Use of ENABL® adjuvant to increase the potency of an adenovirus-vectored foot-and-mouth disease virus serotype A subunit vaccine

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A B S T R A C T

A foot-and-mouth disease (FMD) recombinant subunit vaccine formulated with a lipid/polymer adjuvant was evaluated in two vaccine efficacy challenge studies in steers. The vaccine active ingredient is a replication-deficient human adenovirus serotype 5 vector encoding the FMD virus (FMDV) A24/Cruzeiro/BRA/55 capsid (AdtA24). In the first study, AdtA24 formulated in ENABL® adjuvant was compared to a fourfold higher dose of AdtA24 without adjuvant. Steers vaccinated with AdtA24 formulated in ENABL® adjuvant developed a significantly higher virus neutralizing test (VNT) antibody titer and an improved clinical response following FMDV A24/Cruzeiro/BRA/55 intradermal lingual challenge at 14 days post-vaccination (dpv) than steers vaccinated with the active ingredient alone. In the second study, vaccination with AdtA24 formulated in ENABL® at the same dose used in the first study, followed by FMDV A24/Cruzeiro/BRA/55 intradermal lingual challenge on 7 or 14 dpv, prevented clinical FMD in all steers and conferred 90% protection against viremia. In addition, post-challenge FMDV titers in nasal samples from vaccinated steers compared to unvaccinated steers were significantly reduced. In both studies, none of the AdtA24 vaccinated steers developed antibodies to the FMDV non-structural proteins prior to challenge with FMDV, indicative of the capacity to differentiate infected from vaccinated animals (DIVA). These results demonstrate that administration of AdtA24 formulated in ENABL® adjuvant lowered the protective dose and prevented clinical FMD following exposure of vaccinated steers to virulent FMDV at 7 or 14 dpv.

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Foot-and-mouth disease (FMD) is the most contagious infection in livestock [1,2]. FMD virus (FMDV), an Aphthovirus in the Picornaviridae, causes significant agro-economic loss throughout many parts of the world [1]. FMDV infects cloven-hoofed animals, including cattle, domestic and feral swine, sheep, goats, and buffalo [2]. There are seven FMDV serotypes, and multiple subtypes within each serotype [2]. FMD is enzootic in Africa and Asia [2], and at least 15 other countries in Asia, northern Africa, South America, and Europe have reported sporadic outbreaks [3]. In FMD-free 

1. Introduction
countries, recent outbreaks resulted in economic impacts of >$2.8–16 billion [1,4,5].

To prevent FMD outbreaks in enzootic countries, semi-annual vaccination using chemically inactivated FMDV, purified from large in vitro cell culture batches is often practiced [6]. To prepare for an accidental or intentional FMD outbreak in the United States and enable production of FMD vaccine on the mainland without using FMDV, we developed a replication-defective human adenovirus serotype 5 molecular vaccine platform designed to deliver FMDV capsid and capsid processing genes [7–9]. To produce FMDV molecular vaccines to match circulating viral strains, the only information required is the nucleic acid sequence of the capsid coding region of the target strain(s), which can be chemically synthesized and inserted into the adenovirus vector (adenovector). Cattle and pigs vaccinated with various adenovectored FMD experimental vaccines were protected from clinical FMD following challenge with the homologous FMDV strain [7,10–13].

One version of the adenovectored vaccine, AdtA24, comprises the FMDV A24/Cruzeiro/BRA/55 P1-2A coding region, plus part of FMDV A12/119/Kent/UK/32’s 3B1, and full 3B2, 3B3, and 3C coding regions [8,9,13]. AdtA24 does not replicate in the vaccinated animal [14]. Only the mRNA for the FMDV capsid proteins is expressed and processed, initially in the muscle cells at the injection site, followed by antigen presentation to the immune system in local draining lymph nodes, and eventually in additional lymph nodes, the liver, spleen, and thymus [14].

