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Virome Methodology in Animal Diseases

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Abstract: Virus discovery is a novel and ascending field in science for the last decade. This field is important because of changing host interactions and novel viruses in animals is necessary both for understanding the origin of different virus species that can infect human hosts and for preventing new zoonotic infections. The COVID-19 discovery was shown all around the world, viruses can easily adopt other hosts. The goal of this presentation is to explain the state-of-the-art in virome studies in various animals and in different specimens with different methodologies such as next-generation sequencing with different taqmentation reagents and analyses total raw data in different pipeline. Specifically Chaphamaparvoviruses which belongs to Parvoviridae family have recently been detected in dogs, cats, pigs, rats, red-crowned cranes, turkeys and chickens, macaques, bats, Tasmanian devils, murine and fish. Another chaphamaparvovirus was recently found in the serum of a febrile Brazilian. Some chaphamaparvovirus members have been shown to cause nephropathy in laboratory mice and in tilapia fish. As a result virus discovery has important role in animal and human science and to learn methods in this area give privilege to researchers as well as countries, which may eventually become the epicenter of a new and unpredicted novel virus someday.

Keywords: Next-generation sequencing, virome, metagenomic

Introduction

Virus discovery is a novel and ascending field in science for the last decade. This field is important because of changing host interactions and novel viruses in animals is necessary both for understanding the origin of different virus species that can infect human hosts and for preventing new zoonotic infections. The COVID-19 discovery was shown all around the world, viruses can easily adopt other hosts (Huang et al, 2020). The goal of this presentation is to explain the state-of-the-art in virome studies in various animals and in different specimens with different methodologies such as next-generation sequencing with different taqmentation reagents and analyses total raw data in different pipeline.

Classical methods for detection of known viruses are cell culture, immunological assays, and PCR (Chan J.F et al, 2017) all methods design according to the known viral structure but these methods are not enough for the detection of divergent unknown viruses. The growing rate of virus discovery, caused detailed steadily improved bioinformatic analysis pipeline for each virus discovery groups and public databases such as IDseq. These programs allow to researcher to discovery so divergent viruses base on protein database (Gorbalenya et al,2019).

There are different methodology for virus discovery such as tagmentation and ligation. Traditional library preparation methods include three-step first step is fragmentation for 200~400 bp length DNA and the second step is ligation adaptors and the last step is ligated product amplification. Tagmentation methods do not have fragmentation and ligation step, this method uses transposases for these two-step, will cut the DNA and attach the adaptors (Feng K et al, 2018). This study was compared two different library preparation methods for a novel virus discovery.

Method

Animal and Post-Mortem Examination and PCR

A young, adult female Northern paradise tanager (*Tangara chilensis paradisea)* housed in a large aviary at a zoological institution was found dead with no premonitory signs of disease. Brain tissue was found negative for general protozoa, paramyxovirus, bornavirus, and herpesvirus by PCR. Used all brain tissue sample used for PCR analyses because of that liver tisue was used for viral metagenomic (next generation sequencing -NGS).

Viral Metagenomics

Same extraction and RT-PCR method followed for two different library preparation. Liver which had been frozen at -70C were thawed and homogenized and after centrifugation for 10 min in a table-top microfuge (15 000 × g, 4°C), supernatant was collected and filtered through a 0.45 μm filter (Millipore). Filtered supernatant treated with enzyme coctail to enrich for particle-protected viral nucleic acids. Total nucleic acids were then extracted (MagMAX Viral RNA Isolation Kit, Ambion, Inc, Austin, Tx, USA) (Li L et al, 2015) and amplified by random RT-PCR (Li L et al, 2015).

