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A foot-and-mouth disease SAT2 vaccine protects swine against experimental challenge with a homologous virus strain, irrespective of mild pathogenicity in this species

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ABSTRACT

FMDV serotype SAT2 is most frequently associated with outbreaks in ruminants. However, the risk of it spreading from cattle to pigs cannot be excluded. To assess the efficacy of an SAT2-type FMD inactivated vaccine against homologous challenge in pigs, a suitable challenge strain adapted to pigs was produced. After two passages in two pigs each, a FMDV stock of SAT2 challenge strain was produced. This material was used to infect two groups of five pigs. The first group being vaccinated 28 days before challenge and the other one left as an unvaccinated control. Clinical signs were recorded, virus shedding was assessed on mouth swabs, and neutralising antibody titres were determined. At least 80% of the vaccinated pigs were protected against clinical disease. Furthermore, no virus shedding was observed in any of the vaccinated pigs. This study shows that experimentally inoculated pigs can become infected with a SAT2 serotype. Furthermore, vaccination offers protection against generalisation and viral excretion, confirming the potential of vaccination as an important tool in the control of FMD in pigs.

1. Introduction

Foot-and-Mouth Disease (FMD) is a global problem. It is a contagious viral vesicular disease affecting cloven-hoofed animals worldwide. It has a huge direct economic impact disrupting international trade in animals and animal products. The etiological agent, FMD virus (FMDV), is classified as an *Aphthovirus* within the *Picornaviridae* family. Seven serotypes of FMDV have been identified, namely, O, A, C, Asia 1, South African Territories (SAT) 1, SAT2 and SAT3. Among these, SAT types 1 to 3 were originally restricted to sub-Saharan Africa affecting mainly ruminants. Although they have not become established outside the African continent, incursions into the Middle East have been recorded in recent years. Since 2012, FMD outbreaks of SAT2 have been reported in Egypt, Libya and the Palestinian Autonomous Territories [1]. Emergence of FMD virus SAT2 in Egypt during 2012 was the first known occurrence of this serotype in this country since 1950. It was found that the North African viruses belonged to SAT2 topotype VII. Phylogenetic analysis revealed a close relationship between the recent incursions of FMD in the Middle East and other contemporary and older samples from Eritrea [2]. The isolation of FMDV SAT2 in swine during outbreaks has been rarely reported [3]. The recent incursion of SAT2 in Northern Africa and the Middle East has led to an increased threat of the disease spreading to European nations in the Mediterranean Basin and beyond, affecting FMD free countries densely populated by pigs.

Early reaction contingency plans for a FMD emergency are in force in countries formerly considered free from FMD to target vaccination in relevant domestic population. A few studies have underlined the importance of appropriate vaccine strains in ruminants with particular reference to SAT types [4,5]. To our knowledge it is the first publish study that evaluated protection afforded by vaccination with an SAT2 vaccine in pigs. Considering that swine can play an important role in the dissemination of FMDV [6], it is necessary to determine whether an FMDV SAT2 vaccine strain can prevent clinical disease and reduce the virus being spread by pigs. There are no international standards for FMDV vaccine efficacy testing in pigs, neither in the European Pharmacopoeia monograph 0063 [7], nor in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015 [8].

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In the European Pharmacopoeia, the cattle potency test (PD50 experiment) has been the standard for vaccine quality assessment, but recently the Protection against Podal Generalisation (PPG) test has been added in which the vaccine is only tested at a full dose. It is therefore appropriate to adapt the study design to a protection experiment in pigs, to address the efficacy of vaccination at full dose by challenge with a virulent strain that normally results in generalised disease in the pig. Considering different strains of FMDV can vary greatly in their pathogenicity within different host species, this study focused on the use of a suitable challenge strain, through the investigation of the pathogenicity of FMD type SAT2 in pigs.

In the present work, we report the production of an FMD type SAT2 Saudi Arabia (SAU) challenge strain adapted in pigs and the protection achieved by vaccination with an SAT2 vaccine against a virulent homologous challenge.

2. Material and methods

2.1. Vaccine

An industrial batch of the FMD SAT2 SAU strain (1D sequence of the strain closely related to WRL reference SAT2 isolate SAU/6/00 with GenBank reference number AF367135), double-oil emulsion vaccine, was prepared for the study. A comparable batch was previously tested in cattle and provided a good potency (>6 PD50 per dose).

2.2. Ethics statement and animals

Approval from the Ethics Committee for Animal Experiments at Wageningen Bioveterinary Research, Lelystad, the Netherlands (formerly known as the Centraal Veterinair Instituut [Central Veterinary Institute]), was obtained before the start of both experiments (approval number 2,013,027 for the passage and 2,013,076 for the potency test).

