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Multiple efficacy studies of an adenovirus-vectored foot-and-mouth disease virus serotype A24 subunit vaccine in cattle using direct homologous challenge

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ABSTRACT

The safety and efficacy of an experimental, replication-deficient, human adenovirus-vectored foot-and-mouth disease virus (FMDV) serotype A24 Cruzeiro capsid-based subunit vaccine (AdtA24) was examined in eight independent cattle studies. AdtA24 non-adjuvanted vaccine was administered intramuscularly to a total of 150 steers in doses ranging from approximately 1.0×10^8 to 2.1×10^{11} particle units per animal. No detectable local or systemic reactions were observed after vaccination. At 7 days post-vaccination (dpv), vaccinated and control animals were challenged with FMDV serotype A24 Cruzeiro via the intradermal lingual route. Vaccine efficacy was measured by FMDV A24 serum neutralizing titers and by protection from clinical disease and viremia after challenge. The results of eight studies demonstrated a strong correlation between AdtA24 vaccine dose and protection from clinical disease ($R^2 = 0.97$) and viremia ($R^2 = 0.98$). There was also a strong correlation between FMDV A24 neutralization titers on day of challenge and protection from clinical disease ($R^2 = 0.99$). Vaccination with AdtA24 enabled differentiation of infected from vaccinated animals (DIVA) as demonstrated by the absence of antibodies to the FMDV nonstructural proteins in vaccinates prior to challenge. Lack of AdtA24 vaccine shedding after vaccination was indicated by the absence of neutralizing antibody titers to both the adenovector and FMDV A24 Cruzeiro in control animals after co-mingling with vaccinated cattle for three to four weeks. In summary, a non-adjuvanted AdtA24 experimental vaccine was shown to be safe, immunogenic, consistently protected cattle at 7 dpv against direct, homologous FMDV challenge, and enabled differentiation of infected from vaccinated cattle prior to challenge.

1. Introduction

Foot-and-mouth disease (FMD) is the most highly contagious disease affecting livestock resulting in significant adverse economic impact worldwide [1]. FMD is caused by a *Picornaviridae* virus, foot-and-mouth disease virus (FMDV). FMDV affects domestic and wild-life cloven-hoofed ruminants as well as swine. Typical FMDV clinical signs, although age and species dependent, include fever and lesions on the mouth, hooves, and teats. Most susceptible animals survive infection, but often exhibit decreased production due to debilitation from the lesions. The FMDV infection pathways and host response in cattle are reviewed by Arzt et al. [2].

Abbreviations: FMD, foot-and-mouth disease; FMDV, foot-and-mouth disease virus; AdtA24, replication-deficient human adenovirus vectored FMDV serotype A24 Cruzeiro vaccine; DIVA, differentiate infected from vaccinated animals; PU, particle units; dpv, days post-vaccination; dpc, days post challenge; USDA, United States Department of Agriculture; VNT, virus neutralization test; GMT, geometric mean VNT titer; NSP, nonstructural protein.

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FMD is enzootic in many developing countries in Asia and Africa, and periodic FMD vaccination campaigns are often practiced to prevent outbreaks or mitigate spread. Current FMD serotype-specific vaccines are made by growing live FMDV in cell culture, following by chemical inactivation and purification of 140S virions. Concerns about FMDV introduction into the United States have prompted government research and development into producing next generation vaccines that can be manufactured in the United States.

Mayr et al. initially described the construction of an E1/E3 replication deficient human adenovirus 5 (Ad5) recombinant virus that expressed the FMDV serotype A12 P1 capsid coding region, portions of the nonstructural protein coding regions, and the 3C protease, essential for P1 polyprotein processing [3]. Moraes et al. produced a similar virus containing the capsid coding region of FMDV serotype A24 Cruzeiro (Ad5-A24) that conferred protection in swine [4]. In a subsequent swine study, de Avila Botton et al. showed that higher Ad5-A24 doses resulted in a better clinical outcome, higher serum antiviral activity, no viremia, and lower amounts of FMDV in nasal secretions [5]. Cattle vaccinated with Ad5-A24 were also protected against FMD clinical disease following homologous challenge at one week post-vaccination [6].

Details of the construction of the adenovector E1, E3 deleted backbone (Adt) used in the studies reported herein have been previously described [7,8]. Additionally, a human cytomegalovirus promoter was added to control expression of the target FMDV gene cassette. The FMD vaccine used in our studies (AdtA24) is based on the FMDV strain A24 Cruzeiro P1-2A capsid and serotype A12 3C protease cloned into a replication deficient human adenovirus C, serotype 5 vector [9].