Amplified PCR product separated 2 for library prepation for tagmentation followed by use of the Nextera™ XT Sample Preparation Kit (Illumina) to generate a library for Illumina MiSeq (2 × 250 bases) with dual barcoding as previously described (Li L et al, 2015). For ligation medhodology was used Ovation® Ultralow System V2 DNA-Seq Library Preparation Kit (Nugen) and followed kit instruction for library prepartion. All two libraries runned in Illumina Miseq NGS machine. An in-house analysis pipeline was used to analyze sequence data. Before analyzing, raw data were pre-processed by subtracting human and bacterial sequences, duplicate sequences, and low quality reads. Following de novo assembly using the Ensemble program (Deng X et al, 2015), both contigs and singlets viral sequences were then analyzed using translated protein sequence similarity search (BLASTx v.2.2.7) to all annotated viral proteins available in GenBank. Candidate viral hits were then compared to an in-house non-virus non-redundant (nr) protein database to remove false positive viral hits. To align reads and contigs to reference viral genomes from GenBank and generate complete or partial genome sequences the Geneious R10 program was used.

Phylogenetic Analysis

Paradise Parvovirus NS1 and VP1 protein sequences were aligned using MAFFT in Geneious v10.1.3. and the aa phylogenetic trees of parvoviruses were constructed using the Maximum likelihood method with two substitution models: Le_Gascule_2008 model (LG) with Freqs and gamma distributed, invariant sites (G + I) MEGA software ver. X (Le SQ et al, 2008). Positions containing gaps and missing data were removed. Evolutionary analysis was conducted in MEGAX (Tamura K et al, 2013).

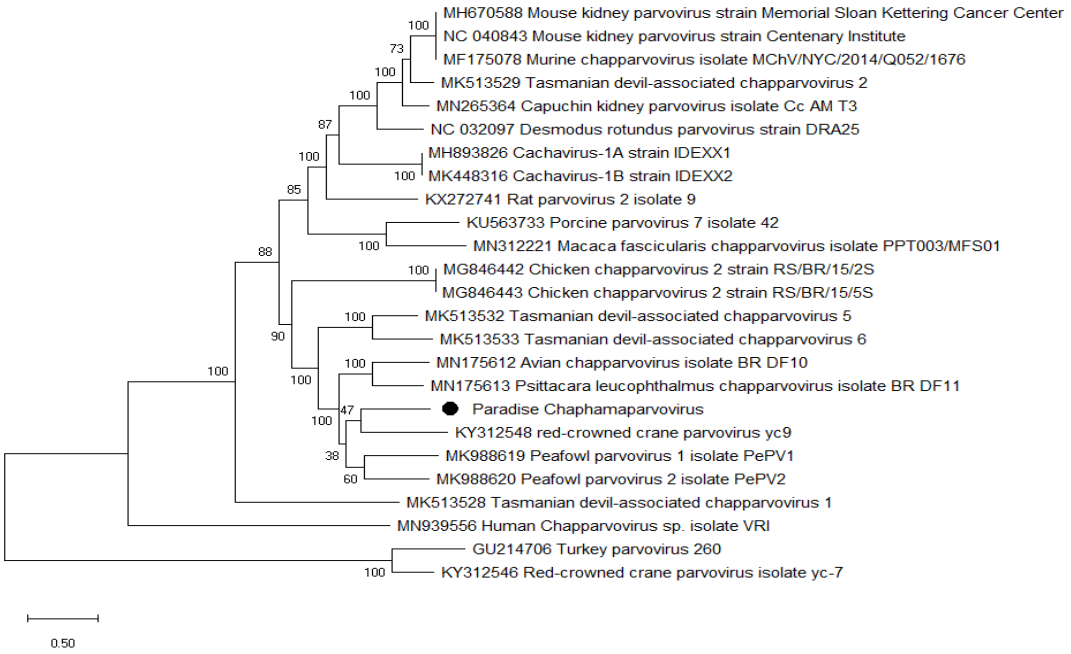
Conclusion

Viral Metagenomics

There wasn't any evident cause of death at necropsy and no gross lesions diagnosed. After all process with two different library preparation had not seen any significant differences in the two methodologies. Both libraries have given the same result and shown the same virus which the length of the paradise bird chaphamaparvovirus genome obtained was 4196 nucleotide (nt) long with typical genome organization of four major ORFs (MT764779).

