Culturing Viruses from Wild Deer in Utah

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Abstract

In the Spring of 2016, two deer showing clinical signs of neurological illness were reported to the Utah Division of Wildlife Resources. The animals were euthanized and post-mortem tissues collected for identification of any disease causing agents. Brain and spleen samples were sent to Dr. Eric Delwart, University of California-San Francisco for metagenomics evaluation. Mobukvirus (described once) and novel picornavirus and bovine parvovirus were tentatively identified. However, cell culture of the viruses is necessary for further characterization and genomic sequencing. If these novel viruses can be cultured, it may be possible to develop serologic tests for additional field investigations in wildlife.

We (USU) received tissue samples in June and have started viral evaluation in a number of cell lines. The cell lines being evaluated include: MDBK cells (bovine kidney), MDCK cells (canine kidney), BHK-21 cells (hamster kidney), Vero cells (African green monkey kidney), ST cells (swine testis), SK-RST cells (swine kidney), RD Cells (human rhabdosarcoma), Hela cells (human adenocarcinoma), and the mosquito C636 cell line.

In this experiment MDBK, SK-RST, and ST cells were used. Lipofection was performed to produce more virus using viral RNA extracted from the brain tissue. Supernatant from the lipofection was used to infect fresh confluent cells. Supernatant from the first infection were used to infect more cells using dilutions. The results are inconclusive as CPE has not been clearly characterized within the samples.

Purpose

To culture putative mobukvirus and picornavirus from clinical samples collected from wild deer.

Introduction

Wildlife diseases not only pose a threat to the conservation of global biodiversity, but also pose a threat to human health.

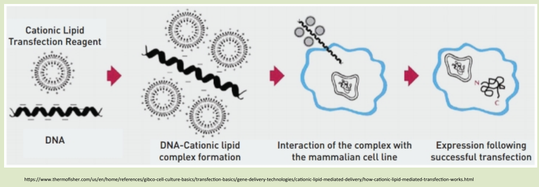
Over the last 30 years, approximately 70% of new and emerging human diseases have been zoonotic (meaning a disease transmitted from animals to people), and many have come from wildlife (Taylor et al., 2001). The Foresight report describes the projected risks from infectious diseases of humans, animals, and plants over 10- and 25-year horizons, and predicts that the highest probability of emergence will be associated with RNA viruses, especially those found at the human-animal interface (Brownlie, et al., 2006). Understanding the mechanisms that underlie newly emerging and reemerging infectious diseases is one of the most difficult scientific problems facing society today. It is critical for the future control of disease outbreaks to understand and respond appropriately to new and emerging disease threats. This will require the paradigm shift from outbreak response, to predicting the outbreak before it has happened.

Methods

Tissue Lysis and RNA Extraction

- Whole frozen deer brain tissue is excised and placed in MEM media and brought to room temperature
- Tissue is Sonicated to disrupt brain cell membranes in preparation for RNA extraction
- Viral RNA extraction is performed on prepared tissue using Qiagen's QIAamp Viral RNA Mini Kit
- RNA concentration and purity is determined using a NanoDrop Spectrophotometer

RNA Lipofection of different Cell Lines



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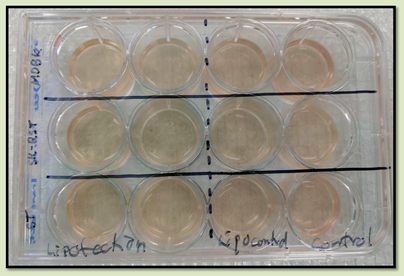
- Cells are cultured and seeded in a 12 well plate at a specific density and incubated for 24 hrs
- Lipofection is performed using Invitrogen's Lipofectamine Transfection Reagent with Plus reagent and extracted viral RNA
- Cells incubate at 37°C for 3 days and are checked for CPE to indicate expression of virus.

Subsequent Infections

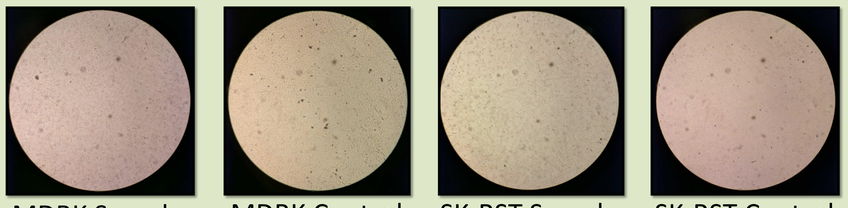
- Cells are cultured and seeded in a 12 well plate and incubated for 24 hrs to achieve confluency
- Cells are infected using supernatant from the Lipofection plate and incubated at 37°C and checked for CPE
- Supernatant from infected cells with observed CPE on the first plate are used to infect a second plate using a 1:3, 1:10, and 1:30 dilution for each cell type.

