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Efficacy of a high potency O_1 Manisa monovalent vaccine against heterologous challenge with a FMDV O Mya98 lineage virus in pigs 4 and 7 days post vaccination

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ABSTRACT

Early protection with a high potency (>6PD_{50}) foot-and-mouth disease (FMD) O_1 Manisa (Middle-East South Asia lineage) vaccine against challenge with O/VIT/2010 (O Mya98 lineage) was tested in pigs. Only two pigs that were vaccinated seven days prior to challenge had any demonstrable antibodies as a result of vaccination at the time of challenge. However, 80% and 60% of pigs that were vaccinated seven and four days prior to coronary band challenge were protected. Vaccination significantly reduced the amount of virus excreted in nasal swabs, saliva and faeces compared to unvaccinated and infected controls. Virus and viral RNA could be detected in some pigs until termination of the experiment 14 days after challenge. Antibodies to the non-structural proteins (NSP) were detected in only one pig that was challenged four days post vaccination (dpv) and transiently in two pigs that were challenged seven dpv at only one time point. For each vaccine and control group, a group of unvaccinated pigs were kept in the same room but with no direct contact with the infected pigs to determine whether vaccination prevented transmission. Despite the presence of live virus and viral RNA in these indirect contact pigs, the groups in contact with the vaccinated and infected pigs did not develop clinical signs nor did they sero-convert. Contact pigs in the same room as unvaccinated challenged controls did show signs of disease and virus infection that resulted in sero-conversion to the NSP. A breach of the wall that separated the two groups at nine days post challenge might have contributed to this finding. This study showed that high potency vaccine can provide protection to pigs soon after vaccination and that aerosol transmission within rooms is a rare event.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease that affects species belonging to the *Artiodactylae*. The disease is characterised by an acute, systemic vesicular disease with lesions developing on areas of friction. Despite low mortality rates in adult animals, FMD severely decreases livestock production and results in devastating trade restrictions.

The causative agent, FMD virus (FMDV), belongs to the *Aphthovirus* genus of the *Picornaviridae* family. Seven serotypes of FMDV (A, O, C, Asia-1, SAT 1, SAT 2 and SAT 3) have been identified on the basis of the ability of viruses to induce cross-protection in animals. This cross-protection is serotype-restricted, and it is not always complete when vaccines contain different subtypes and variants of the same serotype [1].

Highly potent vaccines for emergency use have previously been shown to protect cattle against airborne challenge as early as two

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[2] and four [3] days post immunisation and pigs within four days of vaccination [4,5]. Immunisation of pigs and cattle prior to challenge can dramatically reduce the titre and duration of FMDV excretion [4,6,7]. In these studies the challenge was with a homologous virus. Protection against heterologous challenge has been shown for serotypes A in cattle [8] but similar studies with serotype O did not yield satisfactory results [9,10].

FMD is endemic in many parts of the world and occurs in most countries in South East Asia (SEA) where regular outbreaks of FMDV serotypes O, A, and Asia-1 are reported [11–13]. FMDV serotype O viruses belonging to serotype O/SEA topotype (Mya-98 and Cam-94 strains), O/ME-SA topotype (PanAsia lineage and the derivative Pan-Asia-2 sub-lineage) and O/Cathay topotype, serotype A (ASIA topotype; SEA-97 strain) and serotype Asia-1 have been identified [14–16].

Pigs are seen as the biggest risk of disease dissemination during an outbreak [7]. While it is possible to control the movement of people, animals and their products, it is not possible to control aerosols that have been implicated in previous outbreaks [17–19]. It is therefore important to decrease virus loads and quantify the effect of vaccination on levels of virus excreted during a heterologous challenge. In this paper we report the early protection afforded by the O_1 Manisa high potency vaccine in pigs challenged with a virus isolated in Vietnam in 2010.

2. Materials and methods

2.1. Cell lines, viruses and vaccine

Baby hamster kidney (BHK)-21 cells were used for any virus related work. The challenge virus (O/VIT/2010) belongs to the FMDV O Mya98 lineage [20] and was isolated in 2010 from pig feet tissue and was passaged five times to a titre of $10^{7.05}$ $TCID_{50}$/ml.

A high potency (>6PD_{50}) monovalent O_1 Manisa double oil adjuvant vaccine was prepared by M/s. Merial Company Limited, United Kingdom for this study.

2.2. Preparation of pig challenge virus

The work was performed according to the Australian Animal Ethics Code (AEC1465 and 1497) in the animal facility of NAVETCO, Vietnam. Three month old sero-negative cross-bred Landrace pigs were obtained from a commercial piggery in Vietnam.

Tissue culture adapted virus at $10^{6.5}$ $TCID_{50}$/ml was inoculated either into the foot-pad [21,22] of the left-fore limb at multiple sites (2 ml; 0.1 ml/site in each digit) or intravenously (1 ml) into the ear vein and intramuscularly (1 ml) on the mid neck region in two pigs. Epithelium was collected from lesions other than the inoculation sites and a 10% suspension prepared by homogenizing tissue in PBS (pH 7.4). Two additional pigs were inoculated with 1 ml of the suspension into the foot-pad of left-fore limb at multiple sites and the process repeated in two more pigs. A 10% suspension was prepared from vesicular lesions, titrated and stored at −80 °C.

2.3. Pig immunisation, challenge and sample collection

Three groups of 10 pigs each were divided into sub-groups consisting of five pigs (Fig. 1). Groups O-V7 ($n = 5$) and O-V4 ($n = 5$) were vaccinated intramuscularly in the neck with 2 ml vaccine (21G needle) and challenged seven and four days post vaccination (dpv) respectively. Group O-UV ($n = 5$) was not vaccinated but constituted unvaccinated challenged controls. Groups O-UVC7, O-UVC4 and O-UVC ($n = 5$ each) were used as unvaccinated indirect contacts and housed in the same room as O-V7, O-V4 and O-UV respectively but with a physical partition consisting of a waist-high steel wall between the groups (Fig. 1). Animals shared the same air handling facility but were not in direct physical contact and were provided with separate feed and water troughs.

Animals in O-V7, O-V4 and O-UV were challenged with $10^{5.0}$ $TCID_{50}$ pig derived virus by inoculation in two sites in the left-hind foot pad (0.2 ml/site). Rectal temperatures were recorded daily and animals were observed for 14 days for clinical disease. Nasal secretions, saliva and faeces were sampled daily using cotton swabs for virus isolation (0.5 ml of Eagle's Basal Medium with 10% FCS and field antibiotics) and viral genome detection (0.5 ml of lysis buffer with carrier RNA; Stratec Biomedical, Germany). Swabs were submersed in buffer immediately after collection and stored at −80 °C. Clotted blood for serum was collected on −7, −4, 0, 5, 7, 10 and 14 days post-challenge (dpc) and blood in EDTA buffer on 0–7, 9, 10 and 14 dpc.

Observations and sample collection were performed in O-UVC7, O-UVC4 and O-UVC groups as described above. Clotted blood for serum was collected on days 0, 5, 7, 10 and 14 dpc and unclotted blood on 0, 3, 5, 7, 9, 10 and 14 dpc.

2.4. Virus isolation and antigen ELISA

Virus isolation from the salivary and nasal swabs was performed in 96-well plates by adding 100 μl of $\log_{10}$ dilutions to each well in duplicate and observing for cytopathic effect (CPE) at 24 and 48 h post-infection using standard procedures. Positive samples were confirmed by ELISA [23].

