



Oral delivery of maize-produced porcine epidemic diarrhea virus spike protein elicits neutralizing antibodies in pigs

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Received: 3 January 2020 / Accepted: 30 April 2020 / Published online: 11 May 2020
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Abstract

Porcine Epidemic Diarrhea Virus (PEDV) causes severe diarrhea and mortality in piglets. Robust immunity may break the transmission cycle. Expression of antigens in maize grains is a promising method for producing low-cost vaccines. As a first step, we expressed maize constructs containing PEDV S1 spike protein targeted to various cellular locations including the cell wall, endoplasmic reticulum, and vacuole, and fused to carrier proteins *E. coli* heat labile subunit (LTB) and a dendritic cell (DC) binding peptide, and obtained sufficient antigen for oral immunization. Constructs targeting S1 to the ER or fused to carrier proteins produced high levels of antigen of greater than 20 mg/kg. Oral administration to pigs elicited serum neutralizing antibodies, supporting oral immunization as a practical and cost-effective PEDV vaccine.

Key Message

Transgenic expression of spike protein antigen S1 from Porcine Epidemic Diarrhea Virus in maize accumulated sufficient antigen for oral immunization and elicited serum neutralizing antibodies in pigs.

Keywords PEDV · Maize-expression · S1 antigen · Oral vaccines · Neutralizing antibodies

Abbreviations

PEDV	Porcine epidemic diarrhea virus
COE	Core neutralizing epitope
TGEV	Porcine transmissible gastroenteritis virus
LTB	<i>E. coli</i> Heat labile subunit
SNA	Serum neutralizing antibodies
DC	Dendritic cell
ER	Endoplasmic reticulum
SFE	Supercritical fluid extraction
TCID	Tissue culture infectious dose

Communicated by Degao Liu.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11240-020-01835-0>) contains supplementary material, which is available to authorized users.

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Introduction

PEDV is a disease of swine causing major losses to the industry in the US and worldwide (Gerdtts and Zakhartchouk 2017). Newborn piglets are especially susceptible with attendant high mortality. The disease was first identified in Europe in the early 1970s, then in Asia in 2010, and in the United States in 2013. It continues to be a major problem in the United States, China, and many other countries. PEDV is a positive strand enveloped RNA virus of family *Coronaviridae* with a genome of 28 kb. The virus is divided into phylogenetic genotypes GIa, GIb, GIIa, GIIb, and GIIc (Guo et al. 2019), with GIa containing older strains such as CV777 and GIIa most prevalent in the United States. The spike protein is a transmembrane glycoprotein that binds the cellular receptor aminopeptidase N and recognizes sugar moieties. Although the spike protein is not actually cleaved, it is divided into subunits known as S1 and S2, largely based on homology to other related viruses (Chang et al. 2002; Sun et al. 2008). The S1 moiety contains a high number of neutralizing epitopes and is further subdivided into subdomains S10, S1A, S1B, S1C, and S1D.

A number of vaccines based on inactivated virus are on the market from sources such as Harris vaccines and Zoetis, but are only marginally effective and are largely based on classical strains such as CV777 (Gerdtz and Zakhartchouk 2017). There is a clear need for a low-cost and more effective vaccine for PEDV. In particular, a subunit vaccine based on the spike protein as the primary immunogen is desirable due to its several neutralizing epitopes. A number of prototype vaccines based on different portions of the spike protein have stimulated promising immune responses in animal studies (Oh et al. 2014; Makadiya et al. 2016). These include immunogens based on the S1 moiety (Oh et al. 2014) (Makadiya et al. 2016), the S2 moiety (Okda et al. 2017), and a smaller portion known as the core neutralizing epitope or COE (amino acids 499–638) that has been identified as containing neutralizing epitopes (Chang et al. 2002). However, the existing and many prototype vaccines require injection, which is labor-intensive and not likely to induce the strong mucosal response thought to be required for better protection against transmission across the mucus membranes. In addition, although progress has been made (Van Noi and Chung 2017), the protein has been difficult to produce at high levels in several recombinant systems (Makadiya et al. 2016; Piao et al. 2016).