Previously we demonstrated that AdtA24 administered at relatively high doses without adjuvant prevented clinical FMD and viruria in immunized steers challenged at either 7 or 14 days post-vaccination (dpv) [13]. To support our goal to lower the vaccine cost we formulated AdtA24 with several adjuvants and conducted preliminary cattle serology studies (data not shown). The most promising adjuvant, a lipid/polymer, ENABL®, was formulated with AdtA24 for evaluation of efficacy in cattle challenged via the intradermal route with FMDV A24/Cruzeiro/BRA/55. Results from our first study demonstrated that addition of this ready-to-use ENABL® adjuvant resulted in protection at a 19-fold lower dose than the predicted 90% effective dose of AdtA24 prepared in buffer [13]. The second of our studies reported herein was designed to meet the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Services (APHIS) Center for Veterinary Biologies (CVB) guidelines to fulfill requirements to obtain a product license.

Our goal is to have effective molecular FMD vaccine formulations that are economically attractive, can be manufactured in United States biosafety level-2 facilities, deployed rapidly in the event of an outbreak, and differentiate infected from vaccinated animals (DIVA) against the highest FMDV global threats.

2. Materials and methods

2.1. Animals

An accredited experimental-livestock provider supplied healthy, 200–250 kg Holstein steers, 9–11½ months old. Prior to vaccination, we allocated steers randomly to treatment groups where they moved freely about their biosafety level-3 room. There was no concomitant medication during the studies or the 5–7 day acclimation period. The Plum Island Animal Disease Center (PIADC) Institutional Biosafety Committee and the Institutional Animal Care and Use Committee approved the protocols.

2.2. Vaccine construction, formulation, and administration to steers

AdtA24 contains the FMDV A24/Cruzeiro/BRA/55 P1-2A coding regions, and FMDV A12/119/Kent/UK/32 3B1, minus the coding regions for the first six amino acids, and the complete 3B2, 3B3, and 3C coding regions. GenVec, Inc. (Gaithersburg, MD) constructed AdtA24, and purified, evaluated for purity and processed capsid by Western blot, and stored AdtA24 at ~80 °C [8,13,15]. Adenovector vaccine particle units (PUs) were detected by Absorbance260 following anion exchange HPLC [8]. On the day of vaccination, thawed antigens (37 °C) were mixed with final formulation buffer (FFB; Lonza) or ENABL® adjuvant (No. 7010101, Vax-Liant) diluted 1:10 with FFB. Steers received a single 2 mL injection containing either the control formulation or formulated vaccine in the cleido-occipitalis muscle.

2.3. Design of efficacy study 1. Comparison of AdtA24 vaccine formulated with or without adjuvant inoculated into steers and challenged at 14 dpv

In the first study, designed to compare efficacy of AdtA24 formulated with and without an adjuvant, the vaccines contained 1.2 × 10^10 PU of AdtA24/dose in FFB (treatment group 1, T1, n = 22 steers) and 3.0 × 10^9 PU of AdtA24/dose in ENABL® adjuvant + FFB (T2, n = 10 steers). The difference in the number of steers/group was based on determining whether the AdtA24 vaccine would be licensed by USDA CVB with or without an adjuvant. Five control steers (C1) received FFB. All steers were challenged at 14 dpv (details below).

2.4. Design of efficacy study 2. Evaluation of steers vaccinated with AdtA24 formulated in ENABL® adjuvant and challenged at 7 or 14 dpv

The second study was designed to evaluate a single, adjuvanted vaccine dose to meet the USDA APHIS CVB pivotal immunogenicity study design requirements for vaccine licensure using a 14 dpv challenge model. At 14 days before challenge, AdtA24 was prepared in ENABL® adjuvant + FFB at 2.7 × 10^9 PU/dose for T3, n = 34 steers in two rooms, and six steers in C2 in a different room, were sham-immunized (ENABL® + FFB). Although not required by USDA APHIS CVB, T4 (n = 10) received the same dose of AdtA24 vaccine, 7 days prior to challenge, to understand the early onset of protection at the AdtA24 vaccine minimum protective dose. (T4 was limited in size due to available space.)