2.5. Real-time quantitative RT-PCR for detection of viral RNA

Total RNA from the salivary, nasal and faecal swab samples and blood collected in EDTA 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. The RNA was used for quantitative reverse transcription PCR (RT-qPCR) using the Ambion AgPath-ID MasterMix (Life Technologies, USA) as per standard protocols [24].

In vitro transcribed RNA was prepared to determine a standard curve for each RT-qPCR run using Megascript T7 kit (Ambion, USA) from a plasmid pBluescript KS+, cloned using a 550 base region from the 5′UTR region of the FMDV genome [25].

2.6. Serology for virus antibodies to the structural and non-structural proteins (NSP) of FMDV

The solid phase competition ELISA (SPCE) was performed to detect antibodies to the structural proteins on sera samples at 1:5 dilutions in duplicate following standard procedures [26] using rabbit (O_1 Manisa) and guinea pig (O_1 BFS) antisera and O_1 Manisa inactivated antigen. Final OD values were expressed as the percentage inhibition relative to the mean OD of the OD max control wells i.e. $100 - (100 \times (\text{OD test serum mean/OD OD Max control mean}))$. Samples that showed <50% inhibition of the OD max control were scored negative and those ≥50% were considered positive.

A competitive ELISA (c-ELISA) developed at AAHL to detect antibodies to the NSP was performed in duplicate on the sera samples at 1:5 dilutions as described [27] using baculovirus expressed recombinant 3ABC protein and chicken antibodies raised against the protein. The final OD values were expressed as for the SPCE and cut-off values were the same.

2.7. Statistical analysis

Quantitative data were assessed for normality using commercially available software (MINITAB Statistical Software, Release 13.32, Minitab Inc, State College, Pennsylvania, USA). Quantitative

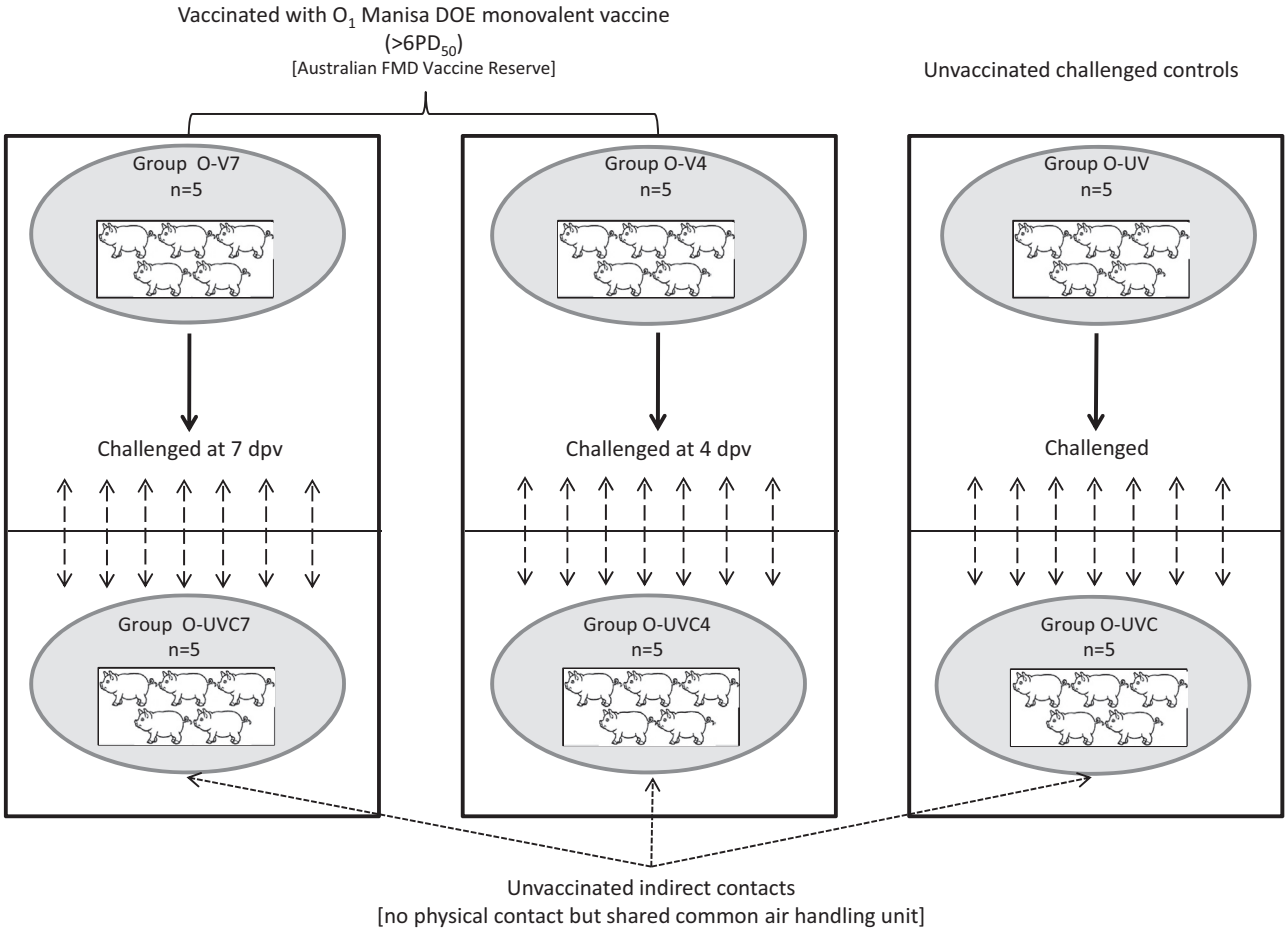

Fig. 1. Experimental lay-out of challenge and indirect contact groups to study the efficacy of high potency O_1 Manisa vaccine against O/VIT/2010 (Mya-98 strain).

data were transformed using the natural logarithm prior to statistical analysis and descriptively presented as the median and range. Categorical variables were compared between groups using chi-square or Fisher exact tests. Quantitative variables were compared among experimental groups at each day using Kruskal–Wallis tests followed by multiple pairwise Mann–Whitney *U* tests with Bonferroni correction of *P* values. Correlation between quantitative variables was estimated using Spearman's rho. Linear mixed models were used to estimate the effect of treatment group on viral titres and rectal temperatures. All models included a random effect term for pig and fixed effect terms for treatment group, experimental day, and barrier status (intact versus breakage). A combined model of multiple specimens also included a fixed effect for sample type. Bonferroni correction was used to adjust *P* values for multiple post-hoc comparisons. Statistical analyses were performed in commercially available software (IBM SPSS Statistics Version 22, International Business Machines Corp., Armonk, New York, USA) and results were interpreted at the 5% level of significance.

3. Results

3.1. Preparation of pig challenge virus

Initial inoculation of the cell culture adapted virus via the footpads did not result in clinical disease. Intravenous infection of two other pigs resulted in lesions in one pig three days post infection (dpi). At the third passage, both animals had disseminated disease 2–3 dpi and material collected from these pigs was used in the vaccine study.