In phylogenetic analyses the bird of paradise chaphamaparvovirus proteins clustered with other bird-associated chaphamaparvoviruses: Peafowl parvovirus 1 and 2 (MK988619-20), red-crowned crane parvovirus (KY312548), Avian chapparvovirus (MN175612), and Psittacara leucophthalmus chapparvovirus (MN175613) (Figure 1A-B). The nonstructural protein (NS1) and the predicted capsid proteins (VP1) proteins showed closest aa identity of 49.85% and 49.78% to the corresponding proteins of Peafowl parvovirus 1 (MK988619).

A



B

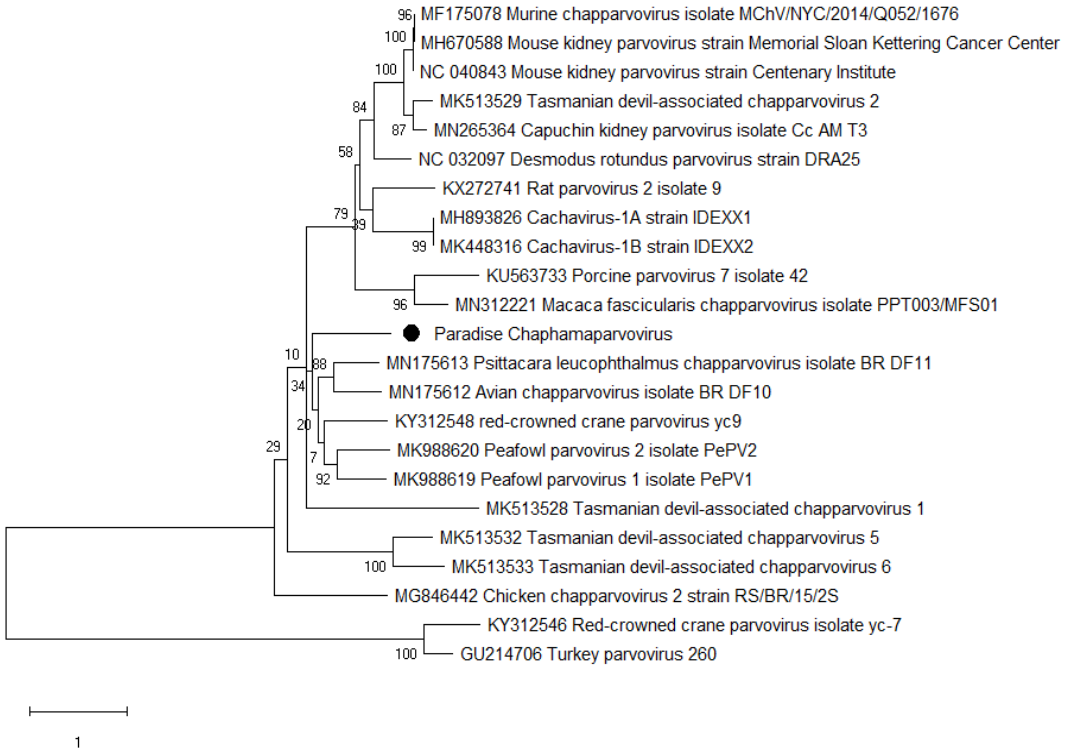


Figure 1. Phylogenetic trees **(A; NS1 and B; VP1)** were constructed using the maximum likelihood method with two substitution models: Le_Gascule_2008 model (LG) with freqs and gamma distributed, invariant sites (G + I) model MEGA software version X.

Recommendations

All countries must be ready for any kind of viral epidemic. Because of that laboratories working virus discovery are necessary for all countries. Specifically working on animal viruses can help to be on alert for any other zoonotic virus.

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