Animal experiments were performed using conventionally reared male pigs, from nine to eleven weeks old. All animals were crossbred pigs, originating from a farm in the Netherlands; a country free of FMDV, and not vaccinating against FMD. The pigs were housed in the Veterinary BSL-4 facilities in Wageningen Bioveterinary Research, Lelystad. First and second pig passage were carried out in separate stables.

2.3. Production of an FMD type SAT2 challenge strain adapted in pigs

2.3.1. Experimental design

For each pig passage, two pigs were anesthetised with a mixture of azaperone (Stresnil®; 1 ml/20 kg BW, IM) and ketamine (Ketamin 10%®; 2 ml/20 kg BW, IM) and subsequently inoculated intradermally with FMD virus into the bulb of the heel, of the outer claw, of the left hind foot, according to the technique described by Pacheco and Mason [9]. Shortly after, FMD SAT2 inoculum was administered using a 0.7 × 32 mm needle, into four locations of the superficial layer of the epidermis starting just above the bulb of the heel (4 × 0.1 ml).

The FMD challenge material used for the first passage was FMDV SAT2 SAU that had been passaged twice in bovine thyroid cells and passaged once in vivo on cattle tongue. It contained 7.8 $\log_{10}$ plaque forming units (PFU) per millilitre when titrated on primary porcine kidney cells. The lesion material harvested in the first passage in pigs (SAT2 SAU P_1) was used for the inoculation of two additional pigs (second passage). For each passage, both pigs were observed for three days after inoculation.

2.3.2. Sample treatment and challenge material preparation

When FMD vesicles were observed in one or both pigs on a site other than the injection site two to three days after infection, after euthanasia, both vesicle and vesicular fluid were collected. At Boehringer Ingelheim Animal Health Netherlands B.V. laboratory, vesicle material was processed. Shortly after, vesicular material was cut into small parts using sterile scalpels. Using a pestle and mortar in combination with sterile silicon dioxide (sea sand), a suspension was made in a minimal essential medium containing Hanks' salts (HMEM) supplemented with antibiotics and 2% foetal bovine serum. The tissue suspension was centrifuged at a speed of 1250*g* for 10 min at 10 °C. The supernatant of the tissue was collected and filtrated sequentially through 0.45 μm and 0.2 μm acrodisk filters. The filtrated virus was divided into small portions and stored at −80 °C.

The prepared virus suspension was titrated by plaque titration on porcine kidney cells. Six-well plates with 2×10^6 secondary porcine kidney cells per well, were prepared and incubated for 24 h. When the cells of the six-well plates showed a 95–100% confluent monolayer, a ten-fold and subsequently a threefold serial dilution of the challenge virus were made. The diluted virus was tested by adding 500 μl/well in duplicate. The virus was fixed by adding methylcellulose solution. After an incubation period of 48 h, the cells were fixed with citric acid and stained with amido black. The quantity of plaques was counted to calculate the titre. Virus titres were expressed as $\log_{10}$ PFU per ml.

Before the inoculation of pigs, the FMDV stock was diluted in HMEM medium supplemented with antibiotics and 2% foetal bovine serum (1:5 and 1:10 dilution applied for the first and second passage, respectively).

2.4. Pig protection test of FMD SAT2 vaccine

2.4.1. Experimental design

Ten male pigs were divided into two groups of vaccinated pigs (group one, n = 5), and unvaccinated controls (group 2, n = 5). Pigs in group one were vaccinated with a full dose (2 ml) of a batch of monovalent FMD SAT2 SAU vaccine. The vaccine was administered by the intramuscular route, behind the ear, on the left side of the neck, using individual syringes (needle 0.8 × 25 mm). The pigs from the control group (group two) remained unvaccinated. Vaccination was given four weeks prior to challenge. All pigs were housed in one single room up to exposure to challenge. The pigs were separated by wooden planks measuring 1.5 m high that prevented contact between pigs from challenge onwards.

The second pig passage SAT2 SAU P_2 was used as challenge virus. After anaesthesia as described above, all the pigs (both groups one and two) were inoculated with a total of 100,000 PFUs of virus by intradermal injections in the left hind-foot (four sites, both bulbs, total of 0.4 ml). To reduce the pain caused by infection, all pigs were treated with an intramuscular administration of flunixin (Finadyne®, 2 ml) for four days. Prolonged treatment with anti-inflammatory drugs was administered when pigs presented signs of pain related to generalised FMD. Pigs which severely suffered from the disease were euthanised.

2.4.2. Monitoring and sample collection

All pigs were monitored for eight days after challenge for their general condition and clinical signs. Blood samples for serum were collected on the day of vaccination, challenge, and at the time of euthanasia (3 or 8 dpi). Mouth swab samples were collected before inoculation and 1, 2, 3, 6 and 7 days after challenge, by inserting a cotton swab (Salivette®, Sarstedt) for approximately 30 s in the mouth using a forceps. Three days after challenge, each pig (groups one and two) was closely inspected under anaesthesia for specific FMD lesions. The presence of vesicles in the feet, mouth and snout