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Protective effects of high-potency FMDV O_1 Manisa monovalent vaccine in cattle challenged with FMDV O/SKR/2010 at 7 or 4 days post vaccination

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ABSTRACT

Serotype O foot-and-mouth disease (FMD) virus belonging to the SEA topotype continues to be a significant problem in the Eastern Asia region, with outbreaks in Japan and South Korea resulting in the culling of over 3.5 million cattle and pigs in recent years. High-potency O1 Manisa vaccine was previously shown to provide protection in cattle 21 days post vaccination (dpv) following challenge with a representative virus, O/SKR/2010. This study tested the ability of the O1 Manisa vaccine to protect cattle from infection and disease with the O/SKR/2010 virus within just 4 or 7 days post vaccination. The vaccine protected 50% of cattle from clinical disease when administered 7 days prior to challenge, but was not protective with just 4 days between vaccination and challenge. Viraemia was significantly reduced in animals challenged 7 dpv but not 4 dpv, compared to unvaccinated controls, however, there were no effects on the level of virus detected in nasal and oral secretions regardless of vaccination time. The level of neutralising antibodies detected in cattle challenged 7 dpv correlated with protection from clinical disease. All animals seroconverted to FMDV non-structural proteins, suggesting no sterile protection. An equal number of animals became persistently infected in both vaccine groups. The results indicated that high-potency O1 Manisa vaccine administered just 7 days prior to challenge should provide partial protection of cattle if an outbreak of O/SKR/2010, or related viruses, occurs, and would be useful to limit spread of FMDV when used in conjunction with other control measures.

1. Introduction

Foot-and-mouth disease (FMD) is a contagious viral disease affecting cloven-hoofed animals. The disease is characterised by rapid onset and high morbidity, with infected animals developing lesions on areas of friction such as the mouth, feet and teats; however, sub-clinical infections can also occur [1]. The disease is endemic in the Middle East, Central and South Asia and Africa. In South America, where vaccination is extensively used and most countries are free, no outbreaks have been recorded since 2012 (WAHIS OIE report, 2016). In FMD-free countries, an outbreak severely impacts the livestock industries due to loss in trade, culling of stock and the costly and lengthy processes to regain disease-free status and market access.

The causative agent of FMD is FMD virus (FMDV), a small, positive-sense RNA virus in the Genus *Aphthovirus*, Family *Picornaviridae*. Foot-and-mouth disease virus is classified into seven distinct serotypes (O, A, C, Asia 1, and Southern African Territories (SAT) 1, SAT 2 and SAT 3). Vaccination with one serotype does not confer protection against the other serotypes and as the virus is rapidly evolving, the genetic and subsequent antigenic variation within each serotype can diminish the ability of vaccines to protect against heterologous strains of the same serotype [2–5].

To effectively control this virus and minimize the economic impact, it is imperative to have effective outbreak response programs that may include emergency vaccination in countries free from the disease. Vaccination using high-potency (>6 protective dose $(PD)_{50}$) vaccines can effectively protect animals challenged as early as 4 days post-vaccination (dpv), reducing the level and

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[1] In memory of our friend and colleague.

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duration of FMDV excretion [6–11], limiting virus transmission and potentially minimising the duration and intensity of an outbreak [12].

During 2009–2011, spread of serotype O virus belonging to the SEA topotype led to outbreaks of FMD in six countries in the Eastern Asia region and the culling of over 3.5 million cattle and pigs in Japan and Korea [13,14]. A representative virus from these outbreaks was O/SKR/2010 [15]. Previously we showed that high-potency O1 Manisa monovalent oil adjuvanted vaccine provided protection in sheep challenged 4 dpv by direct contact with sheep infected with O/SKR/2010 by coronary band inoculation [9]. We have also observed that cattle were protected from disease when challenged with O/SKR/2010 by intra-dermolingual infection (IDL) 21 dpv with the same vaccine [16].

During an emergency response, it is important to know how soon after vaccination cattle will be protected. The aim of the current study was to determine whether or not the O1 Manisa vaccine will provide early protection to cattle challenged with the O/SKR/2010 virus by IDL inoculation just 4 or 7 dpv.

2. Materials and methods

2.1. Ethics statement

All the protocols for experimentation with live cattle were approved by the CSIRO-Australian Animal Health Laboratory Animal Ethics Committee (AEC 1646).

2.2. Experimental animals

Twenty-two unvaccinated male Hereford cattle (12–18 m/o) were obtained from the Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) experimental cattle farm. They were kept at the level 3a animal containment facility at the Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina. Animals were acclimatised in the INTA facility for 3 days and divided into three groups: *V7*: (n = 10) and *V4*: (n = 10) vaccinated 7 and 4 days prior to challenge, respectively; and *UV*: the control unvaccinated group (n = 2). All groups were challenged on the same day.

