

Protocol for ASFV vaccine evaluation in pigs.

Standard protocols and standard techniques are needed:

1. To satisfy the Wellcome Trust who require us to follow UK Home Office Animal Act standards.
2. To compare the results obtained between groups vaccinated with different vaccines and challenged.
3. For quality control and direct comparison between various labs.

1. Pigs used for evaluation (age, sex, breed and weight)
 - Fifteen to 25 Kg (mean around 20 Kg),
 - Large white/Landrace or other European breed.
 - Either sex, but it is recommended to use a single sex within the same experiment and to record the weight of pigs at the time of challenge.
2. The number of pigs per group
 - Preferably 6 pigs per group for the safety test.
 - For the vaccine/challenge experiments 10 pigs should be used. Two non-vaccinated control pigs should be included and kept separate from the vaccinated controls.
 - In preliminary challenge experiments between 6 and 10 pigs may be used but a challenge experiment with 10 pigs must be carried out before proceeding to larger scale experiments.
 - Duration between vaccination and challenge: This depends on the type of vaccine and vaccination protocols. The time between the last vaccination and challenge should be 3 weeks.
 - Dose /amount of challenge virus: Benin 97/1, infected pig spleen derived virus, 10^4 HAD₅₀ Intramuscular into hind leg in 2ml PBS. A common virus stock has been supplied to all labs (spleen homogenate from first pig passage) and we measured the titre of this as 10^7 HAD 50/ml. It is recommended to re-check the virus titre of inoculums by HAD assay.
3. Evaluation.

Data will be collected on the clinical scoring and measurement of the level of challenge virus replication.

 - The clinical scoring system described in the attached sheet should be used.
 - Pigs should be kept for at least 3 weeks following challenge and clinical signs and rectal temperature monitored daily.
 - Blood samples should be taken every day over the period 2 to 7 days post-challenge and thereafter every 2 or 3 days to measure levels of challenge virus replication.
 - The following tissue samples should be collected at the termination of the experiment; spleen, tonsils, paratinoid lymph node, gastro-hepatatic lymph node, mesenteric lymph node, lung, kidney. Virus levels in these tissues will be measured in addition to blood if an additional measure of virus replication is required.

- Post-mortem of pigs will be carried out and presence of haemorrhage in the tissues described above recorded as none, slight, moderate or severe.
- A protocol for real time PCR is being assessed in comparison with virus titration (Pirbright) to quantify virus replication. This will be sent at a later date.

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