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Resumo

Livestock farming is a multifaceted activity that moves billions of dollars, with Brazil having a great relevance. However, there are still few studies aiming to understand the microbiota of cattle, especially regarding the vaginal tract (VT), although it is extremely relevant biotechnologically and in assisted fertilization. To date, only 13 studies have been conducted with metagenomic approaches to know the bovine VT microbiota, the majority highlighting bacteria present in disease. Despite the current space reached by the metagenomics, this tool has not yet been used to explore viral communities of the bovine VT. Knowing the microbial communities in VT, and studying the relationships between animal viruses, bacteria, bacteriophages and their host is relevant to understand the mechanisms of homeostasis related to the microbiota. This work aimed to investigate the bacteriophages diversity of Gyr and Nellore cattle VT using Next Generation Sequencing. For collection, 4 heifers and 4 cows from each breed (Gyr and Nellore) pure by origin and without any clinical signs in the past 12 months were selected. The vaginal wash was collected, lyophilized and total RNA was extracted. The purified RNA was used as template to cDNA synthetization that was pooled and used as input for Nextera XT DNA Library Prep Kit. The libraries were amplified, purified and quantified. Samples were then pooled in equimolar concentrations and sequenced on Illumina HiSeq 2500 machine. Data was processed in Sagarana HPC cluster, CPAD-ICB-UFMG, using Ezmap pipeline. This pipeline was used in default parameters but a customization in host mapping was done, using *Bos indicus* (NC_032650.1) as the reference host genome. As results, we observed a high phage diversity in all the samples, but a low abundance of viral communities. The viral abundance was of 0,05% and 0,33% for Nellore heifers and cows respectively and of 0,36% and 0,60% for Gyr heifers and cows, respectively. The high phage diversity found reinforces the previous concept that vaginal environment in cows has a great bacterial diversity, different than the observed for humans. Among the species found, we highlight species of *Escherichia virus*, *Mycobacterium virus*, *Shigella virus* and *Staphylococcus virus*. Bacteriophage communities seem to be controlling the abundance and diversity of important bacterial pathogens in healthy animals, being extremely relevant to homeostasis, keeping the microbial communities in balance within the VT.

Palavras-chaves: Virome, NGS, Bacteriophages, Bovine, Vaginal Tract

DIFFERENT METHODS OF CELL VIABILITY ANALYSIS OF CULTURES EXPOSED TO A NANOVACCINE FOR THE PREVENTION OF NEWCASTLE DISEASE IN POULTRY

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Resumo

Newcastle disease is a high mortality and notifiable disease of poultry. The lipid envelope of Newcastle disease virus (NDV) contains two surface glycoproteins, fusion protein, and hemagglutinin-neuraminidase (HA-NA). Commercial vaccines are based on attenuated viral strains, which may cause respiratory symptoms in immunocompromised birds. A disadvantage in the use of live attenuated vaccines is that the induction of antibody reactive to the virus interferes with the serological surveillance of the birds in active surveillance programs. Therefore, vaccination strategies are essential in both commercial and backyard poultry production. This study aimed to evaluate the immunogenicity of NDV envelope protein subunit by in vitro assays. A nonpathogenic NDV isolate (No. 209/04) was kindly provided by Lanagro/SP - The Ministry of Agriculture. For the virosomes preparation, sacarose gradient purified virus suspension was diluted in Triton X-100 (1%) to dissolve the viral envelope, followed by ultracentrifugation (1h/100000xg/4°C) to remove the nucleocapsid. Then, a solution of phospholipids was added, and the surfactant was removed with the aid of a hydrophobic resin. The virosomes were characterized by the Zeta potential values between -2.3±0.2 mV, and an average size of 109±11 nm demonstrated good electrostatic suspension stability. The HA assay of the viral suspension from the pre and post treatment remained similar. No virus replication was observed when treated NDV was inoculated into embryonated chicken eggs. The in situ transmission electron microscopy showed a concentration of nanostructures in the membrane of the nanoparticles. Immortalized macrophages lines, RAW 264.7, were used to evaluate the virosome and its influence on cytotoxicity and cell growth. Analyzes of MTT, cytotoxicity and cell counting at dilutions of 1:2-1:256, in 24, 48 and 72h were performed. The rate of cellular apoptosis at different concentrations of virosomes through was evaluated using the LIVE/DEAD®Viability/Cytotoxicity Kit and APO-DIREC assays. The results obtained were satisfactory, with endocytosis of virosomes by macrophages and low cytotoxicity (less than 5%), especially at the dilution of 1:16-1:32. All these results are an indicator of a promising NDV nanovaccine, which will be further evaluated *in vivo* in order to prevent Newcastle disease in poultry.

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Palavras-chaves: virus like particle, virosome, vaccine delivery , chicken

ANTIGENIC AND GENETIC CHARACTERIZATION OF PESTIVIRUSES ISOLATED FROM THE SERA OF BEEF CALVES DESTINED TO EXPORT - RIO GRANDE DO SUL, BRAZIL, 2017.

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Resumo

Bovine pestiviruses include three officially recognized viral species, e.g. bovine viral diarrhea virus 1 (BVDV-1), 2 (BVDV-2) and *HoBi-like* pestivirus (HoBiPeV). Pestivirus field isolates display a high genetic and antigenic variability which hamper diagnostic and production of vaccines. Genetically,