It is apparent that delivery by oral administration to commercial stocks would eliminate the need for injection and greatly facilitate widespread vaccination against PEDV. Precedent for oral immunization for PEDV includes a number of studies expressing PEDV S or N proteins in probiotics such as *Lactobacillus*. Feeding of these products elicits an immune response and protection upon challenge in a mouse model (Di-qiu et al. 2012; Hou et al. 2018). The PEDV spike protein has also been produced in tobacco (8–20 µg/g wet weight or up to 5% total soluble protein (TSP)), rice (0.083% TSP), and other plants, and elicits an immune response with neutralizing activity against the virus in mice (Bae et al. 2003) (Kang et al. 2005) (Huy et al. 2012; Huy and Kim 2019). If this protection could be achieved in a system in which the antigen is stable during production and transport, and that does not require purification of the antigen away from other toxic compounds, a significant reduction in cost would be achieved, which could facilitate adoption of oral vaccines.

Direct oral administration with minimal processing makes maize grain a good option for commercial operations assuming it provides effective protection against the desired pathogen. The maize system has many inherent properties making it amenable to development of practical low-cost oral vaccines for livestock. In addition to high expression levels, a plant-produced antigen should be stable to allow for a low cost of production and storage. Seeds such as maize grain have evolved to maintain proteins in a stable environment, allowing germination after years of dormancy, in contrast to

vegetative tissue or fruits that undergo degradation shortly after harvest. This stability has been demonstrated for recombinant proteins that retain activity after being stored for years in the grain and allows for long-term storage, transport at ambient temperatures, and processing of the grain at will rather than a requirement to process large batches immediately upon harvest (Hayden et al. 2012). Stability can be further increased by removal of oils by supercritical fluid extraction (SFE), a process we have demonstrated for other antigens (Hayden et al. 2014). Bioencapsulation of the antigen in maize grain has been shown to help maintain antigenic determinants during passage through the digestive system leading to a higher immune response relative to purified protein, and the immune response may also be enhanced upon SFE treatment, as has been shown in other cases (Hayden et al. 2014). Early studies with a spike protein from another coronavirus, porcine transmissible gastroenteritis virus (TGEV), demonstrated that an orally-delivered maize-based candidate vaccine elicits an immune response and provides protection on challenge in pigs (Lamphear et al. 2004). Several other maize-based vaccines have also shown efficacy in animal trials (Hayden et al. 2015) and safety in a human clinical trial (Streatfield et al. 2002). There is also one report of expression of the PEDV spike protein COE in maize, which elicited an immune response in mice (Man et al. 2014).

In this study, we describe maize lines targeting expression of the spike protein of Porcine Epidemic Diarrhea Virus (PEDV) to various subcellular locations and in fusion with proteins known to increase immunogenicity, and demonstrate the production of neutralizing antibodies in serum. Relatively high accumulation of the S1 portion of the spike protein was observed using a construct with a signal targeting expression to the endoplasmic reticulum (ER) as well as using fusions of S1 with LTB and DC peptide that may act as carriers to increase immunogenicity. Maize flour containing S1 with an ER-targeting signal was orally administered to piglets and elicited a neutralizing serum antibody response. To our knowledge, this is the first report of an immune response in pigs using plant-produced material expressing PEDV S1 to elicit neutralizing antibodies on oral delivery. This proof-of-concept provides encouragement for this approach in the development of an effective oral vaccine against PEDV.