2.5. Preparation of FMDV challenge

At 7 or 14 dpv (designated per protocol), we challenged sedated steers according to the OIE guidelines via the intradermal route (IDL) route using FMDV A24/Cruzeiro/BRA/55 stock produced and titrated in bovine tongue [13,16]. The challenge dose was 1 × 10^4 bovine infectious dose 50%/0.4 mL, with titers ranging from 5.625 to 6.0 log_{10} tissue culture infective dose 50% (TCID50)/mL after back titration of inocula on LFBK-α_v cells, kindly provided by M. LaRocco, USDA Agricultural Research Service, PIADC [17,18]. All samples collected on the day of challenge occurred prior to FMDV inoculations.

Blinding of immunizations, clinical observations (pedal lesions), and laboratory analyses occurred through masked treatment allocation. The primary outcome included the prevention of clinical FMD and lesion development on hooves, assessed on 3, 7, 10, and 14 days post-challenge (dpc) [13].

2.6. Serum virus neutralization test (VNT)

Serum samples from each steer were collected weekly prior to administration of any treatments starting on the day of vaccination. Antibody titers to FMDV A24/Cruzeiro/BRA/55 and to adenovirus serotype 5 (Ad5) were determined by VNT using heat-inactivated serum samples (56 °C, 30 min) [13,16]. FMDV
VNT titers were determined in BHK21 [C13] (ATCC® CCL105™) cells, and Ad5 VNT titers in HEK293 (ATCC® CRL1573™) cells. VNT titers were calculated using the Spearman–Kärber method based on cytotoxic effect (CPE) using 50% as the neutralization endpoint. A positive VNT titer was >0.6 log₁₀, and the percent positive was the fraction of serum samples with VNT titers >0.6 log₁₀. The lower limit of detection, 0.6 log₁₀, was used for calculations of samples with no CPE [10,19].

2.7. Detect antibody production to the FMDV 3ABC non-structural protein (NSPs)

The PrioCHECK® FMDV NS blocking ELISA (ThermoFisher Scientific) was used to detect antibodies to the nonstructural 3ABC protein, per manufacturer’s instructions. A positive serum sample has ≥50% inhibition.

2.8. FMDV detection in plasma and nasal secretions

Heparinized blood (plasma) samples collected on 0–5 dpc from all steers were tested for the presence of infectious FMDV on LFBK-αβγδ6 cells [13,16] and for the presence or absence of FMDV RNA by rRT-PCR [16,20]. RNA was extracted using the MagMAX™ Viral RNA Isolation Kit (ThermoFisher Scientific). A Ct value ≤40 was scored positive. A positive result in either test was scored as viremia. In Study 2, per USDA APHIS CVB regulatory feedback on Study 1 results, we collected nasal swabs (polyester fiber) prior to challenge on 0 dpc and daily on 1–8 dpc, and detected infectious FMDV on LFBK-αβγδ6 cells [13]. The limit of detection for FMDV from nasal secretions was 1.5 log₁₀ TCID₅₀/mL.

2.9. Data analysis

For the positive virus isolation data obtained from nasal swabs in Study 2, we determined daily (2, 3, and 4 dpc) mean and standard deviation of FMDV titers (TCID₅₀/mL) for each group. VNT titers were analyzed using unpaired two-tailed T-tests (Welch correction; Excel and GraphPad). Other analyses used the Fisher’s Exact Test (GraphPad). For all analyses, a P ≤ .05 was considered statistically significant.

3. Results

3.1. Efficacy study 1. Comparison of AdtA24 vaccine formulated with or without adjuvant inoculated into steers and challenged at 14 dpv

In the first study, we assessed the effect of AdtA24 formulated with and without an adjuvant on prevention of clinical FMD and viremia and on the production of functional neutralizing antibodies to FMDV and the adenovector (Table 1). All five C1 control steers developed clinical FMD and viremia. Lesions developed on all four hooves of all non-vaccinated controls by 3 dpc. All 10 steers in T2 (3.0 x 10⁸ PU + ENABL® adjuvant) were protected from both generalized FMD and viremia compared to 82% of 22 steers in T1 (1.2 x 10⁹ PU in FFB). Protection from clinical FMD and viremia for T1 and T2 were not different from each other (P = .28), but were different from results in C1 (P < .01). Due to the relatively high protection against viremia in both T1 (82%) and T2 (100%) differences in onset to viremia could not be assessed.

