.4"W) [EEC in yellow highlighting; P1 at P5 in red in Fig. 1]. All samples were aseptically collected in 500mL sterile flasks, packed in isothermal boxes and sent to the Laboratory of Molecular Microbiology (LMM) of Feevale University to be processed.

The dog's stools were collected in the kennel as soon as they were excreted (see Fig. 1 and the epidemiological aspects in Table 3). The use, handling and disposal of the stools used in this project are in agreement with the ethical principles for animal experimentation. The Ethics Committee of Animal Use of Feevale University (CEUA/Feevale) approved this research under registration number 01.17.055/2016.

Lettuce (*Lactuca sativa)* was collected in the EEC's garden (closer to EEC in the Fig. 1). Lettuce samples were named H1 to H4, and three parts of each were analyzed (leaf, stem and root), renamed: H1L, H1S and H1R.

Sample were filed and named LMM with their respective number, following the sampling protocols.

Coliforms assay

Detection of TC and FC assay were performed by the Colilert® substrate enzyme method (Idexx®, USA) according to the manufacturer's instructions. All samples (from P1 to P5) were assayed within 24 h after collection. The sample was considered positive for TC based on the yellow indicator. F or FC was considered positive if fluorescing blue following exposure to 300 nm UV light and negative in the absence

of color and/or fluorescence. Results are expressed as most probable number in 100 mL of water (MPN/100mL), according to the table provided by the manufacturer.

Processing samples at viral analyzes

Water

Samples were concentrated by applying the ultracentrifugation method [16], in which 36mL of each was used and centrifuged at a rate of 21,000 x g at 8° C for 3 hours. Products were resuspended in 1mL of Tris-EDTA buffer (pH 8.0) and homogenized under vigorous agitation for 1 minute, according to the standard protocol described in previous studies [16]. The final volume was aliquoted and the DNA extracted.

Vegetables

Lettuce (from H1 to H4) from both collections (2017 and 2018) were organized in: leaves (HL), stem (HS) and roots (HR). Each one generated three samples, totaling 12 samples per collection and 24 in total. Each part was chopped and macerated separately. Subsequently, one gram of each was placed in 15 mL falcons and 9 mL of PBS buffer was added (Phosphate-buffered saline), then brought into the incubator at 22°C with constant stirring at 173 RPM for 1 hour. After, 1mL of supernatant was aliquoted into reaction microtubes and sent for DNA extraction using the protocols already described previously in the LMM at food processed pork analyzes that have been detected HEV [20].

Stool dogs

For stool sample processing, 0.2 grams of diluted stools in 1 mL of Eagle's Minimum Essential Medium (MEM) were used, then vortexed for 1 minute and centrifuged for 3 minutes at 120,000 RPM. From the supernatant, 1mL was used for viral DNA extraction, according to Heldt et al. [20].

Viral DNA extraction

Viral extraction of samples mentioned below was carried out using the Promega® extraction kit, following manufacturer's instructions. Aliquots of 200µL of each sample were used, and the final elution was performed in microtubules free of DNAse and RNAse at analysis by Polymerase Chain Reaction (PCR).

Polymerase Chain Reaction

Nested-PCR, which DNAPol target gene, was used for the detection of different AdVs in all samples. In the first round, 1µL of each primer pol-F (5'-CAGCCKCKGTTRTGYAGGGT-3') and pol-R (5'-GCHACCATYAGCTCCAACTC-3') were used, both with 20 pmol concentration, 18µL of DNAse free water and RNAse, 25µL of mix (Promega®) and 5µL of extracted DNA, totaling 50µL of reaction volume. At the second round, the same reagents and volume of the first one were used. Only the 5µL of DNA extracted by the product of the first PCR reaction was replaced, as well as the set of oligonucleotides for the

oligonucleotides sense pol-nF (5'GGGCTCRTTRGTCCAGCA-3') and reverse pol-nR (5'-TAYGACATCTGYGGCATGTA-3') [28]. The generated PCR product is approximately 300 bp. Each reaction had negative and positive control, water RNAse and DNAse free and HAdV-41 respectively.

