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Efficacy of a high quality O_1/Campos foot-and-mouth disease vaccine upon challenge with a heterologous Korean O Mya98 lineage virus in pigs

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ABSTRACT

In 2010 serotype O foot-and-mouth disease virus of the Mya98 lineage/SEA topotype spread into most East Asian countries. During 2010–2011 it was responsible for major outbreaks in the Republic of Korea where a monovalent O/Manisa vaccine (belonging to the ME-SA topotype) was applied to help control the outbreaks. Subsequently, all susceptible animals were vaccinated every 6 months with a vaccine containing the O/Manisa antigen. Despite vaccination, the disease re-occurred in 2014 and afterwards almost annually. This study focuses on the *in vivo* efficacy in pigs of a high quality monovalent commercial O_1/Campos vaccine against heterologous challenge with a representative 2015 isolate from the Jincheon Province of the Republic of Korea. Initially, viral characterizations and r_1 determinations were performed on six viruses recovered in that region during 2014–2015, centering on their relationship with the well characterized and widely available O_1/Campos vaccine strain. Genetic and antigenic analysis indicated a close similarity among 2014–2015 Korean isolates and with the previous 2010 virus, with distinct differences with the O_1/Campos strain. Virus neutralisation tests using O_1/Campos cattle and pig post vaccination sera and recent Korean outbreak viruses predicted acceptable cross-protection after a single vaccination, as indicated by r_1 values, and in pigs, by expectancy of protection. In agreement with the *in vitro* estimates, *in vivo* challenge with a selected field isolate indicated that O_1/Campos primo vaccinated pigs were protected, resulting in a PD50 value of nearly 10. The results indicated that good quality oil vaccines containing the O_1/Campos strain can successfully be used against isolates belonging to the O Mya98/SEA topotype.

1. Introduction

Foot-and-Mouth Disease (FMD) is a highly transmissible and economically devastating vesicular disease of cloven-hoofed animals [1,2]. Its presence severely constrains international trade of livestock and animal products and poses a constant threat to FMD-free countries. The causative agent, FMD virus (FMDV), belongs to the genus Aphthovirus within the Picornaviridae family [2].

There are seven immunologically distinct serotypes, and new variants arise continuously [3–4] that are grouped in intratypic genetic lineages within topotypes. Infection or vaccination with one serotype does not cross-protect against the other serotypes and may fail to protect fully against some strains within serotypes [5,6].

Inactivated vaccines are widely used to control, eradicate and prevent FMD [7,8]. Historically, serotype O vaccine strains can be included within two main groups. One represented by the South American strain O_1/Campos, selected and harmonized for use in the region, as well as by the related viruses: O/Lausanne, OBFS/1860 (UK1967) and O/Kaufbeuren, which were widely used in Europe. The second group, represented by the O/Manisa strain,

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was used mainly in Middle East and Asia, as well as in North and South Africa [9]. However, the vaccine has not provided effective protection against recent viruses from the Middle East [10], requiring the testing of alternative vaccine strains. In this regard, preliminary studies were addressed with some candidate vaccine strains, establishing antigenic correlations through r_1 values [11].

In 2010 a FMDV serotype O, Southeast Asia (SEA) topotype/Myanmar 98 (Mya98) lineage, endemic in Southeast Asia, expanded into most eastern Asian countries [12,13]. In 2010 major outbreaks occurred in the Republic of Korea where the culling of hundreds of thousands of pigs took place [14,15]. A monovalent O/Manisa vaccine was applied to assist in controlling the episode. Subsequently, to help prevent recurrence of the disease, it was mandatory to vaccinate all susceptible animals twice a year with trivalent vaccines containing O/Manisa, A Malaysia 97, and Asia 1 Shamir viruses [16,17].

Despite nationwide immunization, the Mya98 lineage of serotype O reappeared in 2014 and afterwards almost every year [17]. These recurrences revived the controversy over the efficacy of the O/Manisa vaccine [11,15,16], backed by the low or moderate serological relationship between O/Manisa and Korean 2010 stains described in some reports (r_1 value of approximately 0.3) [15,16]. It also reinforced the need to search for an alternative vaccine strain, preferably a well-established and well-characterized strain with a broad antigenic spectrum.

The antigen derived from O_1/Campos strain blended in oil adjuvanted vaccines gave effective and broad immunological coverage against South American strains [18,19]. In addition, the strain was successfully used to assist in controlling a widespread epidemic in pigs caused by serotype O in Taiwan in 1997 [20,21].

In a previous *in vitro* study we suggested that good quality vaccines containing the O_1/Campos strain can be used against representative viruses of three currently circulating topotypes in Asia (SEA, ME-SA and CATHAY), including a 2010 Korean isolate belonging to the SEA topotype, Mya98 lineage [22]. The results supported the application of O_1/Campos vaccines in emergency vaccination programs in pigs in the Republic of Korea, since 2016.

