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A Malaysia 97 monovalent foot-and-mouth disease vaccine (>6PD$_{50}$/dose) protects pigs against challenge with a variant FMDV A SEA-97 lineage virus, 4 and 7 days post vaccination

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ABSTRACT

Pigs play a significant role during outbreaks of foot-and-mouth disease (FMD) due to their ability to amplify the virus. It is therefore essential to determine what role vaccination could play to prevent clinical disease and lower virus excretion into the environment. In this study we investigated the efficacy of the double oil emulsion A Malaysia 97 vaccine (>6PD$_{50}$/dose) against heterologous challenge with an isolate belonging to the A SEA-97 lineage at 4 and 7 days post vaccination (dpv). In addition, we determined whether physical separation of pigs in the same room could prevent virus transmission. Statistically there was no difference in the level of protection offered by 4 and 7 dpv. However, no clinical disease or viral RNA was detected in the blood of pigs challenged 4 dpv, although three of the pigs had antibodies to the non-structural proteins (NSPs), indicating viral replication. Viral RNA was also detected in nasal and saliva swabs, but on very few occasions. Two of the pigs vaccinated seven days prior to challenge had vesicles distal from the injection site, but on the inoculated foot, and two pigs had viral RNA detected in the blood. One pig sero-converted to the NSPs. In contrast, all unvaccinated and inoculated pigs had evidence of infection. No infection occurred in any of the susceptible pigs in the same room, but separated from the infected pigs, indicating that strict biosecurity measures were sufficient under these experimental conditions to prevent virus transmission. However, viral RNA was detected in the nasal swabs of one group of pigs, but apparently not at sufficient levels to cause clinical disease. Vaccination led to a significant decrease in viral RNA in vaccinated pigs compared to unvaccinated and infected pigs, even with this heterologous challenge, and could therefore be considered as a control option during outbreaks.

1. Introduction

Foot-and-mouth disease (FMD) directly impacts livestock production due to loss in productivity and usually affects the economy further due to quarantine and import restrictions on live animals and their products. Vaccination has been used successfully in a number of previously endemic countries to control the disease and most countries free from FMD will consider emergency vaccination if an outbreak should occur.

Susceptible domestic species include cattle, sheep, goats, pigs and water buffalo. All may demonstrate lesions on areas of friction such as the mouth, feet and teats in lactating animals, but subclinical infections can also occur, especially in sheep and goats [1]. Pigs are the amplifier hosts of the disease and excrete large amounts of virus in all secretions and excretions [2,3]. For this reason it is imperative to prevent them from becoming infected or to decrease viral shedding using vaccination.

There are seven serotypes of FMD virus (FMDV—A, O, C, Asia-1, SAT 1, SAT 2 and SAT 3) and large numbers of variants exist

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within each. Since cross protection between serotypes does not exist [4,5], vaccines need to contain multiple strains to ensure immunity to more than one serotype. Even within serotypes, protection is not complete [6,7]. FMDV serotype A viruses have always been considered to be antigenically the most diverse [8,9], and have genetically been classified under three broad genotypes [10]. The Asian genotype consists of several lineages and sub-lineages with viruses belonging to the lineage A SEA-97 being endemic to South East Asia (SEA) and new clusters emerging in the region [10,11]. These viruses have recently spread beyond the SEA region to cause outbreaks in countries that were previously free of serotype A [12].

FMD is endemic in many parts of the world and occurs in most countries in SEA. Through their proximity and the amount of trade and travel, these countries pose the biggest perceived risk to Australia's livestock industries and agricultural economy. Australia's last suspected outbreak was in 1872 [13] and having FMD-free status, together with the absence of several other diseases, has provided the country with a significant trade advantage. The local pork industry is small compared to high producing countries such as China, South Korea and Japan, but the potential overall losses due to a large outbreak of FMD could reach 50 billion Australian dollars over a 10 year period [14]. For this reason it is important to determine whether the strains in the Australian vaccine bank will provide early protection in pigs against the serotype A viruses that are currently circulating in SEA.

2. Materials and methods

2.1. Cell lines, viruses and vaccine

Baby hamster kidney-21 (BHK-21) cells were used for all virus culture. The challenge virus (A/VIT/08/2005) belongs to the FMDV A SEA-97 topotype, circulating in Vietnam and other SEA countries, and has a relative homology (r1) of 0.51 to the A Malaysia 97 (A/MAY/97) vaccine strain (WRL Report 2006; http://www.wrlfmd.org/ref_labs/ref_lab_reports/OIE-FAO%20FMD%20Ref%20Lab%20Network%20Report%202006.pdf). The virus was passaged three times in BHK-21 cells before preparation of the pig-derived challenge virus.

A monovalent double oil emulsion A/MAY/97 vaccine (>6PD_{50}/dose) was prepared by Merial, United Kingdom.