Materials and methods

Preparation of constructs

The sequence of the spike protein from the PEDV strain Colorado 2013 (Genbank KF272920) was optimized for maize codon usage. The nucleotide sequence of the coding

region was outsourced for commercial gene synthesis by GenScript. Six different constructs were prepared (Fig. 1) with varying subcellular targets and fusions. The native signal sequence was replaced with a barley alpha amylase signal sequence (BAASS) for cell wall targeting in constructs PDA, PDC, PDD, PDK, and PDM, and with a vacuolar targeting sequence in construct PDB. The S1 coding region (aa 23–738 for constructs PDA, PDB, PDC, and PDB or aa 23–789 for constructs PDK and PDM) was synthesized for transfer into the maize transformation vector pSB11 (Komari et al. 1996) using an NcoI site overlapping the initiating ATG and a PstI restriction site in the terminator region to add the coding region downstream of the promoter. For the other constructs, smaller portions of the coding region containing, for example, the KDEL ER targeting sequence, were commercially synthesized to exchange with portions of the full-length coding region by restriction digestion with EcoRI and HindIII and ligation. All constructs except one, PDD, incorporated the S1 coding region under control of a promoter derived from the maize globulin-1 gene (pr25 or pr44) which target expression to the embryo. In construct PDM an engineered version of the pr25 promoter, pr44, contains two extra copies of the 5' region of pr25 (Hayden et al. 2012). One construct (PDD) incorporated the S1 protein under control of a promoter (pr39, derived from the maize 27 kDa gamma zein gene) targeting expression to the endosperm. Each transcription unit also incorporated the terminator from potato proteinase inhibitor II. Subcellular localization and terminator sequences were as previously described

(Hayden et al. 2012). Further details are included in supplementary Material S1.

Maize transformation

Maize transformation was carried out as previously described with modifications (Ishida et al. 1996). In brief, the constructs were transferred into the LBA4404 *Agrobacterium* strain containing the vector pSB11 by a triparental mating procedure (Komari et al. 1996). The cointegrate DNA was then electroporated into *Agrobacterium tumefaciens* strain EHA101 (Hood et al. 1986). HiII maize embryos roughly 1.5 to 3 mm in length were mixed with *A. tumefaciens* EHA101 with the appropriate vector for transformation (Ishida et al. 1996). Plants from events selected on bialaphos were grown to maturity in the greenhouse and pollinated with HiII to produce T1 generation seed. Further details are included in supplementary Material S2.

Western blot analysis

A small antigenic portion of the S1 protein known as the core neutralizing epitope (COE, aa 494–641) was cloned, expressed and purified by GenScript. The resulting protein was used as an immunogen to prepare polyclonal anti-S1 antibodies in rabbit by Pacific Immunology. Proteins were extracted from ground maize seed with 1 X PBS + 1% SDS, loaded onto a 4–12% bis–tris gel (LifeTech), and transferred to PDVF membrane by iBlot. The blot was incubated in Pacific Immunology's custom rabbit anti-PEDV

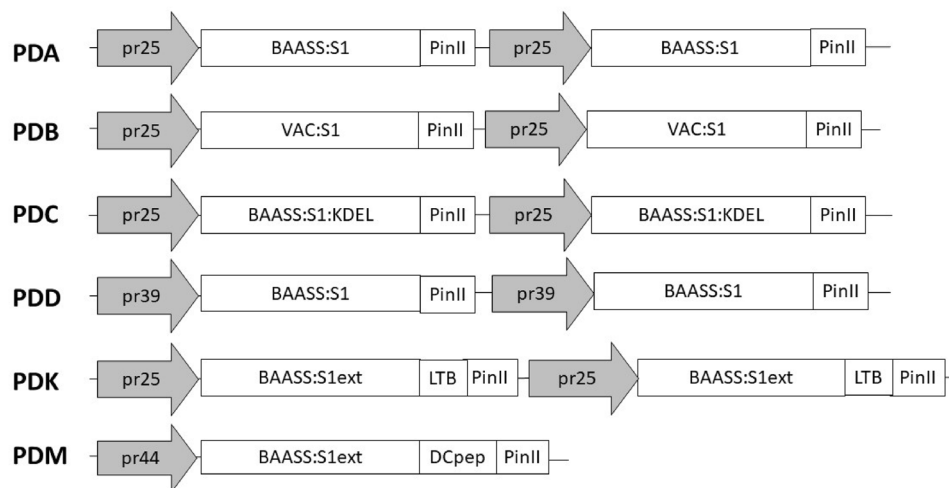


Fig. 1 Diagram of constructs. The layout of six transformed constructs is shown. Promoters pr25 and pr44 target expression to the embryo and promoter pr39 targets expression to the endosperm. The BAASS signal sequence targets expression to the cell wall but addition of the KDEL sequence causes the protein to be retained in the ER. Construct PDB contains a signal sequence targeting expression

to the vacuole. The spike protein coding region from the Colorado 2013 strain is either amino acids 23–738 (S1) or amino acids 23–789 (S1ext). All constructs contain the PinII terminator sequence. Constructs PDK and PDM have the LTB or DC peptides, respectively, fused to the carboxy terminus

