Detection of Bovine Respiratory Disease Viruses using Viral Neutralization Assays

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Introduction

Bovine respiratory disease (BRD) is the primary cause of feedlot mortality and economic loss in the North American beef industry and majorly affects dairy industry despite 50 years of research efforts. A combination of stress compromising respiratory defenses mechanisms and infection with one or more viruses leads to BRD. These viruses include bovine parainfluenza-3 virus, or PIV3. The viral infection suppresses physiological function and allows for further respiratory infection, pneumonia, and death. Proper control and treatment of BRD relies on diagnosis and treatment of viral contributors in early stages. We need to develop an effective model, and eventually use that model to test potential compounds against PIV3 using viral neutralization assays on serum from inoculated bovine. We preformed a blind test on series of serum to determine efficacy of our proposed model in identifying PIV3 exposed cattle.

Methods

- Madin-Darby Bovine Kidney (MDBK) cells provide manageable and robust cellular models for studying viruses.
- Using Madin-Darby Bovine Kidney cells we tested the effects of various bovine obtained serum to suppress PIV3 infection rate, via virus neutralization assays.
- Evaluated the effectivity of different individual bovine serum dilutions on a logarithmic scale by utilizing statistical methodology to validate our model.

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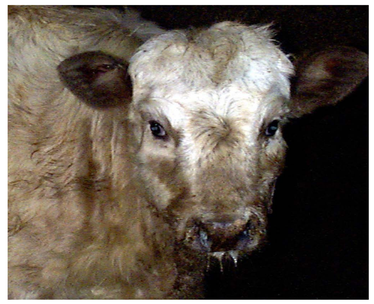
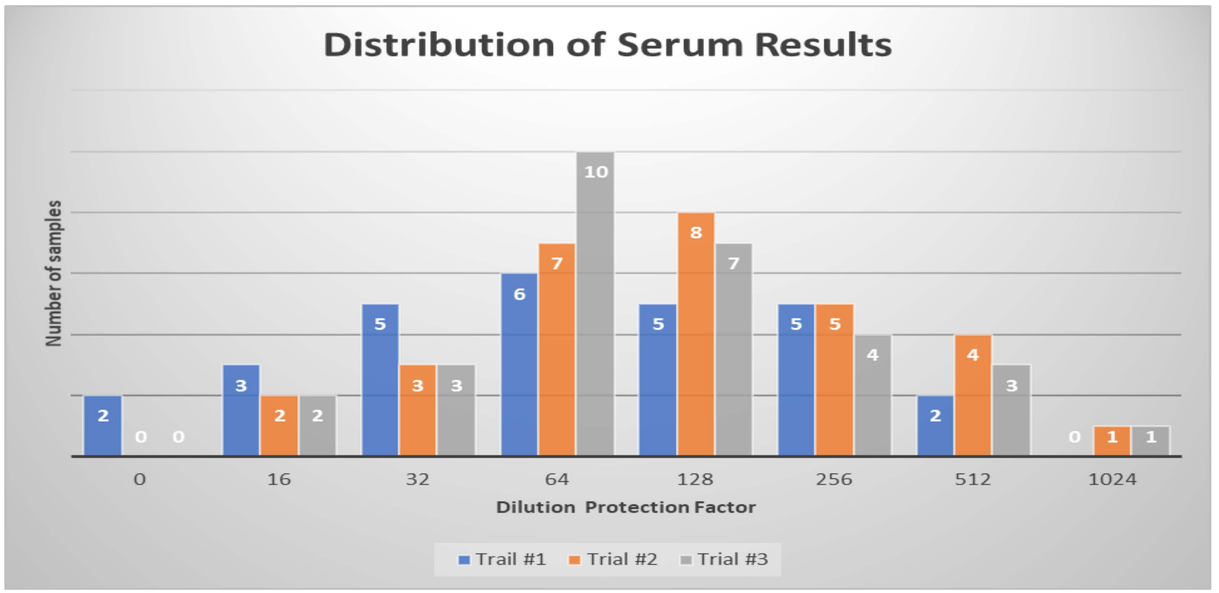
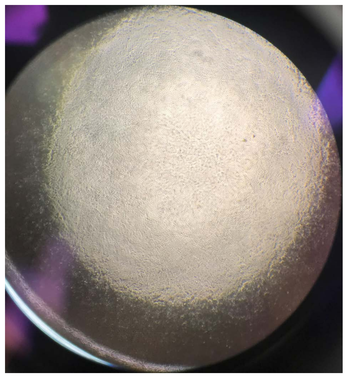
Figure 1: Qualifying Parainfluenzia-3 Assay



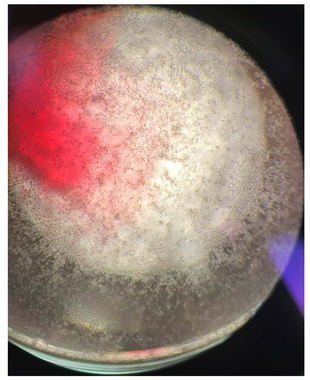
Stock images of Viral Assay development (left) and a bovine infected with PIV3 (right).





Interpretation

The image to the left shows a normal healthy well of MDCK where the serum protected after five days. On the right we see a well that shows a serum dilution that failed to protect against PIV3. It was found that a five day incubation with non heat-inactivated serum was best with a 100,00 virus particle concentration and a 4.4e^4 cell per well was optimal.



Results

Using viral neutralization assays, we found vital evaluation parameters were cell line, virus concentration, and incubation time. Using the establish protocol above we found that the average of the numerical protection average from the serums tested was around 169.24. An average standard deviation of 64.65 was obtained, which is a 33 % of test varied by about one dilution step from trial to trial.

Conclusions

The protocol for evaluation and testing we developed appeared to have worked efficiently and minimized a lot of random statistical variance. It allowed us to isolate which samples were best to develop our assays, minimize errors, and isolate what conditions were favorable in a laboratory setting for testing. The development of the assay shows potential to possibly provide a reasonably quick and high-throughput testing measure to screen various compounds for protection against PIV3 as well as the presence of cytotoxic effects. Because the samples came from living creatures that have an inherent random error that is not minimizable. Despite this, the Gaussian distribution shown in the middle chart was sufficient for statistical modeling. With these results, the logical conclusion would be to report our finding to our sponsors, and see if the results are sufficient for their standards. If this is the case establishing the next step would be to start the qualification steps of this assay and experimental testing before it goes into use.