The DNA extracted from the dog stool samples were concomitantly submitted to PCR, specific for CAV and CPV. A positive control was used for CAV analyses detected by the previous LMM. The standard PCR reaction for CAV was performed in a final volume of 50 µL containing 5 µL of DNA diluted in 25 µL of mix (Promega®), 18 µL of water and 20 pmol of each primer. The primer utilized for canine AdV was CAV-F1, 5'-CACGATGTGACCACTGAGAG-3' and CAV-R1, 5'-GGTAGGTATTGTTTGTGACAGC-3 (20 pmol dilution). The amplicon resulted 300 to 350 bp of the gene encoding a CAV-1 and CAV-2 hexon protein, respectively [30].

For CPV detection, the Vanguard® HTLP 5/CV-L vaccine was used as positive control, which contains the viruses of interest. Reactions of the conventional PCR for CPV were performed with the same final volume used for the CAV. The primer for CPV was: CPV-555-F, 5'-CAGGAAGATATCCAGAAGGA-3 'and CPV-555-R, 5'-GGTGCTAGTTGATATGTAAT3ACA-3' [8]. The generated PCR product was 555 bp of the capsid protein gene (VP2 – viral protein).

Amplicon purification, sequencing and phylogenetic analyzes

All positive PCR samples were submitted at purified assays by the PureLink® kit (Invitrogen), according to manufacturer's instructions, and then subjected to sequencing. For that, ABIPrism 3100 system/company ACTGene equipment was used for to characterize species and/or type.

Nucleotide sequences resulting were analyzed by the CAP3 program implemented by BioEdit 7.0.5. The alignments were performed using Clustal Omega [38]. The phylogenetic trees were obtained through the Neighbor Joining method [37] combined with Kimura 2 [24], MEGA7 software [27].

Results

Survey samples

Water samples collected were named as LMM 3940 - 3944 (P1 - P5) [2017] and LMM 4160 - 4164 (P1 - P5) [2018] (Table 1 and 2). Fig. 2 and 3 present the species and types of viruses characterized (LMM). Dog stools were named LMM 3948 - 3965, 3970 at 3971 (2017); and LMM 4165 - 4177 (2018). Table 3 shows the total of dog stool samples, including epidemiological aspects and detected viruses. Fig. 3 demonstrates the organization of phylogenetic results (LMM). Lettuce samples were named LMM 3966 – 3969 and 3972 – 3979 (2017) and LMM 4312 – 4323 (2018). Fig. 2 presents the LMM number of one lettuce sample in which viruses were detected (LMM 4319).

Fifty-one samples collected, including 33 of stools, 10 of surface water and 8 of lettuce. However, 67 samples were analyzed, because for each lettuce sampling generated three samples, totaling 12 samples

per collection and 24 in total. In 29.8% (20/67) of all samples analyzed were detected at least one virus. Co-contamination was observed in one of the water samples (CAV-1 with *Raccoon* CPV-like). There was co-detection in two dog stool samples (CAV-1 with CPV-2a).

FC and TC were detected in all water sampling in at least one of the collections.

Coliforms assay

Different concentrations of each sampling (2017 and/or 2018) and at points (P1 to P5) were observed. In samples in which it was impossible to determine the MPN for FC and TC, the test was performed without diluting (see Table 1).

Viral detection

DNA AdV was detected in all samples collected and can be observed in nine of them. Human AdV type C was found in one water sample and E (HAdV-C and E) was detected in three water samples. The sample positive to HAdV-E was co-contamined with HAdV-C. HAdV-E was also detected in one lettuce sample (stem). CAV-1 was detected in two stool samples and in one water sample. DNA of animal *Protoparvovirus* (domestic and wildlife) was detected in 13 stool samples and in one water sample. In stools, it was characterized as CPV-2a and in water as *Raccoon* CPV-like. Co -contamination were observed in two stool samples (CAV-1 with CPV-2a) and in one water sample, CAV-1 associated with *Raccoon* CPV-like (Tables and Fig. 2 and 3).

Water analyzes

Table 2 shows the PCR results for water samples. P1 and P2 were negative in both samples. In the 2007 collection, HAdV-C was detected at P3, P4 and P5. In 2018, HAdV-E was found at P3 and co-contamination CAV-1 and a virus from wild animal species (*Raccoon* CPV-like) was observed at P5.