This study extends to the 2014-2015 O/Mya98 Korean viruses the previous *in vitro* assessments and confirms the accuracy of such predictions by an *in vivo* vaccination and challenge study in pigs with a representative 2015 Korean isolate.

2. Materials and methods

2.1. Cell lines and FMDV strains

Baby hamster kidney (BHK)-21 cells were used for all virus related work. O_1/Campos South American vaccine virus was provided by SENASA. Korean isolates were received from the Animal and Plant Quarantine Agency in the Republic of Korea (APQA). Viruses O/SKR/02 D1-2, O/SKR/02 D6-2, O/SKR/02 D11-1, were isolated in the year 2014 and virus O/SKR/84 YDM in 2015 from pig feet tissue and viruses O/SKR/71 GHW, O/SKR/35 LYC in the year 2015 from cattle tongue. For serological assays, viruses were amplified in cell monolayers, clarified and stored at -70 °C. Dulbeccós modified minimal essential medium without serum was used for cell infection. All field viruses were passaged seven times to reach titres around 10^7 $TCID_{50}$.

2.2. Genetic characterization: Phylogenetic analysis

RNA extraction, amplification and sequencing conditions to determine the sequence of the complete VP1-coding region of the isolates were as described [23]. Phylogenetic analysis was performed using the program MEGA, version 7.0 [24], applying the General Time Reversible evolutionary model to construct unrooted trees, with evolutionary distances calculated using the Kimura two-parameter method and a bootstrap resampling analysis performed with 1000 replicates.

2.3. Antigenic characterization

Monoclonal antibody profiling, was determined through a trapping ELISA using a panel of 21 monoclonal antibodies (MAbs), characterized as described [25]. MAbs were raised against FMDV strains O_1/Campos (1H10, 1B9-3, 17, 2B3, 3H10), O_1/Caseros (8G, 3, 74, 69, 2-6F) and O/Taiwan (3A1, 3D1, 4B2, 1A11, 3A2, 2F8, 1B3, 2D4, 1B9, 2C9, 3G10). To obtain a relationship between viruses, coefficient of correlation (cc) of their MAb reactivity values was calculated by applying a linear regression to fit the best straight line. cc values = 1 correspond to identical profiles; cc values close to 0 indicate totally dissimilar antigenic profiles [25].

2.4. In vitro vaccine matching studies

2.4.1. r_1 determination

Virus neutralization (VN) titers against the homologous O_1/Campos vaccine strain and the heterologous field viruses were obtained by two-dimensional assays performed as described in the World Organization for Animal Health (OIE) Manual [26] using pools of five medium to high titer serum samples collected from cattle or pigs vaccinated with a 2 ml dose of O_1/Campos monovalent vaccine. The r_1 values were calculated as the reciprocal serum titer against heterologous virus/reciprocal serum titer against homologous vaccine virus.

2.4.2. Expectancy of protection (EPP)

EPP estimates the likelihood of protection by correlating VN antibody titers in vaccinated animals with clinical protection against challenge with 10,000 infective doses, based on predetermined tables established in cattle for the O_1/Campos vaccine strain [27]. Titers were expressed as $\log_{10}$ of the reciprocal sera dilution. Four different commercial monovalent vaccine batches were assessed using groups of 10 pigs for each batch tested.

2.5. Vaccine formulation and approval

The commercial vaccines (Aftogen Oleo®) were produced by Biogénesis Bagó (Argentina) according to good manufacturing practices as single-in-oil emulsion vaccines (PD50 > 6) using O_1/Campos purified antigen [28]. They were approved by SENASA (Argentine Animal Health Authorities) for safety, purity and potency in swine following local [29,30], OIE [26], European Pharmacopeia (Ph.Eur.) [31], and specific Korean standards before release to the market. The batches were also approved by APQA (Animal and Plant Quarentine Agency) and applied during the 2016 vaccination program in Korea.

2.6. In vivo challenge test

2.6.1. Challenge virus stock production

The work was performed according to the Argentine Animal Ethics Code in the animal facility of SENASA, Argentina. BHK adapted O/SKR/84 YDM virus was passaged twice by intradermal inoculation of $10^{6.8}$ TCID50/ml in the heel bulb of each mayor digit of the left forefoot in 2-month-old seronegative Landrace x Large White pigs (approximately 30 kg) obtained from a commercial farm. Epithelia recovered from the second passage were mortared in DMEM (proportion 1:5), clarified by centrifugation, aliquoted and stored at −80 °C. Aliquots were further titrated.

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