The percentage of steers with positive VNT titers to FMDV A24/Cruzeiro/BRA/55 was greater for T2 (90%) than for T1 (64%) steers on the day of challenge (14 dpv), but not statistically significant (P = .2) (Table 1). The geometric mean FMDV VNT titers (GMTs) for the T2 steers were significantly higher compared to the T1 steers on both 7 dpv (P = .04) and 14 dpv (P = .009) (Fig. 1A and Table 1). By 7 and 14 dpv, the GMTs to FMDV for all groups were ≥2.2 and ≥2.5 log₁₀ (Fig. 1A).

The adenovirus GMT increased from 7 to 14 dpv by >10-fold for the AdtA24 plus adjuvant group (T2) and remained at that level through 14 dpv (Fig. 1B). The adenovirus GMT was significantly higher in T2 steers compared to T1 steers; P < .001 for serum samples collected at 14, 21, or 28 dpv (Fig. 1B).

3.2. Efficacy study 2. Evaluation of steers vaccinated with AdtA24 formulated in ENABL® adjuvant and challenged at 7 or 14 dpv

In the second study, the same adjuvanted vaccine dose that was effective in the first study was administered to two groups of steers, challenged at 14 or 7 dpv (T3 and T4). Protection from clinical FMD for steers challenged at 14 dpv was similar between both studies for the AdtA24/ENABL® T2 and T3 groups (Table 1). All 10 steers challenged at 7 dpv (T4) were protected from clinical FMD. Unexpectedly, one of the six control steers in C2 did not develop FMD (Table 1), but the other five control steers developed lesions on all hooves by 3 dpv. Protection from clinical FMD and viremia was significantly different between C2 and each of the vaccination groups (P < .01), but not between T3 and T4 (P > .99).

During the first 5 dpc, viremia was prevented in 91% and 90% of the steers in the T3 and T4 vaccine groups (Table 1); therefore, differences in onset to viremia could not be evaluated. In C2, five naive steers were positive for viremia on 1–3 dpc, and four remained positive at 4 dpc (data not shown). The C2 calf that was free of clinical FMD and viremia developed lesions only on the tongue and antibodies to FMDV (1.2 log₁₀) and the FMDV NSPs by 7 dpc, indicating that this steer may not have received a sufficient challenge.

Prior to challenge (0 dpc) and on 5–8 dpv, all samples from each steer were negative for FMDV in nasal secretions. Nasal shedding of FMDV occurred on 1–4 dpc (Fig. 2). The prevalence of nasal shedding over 1–4 dpc was significantly lower in T3 (17%, 23/136) and T4 (23%, 9/40) compared to C2 (63%, 15/24) (P < .003). The positive FMDV titers in C2 secretions on 1–4 dpc were statistically different from those in either T3 or T4 (P < .02). However, there was no difference between T3 and T4 on 1–4 dpc (P = .3). Additionally, 67% of both T3 and T4 vaccines were FMDV positive on 2 dpc, suggesting that the time between vaccination and challenge did not impact the onset of nasal shedding.

The GMT to FMDV A24/Cruzeiro/BRA/55 was higher on the day of challenge for T3 (14 dpv/0 dpc) than for T4 (7 dpv/0 dpc), and the GMTs were significantly different (P = .005) (Fig. 3A and Table 1). At 7 dpv, there was a significant difference between the VNT titers in C2 compared to either T3 or T4 (P < .001 and P = .03). However, there was no difference between the FMDV VNT titers at 7 dpv for T3 compared to T4 (P = .96). All steers had positive FMDV VNT titers by 7 dpv.

By 7 dpv, 94% of the T3 samples were positive by VNT to the adenovector, and all were positive at 14, 21, and 28 dpv. Similarly, all T4 samples were positive for antibodies to the adenovector by 7 dpv/0 dpc, and remained positive through 14 dpv. There were no significant differences between the adenovector VNT titers in C2 compared to T4 at 7, 14, and 21 dpv (Fig. 3B). No antibodies to the adenovector were detected in control steers, despite being co-mingled with 44 vaccinated steers for 21 or 28 days.