S1 overnight at a dilution of 1:2000 and developed with anti-rabbit-alkaline phosphatase conjugate at a dilution of 1:2000 (Jackson ImmunoResearch #111–055-003) and BCIP-NBT liquid substrate (Sigma #B1911). The positive control was 10 ng of COE standard synthesized by Genscript and the concentration of S1 was estimated visually using this standard.

Preparation of material

Several high-expressing maize lines were identified for construct PDC. Seed from the first generation of plants (T1) was planted to obtain additional grain (T2) for pig studies. T1 and T2 generation material was pooled and used for the animal studies. Since expression was targeted to the embryo, the S1 antigen was further concentrated by enriching for the germ fraction using customized small-scale processing equipment. The germ fraction was then dried to obtain a moisture content of 11% and ground such that > 80% of the material could pass through a 20-mesh screen.

Pig study

Young pigs were purchased specifically for this study and were commercial cross-bred animals of mixed gender born on the same day, and which were 21 days old upon arrival to the test facility. They were sourced from a high health status herd in Wisconsin (Wilson's Prairie View Farms, Burlington, WI) that has never been diagnosed with PEDV and does not vaccinate for PEDV, and determined to be free from PEDV by PCR analysis of fecal material and by serum neutralizing tests performed by South Dakota State University Diagnostics Laboratory. Prior to weaning and delivery to the test facility, all pigs received a dose of PCV2 vaccine (Circoflex®), antibiotic (0.3 mLs Excede®) and a dose of Vitamin E (Vital E®). Piglet weights ranged from 5.1 to 8.0 kg at Day -5 (one day after arrival). The weights of the pigs at different stages of the study are included in Supplementary Material S3.

Pigs were housed at a BSL-2 facility. All pigs were housed in one hepa-filtered isolation room. Pens were raised plastic tube (4ft × 5ft) with plastic slatted flooring. Four pigs per pen (five pens total) were used. Feed was a commercially available Purina® brand type that was appropriate for age/size of pigs. Feed was provided ad libitum via a six-hole plastic nursery feeder with immunization as described below. Water was provided ad libitum via one nipple waterer. Water was sourced from the on-site rural well. Photoperiod was controlled by a timer and provided 15 h of light and 9 h of dark. Room temperature was monitored daily with a high/low thermometer (range of 68°–83°F).

All pigs were observed daily for general health by trained personnel. No abnormal health observations occurred during

the conduct of the study. Standard Operating Procedures (SOPs) at VRI describing how animals are to be treated were followed. All personnel involved with the study are required to review the SOPs and are reviewed by management to be proficient at performing the pertinent SOPs.

All pigs were euthanized via an overdose of barbiturate (euthanasia solution with active ingredients 390 mg/mL pentobarbital sodium and 50 mg/mL phenytoin sodium). Each pig was restrained by snaring and a bolus of approximately 10 mLs of the solution was intravenously administered in the jugular vein. Each animal was tested for ocular reflexes and observed for respiratory movements. Upon absence of these movements, each animal was exsanguinated by incising the jugular area.

No IACUC review was done for this project. VRI reviews projects based on request by sponsors. According to the 9CFR, Animal Welfare section—agricultural animals are exempt from IACUC approval. VRI does have SOPs in place for the conduct of animal trials. The “Guide for the Care and Use of Laboratory Animals, National Research Council, Eighth Edition, 2011” was used as a resource for the basis of these SOPs. SOPs are reviewed every two years and personnel are also reviewed for proficiency in following the SOPs.