As the detection of *Raccoon* CPV-like was a new finding, isolation in culture cells was performed. African green monkey kidney cells (VERO) and Mardin-Darby kidney canine (MDKC) line cells were selected. The original positive sample (1mL water) plus 1 ml of MEM was filtered with a specific membrane viral filter (0.2 µm) and inoculated into VERO and MDCK cells. Both cells were cultured in culture flasks with Eagle's MEM, supplemented with fetal bovine serum (FBS) and antibiotics (PS - Penicillin-Streptomycin solution). The cells were cultured and conditioned in CO_2 incubators at 37° C and then placed in 6-well cell culture plates. For viral inoculation, 500µL of the viral sample filtered was utilized, being inoculated into the two 6-well cell culture plates, one containing MDCK cells and the other one containing VERO cells. These plates were incubated at 37° C for 2 hours. Subsequently, 2mL of MEM with 1% PS was added. The CPE effect (cytopathic effect) has been observed in the fourth day after inoculation at the first passage in both culture cells. DNA extraction was performed with BioPur® kit. PCR was carried out using primers CPV-555-F and CPV-555-R at CPV, as previously described.

Dogs Stool samples

Viruses were detected in 57.5% (16/33) of the samples. Considering the number of animals, this number falls to 42.4% (14/33), because two animals (one female, one male) presented co-infection by CAV-1 and CPV-2a (see Table 3).

Vegetable samples

Three of the four lettuce samples were irrigated with water from P2. The lettuce named “control” was irrigated with cistern water from its planting (EEC in Fig. 1). Only one sample (stem) irrigated with water from P2 collected in 2018 tested positive for HAdV-E (LMM 4319).

Sequencing and phylogenetic analyses

The nucleotide sequences resulting from the sequencing of positive samples for *Mastadenovirus* and *Protoparovovirus* were respectively compared to 38 and 24 sequences were deposited in GenBank data bases. The phylogeny tree from eight AdV (human and canine species) and 14 *protoparvovirus* characterized are shown in Fig. 2 and 3, respectively. One AdV that could not be characterized and was not included in this analyzes.

Discussion

This study shows the presence of viruses in water, dog stools and lettuce. In addition, this research detected TC and FC in water samples collected in an EEC where the city kennel shelter is located. As far as we know, this is the first description in Brazil about detection of animal *Protoparvovirus* in surface water samples and HAdV-E in lettuce.

TC and FC detected in this study have shown different values according to the period they were collected. There had been some rain prior to the October collection period, which is the most probable hypothesis to account for the values observed [see P1 in Table 1]. Not only due to the percolation of soil surface residues; but also the source of this stream (P1) is located in a seaside resort whose upper bed covers properties that breed pigs, sheep and birds. Pigs are an environment-degrading species due to their large volume of excretion [18]. On the other hand, before the second collection in 2018, there was a drought period, which may be related to the cutting of the TC and FC levels (Table 1). Nevertheless, it was surprising that no virus was detected then. The same reduction of TC and FC values in the second collection was observed in P2 (weir – irrigation pond), corroborating with the same pluviometric influence observed in P1. P2 is a nursery for carp fish (*Ciprinus carpio*), which feed on organic matter from vegetation and sediments [35]. Although it is a species with varied eating habits and widely used for aquatic macrophytes control [29], its presence may have influenced the low rate of coliforms and even the viral absence. However, P2 was the origin of the lettuce irrigation system, in which DNA of HAdV-E was detected in the stem of a sample (LMM4319). This could be explained due to characteristics of the soil used for growing, however this research has not analyzed this aspect.

Lettuce is normally consumed raw, so its ingestion may represent a potential risk of infection, according to this study and others. A study performed in South Korea about enteric viruses in raw vegetables and groundwater used for irrigation also detected DNA of AdV in lettuce, chicory and spinach. Infectivity assay by cell-culture PCR (ICC-PCR) was positive only for spinach species [14]. The present study has not used this tool. This outlook highlights the questions about standards of the Brazilian legislation for irrigation system, because green leafy vegetables with viruses may result in clinical or subclinical waterborne diseases, impacting in health public.

In P3 and P4, coliforms presented lower rates in the first sample collection (2017) than other points. Even so, HAdV-C was detected in both, and in P3 HAdV- E was detected in the second sample collection (see Table 2). This non-qualitative relationship of bacteria with viral detection has been commonly reported [14, 15]. In one study, the occurrence of enteric viruses in groundwater was not correlated with water temperature [14]. This relationship probably also does not happen here. Presence of HAdVs in these waters may be related to anthropogenic circulation in this rural area, such as employees, visitors and hikers. As well as,the oscillation in water volume due to rainfall rates and the presence of *Eichhornia crassipes* (water hyacinth), which are common at P3 and P4. This emergent aquatic plant has a high nutrient retention capacity, which may cause eutrophication of aquatic environments. It can provide the protection of the viral particles against environmental adversities, such as solar rays, temperature and pH [3], which can justify the change of appearance of water (data observed by first author). The intense rainfall may have contributed to water dilution. P3 and P4 are located in a better preserved area, which can have led to lower TC and FC values. The absence or scarce rain in February 2018 may have been the cause of concentration of microorganisms, thus raising the TC and CF levels per mL of the sample.