3.2. Vaccine efficacy study

All animals in O-UV showed generalised disease, defined as lesions at locations other than the inoculation site, within 48–72 h post-challenge (Table 1). One pig (no. 5) died 2 dpc and necropsy showed infarction in the epicardium. The other four animals (nos. 1–4) showed lesions on all sites between 2 and 4 dpc. On 9 dpc the pigs breached the steel wall and a small hole was created where pigs from O-UV and O-UVC could have direct contact. None of the contact animals in O-UVC showed disease until 13 dpc, when one pig (no. 9) showed lesions on the feet and tongue and was removed. At 14 dpc, when the trial was terminated, necropsy examination of pig no. 10 showed heart lesions but no other lesions were noticed in this or the three remaining pigs (Table 1).

In O-V7, one pig (no. 21) showed secondary lesions on all three feet other than the site of inoculation at 2 dpc. One animal (no. 22) showed lines of infarction on the heart musculature upon necropsy at 14 dpc, while O-UVC7 remained clinically normal.

One pig (no. 12) in O-V4 had secondary lesions on the non-inoculated feet and lower lip at 3–4 dpc. Pig no. 15 developed a lesion on the tongue at 4 dpc that resolved quickly. None of the contact animals in O-UVC4 showed any clinical signs (Table 1). The cumulative incidence of clinical signs over the 14 day study period is presented in Supplementary Table 1.

3.3. Pyrexia and viraemia in pigs as determine by RT-qPCR

Pyrexia was intermittent in most cases (Table 1). Three of the pigs in O-UV had detectable RNA in the blood between 3 and 7 dpc;

Table 1
Summary of the clinical outcome and presence of genomic material and live virus after challenge with O/VIT/05/2010.

Days post challenge		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Groups	ID															
O-UV	**1**		N^{GV} S^{V}	♦ N^{G} S^{GV}	■ N^{GV} S^{GV}	N^{GV} S^{GV}	N^{G} S^{GV}	N^{GV} S^{G}	N^{G}	N^{G}	N^{G} S^{G}	N^{G} S^{G}	N^{G}			ψ
	2		♦ N^{G} S^{G}	■ N^{GV} S^{GV}	N^{G} S^{GV}	N^{GV} S^{GV}	N^{G} S^{GV}	N^{GV} S^{G}	N^{GV}	N^{G} S^{G}	N^{G} S^{G}	N^{G} S^{G}	N^{G}	S^{G}		N^{G}ψ
	3		S^{V}	N^{GV} S^{GV}	■ N^{G} S^{GV}	N^{G} S^{G}	N^{G} S^{G}	N^{GV} S^{GV}	N^{V} S^{G}	N^{G} S^{G}	N^{G} S^{G}	N^{G} S^{G}				ψ
	4		S^{V}	N^{GV} S^{GV}	N^{GV} S^{GV}	N^{G} S^{GV}	■ N^{GV} S^{G}	N^{GV} S^{GV}	N^{GV} S^{G}	N^{G} S^{G}	N^{G} S^{G}	N^{G} S^{G}	S^{G}	N^{G} S^{V}		N^{G} S^{G}ψ
	5		Hψ													
O-UVC	**6**			S^{V}		S^{GV}	S^{G}	N^{V} S^{V}	S^{V}				S^{V}	S^{GV}		N^{G} S^{G}ψ
	7			S^{V}		S^{V}	N^{V}	S^{GV}	S^{G}	S^{GV}	S^{V}			S^{G}		S^{G} ψ
	8				S^{V}	N^{V}	S^{GV}	S^{V}		S^{GV}						S^{G} ψ
	9			N^{V} S^{V}					S^{G}				S^{G}	S^{G}	■ S^{GV}ψ	
	10			S^{V}			S^{V}	S^{G}	N^{V}							H S^{G} ψ
O-V4	**11**		N^{V}	N^{GV} S^{V}	N^{GV} S^{G}	N^{GV} S^{G}	N^{G} S^{G}	N^{G}	N^{G} S^{G}	N^{G} S^{GV}	N^{G}	N^{GV} S^{G}		N^{G} S^{G}	N^{G}	N^{G}ψ
	12			♦ N^{G} S^{G}	■ N^{GV} S^{GV}	N^{G} S^{G}	N^{G}	N^{G} S^{G}	N^{G} S^{G}	N^{G} S^{GV}		N^{G}			N^{G}	ψ
	13		S^{V}	N^{GV} S^{G}	N^{G} S^{G}	N^{G} S^{G}	N^{G} S^{G}		N^{G} S^{G}	N^{G} S^{G}	N^{G}	N^{G} S^{G}	N^{G}		N^{G}	ψ
	14			N^{G} S^{G}	N^{G} S^{G}	N^{G} S^{G}	N^{G}	N^{G} S^{G}		N^{GV} S^{G}		N^{G}	N^{G} S^{G}	N^{G}	N^{G} S^{G}	N^{G}ψ
	15			♦■ N^{G} S^{G}	N^{G} S^{G}	M S^{G}	N^{G} S^{G}	N^{G}	S^{G}	N^{G} S^{G}		S^{G}	N^{G}	N^{V}		N^{G} S^{G}ψ
O-UVC4	**16**		N^{V}	S^{V}	N^{V} S^{V}	N^{GV} S^{V}	N^{G} S^{V}	N^{V}	N^{G}	S^{V}	N^{V} S^{V}	N^{V}	S^{V}	N^{G}	N^{V}	S^{GV}ψ
	17			S^{V}			S^{V}		N^{GV}	N^{GV} S^{V}		N^{G} S^{G}	S^{G}			N^{G}ψ
	18		S^{V}				N^{G}	N^{V}	N^{G}	N^{G} S^{V}		N^{V}	S^{G}	N^{V}	N^{G} S^{G}	ψ
	19			N^{V}	N^{V}	S^{G}	N^{G}	S^{V}	N^{V}	N^{G}	N^{V}	N^{V} S^{V}	S^{G}		N^{G} S^{G}	N^{G}ψ
	20		N^{GV}		N^{G}	S^{V}	S^{V}	N^{GV}	N^{G}	N^{G}		N^{V}				ψ
O-V7	**21**		♦N^{GV} S^{GV}	■ N^{G} S^{G}	N^{G} S^{GV}	N^{GV} S^{G}	N^{GV} S^{G}	N^{G} S^{V}			N^{G}	N^{V} S^{V}	S^{V}	S^{V}	N^{G} S^{V}	N^{V}ψ
	22		♦N^{GV} S^{GV}	N^{GV} S^{GV}	N^{G} S^{G}	N^{G}	N^{V}		N^{G} S^{GV}	N^{G}		N^{G}	S^{V}			Hψ
	23		N^{G} S^{G}	N^{G} S^{G}	N^{G} S^{G}	N^{G}	N^{GV}		N^{G} S^{GV}	N^{GV}	N^{GV} S^{G}	S^{V}	N^{G}		N^{G} S^{G}	S^{V}ψ
	24		♦ ■ N^{G} S^{G}	N^{G} S^{GV}	N^{G}	S^{V}	S^{GV}	N^{G} S^{V}	S^{V}	N^{G} S^{G}			S^{G}		N^{G} S^{V}	N^{G}ψ
	25		N^{G} S^{G}	N^{G} S^{G}	N^{G}	N^{G}	N^{G}	N^{GV}		N^{G}	N^{G}	S^{V}	S^{V}	S^{V}	N^{G}	ψ
O-UVC7	**26**		N^{V}	S^{V}												ψ
	27															ψ
	28			S^{V}												ψ
	29		S^{V}		N^{V} S^{V}											ψ
	30															ψ

♦ Lesion at site of inoculation; ■ Lesion at any other site including feet, mouth, tongue and snout, indicating disseminated disease; H—lesion in heart muscle; ψ Animal died/Euthanised; shaded boxes—temperature ≥40 °C; N^{G} S^{G}—Viral RNA detected in nasal and saliva swabs respectively by RT-PCR; N^{V} S^{V}—virus detected in nasal and saliva swabs respectively by virus isolation on cell culture; N^{GV} S^{GV}—both RNA and live virus detected in the nasal and saliva swabs, respectively.

pig no. 2 was positive at 3 dpc, pig no. 3 at 7 dpc, with only pig no. 4 showing RNA on two consecutive days (4–5 dpc). One pig in O-V4 tested positive at 7 dpc. The levels of RNA were variable (8.7×10^3–2.7×10^6 copy numbers). Surprisingly, one pig in O-UVC had 7.9×10^5 copy numbers in the blood at 14 dpc (results not shown).