3.3. Differentiation of infected from vaccinated animals (DIVA)

No vaccinated steers produced antibodies to the FMDV 3ABC NSPs prior to challenge in either study (Fig. 4A and B). In Study 1, all five control steers (C1) were positive for antibodies to the NSPs by 7 dpv, while 45% and 30% of the steers in T1 and T2 were positive, which increased by 14 dpv for T1 (73%) and T2 (60%).
In Study 2, all C2 and T4 steers were positive for antibodies to the NSPs on 7 dpc, and 53% and 85% of T3 steers were positive on 7 and 14 dpc (Fig. 4B).

4. Discussion

In two studies, we evaluated the effectiveness of single dose vaccination of steers with AdtA24 formulated with the adjuvant, ENABL/C210, against homologous challenge with FMDV A24/Cruzeiro/BRA/55. In Study 1, a fourfold lower dose of AdtA24 with ENABL/C210 conferred higher protection rates against clinical FMD and viremia, and elicited higher post-vaccination/pre-challenge VNT titers, compared to AdtA24 without adjuvant. In the second study, the AdtA24/ENABL/C210 formulation administered to steers either 7 or 14 days before FMDV challenge, prevented clinical FMD in all but one of the 44 steers and prevented viremia in 40 of 44 vaccinates. The AdtA24/ENABL/C210 formulation was safe as evidenced by an absence of an injection site and gross physical reaction, lack of febrile response within the first three days, and lack of vaccine vector shedding (data not shown; consistent with previous results [13,21]). The AdtA24/ENABL post-vaccination/pre-challenge antigenicity (FMDV A24/Cruzeiro/BRA/55 VNTs) and efficacy results are higher and more consistent versus our previous reported findings with AdtA24 vector alone [13,21]. The antigenicity and efficacy results reported herein using the AdtA24/ENABL formulation are consistent with results using a related adenovirus vector expressing the FMDV A24/Cruzeiro/BRA/55 capsid coding region [12] or the FMDV O coding region [22]. Other adenovirus-vectored FMD vaccines using FMDV serotype A, O, or Asia capsid coding regions, administered at 5 × 10^9 plaque forming units/dose (approximately 5 × 10^10 PU), elicited FMDV VNT titers at 14 dpv that were lower than the current study, although VNT titers to the adenovirus vector were similar [23]. Our antigenicity and efficacy results are similar to reported results using conventional inactivated vaccines [24–27].

The AdtA24/ENABL formulation, administered 7 or 14 days before challenge, reduced the estimated 90% bovine protective dose (BPD90) by 19-fold compared to the estimated BPD90 of 5.6 × 10^10 PU for AdtA24 without adjuvant administered 7 days before challenge.

<table>
<thead>
<tr>
<th>Study No.</th>
<th>AdtA24 dose (PU/steer)</th>
<th>N</th>
<th>% protection from clinical FMD</th>
<th>% protection against viremia</th>
<th>% protection against nasal virus shedding (per animal)</th>
<th>FMDV VNT Titer</th>
<th>Day of challenge (Mean ± standard deviation; range; log10)</th>
<th>% Positive 1 week post-vaccination</th>
<th>% Positive 2 weeks post-vaccination</th>
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<tr>
<td>1</td>
<td>C1: FFB 5 0% 0% ND 0.6 ± 0.0 0% 0%</td>
<td>5 22 82% 82% ND 1.0 ± 0.4 (0.6–1.8) 50% 64%</td>
<td>T1: 1.2 × 10^10 in FFB, challenged 14 dpv</td>
<td>T2: 3.0 × 10^9 + ENABL/C210; challenged 14 dpv</td>
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<tr>
<td>2</td>
<td>C2: FFB + ENABL/C210 6 17% 17% 0% 0.6 ± 0.0 (0.6–2.1) 50% NA</td>
<td>6 34 97% 91% 47% 1.3 ± 0.4 (0.6–2.1) 71% 94%</td>
<td>T3: 2.7 × 10^9 + ENABL/C210; challenged 14 dpv</td>
<td>T4: 2.7 × 10^9 + ENABL/C210; challenged 7 dpv</td>
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IDL, intradermolingual; PU, particle units; VNT, virus neutralization test; FFB, final formulation buffer; NA, not applicable; ND, not determined.

