

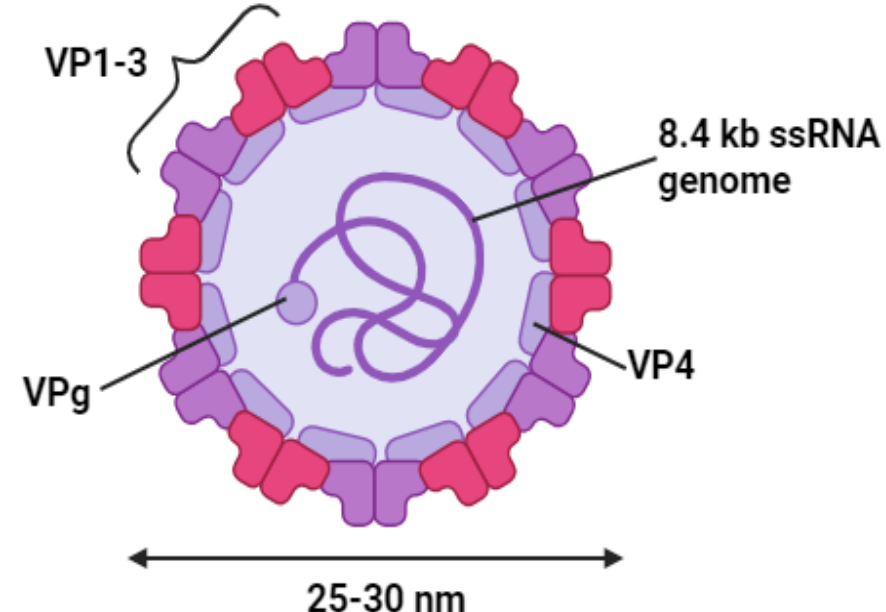
Investigation of novel methods to study the survival of foot-and-mouth disease virus in aerosols

Charlotte Reston

Supervisors: Dr. Claire Colenutt and Prof. Jonathan Reid

1. Foot and Mouth Disease Virus

- Non-enveloped, icosahedral +ssRNA picornavirus, as seen in diagram
- Infects cloven-hooved animals
- Endemic to Africa, Asia, the Middle East, and South America
- Highly transmissible – up to 100% morbidity
- A vesicular disease typically transmitted via direct contact, however a low probability-high consequence transmission event is via aerosols
- Highly economic disease; annually, the cost of FMDV is approximately £5.4-17.3 billion



4. Project Aims

Set-Up



1. Build a CELEBS instrument suitable for a SAPO4 virus
2. Establish levitation and subsequent survival assays using a surrogate BSL-2 virus with this CELEBS instrument

Investigating FMDV



3. Culture and titre contemporary FMDV strains
4. Investigate optimal survival of each strain using methodology developed in set-up
 - a. Vary temperature during aerosolisation while controlling other environmental conditions
 - b. Repeat, varying RH and controlling other environmental factors
 - c. Repeat, varying pH of the droplet the virus particles are suspended in

Analysis



5. Establish survival parameters for each environmental condition for each strain
6. Investigate links between strain survival (virulence, evolutionary similarity)

6. Responsible Innovation

- There always must be the consideration of dual use research concerns when working with a highly transmissible pathogen, primarily use as a bioterrorism agent
- Strains are housed at the Pirbright Institute with security and biosafety procedures in place, and containment protocols are activated if FMD is suspected on a farm

Key References

- Brown, E., et al., *Airborne Transmission of Foot-and-Mouth Disease Virus: A Review of Past and Present Perspectives*. Viruses, 2022. 14(5).
- Oswin, H.P., et al., *Measuring stability of virus in aerosols under varying environmental conditions*. Aerosol Science and Technology, 2021. 55(12): p. 1315-1320.
- Fernandez, M.O., et al., *Assessing the airborne survival of bacteria in populations of aerosol droplets with a novel technology*. J R Soc Interface, 2019. 16(150): p. 20180779.
- Haddrell, A., et al., *Differences in airborne stability of SARS-CoV-2 variants of concern is impacted by alkalinity of surrogates of respiratory aerosol*. J R Soc Interface, 2023. 20(203): p. 20230062.

2. CELEBS Instrument

- Controlled Electrodynamics Levitation and Extraction of Bioaerosols onto a Substrate
- Environmental conditions (e.g., temperature and relative humidity) can be controlled using the airflow within the instrument
- Individual aerosols can be generated, maintained under chosen conditions, and deposited into appropriate media