3.4. Detection of FMDV by RT-qPCR and virus isolation from nasal and saliva swab samples

Samples were deemed positive when either RNA was detected or virus isolated. Saliva swabs from all surviving pigs in O-UV were positive from 1 to 10 dpc, while pig no. 4 tested positive for viral RNA until 14 dpc (Table 1). Nasal swabs were positive between 1 and 7 dpc, while pigs nos. 2 and 4 tested positive until 14 dpc. Pigs in O-UVC tested positive for virus in the saliva swabs from 2 dpc. Before the breach at 9 dpc, three of the five pigs had live virus isolated from the saliva at one or more time points.

All pigs in O-V7 tested positive in both oral and nasal swabs at 1 dpc. From 3 dpc, the recovery from swabs was intermittent but present in at least three pigs daily until 14 dpc. Live virus was isolated from the nasal swab of one pig until 9 dpc. Viral RNA was detected in the nasal and saliva swabs of three of the contact pigs (O-UVC7) between 1 and 3 dpc but not subsequently.

Virus was detected in either the nasal or saliva swabs of pigs nos. 11 and 13 in O-V4 at 1 dpc. At 2 dpc, all samples were positive. From 6 dpc the detection was intermittent, but RNA was still detected in swabs at 14 dpc. Contact pigs (O-UVC4) were also positive from 1 dpc, with live virus found in nasal and saliva swabs of three pigs on that day. Live virus was also recovered from the saliva of one pig at 14 dpc (Table 1).

3.5. Antibody responses in the different groups of pigs

Only two pigs that were vaccinated seven days prior to challenge (O-V7: nos. 23 and 24) were sero-positive to the structural proteins on the day of challenge (Table 2). At 5 dpc, all five pigs in O-V7 and O-V4 had sero-converted including one pig (no. 2) in O-UV that had not been vaccinated, but challenged. By 10 dpc, all the challenged pigs were sero-positive. In the contact groups, sero-conversion was detected in a single pig (no. 7) in O-UVC between 10 and 14 dpc.

None of the pigs had detectable antibodies to FMDV NSP until 10 dpc when all pigs in O-UV were positive and remained so until 14 dpc. One of the contact pigs (no. 7) in O-UVC had antibodies to the NSP at 14 dpc. One pig (no. 12) in O-V4 was sero-positive on both 10 and 14 dpc, while two pigs in O-V7 (nos. 24 and 25) were sero-positive only on 10 dpc (Table 2).

3.6. Comparison of the viral RNA recovered from samples across the different groups

The amount of viral RNA detected from the saliva swabs was statistically different among all treatment groups when evaluated for all sampling times (Table 3; $P < 0.001$). Furthermore, both vaccinated groups had significantly less RNA compared to the unvaccinated challenged O-UV; however there was no significant difference between O-V4 and O-V7. Pigs in O-UV had significantly more detectable RNA than O-V7 at 4, 6, 9, 10, and 14 dpi. There were no significant differences observed between O-UV

Table 2
Results of cELISA for antibodies against structural proteins against FMDV and NSP antibody ELISA.

Group	Pig ID	cELISA					NSP antibody ELISA				
		0 dpc	5 dpc	7 dpc	10 dpc	14 dpc	0 dpc	5 dpc	7 dpc	10 dpc	14 dpc
O-UV	1	Neg	Neg		Pos	Pos	Neg	Neg		Pos	Pos
	2	Neg	Pos		Pos	Pos	Neg	Neg		Pos	Pos
	3	Neg	Neg		Pos	Pos	Neg	Neg		Pos	Pos
	4	Neg	Neg		Pos	Pos	Neg	Neg		Pos	Pos
	5	Neg	–	–	–	–	Neg	–	–	–	–
O-UVC	6	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
	7	Neg		Neg	Pos	Pos	Neg		Neg	Neg	Pos
	8	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
	9	Neg		Neg	Neg	Neg	Neg		Neg	Neg	–
	10	Neg		Neg		Neg	Neg		Neg		Neg
O-V4	11	Neg	Pos		Pos	Pos	Neg	Neg		Neg	Neg
	12	Neg	Pos		Pos	Pos	Neg	Neg		Pos	Pos
	13	Neg	Pos		Pos	Pos	Neg	Neg		Neg	Neg
	14	Neg	Pos		Pos	Pos	Neg	Neg		Neg	Neg
	15	Neg	Pos		Pos	Pos	Neg	Neg		Neg	Neg
O-UVC4	16	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
	17	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
	18	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
	19	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
	20	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
O-V7	21	Neg	Pos		Pos	Pos	Neg	Neg		Neg	Neg
	22	Neg	Pos		Pos	Pos	Neg	Neg		Neg	Neg
	23	Pos	Pos		Pos	Pos	Neg	Neg		Neg	Neg
	24	Pos	Pos		Pos	Pos	Neg	Neg		Pos	Neg
	25	Neg	Pos		Pos	Pos	Neg	Neg		Pos	Neg
O-UVC7	26	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
	27	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
	28	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
	29	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg
	30	Neg		Neg	Neg	Neg	Neg		Neg	Neg	Neg

Pos: positive; Neg: negative; – animal removed.

and O-V4 or between O-V4 and O-V7 at any individual sampling day.

In nasal swabs, the amount of viral RNA detected was statistically different among all treatment groups when evaluated over all days (Table 4; $P < 0.001$). Furthermore, O-V7 group had significantly less detectable RNA compared to both O-UV and O-V4. The amount of detectable RNA was not different between O-UV and O-V4. The amount of detectable RNA was different between challenged and contact groups for multiple experimental days but there were no detectable differences among O-UV, O-V4 and O-V7 at any individual experimental day.

Levels of RNA detected in faecal swabs were statistically different between all treatment groups when evaluated over all sampling times (Supplementary Table 2; $P < 0.001$). Furthermore, O-UV had significantly more detectable RNA compared to O-V4 but not O-V7.

When adjusting for the observed barrier breakage in a multivariable model, the amount of RNA detected was different among the treatment groups (Table 5; $P < 0.001$). Pigs within both vaccinated groups had less detectable RNA than O-UV. O-V7 had less detectable RNA compared to O-V4 but the overlapping confidence intervals suggested that the difference was not statistically different. The amount of FMDV RNA detected varied by specimen type with the lowest levels in blood and the highest in nasal swabs. Overlapping confidence intervals for nasal and oral swabs suggested that the difference between these two specimen types was not statistically significant.

The amount of FMDV RNA detected in the blood was positively correlated with the presence of clinical lesions (rho = 0.211, $P < 0.001$).