Fig. 1. Efficacy Study 1. Serum virus neutralizing test (VNT) titers against FMDV A24/Cruzeiro/BRA/55 (A) and adenovirus serotype 5 (B). Number of steers per treatment group: C1, 5; T1, 22; T2, 10. Geometric mean VNT titers ± standard deviation for each treatment group at each time are presented. Differences between the FMDV A24/Cruzeiro/BRA/55 (A) results obtained on 7 dpv and 14 dpv/0dpc for the VNT titers in T1 and T2 were statistically significant (P = .04; P = .009). Differences between the adenovirus serotype 5 (B) results obtained on 14 dpv/0dpc, 21 dpv/7 dpc, and 28 dpv/14 dpc for the VNT titers in T1 and T2 were statistically significant (P < .001 for each). The range of positive values for both assays was >0.6–3.3 log10. The dotted line represents the limit of detection for the assay, 0.6 log10.
限检测，1.5 log10 TCID50/mL。水平线表示每组的挑战）到8 dpc；没有在0和5–8 dpc采集样品的FMDV阳性。

推测未来的研究将证明更低的最小有效剂量

IDL挑战 [13]。类似地，3 × 10^9 PU AdtA24/ENABL/C210 预防90–100%的病毒血症，一个单位的估计BPD90为3 × 10^10 PU 对AdtA24单独 [13]。基于估计的最小保护性

剂量，具有其他Adt-vectored serotype A和O subtype的ENABL，我们已经测试了（手稿提交准备中）。我们推测，未来的研究将证明一个更低的最小保护性

剂量的AdtA24/ENABL与>90%的效力。另一种化合物作为腺病毒作为佐剂，低聚ICLC，降低了保护

剂量的腺病毒5-vectored疫苗 [28]。

我们推测AdtA24疫苗促进免疫应答

希望，促进AdtA24疫苗的呈现与组装

FMDV A24/Cruzeiro/BRA/55空壳子或病毒样

颗粒的免疫反应 [23,30]。

虽然免疫反应对腺病毒样疫苗是更高的，但是我们不能期待一个抗原

效果的进一步研究，这些疫苗已经被报道 [13,21]。尽管AdtA24

疫苗的构建包含部分的FMDV NSP编码区域：3B1，除了编码区域的前六个氨基酸，和

完整的3B2，3B3，和3C编码区域，没有抗体被检测到7 dpc在非免疫的FMDV

或腺病毒样FMD或无活性FMDV接种，

在AdtA24-vaccinated steers. Sera from non-vaccinated FMDV-

infected animals are usually positive by 7 days post challenge

[13,31,32] (Chung, submitted; unpublished results). Because FMDV

mRNA transcripts in the tongue following the IDL inoculation

adenovirus vectored FMD or inactivated FMDV vaccinated,

FMDV-challenged cattle develop antibodies to the NSPs [12,32].

The PrioCHECK® FMDV NS ELISA uses a monoclonal antibody to

an unspecified epitope of FMDV 3B [33–35]，尤其是3B1，包括在至少删除了3B1编码区域

AdtA24（个人通信）。管理的相关的腺

病毒样抗原与FMDV capsid P1-2A和3C蛋白编码

区域导致在免疫或非免疫抗体在PrioCHECK® assay prior to infection, indicating that antibodies
to FMDV 3C protein are not recognized [11,12]。因此，AdtA24

does not contain the coding region for the epitopes recognized by

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Fig. 2. Daily FMDV titers in FMDV-positive nasal samples in Study 2. No. of positive samples/no. samples collected per treatment group on 1–4 dpc: C2, 15/24; T3, 23/136; T4, 9/40. FMDV titers, expressed as the tissue culture infective dose 50% (log10 TCID50/mL), were measured from nasal samples collected daily from 0 (prior to challenge) to 8 dpc; no samples collected on 0 and 5–8 dpc were positive for FMDV. Limit of detection, 1.5 log10 TCID50/mL. Horizontal lines for each group indicate the geometric mean FMDV titer and the standard deviation. There was no difference

in the positive titers between T3 and T4, P = 3, and there was a significant difference between the positive titers in C2 and T3 or T4, P ≤ .02.