The most critical point in both sample periods was P5 (animal shelter's septic tank). It has presented high levels of TC and FC (see Table 2). Besides, P5 presented viruses not yet detected at others points, such as CAV-1 and *Raccoon* CPV-like - LMM4164 sample (Table 2 and Fig. 3). A study through biological samples (intestinal tracts issue *post mortem* records) with dead wild raccoons (*Procyon lotor*) in Canada (2009-2017) resulted in an epidemiological and molecular characterization of *Protoparvoviruses* infecting this population [11]. In that study, the isolates originated from raccoons was named CPV-like [11]. The phylogenetic tree constructed in the present study has grouped LMM4164 in the same clade (see Fig. 3) as MF069443 and MF069444 which had been characterized as CPV-like in this study [11]. Therefore, the most probable hypothesis is that the isolated LMM4164 comes from raccoons, even so it has been coming from water sources. On the other hand, there is a virus named *Raccoon protoparvovirus* (RPV) member of *Protoparvovirus* that is isolated from *post mortem* or other biological samples of these animals [22]. Nonetheless, once this virus has been detected in water and its real origin is not clear (domestic or wildlife animals, carnivore or not), this study poted to name it as *Raccoon* CPV-like.

In Southern Brazil, raccoon is known as *guaxinim*. It is a nocturnal omnivore that has adapted well to urban habitats. Raccoons prefer to live near river banks that provide shelter and food. In urban settings they may interact with dogs and cats. They are susceptible to some diseases that affect carnivores, especially canine distemper, rabies, canine adenovirus, leptospirosis and parvovirus [23]. This description

strengthens the hypotheses that the *Raccoons* CPV-like detected comes from raccoon species and not from domestic dogs that were infected by it.

Raccoons clearly are the main hosts for the perpetuation, diffusion and evolution of *Carnivore protoparvoviruses* highlighting role in the emergence of CPV-2a in dogs [11] due two key mutations in VP2 (87Leu and 101Thr) [1]. These CPV-like strains originated from multiple cross-species transmission episodes followed by sustained transmission and evolution in raccoons [2].

Regarding to the stools of the 33 dogs, CAV-1, AdV uncharacterized and CPV-2a were detected. Although these viruses have been detected, no animals were sick (it was observed by author and reported by caregivers). Prior to the 2017 sample collection, the animals had been immunized against the target pathogens of the study (CPV mainly). This can be the main cause for the outcome, which found only one positive animal for viruses (Table 3). This practice normal happens once a year, near spring, according to caregivers.

One important finding that received our attention, is the detection of carnivore viruses in P5 also in 2018 (the second sampling). Two animals tested positive for CAV-1, also detected in water (Table 2 and 3). This result to the main hypothesis that the origin of CAV-1 in P5 has come from dogs keeping in shelter. On the other hand, the CPV-2a detected in dog in the second sampling was not detected in P5, just a *Raccoon* CPV-like. It reinforces our hypothesis that this founded is from wildlife animal and not from a domestic dog. In addition, the detection of CAV-1 in surface water and in dogs stools in the same period strengthens the idea that viruses were detected in water because there has host infected with virus (symptomatic or asymptomatic) in around. Corroborating that, the use of water sources as tracking environmental monitoring at human and animal public health can be helping the epidemiological surveillance.

The presence of CPV-2a is not surprising, because this virus is highly prevalent in canine population around the world, including Brazil, indifferent of the vaccination status. Besides, it is frequently detected in young dogs. Gender predilection was not found in this study (Table 3)

Occurrence of viruses from human, canine and wildlife hosts in water is a concern with waterborne diseases for humans and animal. It reinforces what has already been widely discussed, pointing the urgency of growing public policies at conserve water sources and effluent treatment in the urban a countryside.