2.2. Animal ethics and pigs used in the study

The animal studies were performed according to the Australian code of practice for the care and use of animals for scientific purposes (AEC1514 and 1571). Sero-negative three-month-old cross-bred Landrace pigs were obtained from a commercial piggery in Vietnam.

2.3. Preparation of challenge virus

Five healthy pigs were used to prepare pig-derived challenge virus. Two pigs were administered 1 ml of A/VIT/08/2005 intravenously into the ear vein, 1 ml intramuscularly on the dorsal aspect just behind the left ear and 2 ml intradermally into the foot-pad of the left-hind limb at multiple sites (0.1 ml/site in each digit). The animals were monitored for the appearance of lesions for three days. A 10% (w/v) suspension of tissue homogenate was prepared in phosphate buffered saline using the epithelial tissue from the coronary band and foot lesions and three more pigs were inoculated intradermally with 0.1–0.2 ml of a 10% (w/v) suspension in the foot pad of the left-fore limb. Epithelial tissue from the coronary band and foot lesions was collected and a 10% (w/v) suspension of tissue homogenate was prepared and stored at −80 °C.

2.4. Titration of A/VIT/08/2005 pig-derived virus

Four healthy pigs were used for titrating the pig-derived virus at $\log_{10}$ dilutions (10^{-1} to 10^{-8}) in basal medium eagles (BME) cell culture medium supplemented with 1% foetal calf serum (FCS). Two pigs received 0.1–0.2 ml of inoculum dilutions −2, −3, −4 and −5, whereas two other pigs were administered dilutions −4, −5, −6 and −7, intradermally in the footpad. Each dilution was administered to two feet. Lesions at the inoculation sites were scored at 24, 36, 48, 60 and 72 h post inoculation. The 50% pig infective dose per ml (PID_{50}/ml) was calculated using the Spearman-Kärber method [15].

2.5. Pig immunisation and challenge

The experiment consisted of three groups of eight pigs each in separate rooms. One group was vaccinated intramuscularly in the mid neck region with 2 ml of vaccine (0.82 mm × 38.1 mm) seven days prior to challenge (A-V7), another four days before challenge (A-V4) and the last group was left unvaccinated and was challenged on day 0 (A-UV). Vaccinations were staggered so that the virus challenge occurred on the same day. For each of these groups, five additional non-vaccinated pigs were kept in the same room (comprising groups A-UVC7, A-UVC4 and A-UVC), but were separated by a waist-high steel wall that prevented direct contact with the challenged animals.

Groups A-V7, A-V4 and A-UV were challenged with $10^{5.0}$ PID_{50} of the pig-derived virus by inoculation in two sites in the left-hind foot pad (0.2 ml/site). The animals were observed and sampled daily for 14 days, and rectal temperatures recorded. Clinical scores were determined by giving each site of lesion development, except the inoculation site, one point (four feet, tongue, mouth and snout); the maximum score was therefore seven. Nasal secretions, saliva and faeces were collected in duplicate using cotton swabs (diameter: 2.7 mm; length: 150 mm); one swab was used for virus isolation (0.5 ml of BME with 10% FCS and antibiotics) and the other for viral genome detection (0.5 ml of lysis buffer with carrier RNA and proteinase K; Startec Biomedical AG, Germany). Swabs were submersed in the buffer and stored at −80 °C. Clotted blood for serum was collected on −7, −4, 0, 5, 7, 10 and 14 days post-challenge (dpc). Whole blood was collected in EDTA tubes on 0, 1–7, 9, 10 and 14 dpc.

The animals in groups A-UVC7, A-UVC4 and A-UVC were observed and sampled as described above. Clotted blood for serum was collected on days 0, 5, 7, 10 and 14 dpc. Whole blood was collected in EDTA tubes on 0, 3, 5, 7, 9, 10 and 14 dpc.

2.6. Quantitative real-time reverse transcriptase PCR (RT-qPCR) for detection of FMD viral RNA

Total RNA from samples was extracted using the InviMag Virus RNA Mini kit/KF96 (Stratec Molecular, Germany) on an automated nucleic acid extraction system (KingFisher Flex Magnetic Particle Processor, ThermoFisher Scientific, USA) following the manufacturer's protocol. RT-qPCR was carried out using Ambion AgPath-ID MasterMix (Life Technologies, USA) using the assay previously described by [16].

In vitro transcribed RNA was prepared using the Megascript T7 kit (Ambion, USA) from a pBluescript KS+ plasmid containing the FMDV IRES region [17]. The RNA was purified and checked for integrity by RT-PCR using the specific primers that would be used for the RT-qPCR [18], and by sequencing. RNA standards were prepared to determine a standard curve for each RT-qPCR run.