Fig. 3. Efficacy Study 2. Serum virus neutralizing test (VNT) titers against FMDV A24/Cruzeiro/BRA/55 (A) and adenovirus serotype 5 (B). Number of steers per treatment

group: C2, 6; T3, 34; T4, 10. Geometric mean VNT titers ± standard deviation for each treatment group at each time are presented. Differences between the FMDV A24/

Cruzeiro/BRA/55 (A) results obtained on 7 dpv (-7 dpc) for C2 and T3, and for C2 on 14 dpv (0 dpc) and T4 on 7 dpv (0 dpc) were statistically significant (P < .001 and P = .03), and the 7 dpv data for T3 (-7 dpc) and T4 (0 dpc) were not different (P = .96). Differences between the adenovirus serotype 5 (B) results obtained on 7 dpv (-7 dpc) for C2 and T3, and for C2 on 14 dpv (0 dpc) and T4 on 7 dpv (0 dpc) were statistically significant (P < .001 for each). The 7, 14, and 21 dpv data for T3 and T4 were not different (P = .14, P = .11, and P = .32). The range of positive values for both assays was 0.6–3.3 log10. The dotted line represents the limit of detection for the assay, 0.6 log10.
the monoclonal antibody in the PrioCHECK® FMDV NS ELISA, resulting in the basis of the DIVA capability for the AdtA24 vaccine. Additionally, data from > 400 cattle that have been vaccinated with AdtFMD vaccines with different FMDV serotype P1 coding regions have always been negative in this ELISA assay prior to infection with FMDV, i.e. at 7, 14, 21 or 29 days post-vaccination (unpublished results). The only exception is the 1–2% false positive rate reported for the assay [33,36]. Using a different FMDV 3B ELISA (VMRD, Inc.) with a high correlation to results with the PrioCHECK® FMDV NS ELISA, no antibodies to FMDV 3B NSPs were detected in 52 AdtFMD-vaccinated cattle and pigs at 7–29 dpv (Chung, manuscript submitted). On a longer term, at 9–10 months after AdtA24 vaccination, uninfected cattle were negative in the newer FMDV 3B ELISA, although they were positive for antibodies to the FMDV A24/Cruzeiro/BR/55 capsid proteins and adenovirus vector [21].

Future research involves developing effective molecular FMD vaccines with or without an adjuvant for pigs. In our laboratory, using an oro-pharyngeal FMDV swine challenge model [37,38], AdtA24/ENABL® vaccine conferred 80% efficacy against clinical FMD, a result identical to that using a commercial FMDV A24/Cruzeiro/BR/55 vaccine (unpublished results). However, this same AdtA24/ENABL® vaccine dose was ineffective in pigs challenged via the intradermal heel bulb route. We are currently collaborating with federal and industry scientists to improve AdtFMD antigenicity and efficacy in swine.

Licensed FMD vaccines used worldwide are made from tissue culture adapted field isolates that are amplified, chemically inactivated, purified, and typically formulated with aqueous adjuvants (aluminum hydroxide/saponin) or as an oil emulsion (water-in-oil or water-in-oil-in water) [39–41]. Adjuvants used with inactivated FMDV vaccines enhance storage stability, provide a good antibody response, improve protection, and can shorten onset of protection [26,42–45]. Our results demonstrated that the addition of a ready-to-use adjuvant, ENABL®, with the recombinant AdtA24 DIVA FMD vaccine, lowered the cattle minimum protective dose and increased the antibody response significantly compared to AdtA24 alone, to provide a cost effective FMD vaccine.

Conflict of interest statement


Acknowledgments

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