4. Discussion

Most vaccine efficacy studies are performed with homologous challenge and there is a dearth of information on protection when there are genetic and antigenic differences between the vaccine strain and challenge virus. Antigenic matching results between the challenge virus and the O_1 Manisa vaccine are not available, however, comparisons between another virus that was isolated during 2010 in Vietnam indicated an acceptable match (Annual reports of the World Reference Laboratory 2010, 2011). In this study, there was 80% and 60% protection in pigs vaccinated seven and four days prior to a heterologous challenge, respectively, indicating that vaccination can provide some protection at early time points. Similar results were obtained when pigs were challenged by exposure to aerosols generated by infected pigs [4], a method that is seen as less traumatic and a more natural route of exposure compared to the coronary band route used in our study. However, pigs vaccinated four and seven days prior to exposure could infect cohorts with which they had direct contact [4,28,29] indicating that vaccination does not fully prevent disease transmission within a pen soon after vaccination whereas those vaccinated 14 days prior to infection did not infect their cohorts [28].

More importantly, vaccination lowered the amount of virus excreted compared to the unvaccinated challenged controls, although there was not a statistically significant difference between the two vaccine groups. This is in agreement with other vaccine experiments [28–32] although in at least one experiment the pigs vaccinated seven days before challenge excreted more virus than the infected controls on some occasions [28]. Therefore although vaccinated pigs may still become infected, the overall viral load

Table 3
Median (range) viral RNA copy numbers recovered from saliva swabs via PCR. All contact animals were unvaccinated.

Day PC	Unvaccinated		Exposed 4 days post vaccination		Exposed 7 days post vaccination		*P* value*
	Challenged (O-UV)	Contact (O-UVC)	Challenged (O-V4)	Contact (O-UV4)	Challenged (O-V7)	Contact (O-UV7)	
1	NGD[a,b] (NGD, 1.3E+2)	NGD[b]	NGD[b]	NGD[b]	2.8E+4[a] (NGD, 9.2E+4)	NGD[b]	0.002
2	6.7E+5[a] (6.1E+3, 2.5E+6)	NGD[b]	1.8E+4[a,b] (NGD, 1.2E+5)	NGD[b]	1.0E+5[a] (6.0E+3, 1.0E+7)	NGD[b]	<0.001
3	6.7E+5[a] (2.9E+5, 2.3E+6)	NGD[b]	4.0E+5[a,b] (7.4E+4, 1.8E+6)	NGD[b]	1.2E+4[a] (NGD, 2.4E+7)	NGD[b]	0.001
4	7.9E+5[a] (4.0E+5, 1.2E+6)	NGD[b] (NGD, 1.0E+3)	5.2E+3[a,b] (5.3E+2, 7.5E+4)	NGD[b] (NGD, 4.3E+4)	NGD[b] (NGD, 2.2E+5)	NGD[b]	0.001
5	7.5E+5[a] (1.5E+5, 5.8E+6)	NGD[a,b] (NGD, 8.9E+3)	8.6E+2[a,b] (NGD, 5.7E+3)	NGD[b]	NGD[a,b] (NGD, 3.5E+4)	NGD[b]	0.004
6	7.8E+4[a] (3.2E+4, 2.4E+5)	NGD[a,b] (NGD, 1.0E+7)	5.1E+2[a,b] (NGD, 2.1E+4)	NGD[b]	NGD[b]	NGD[b]	0.003
7	9.4E+4 (NGD, 7.2E+6)	NGD (NGD, 2.5E+6)	1.2E+3 (NGD, 1.9E+4)	NGD	NGD (NGD, 4.7E+3)	NGD	0.131
8	3.4E+3[a] (NGD, 2.4E+4)	NGD[a] (NGD, 4.0E+5)	1.4E+3[a] (4.2E+2, 4.4E+3)	NGD[a]	NGD[a] (NGD, 2.8E+3)	NGD[a]	0.018
9	1.3E+3[a] (1.6E+2, 1.2E+4)	NGD[b]	NGD[b]	NGD[b]	NGD[b] (NGD, 3.5E+3)	NGD[b]	0.001
10	1.1E+4[a] (1.2E+3, 1.4E+4)	NGD[b]	3.7E+2[a,b] (NGD, 9.5E+3)	NGD[a,b] (NGD, 2.0E+3)	NGD[b]	NGD[b]	0.002
11	NGD (NGD, 1.9E+2)	NGD (NGD, 5.3E+2)	NGD (NGD, 7.6E+2)	1.7E+3 (NGD, 2.9E+3)	NGD (NGD, 1.7E+3)	NGD	0.237
12	NGD (NGD, 2.2E+2)	1.3E+3 (NGD, 1.5E+5)	NGD (NGD, 1.9E+3)	NGD	NGD	NGD	0.087
13	ND	4.4E+4 (4.4E+4, 4.4E+4)	NGD (NGD, 3.5E+2)	NGD (NGD, 2.6E+3)	NGD (NGD, 5.2E+3)	NGD	0.148
14	NGD[a] (NGD, 2.0E+2)	1.6E+4[a,b] (2.4E+3, 2.5E+4)	NGD[a,b] (NGD, 6.2E+4)	NGD[a,b] (NGD, 2.6E+3)	NGD[b]	NGD[b]	0.010
Overall	1.3E+3[a] (NGD, 7.2E+6)	NGD[b,d] (NGD, 1.0E+7)	NGD[c] (NGD, 1.8E+6)	NGD[b] (NGD, 4.3E+4)	NGD[c,d] (NGD, 2.4E+7)	NGD[b]	<0.001†

PC = post-challenge. NGD = no genome detected. ND = no testing done.
* Based on Kruskal–Wallis tests for a difference among the 6 treatment groups. Medians without superscripts in common are significantly different based on pairwise Mann–Whitney *U* tests after Bonferroni correction of *P* values.
† Based on mixed-effects linear regression over all days. Medians without superscripts in common are significantly different based on model results after Bonferroni correction of *P* values.

and infectious period may be significantly reduced in a facility [28,32,33] thereby assisting in disease control.

Live virus and/or viral RNA could be detected in the excretions intermittently until the study was terminated at 14 dpc, similar to other short term experiments where detection lasted until 11 dpc [28–30]. In one long term study, RNA was detected up to 28 dpc in the saliva and probang of a vaccinated pig [31]. The implications of these findings are not clear, as in most instances no live virus could be isolated.

The study furthermore aimed to determine if transmission would occur to unvaccinated pigs when kept in close, but indirect contact with vaccinated and infected pigs. The pigs kept next to the unvaccinated challenged controls only showed disease at 13 and 14 dpc. This followed a break in the wall that allowed pigs to have direct contact from 9 dpc. However, live virus and viral RNA were detected in these pigs from 2 dpc indicating that despite the absence of clinical signs and lack of sero-conversion, these pigs were exposed to live virus. Van Roermund [32] showed that walls between groups of pigs reduced transmission 10- to 20-fold compared to within pen transmission. In addition, when pigs were separated by a wooden wall, transmission was delayed to three days after exposure, compared to within pen transmission that usually occurred within one day. Airborne transmission seems to be relatively rare when pigs don't have direct contact [34] and it is speculated that faeces and urine may also cause transmission [32]. In our experiment it was similarly possible that these excretions may have run between the pens, and this was certainly observed with groups O-UV and O-UVC. The delayed infection could therefore be due to the lower amount of RNA found in faeces compared to nasal and saliva samples.