2.3. Vaccination

Monovalent O1 Manisa double oil adjuvant vaccine at an antigen payload of > 6 PD_{50} was prepared by M/s. Merial Company Limited, United Kingdom (Merial). Cattle were vaccinated with 2 ml vaccine, administered intramuscularly in the anterior neck region.

2.4. Challenge

The challenge virus, O/SKR/4/2010 of the SEA topotype (Mya98 lineage) [15], was provided by the Pirbright Institute, UK. Vaccine matching studies carried out by the World Reference Laboratory, Pirbright indicated that the r1 value was 0.42 and 0.57 based on liquid phase ELISA and VNT respectively (OIE/FAO FMD Reference Laboratory Network Annual Report 2010). This virus was passaged twice in suckling mice then once in cattle tongue at SENASA (the VP1 region of all of these viruses was sequenced and no changes were observed). The titre of the cattle tongue virus was determined to be $10^{8.3}$ suckling mouse lethal dose $(SMLD_{50})$/ml (equivalent to $10^{6.3}$ tissue culture infectious dose $(TCID)_{50}$/ml when titrated in baby hamster kidney (BHK) cells). All cattle were challenged by IDL inoculation with 10^4 $SMLD_{50}$ in 1 ml, administered to approximately four locations in the tongue.

2.5. Monitoring and sample collection

Cattle were monitored for the development of clinical signs such as lameness, nasal discharge and the development of vesicles. The animals were sampled on designated days post-vaccination and post-challenge (dpc) to assess the humoral immune responses, viraemia, virus shedding in saliva, nasal secretions, and oro-pharyngeal fluid (OPF) up to 28 dpc. Clotted blood for RT-qPCR and serology and nasal and saliva swabs were collected at -7 dpc (V7 cattle only), -4 dpc (V7 and V4 cattle only) and at 0, 1, 3, 5, 7, 10, 14, 21 and 28 dpc (all cattle). Salivette® swabs were used to collect nasal and saliva secretions. Phosphate buffered saline (PBS) (500 μl) was added to the swabs prior to centrifugation for recovery of the samples. Oro-pharyngeal fluid was collected at 0, 7, 10, 14, 21 and 28 dpc, using a probang sampling cup and mixed with 50 ml virus transport media. All samples were stored at -70 °C until processing.

2.6. Virus isolation

Samples were examined for the presence of infectious virus by inoculating 250 μl sample onto monolayers of BHK C-13 cells grown in 24-well cell culture trays, and incubated for 30 min at 37 °C. The cells were washed with PBS and overlaid with Dulbecco's modified Eagle's Medium (DMEM) containing 10% foetal bovine serum and antibiotics (catalog No. 15240062; Gibco), then examined for cytopathic effect (CPE) after 24, 48 and 72 h incubation at 37 °C with 5% CO_2. If no CPE was observed, cells and supernatant were collected, freeze-thawed and inoculated onto fresh BHK monolayers.

2.7. Detection of FMDV RNA by RT-qPCR

The amount of viral RNA in blood, nasal and oral swab samples and in OPF was quantified by RT-qPCR, as described previously [17]. The viral RNA was extracted from samples using the RNeasy Kit™ (Qiagen®), following the manufacturer's instructions. One-step RT-qPCR was performed using a primer and Taqman probe set targeting the internal ribosomal entry site of FMDV O UKG 34/2001 [18] and the AgPath ID One-Step RT-PCR reagents (Life Technologies). Reactions were performed on the Applied Biosystems 7500 Real-Time PCR Instrument. Samples with a C_T>38 were considered negative.

2.8. Determination of neutralising antibody titre

Serum samples were heat inactivated (56 °C, 30 min) and used for virus neutralisation test (VNT) to measure antibodies to both O1 Manisa and O/SKR/2010 using standard procedures [19]. The titres were calculated as the reciprocal of the last serum dilution to neutralise 100 $TCID_{50}$ of virus in 50% of the wells. Titres ≥1.2 $\log_{10}$ (1:16) were considered positive (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals).

2.9. Detection of antibody to non-structural proteins

Antibodies to the non-structural proteins (NSP) of FMDV were detected on duplicate serum samples, diluted at 1:5, using the PrioCHECK® FMDV-NS kit (Prionics, Sweden). A "per cent inhibition" of 50 or more was considered a positive result. Samples diluted 1:20 were also tested using the NCPanaftosa-Screening Test 3ABC ELISA [20]. Results were expressed as T/C, being the quotient between the absorbance obtained from the serum sample (T) and the absorbance obtained from the control serum (C). Doubtful and reactive sera were tested with the NCPanaftosa–Confirmatory Test [21].

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