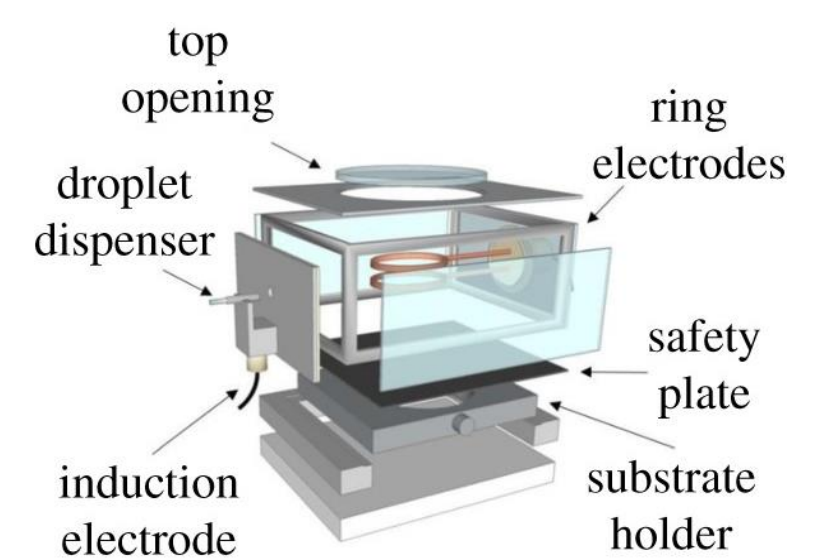


Figure 1: The CELEBS instrument

3. Gaps in Current Knowledge

- The effects of relative humidity, temperature, and pH on the survival of FMDV within aerosols - research that has been completed is significantly outdated, e.g., humidity effects studied over 50 years ago
- Investigation of aerosolised FMDV using updated instrumentation (CELEBS)
- Investigation of contemporary strains of aerosolised FMDV (most up to date strains housed at the Pirbright Institute)

5. Methodology

- At SAPO4, FMDV particles will be aerosolised into droplets of 5-10 μm radius for 5 s – 20 min and collected into 6 mL 2% FBS MEM culture media
- This will be added immediately to 60 wells seeded with LFBK- $\alpha\text{V}\beta\text{6}$ cells for a 3–5 day incubation
- Microscopy will be used to determine cytopathic effects – how well has the virus maintained its infectivity after levitation within aerosols?
- Poisson statistics will describe the specific number of infectious particles
- Staining will additionally be used to determine infection using plaque counts
- This can be repeated with varying temperature, relative humidity, and pH of droplet media for each contemporary strain under investigation to determine unique survival parameters

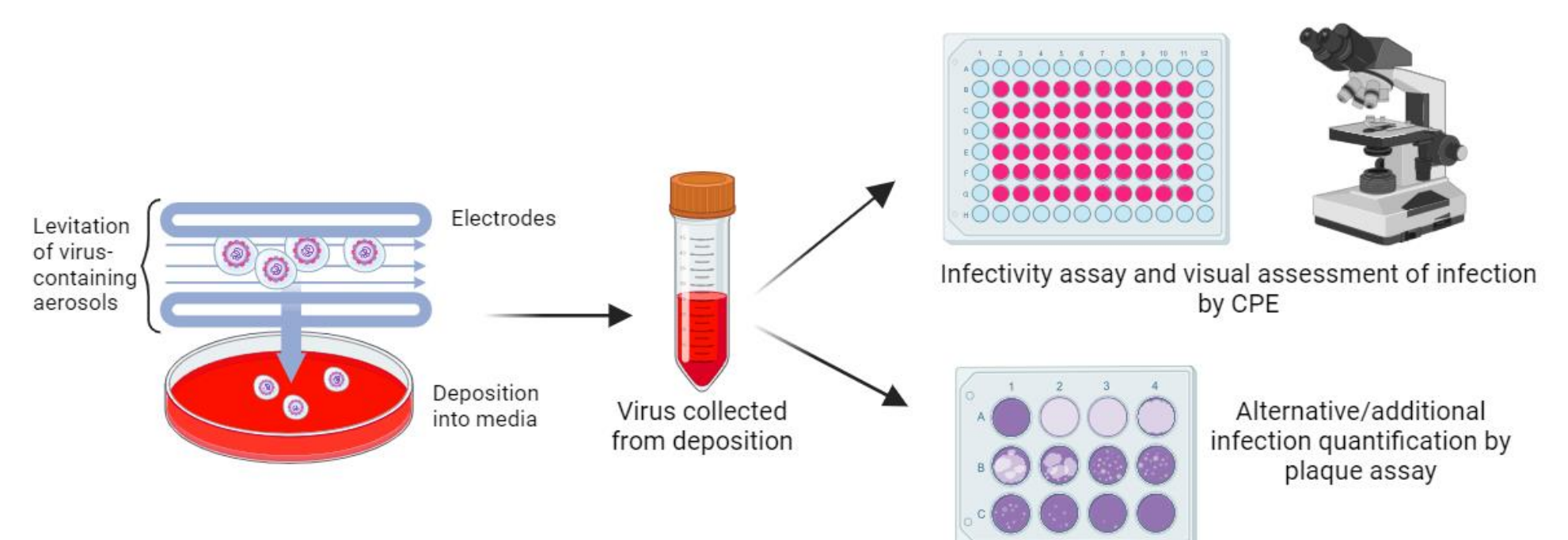


Figure 2: Workflow for determination of infection from levitation using CELEBS instrument.

7. Policy and Scientific Innovation

- The CELEBS instrumentation was only introduced in 2019, and has not yet been applied to picornaviruses
- New survival parameters for contemporary strains could inform updated quarantine policy, taking into consideration certain environmental conditions allowing greater distances to be travelled in the aerosol phase