Temperature was positively correlated with the presence of clinical lesions (rho = 0.141, $P = 0.003$) but not viraemia (rho = 0.081, $P = 0.193$). Three pigs had elevated temperatures on 0 dpc, possibly due to stress. Viral RNA was detected only in the blood of needle infected pigs on very few occasions between 3 and 7 dpc and not in any of the pigs in group O-V7. The contact pig in O-UVC that had RNA in the blood at 14 dpc, also showed evidence of heart lesions and viral RNA in the saliva on that day. All pigs in O-UV had antibodies to the NSP from 10 to 14 dpc indicating that sufficient virus replication had occurred to stimulate antibodies.

The detection of antibodies to the NSP is an important tool during post-outbreak surveillance. Two pigs in O-V7 (nos. 24 and

Table 4
Median (range) viral RNA copy numbers recovered from nasal swabs via PCR. All contact animals were unvaccinated.

Day PC	Unvaccinated		Exposed 4 days post vaccination		Exposed 7 days post vaccination		*P* value*
	Challenged (O-UV)	Contact (O-UVC)	Challenged (O-V4)	Contact (O-UV4)	Challenged (O-V7)	Contact (O-UV7)	
1	NGD[a,b] (NGD, 1.2E+4)	NGD[a]	NGD[a]	NGD[a] (NGD, 1.3E+3)	2.7E+5[b] (1.1E+4, 4.9E+6)	NGD[a]	0.001
2	1.8E+5[a] (4.2E+4, 2.1E+5)	NGD[b]	1.7E+3[a,b] (3.4E+2, 8.6E+4)	NGD[b]	5.5E+5[a] (2.8E+4, 6.9E+5)	NGD[b]	<0.001
3	1.4E+5[a] (3.4E+3, 6.1E+5)	NGD[b]	4.9E+4[a] (1.1E+3, 6.9E+5)	NGD[a,b] (NGD, 1.4E+3)	3.9E+3[a,b] (2.1E+3, 1.5E+5)	NGD[b]	<0.001
4	1.2E+5[a] (7.4E+3, 3.8E+5)	NGD[b]	1.1E+4[a,b] (NGD, 1.0E+5)	NGD[a,b] (NGD, 3.5E+3)	2.4E+3[a,b] (NGD, 1.1E+4)	NGD[b]	0.001
5	2.8E+5[a] (5.4E+4, 3.1E+5)	NGD[b]	1.2E+3[a,b] (6.4E+2, 2.0E+5)	4.8E+3[a,b] (NGD, 5.6E+4)	1.2E+4[a,b] (NGD, 4.1E+4)	NGD[b]	0.002
6	5.8E+5[a] (1.7E+5, 7.8E+5)	NGD[b]	5.1E+2[a,b] (3.1E+2, 1.5E+4)	NGD[b] (NGD, 5.2E+3)	1.2E+3[a,b] (NGD, 4.6E+4)	NGD[b]	0.001
7	1.3E+3 (NGD, 1.4E+5)	NGD	2.6E+3 (NGD, 1.0E+5)	3.7E+3 (NGD, 1.2E+4)	NGD (NGD, 3.7E+4)	NGD	0.071
8	2.9E+3[a] (7.2E+2, 4.4E+5)	NGD[a]	7.4E+2[a] (2.9E+2, 3.1E+4)	2.8E+3[a] (NGD, 1.1E+4)	4.2E+3[a] (NGD, 3.8E+5)	NGD[a]	0.005
9	8.5E+3[a] (5.6E+3, 2.9E+4)	NGD[b]	NGD[a,b] (NGD, 2.5E+2)	NGD[b]	9.2E+2[a,b] (NGD, 4.8E+3)	NGD[b]	0.001
10	4.4E+3[a] (1.9E+3, 1.2E+4)	NGD[b]	2.8E+3[a,b] (NGD, 1.5E+7)	NGD[a,b] (NGD, 2.1E+3)	NGD[a,b] (NGD, 4.3E+3)	NGD[b]	0.003
11	7.8E+1 (NGD, 1.4E+3)	NGD	2.4E+2 (NGD, 4.1E+3)	NGD	NGD (NGD, 1.5E+4)	NGD	0.084
12	NGD (NGD, 3.8E+2)	NGD	NGD (NGD, 4.5E+2)	NGD (NGD, 2.4E+4)	NGD (NGD, 1.2E+4)	NGD	0.611
13	ND	NGD	8.7E+2 (NGD, 6.8E+3)	NGD (NGD, 5.8E+3)	6.0E+3 (NGD, 5.0E+4)	NGD	0.054
14	1.0E+2 (NGD, 3.4E+2)	NGD	2.5E+2 (NGD, 2.8E+3)	NGD (NGD, 2.2E+3)	NGD (NGD, 1.6E+3)	NGD	0.380
Overall	4.1E+3[a] (NGD, 7.8E+5)	NGD[b]	5.2E+2[a,c] (NGD, 1.5E+7)	NGD[d] (NGD, 5.6E+4)	2.1E+3[c] (NGD, 4.9E+6)	NGD[b]	<0.001†

PI = post-challenge. NGD = no genome detected. ND = no testing done.
* Based on Kruskal–Wallis tests for a difference among the 6 treatment groups. Medians without superscripts in common are significantly different based on pairwise Mann–Whitney *U* tests after Bonferroni correction of *P* values.
† Based on mixed-effects linear regression over all days. Medians without superscripts in common are significantly different based on model results after Bonferroni correction of *P* values.

Table 5
Multivariable model results including viraemia.

Variable	Estimate (95%CI)	*t* Statistic	*P* value
Experimental group			<0.001
Contact with unvaccinated (O-UVC)	−6.95 (−8.06, −5.85)	−12.353	<0.001
Contact with vaccinated pigs challenge 4 dpv (O-UVC4)	−5.38 (−6.31, −4.44)	−11.309	<0.001
Contact with vaccinated pigs challenged 7 dpv (O-UVC7)	−7.13 (−8.06, −6.20)	−14.996	<0.001
Vaccinated 4 days prior to challenge (O-V4)	−1.99 (−2.90, −1.09)	−4.321	<0.001
Vaccinated 7 days prior to challenge (O-V7)	−2.79 (−3.69, −1.88)	−6.049	<0.001
Unvaccinated challenged (O-UV)	Referent		
Barrier			
Breakage	3.30 (1.82, 4.77)	4.392	<0.001
Intact	Referent		
			<0.001
Sample type			
Nasal swab	5.22 (4.50, 5.95)	14.092	<0.001
Oral swab	4.38 (3.66, 5.11)	11.825	<0.001
Whole blood	Referent		

CI = confidence interval. Model estimates for the variables associated with days post-inoculation are not presented in the table. Pig sex was not significant ($P = 0.198$) so it was removed from the final model. All treatment groups were significantly different ($P < 0.05$) except for the comparisons of O-UVC and O-UVC4 ($P = 0.07$). Nasal and oral swab values were significantly different ($P = 0.018$).

25) were sero-positive for NSP antibodies on only 10 dpc but not on 14 dpc, probably indicating a low level of virus replication, confirming findings from other studies that showed vaccination resulted in a lower and shorter duration of responses to NSP [29]. Both these pigs had lesions only at the site of inoculation. One pig in O-V4 that had generalised disease, sero-converted on 10 dpc and remained positive until 14 dpc whilst one other pig in that group that had lesions on the inoculated foot away from the inoculation site, remained negative. None of the indirect contact pigs in O-UVC4 and O-UVC7 sero-converted to NSP antibodies. However, viral RNA and on occasion live virus, could be detected in saliva and nasal swabs of most of these pigs. It is therefore possible that the NSP tests may fail to identify all infected pigs during an outbreak.