Results

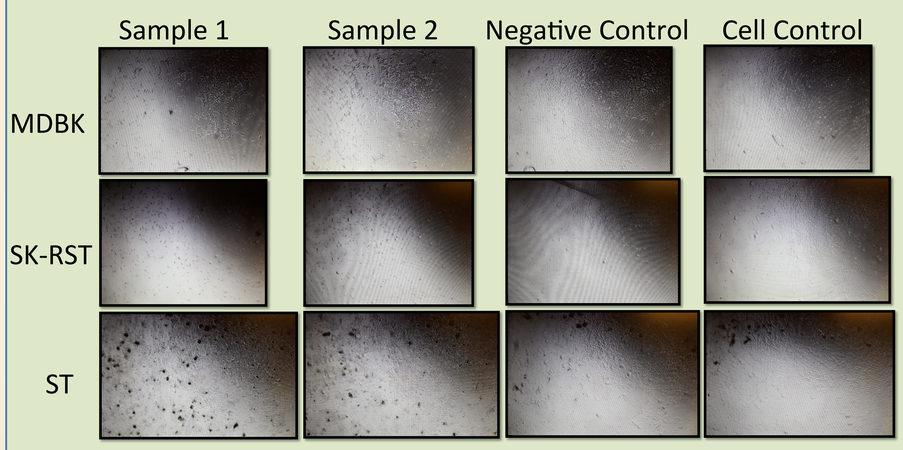


Lipofection

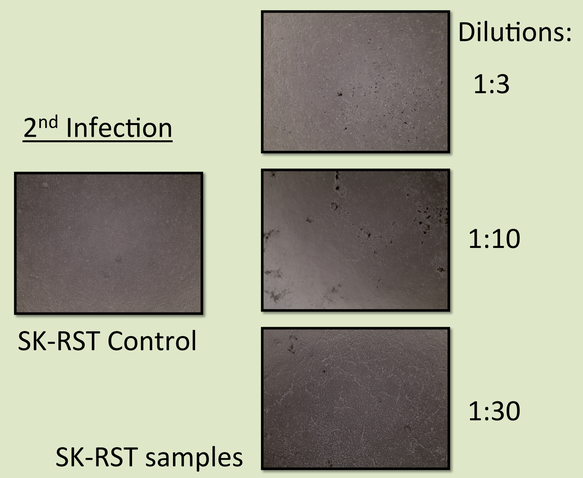
1st Infection



MDBK Sample | MDBK Control | SK-RST Sample | SK-RST Control



2nd Infection



Conclusion/Discussion

The results of this experiment are inconclusive concerning whether or not any virus derived from the deer brain have been cultured using these methods. Further testing is necessary to determine whether cells are dying of toxicity or viral infection.

Cytopathic affect (CPE) occurs when a host cell is infected with virus and the cell dies or is lysed open (broken open) as a result of infection. This is able to be visualized under a microscope to determine whether cells are potentially infected with viral material. This visualization was used to determine the successfulness of the experiments.

Lipofection was first used to introduce extracted viral RNA from the deer brain into cultured cells in order to produce more virus; visible CPE should have been apparent. Cell density in the 12-well plate was too low to determine whether the lipofection successfully worked and CPE could not be ascertained. Cell death may have consequentially resulted from low cell density.

The first infection has some visible cell death. This result could be due to toxicity of residual lipofection reagent and dead cells. The second infection also shows inconsistent results as the 1:10 dilution appears to have more cell death than the 1:3 dilution. Although virus could be producing CPE, the results are indefinite.

References

- Taylor, L.H., Latham, S.M., Woolhouse, M.E. (2001) Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci. 356:983-9.
- Brownlie, J., Peckham, C., Waage, J., Woolhoose, M., Lyall, C., Meagher, L., Tait, J., Baylis, M., Nicoll, A. (2006) *Foresight. Infectious Diseases: Preparing for the Future. Future Threats*. Office of Science and Innovation, London, United Kingdom.

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