Conclusion

The epidemiological tracking of contamination sources in rural environments is extremely delicate since sanitation and environmental education are little effective or even non-existent. Humans relationship with nature, the occupation of areas that should be better preserved, care of soil and water are of primary importance. Efficient basic sanitation, whether in urban areas with effluent treatment or in rural areas with a filter, septic and sink system, and ecological solutions with biotreatment are options that should be

considered and put into practice. Regardless of the scenario being urban or rural, the high degree of infectivity existent compromises the human and animal health indexes, with the spread of pathogens that propagates in the environment. The detection of wild animal virus indicates that their sympatry with domestic canines potentiates diseases transmission, once both species become viruses' carriers, thus playing an important role in epidemiology.

Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Allison AB, Harbison CE, Pagan I, Stucker KM, Kaelber JT, Brown JD, Ruder MG, Keel MK, Dubovi EJ, Holmes EC, Parrish CR (2012) Role of multiple hosts in the cross-species transmission and emergence of a pandemic parvovirus. J Virol 86:865– 872. https://doi.org/10.1128/JVI.06187-11
2. Allison AB, Kohler DJ, Fox KA, Brown JD, Gerhold RW, Shearn-Bochsler VI, Dubovi EJ, Holmes EC, Parrish CR (2013) Frequent cross-species transmission of parvoviroses among diverse carnivore hosts. J Virol 87:2342-2347. https://doi.org/10.1128/JVI.02428-12
3. Alves E, Cardoso LR, ScavroniJ LR, Ferreira LC, Boaro CSF, Cataneo AC (2003) Avaliações fisiológicas e bioquímicas de plantas de aguapé (Eichhornia crassipes) cultivadas com níveis excessivos de nutrientes. Plant Dan 21:27-35. https://www.scielo.br/pdf/pd/v21nspe/a05v21ns.pdf
4. Bacci DC, Pataca EM (2008) Educação para a água-Estudos Avançados. J USP 22:211-226. http://www.revistas.usp.br/eav/article/view/10302
5. Benkő M, Élö P, Ursu K, Ahne W, La Patra SE, Thomson D, Harrach B (2002) First molecular evidence for the existence of distinct fish and snake adenoviruses. J Virol 76:10056-10059. https://10.1128/JVI.76.19.10056-10059.2002
6. Bibby K, Peccia J (2013) Prevalence of respiratory adenovirus species B and C in sewage sludge. Environ Sci Process Impacts 15:336-338. https://doi.org/10.1039/C2EM30831B
7. Blinkova O, Rosario K, Li L, Kappor A, Slikas B, Bernardin F, Breitbart M, Delwart E (2009) Frequent detection of highly diverse variants of cardiovirus, cosavirus, bocavirus, and circovirus in sewage samples collected in the United States. J Clinical Microbiol 47:3507–3513. http://dx.doi.org/10.1128/JCM.01062-09.