Although vaccination did not protect all the challenged pigs, it decreased the amount of virus excreted and remains an important tool for control during an outbreak.

Conflict of interest statement

All the authors have read and approved the manuscript and there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.04.045

References

[1] Sobrino F, Saiz M, Jimenez-Clavero MA, Nunez JI, Rosas MF, Baranowski E, et al. Foot-and-mouth disease virus: a long known virus, but a current threat. Vet Res 2001;32:1–30.
[2] Salt JS, Williams L, Statham R, Barnett PV. Further studies on the rate of development of protection in cattle given emergency vaccination against FMD. In: Report of the session of the FAO research group of the standing technical committee of the European Commission of Foot and Mouth Disease, September 19–22. Vienna, Austria: Moedling; 1995. p. 90–7. Appendix 17.
[3] Doel TR, Williams L, Barnett PV. Emergency vaccination against foot-and-mouth disease: rate of development of immunity and its implications for the carrier state. Vaccine 1994;12:592–600.
[4] Salt JS, Barnett PV, Dani P, Williams L. Emergency vaccination of pigs against foot-and-mouth disease: protection against disease and reduction in contact transmission. Vaccine 1998;16:746–54.
[5] Barnett PV, Cox SJ, Aggarwal N, Gerber H, McCullough KC. Further studies on the early protective responses of pigs following immunisation with high potency foot and mouth disease vaccine. Vaccine 2002;20:3197–208.
[6] Garland AJM. The inhibitory activity of secretions in cattle against FMDV. London: London School of Hygiene and Tropical Medicine; 1974 (PhD thesis). ⟨http://researchonline.lshtm.ac.uk/878722/⟩.
[7] Sellers RF, Herniman KA, Gumm ID. The airborne dispersal of foot-and-mouth disease virus from vaccinated and recovered pigs, cattle and sheep after exposure to infection. Res Vet Sci 1977;23:70–5.
[8] Brehm KE, Kumar N, Thulke HH, Haas B. High potency vaccines induce protection against heterologous challenge with foot-and-mouth disease virus. Vaccine 2008;26:1681–7.
[9] Nagendrakumar SB, Srinivasan VA, Madhanmohan M, Yuvaraj S, Parida S, Di Nardo A, et al. Evaluation of cross-protection between O_1 Manisa and O_1 Campos in cattle vaccinated with foot-and-mouth disease virus vaccine incorporating different payloads of inactivated O_1 Manisa antigen. Vaccine 2011;29:1906–12.
[10] Maradei E, Perez Beascoechea C, Malirat V, Salgado G, Seki C, Pedemonte A, et al. Characterization of foot-and-mouth disease virus from outbreaks in Ecuador during 2009–2010 and cross-protection studies with the vaccine strain in use in the region. Vaccine 2011;29:8230–40.
[11] Gleeson LJ. A review of the status of foot and mouth disease in South-East Asia and approaches to control and eradication. Rev Sci Tech OIE 2002;21:465–75.
[12] Rweyemamu M, Roeder P, Mackay D, Sumption K, Brownlie J, Leforban Y, et al. Epidemiological patterns of foot-and-mouth disease worldwide. Transboundary Emerg Dis 2008;55:57–72.
[13] Knowles NJ, He J, Shang Y, Wadsworth J, Valdazo-Gonzalez B, Onosato H, et al. Southeast Asian foot-and-mouth disease viruses in Eastern Asia. Emerg Infect Dis 2012;18:499–501.
[14] Le VP, Nguyen T, Lee KN, Ko YJ, Lee HS, Nguyen VC, et al. Molecular characterization of serotype A foot-and-mouth disease viruses circulating in Vietnam in 2009. Vet Microbiol 2010;144:58–66.
[15] Le VP, Nguyen T, Park JH, Kim SM, Ko YJ, Lee HS, et al. Heterogeneity and genetic variations of serotypes O and Asia 1 foot-and-mouth disease viruses isolated in Vietnam. Vet Microbiol 2010;145:220–9.
[16] Abdul-Hamid NF, Firat-Sarac M, Radford AD, Knowles NJ, King DP. Comparative sequence analysis of representative foot-and-mouth disease virus genomes from Southeast Asia. Virus Genes 2011;43:41–5.
[17] Donaldson AI, Gloster J, Harvey LD, Deans DH. Use of prediction models to forecast and analyse airborne spread during the foot-and-mouth disease outbreaks in Brittany, Jersey and the Isle of Wight in 1981. Vet Rec 1982;110:53–7.
[18] Gloster J, Sellers RF, Donaldson AI. Long distance transport of foot-and-mouth disease virus over the sea. Vet Rec 1982;110:47–52.
[19] Gloster J, Blackall RM, Sellers RF, Donaldson AI. Forecasting the airborne spread of foot-and-mouth disease. Vet Rec 1981;108:370–4.
[20] Abdul-Hamid NF, Hussein NM, Wadsworth J, Radford AD, Knowles NJ, King DP. Phylogeography of foot-and-mouth disease virus types O and A in Malaysia and surrounding countries. Infect Genet Evol 2011;11:320–8.
[21] Burrows R. The behaviour of some modified strains of foot-and-mouth disease virus in the pig. I. Innocuity. Bull Off Int Epiz 1964;61:1251–75.
[22] Burrows R. The infectivity assay of foot-and-mouth disease virus in pigs. J Hyg (Lond) 1966;64:419–29.
[23] Roeder PL, Le Blanc Smith PM. Detection and typing of foot-and-mouth disease virus by enzyme-linked immunosorbent assay: a sensitive, rapid and reliable technique for primary diagnosis. Res Vet Sci 1987;43:225–32.
[24] Shaw AE, Reid SM, Ebert K, Hutchings GH, Ferris NP, King DP. Implementation of a one-step real-time RT-PCR protocol for diagnosis of foot-and-mouth disease. J Virol Methods 2007;143:81–5.
[25] Boyle DB, Taylor T, Cardoso M. Implementation in Australia of molecular diagnostic techniques for the rapid detection of foot and mouth disease virus. Aust Vet J 2004;82:421–5.
[26] Mackay DK, Bulut AN, Rendle T, Davidson F, Ferris NP. A solid-phase competition ELISA for measuring antibody to foot-and-mouth disease virus. J Virol Methods 2001;97:33–48.
[27] Foord AJ, Muller JD, Yu M, Wang LF, Heine HG. Production and application of recombinant antibodies to foot-and-mouth disease virus non-structural protein 3ABC. J Immunol Methods 2007;321:142–51.
[28] Eble PL, Bouma A, de Bruin MG, van Hemert-Kluitenberg F, van Oirschot JT, Dekker A. Vaccination of pigs two weeks before infection significantly reduces transmission of foot-and-mouth disease virus. Vaccine 2004;22:1372–8.
[29] Eble PL, Bouma A, Weerdmeester K, Stegeman JA, Dekker A. Serological and mucosal immune responses after vaccination and infection with FMDV in pigs. Vaccine 2007;25:1043–54.
[30] Orsel K, de Jong MC, Bouma A, Stegeman JA, Dekker A. Foot and mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. Vaccine 2007;25:6381–91.
[31] Parida S, Fleming L, Oh Y, Mahapatra M, Hamblin P, Gloster J, et al. Reduction of foot-and-mouth disease (FMD) virus load in nasal excretions, saliva and exhaled air of vaccinated pigs following direct contact challenge. Vaccine 2007;25:7806–17.
[32] van Roermund HJ, Eble PL, de Jong MC, Dekker A. No between-pen transmission of foot-and-mouth disease virus in vaccinated pigs. Vaccine 2010;28:4452–61.
[33] Eble PL, de Koeijer AA, de Jong MCM, Engel B, Dekker A. A meta-analysis quantifying transmission parameters of FMDV strain O Taiwan among non-vaccinated and vaccinated pigs. Prev Vet Med 2008;83:98–106.
[34] Alexandersen S, Donaldson AI. Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs. Epidemiol Infect 2002;128:313–23.