Based on the working hypothesis that a lead vaccine candidate and method of production could be identified for transition to a full development regulatory program, we conducted eight independent AdtA24 vaccine safety and efficacy studies using FMDV A24 Cruzeiro experimental challenge at one week post-vaccination. The primary goals were prevention of FMD clinical disease without adverse effects. The study series started with initial proof-of-concept studies using a research restricted vaccine production method and culminated with the identification of a vaccine purification method that could be scaled up and used in a manufacturing process and a vaccine dose that met the requirements for advancement to a regulatory-based product licensing program.

2. Materials and methods

2.1. Animals

Healthy Holstein cross-bred steers four to ten months of age and 160–260 kg were purchased from an Association for the Assessment and Accreditation of Laboratory Animal Care accredited livestock facility. Steers were acclimated and housed in the Plum Island Animal Disease Center (PIADC) BSL-3Ag animal facility. Prior to vaccination, steers were randomly allocated to treatment groups and allowed to freely co-mingle in assigned rooms throughout the duration of the study. Animal care and study protocols were in accordance with the institutional guidelines of the PIADC Institutional Animal Care and Use Committee.

3. Experimental AdtA24 vaccines

The AdtA24 vaccine vector was constructed by GenVec, Inc. (Gaithersburg, MD) as summarized by Brake et al. [9] and grown in the M2A cell line in adherent flasks, shaker flasks, or bioreactors. Following lysis of AdtA24-infected host cells, sequential downstream purification steps were used to prepare the four experimental vaccines of varying purity used in these studies as follows: (1) three cycles of centrifugation on cesium chloride (CsCl) gradients (research restricted method); (2) benzonase treatment followed by clarification by centrifugation to produce downstream fraction 3 (DS3); (3) DS3 purification using ultrafiltration/diafiltration to produce downstream fraction 5 (DS5); (4) DS5 purification through anion exchange chromatography (Cipheron Q Hyper-D) to produce downstream fraction 7 (DS7). For each vaccine lot, FMDV capsid expression and processing was confirmed by Western blot of transfected cell lysates using a VP2-specific monoclonal antibody [10] (kindly provided by CFIA, Winnipeg, Canada), and particle units (PU) were quantified [8]. AdtA24 preparations were stored at −80 °C. On the day of vaccination, thawed vaccines were diluted with final formulation buffer (FFB; Lonza, Walkersville, MD) to the final target dose. For each study, baseline serum samples from each animal were collected immediately prior to vaccination (Day 0). Steers were inoculated intramuscularly (IM) in the cleidooccipitalis muscle with a single 2 ml injection in the right side of the neck with either placebo (FFB alone or an Adt.Null vector) or AdtA24 (Table 1). Individual blood samples were collected weekly and used for serum virus neutralization tests or plasma virus isolation. Steers were assessed for potential adverse reactions, including core body temperature and observations of overall gross physical and injection site reactions.

4. Challenge virus preparation and administration

FMDV serotype A24 Cruzeiro (SGD variant) (isolate originated in Brazil, approximately 1950) challenge virus stock (1×10^6 bovine infectious dose 50% [BID_{50}]/ml) was prepared following one time passage in BHK-21 cell culture [11] and two amplifications in bovine tongue. For challenge, virus stock was diluted 1:40 in Dulbecco's Modified Eagle Medium (DMEM) with 1% antibiotics/antimycotics to obtain the target titer, 5.6–6.0 $\log_{10}$ tissue culture infective dose 50% ($TCID_{50}$/ml), based on titration on a porcine cell line highly permissive for FMDV, LF-BK [12], or LF-BK $\alpha_V\beta_6$, [13,14].

One week post-vaccination, steers were sedated with 0.22 mg/kg of xylazine IM in the hindquarter and challenged via the intradermal lingual (IDL) route using a minor modification of the World Organisation for Animal Health (OIE) guidelines [15]. FMDV challenge (1×10^4 BID_{50}/0.4 ml) was delivered by inoculation of 0.1 ml into each of four sites on the upper surface of the tongue. Sedation was reversed by administering 2–4 mg/kg of tolazoline intravenously.

5. Clinical observations

Individuals, through masked treatment allocation, performed immunizations, clinical observations (lesions), and laboratory assays (plasma virus isolation, plasma rRT-PCR, and virus neutralization tests). We assessed the presence or absence of FMD clinical disease in sedated steers at 3, 7, 10, and 14 dpc. We noted FMD clinical signs and lesions using the following criteria: negative, no pedal or secondary (lip, mouth, or nose) vesicular lesions; positive, one or more pedal vesicular lesions on one or more feet or any secondary vesicular lesions. For dose response studies using economically feasible purification methods, we calculated the bovine protective dose for 50% or 80% of steers (BPD_{50} or BPD_{80}) using the Spearman/Kärber method with endpoint generalized lesion data obtained on 14 dpc [16].

5.1. Virus neutralization test (VNT)

FMDV serotype A24 strain Cruzeiro and adenovirus serotype 5 (Ad5) antibody titers were determined using heat-inactivated

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