8. Buonavoglia C, Martella V, Pratelli A, Tempesta M, Cavalli A, Buonavoglia D, Bozzo G, Elia G, Decaro N, Carmichael L (2001) Evidence for evolution of canine parvovirus type 2 inn Italy. J Gen Virol 82:3021-3025. https://doi.org/10.1099/0022-1317-82-12-3021
9. Buonavoglia C, Martella V (2007) Canine respiratory viruses. Vet Res 38:355-373. http://dx.doi.org/10.1051/vetres:2006058.
10. Buzanello EB, Martinhago MW, Almeida MM, Pinto FGS (2008) Determinação de coliformes totais e termotolerantes na água do Lago Municipal de Cascavel, Paraná. Rev Bras Bioc 6:59-60. http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/1087/811
11. Canuti M, Britton AP, Graham SM, Lang AS (2017) Epidemiology and molecular characterization of protoparvoviruses infecting wild racoons (Procyon lotor) in British Columbia, Canada. J Virus Res 242:85-89. http://dx.doi.org/10.1016/j.virusres.2017.09.015
12. Carratalá A, Rusinol M, Hundesa A,Biarnes M,Rodriguez-Manzano J, Vantarakis A, Kern A, Suñen E, Girones R, Bofill-Mas S (2012) "A novel tool for specific detection and quantification of chicken/turkey parvoviruses to trace poultry fecal contamination in the environment." Appl Environmental Microbiol 78:7496-7499. http://doi.org/10.1128/AEM.01283-12
13. Carvalho SVC, Rogovski P, Cadamuro RD, Viancelli A, Michelon W, Reis DA, Chagas IAS, Assenço R, Lanna MCS, Treichel H, Fongaro G (2020) Co contamination of food products from family farms in an environmental disaster area in Southeast Brazil with pathogenic bacteria and enteric viruses. Arch Virol 165:715–718. https://doi.org/10.1007/s00705-019-04501-9
14. Cheong S, Lee C, Song SW, Choi WC, Lee CH and Kim SJ (2009) Enteric viruses in raw vegetables and groundwater used for irrigation in South Korea. Appl Environmental Microbiol 75: 7745-7751. https://doi.org/10.1128/AEM.01629-09
15. Comerlato J, Oliveira LK, Spilki FR (2011) Enterovírus como indicadores de qualidade da água. Rev Bras Bioc Porto Alegre 9:114-125. http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/1593
16. Dalla Vecchia, Fleck JD, Comerlato, J, Kluge M, Bergamaschi B, da Silva JVS, da Luz RB, Teixeira TF, Garbinatto GN, Oliveira DV, Zanin JG, Van der Sand S, Frazzon APG, Franco AC, Roehe PM, Spilki FR (2012) First description of Adenovirus, Enterovirus, Rotavirus and Torque teno virus in water samples collected from the Arroio Dilúvio, Porto Alegre, Brazil. Brazilian J Biol 72:1-7. https://www.scielo.br/pdf/bjb/v72n2/13.pdf
17. Decaro N, Desario C, Addie DD, Martella V, Vieira MJ, Elia G, Zicola A, Davis C, Thompson G, Thiry E, Truyen U, Buonavoglia C (2007) Molecular epidemiology of Canine Parvovirus, Europe. J Emerging Infect Disease 13:1222-1224. https://doi.org/10.3201/eid1308.070505
18. Druzian JI, Marchesi CM, Scamparini ARP (2007) Perfil dos ácidos graxos e composição centesimal de carpas (Cyprinus carpio) alimentadas com ração e com dejetos suínos. Rev Ciência Rural 37:539-544. https://www.redalyc.org/pdf/331/33137238.pdf
19. Han G, Niu H, Zhao S, Zhu B, Wang C, Liu Y, Zhang M, Yang S, Liu F, Wan C, Zhang Q (2013) Identification and typing of respiratory adenoviruses in Guangzhou, Southern China using a rapid and simple method. Virol Sin 28:103–108. https://doi.org/10.1007/s12250-013-3308-7

20. Heldt FH, Staggmeier R, Gularte JS, Demoliner M, Henzel A, Spilki FR (2016) Hepatitis E Virus in Surface Water, Sediments, and Pork Products Marketed in Southern Brazil. Food Environ Virol 8:1-6. https://doi.org/10.1007/s12560-016-9243-7
21. Hu RL, Huang G, Qiu W, Zhong ZH, Xia XZ, Yin Z (2001) Detection and differentiation of CAV-1 and CAV-2 by polymerase chain reaction. Vet Res Communications 25:77-84. https://doi.org/10.1023/A:1006417203856
22. Internacional Commitee on Taxonomy of Viruses (2019) Family: Adenoviridae and Parvovirinae. Virus Taxonomy: 2019 release. Disponível em: https://talk.ictvonline.org/ictv-reports/ictv_online_report/> . Acesso em: dezembro 2019.
23. Kapil S, Rezabek G, Germany B, Johnston L (2015) Isolation of a virus related to canine parvovirus type 2 from a raccoon (Procyon lotor). Vet Record 166:24-25. http://dx.doi.org/10.1136/vr.b5587
24. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evolution 16:111-120. https://doi.org/10.1007/BF01731581
25. Kluge M, Henzel A, Spilki FR (2013). Detection of Human Bocavirus in raw water samples of Rio dos Sinos watershed, Rio Grande do Sul, Brazil. In:_XXIV Congresso Brasileiro de Virologia, Porto Seguro, Bahia, Brazil.
26. Kokkinos P, Kozyra I, Lazic S, Bouwknegt M, Rutjes S, Willems K, D'Agostino M (2012) Harmonized investigation of the occurrence of human enteric viroses in the leafy green vegetable supply chain in three European countries. Food Environ Virol 4:179–191. https://doi.org/10.1007/s12560-012-9087-8
27. Kumar S, Stecher G, Tamura K (2016) Mega 7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biol Evolution 33:1870-1874. https://doi.org/10.1093/molbev/msw054
28. Li Y, Ge X, Zhang H, Zhou P, Zhu Y, Zhang Y, Yuan J, Wang LF, Shi Z (2010) Host Range, Prevalence, and Genetic Diversity of Adenoviruses in Bats. J Virol 84:3889-3897. https://doi.org/10.1128/JVI.02497-09
29. Marques NR, Hayashi C, Souza SR, Soares T (2004) Efeito de diferentes níveis de arraçoamento para alevinos de carpa-capim (Ctenopharyn godonidella) em condições experimentais. B Inst Pesca São Paulo 30:51-56. https://www.pesca.sp.gov.br/Marques30_1.pdf
30. Monteiro GS, Fleck JD, Kluge M, Rech NK, Soliman MC, Staggemeier R, Rodrigues MT, Barros MP, Heinzelmann LS, Spilki FR (2015) Adenovirus of canine and human origins in stool samples from free-living pampas foxes (Lycalopex gymnocercus) and crab-eating foxes (Cerdocyon thous) in São Francisco de Paula, Rio dos Sinos basin. Braz J Biol 75:s11-16. http://dx.doi.org/10.1590/1519-6984.0313
31. Prado T, Miagostovich MP (2014) Virologia ambiental e saneamento no Brasil. I.O.C. Cad Saúde Pública, Rio de Janeiro 30:1367-1378. http://dx.doi.org/10.1590/0102-311X00109213