Supplementary Table 1. Cumulative incidence (frequency) of clinical signs during the 14 day study period.

	Unvaccinated		Exposed 4 days post vaccination		Exposed 7 days post vaccination		
Clinical outcome	**Challenged**	**Contact**	**Challenged**	**Contact**	**Challenged**	**Contact**	**P value***
Temp ≥40	3/5	1/5	2/5	2/5	1/5	3/5	1.0
Lesion RF	4/5	0/5	0/5	0/5	0/5	0/5	0.100
Lesion LF	3/5	0/5	1/5	0/5	1/5	0/5	0.042
Lesion RH	4/5	0/5	1/5	0/5	1/5	0/5	0.017
Lesion LH	3/5	1/5	1/5	0/5	1/5	0/5	0.093
Lesion tongue	3/5	0/5	2/5	0/5	0/5	0/5	0.042
Lesion mouth	1/5	0/5	1/5	0/5	0/5	0/5	0.483
Lesion snout	1/5	0/5	0/5	0/5	0/5	0/5	1.0
Lesion heart	1/5	0/5	0/5	0/5	1/5	0/5	1.0
Death	1/5	1/5	0/5	0/5	0/5	0/5	1.0
Any lesion or death	5/5	1/5	2/5	0/5	2/5	0/5	0.002
cELISA positive	4/5†	1/5	5/5	0/5	5/5	0/5	<0.001
NSP ELISA positive	4/5†	1/5	1/5	0/5	2/5	0/5	0.019
Oral swap VI positive	4/5†	5/5	3/5	5/5	5/5	3/5	1.0
Nasal swab VI positive	4/5†	5/5	5/5	5/5	5/5	2/5	0.598
Either specimen VI positive	4/5†	5/5	5/5	5/5	5/5	4/5	1.0

VI = virus isolation.

*Chi-square or Fisher exact tests comparing frequency of clinical outcomes in experimentally challenged versus in-contact pigs.

†One pig died prior to seroconversion or isolation of the virus

Supplementary Table 2. Median (range) viral RNA copy numbers recovered from fecal swabs via PCR. All contact animals were unvaccinated.

Day	Unvaccinated		Exposed 4 days post vaccination		Exposed 7 days post vaccination		P
PC	Challenged (O-UV)	Contact (O-UVC)	Challenged (O-V4)	Contact (O-UV4)	Challenged (O-V7)	Contact (O-UV7)	value*
1	NGD[a]	NGD[a]	NGD[a]	NGD[a]	1.3E+3[b] (NGD, 3.8E+3)	NGD[a]	<0.001
2	NGD (NGD, 1.0E+4)	NGD	NGD	NGD	2.2E+3 (NGD, 1.5E+4)	NGD (NGD, 4.2E+2)	0.083
3	8.2E+2 (2.2E+2, 2.1E+3)	NGD	4.6E+2 (NGD, 5.5E+3)	NGD (NGD, 1.3E+5)	6.1E+1 (NGD, 7.1E+2)	NGD (NGD, 1.2E+3)	0.076
4	2.2E+3 (3.6E+2, 8.3E+3)	NGD	3.9E+2 (NGD, 2.8E+3)	NGD (NGD, 3.3E+3)	NGD (NGD, 9.8E+2)	1.5E+3 (NGD, 2.2E+4)	0.072
5	2.2E+3[a] (1.5E+2, 6.8E+3)	NGD[a] (NGD, 4.2E+2)	NGD[a] (NGD, 4.6E+2)	5.7E+3[a] (6.8E+2, 1.1E+4)	2.1E+2[a] (NGD, 1.1E+3)	2.3E+3[a] (NGD, 7.5E+4)	0.020
6	2.2E+2[a] (7.0E+1, 6.4E+3)	NGD[a]	NGD[a] (NGD, 3.7E+2)	NGD[a] (NGD, 6.0E+3)	3.4E+1[a] (NGD, 1.8E+2)	1.5E+3[a] (NGD, 8.5E+6)	0.041
7	ND	NGD	NGD (NGD, 2.0E+1)	2.0E+3 (NGD, 4.5E+3)	NGD (NGD, 1.1E+2)	NGD (NGD, 8.2E+2)	0.177
8	1.0E+0 (1.0E+0, 1.0E+0)	NGD (NGD, 4.6E+2)	NGD (NGD, 1.3E+2)	1.9E+3 (NGD, 4.1E+3)	NGD (NGD, 5.3E+1)	NGD (NGD, 2.1E+3)	0.187
9	9.4E+1 (1.0E+0, 4.2E+2)	NGD (NGD, 9.4E+3)	NGD	NGD (NGD, 9.6E+2)	NGD (NGD, 1.3E+2)	NGD (NGD, 1.1E+3)	0.097
10	1.0E+0[a] (1.0E+0, 1.0E+0)	NGD[a]	NGD[a]	5.4E+2[a] (NGD, 2.8E+3)	NGD[a] (NGD, 3.4E+1)	3.8E+2[a] (NGD, 1.7E+4)	0.029
11	1.0E+0[a] (1.0E+0, 1.0E+0)	NGD[b]	NGD[b]	NGD[a,b] (NGD, 3.2E+3)	NGD[b]	NGD[a,b] (NGD, 1.5E+3)	0.012
12	1.0E+0[a] (1.0E+0, 1.0E+0)	NGD[b]	NGD[b]	NGD[a,b] (NGD, 9.8E+2)	NGD[b]	NGD[a,b] (NGD, 1.2E+3)	0.007
13	ND	NGD[a]	NGD[a]	1.8E+3[a,b] (1.1E+3, 3.1E+3)	NGD[a]	1.3E+4[b] (8.2E+2, 6.9E+4)	<0.001
14	1.0E+0[a,b] (1.0E+0, 1.2E+0)	1.0E+0[a,b] (NGD, 5.0E+2)	NGD[a]	NGD[a,b] (NGD, 1.7E+3)	NGD[a]	1.2E+3[b] (NGD, 2.5E+4)	0.011
Overall	1.0E+0[a] (NGD, 1.0E+4)	NGD[b] (NGD, 9.4E+3)	NGD[b,c] (NGD, 5.5E+3)	NGD[a] (NGD, 1.3E+5)	NGD[a,c] (NGD, 1.5E+4)	NGD[a] (NGD, 8.5E+6)	<0.001†

PI = Post-challenge. NGD = no genome detected. ND = no testing done.

*Based on Kruskal-Wallis tests for a difference among the 6 treatment groups. Medians without superscripts in common are significantly different based on pairwise Mann-Whitney U tests after Bonferroni correction of P values.

†Based on mixed-effects linear regression over all days. Medians without superscripts in common are significantly different based on model results after Bonferroni correction of P values.