32. Prez VE, Martinez LC, Victoria M, Giordano MO, Masachessi G, Ré VE, Pavan JV, Colina R, Barril PA, Nates SV (2018) Tracking enteric viruses in green vegetables from central Argentina: potential association with viral contamination of irrigation waters. Science Total Enviroment 637-638:665-671. http://dx.doi.org/10.1016/j.scitotenv.2018.05.044
33. Purpari G, Macaluso G, Bella S, Gucciardi F, Mira F, Marco P, Lastra A, Petersen E, Rosa G, Guercio A (2019) Molecular characterization of human enteric viruses in food, water samples, and surface swabs in Sicily. Int J Infect Dis 80:66-72. https://doi:10.1016/j.ijid.2018.12.011
34. Rames E, Roiko A, Stratton H, Mac Donald J (2016) Technical aspects of using human adenovirus as a viral water quality indicator. Water Res 96:308-326. https://doi.org/10.1016/j.watres.2016.03.042
35. Reidel A, Oliveira LG, Piana PA, Lemainski D, Bombardelli RA, Boscolo WR (2004) Avaliação de rendimento e características morfométricas do curimbatá Prochilodus lineatus (Valenciennes, 2836), e do piavuçu Leporinus macrocephalus (Garavello&Britski, 1988) machos e fêmeas. Rev Varia Scientia 4: 71-78. http://e-revista.unioeste.br/index.php/variascientia/article/view/724/613
36. Roy S, Vandenberghe LH, Kryazhimskiy S, Grant R, Calcedo R, Yuan X, Keough M, Sandhu A, Wang Q, Jaszek CAM, Plotkin JB, Wilson JM (2009) Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. PLoS Pathog 5: https://doi.org/10.1371/journal.ppat.1000503
37. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
38. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWillian H, Remmert M, Söding J, Thopson JD, Higgings DG (2011) Fast scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 11:539. https://doi.org/10.1038/msb.2011.75
39. Smith JG, Wiethoff CM, Stewart PL, Nemerow GR (2010) Adenovirus. Curr Top Microbiol Immunol 343:195–224. https://doi.org/10.1007/82_2010_16
40. Steinel A, Parrish CR, Bloom ME, Truyen U (2001) Parvovirus infections in wild carnivores. J Wildl Dis 37:594-607.https://doi.org/ 10.7589/0090-3558-37.3.594
41. Straus JH, Straus EG (2008) Viruses and human disease. Journals books Science Direct Published by Elsevier. 2ª edition- chapter 1-11. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7149408/pdf/main.pdf
42. Strottmann DM, Scortegagna G, Kreutz LC, Barcellos LJG, Frandoloso R, Anziliero D (2008) Diagnóstico e estudo sorológico da infecção pelo parvovírus canino em cães de Passo Fundo, Rio Grande do Sul, Brazil. Ver Ciência Rural 38:400-405. https://www.redalyc.org/articulo.oa?id=33138217

Tables And Figures

Tables 1-3 and Figures 1-3 have not beeen provided in this version.