Animals and treatment groups

A double-blind study was conducted by Veterinary Resources Inc. (VRI, Ames, IA). Twelve pigs total, allotted to 3 treatment groups (Gps 1, 2, 3) with four pigs per treatment group were made. For the assignment to groups, pigs were blocked by body weight/assigned a random number using Excel® random number generator/sorted by ascending random number. The pig with the lowest random number was assigned to group A, the next lowest random number to group B and so on. Pigs were fed Ultra Care 240 or 500 Med. PDC maize material was fed to pigs as a prime and booster dose (Table 1). A primary dose was administered after six days acclimation. Another treatment consisted of pigs that were injected with a commercial virus-based vaccine, while a third group was administered untransformed ground maize germ. Animals (4 per treatment) were either individually fed recombinant or control ground maize material (29 g/day on three successive days for a total of 87 g) or injected intramuscularly with a commercially available killed virus vaccine (Zoetis). The animals were fasted for

Table 1 Pig study design

Group	Prime	Boost
1	Injected	Injected
2	Oral S1 maize	Oral S1 maize
3	Oral control maize	Oral control maize

four hours before being offered the maize material and returned to their normal diet an hour after administration. Each animal was hand fed and consumed the full dose of maize material offered. At day 28 a booster dose of oral material or injection was administered.

Challenge, sample collection, and analysis of serum response

Virus (isolate PEDV USA/NC49469/2013) for challenge was obtained from Dr. Jianqiang Zhang at Iowa State University. Challenge by intragastric inoculation was on day 42 after primary immunization with 10 mL at 10^4 to 10^5 TCID₅₀/mL, the recommended dose based on experience with this isolate. Serum samples were collected on day 0 (pre-screen), day 42 (pre-challenge), and 15 days after challenge. Serum neutralizing antibody (SNA) response was determined by fluorescent focus neutralization assay at the South Dakota State University Diagnostics Laboratory. Vero-76 cells were seeded onto 96 well microplates and cultured for 3–4 days. Serum samples were added to the cells in serial 1:2 dilutions and PEDV virus stock was added at approximately 100 FFU/well. After overnight incubation in MEM media with trypsin cells were fixed by addition of 80% acetone. Monoclonal antibody SD6-29 conjugated to FITC was added and binding was assessed with fluorescent microscope. A sample was considered positive if 90% inhibition of fluorescent foci were observed and the titer was reported as the highest dilution that has $\geq 90\%$ inhibition. A more detailed protocol is available on request.

Statistics

A mixed effects analysis of variance (ANOVA) was used to analyze the data with the final titer as response variable of interest and the treatment group and the weight group as factors. Weight group was included in the analysis as a random effect to account for any variation in the titers due to animal weight. Differences in the treatment groups were compared using Tukey's HSD procedure with a 5% significance level. The data were analyzed on a base-2 log scale. Comparing the titers on the log scale detects significant differences between the treatment groups (p -value = 0.05). The differences between groups were determined using the Tukey HSD method.

Results

Expression of S1

A number of constructs coding for the PEDV S protein S1 subunit were prepared and transformed into maize to

produce the subunit vaccine (Fig. 1). Three constructs, PDC, PDK, and PDM, showed the highest levels of expression of PEDV S1 (> 20 $\mu\text{g/g}$ seed), based on Western blot analysis of single seeds (Fig. 2). Construct PDC codes for a portion of the spike protein S1 region incorporating amino acids 23–738 with a BAASS signal sequence at the N-terminus and a KDEL sequence for endoplasmic reticulum (ER) retention at the C-terminus. Construct PDK contains an extended S1 protein incorporating amino acids 23–789, with the BAASS signal sequence replacing the signal sequence at the N-terminus and fused to LTB at the carboxy terminus. Construct PDM contains the extended S1 protein fused to a DC-targeting peptide at the carboxy terminus. In each case expression is under control of a promoter derived from the maize globulin 1 gene that directs expression to the embryo portion of the maize kernel. Also, in constructs PDC and

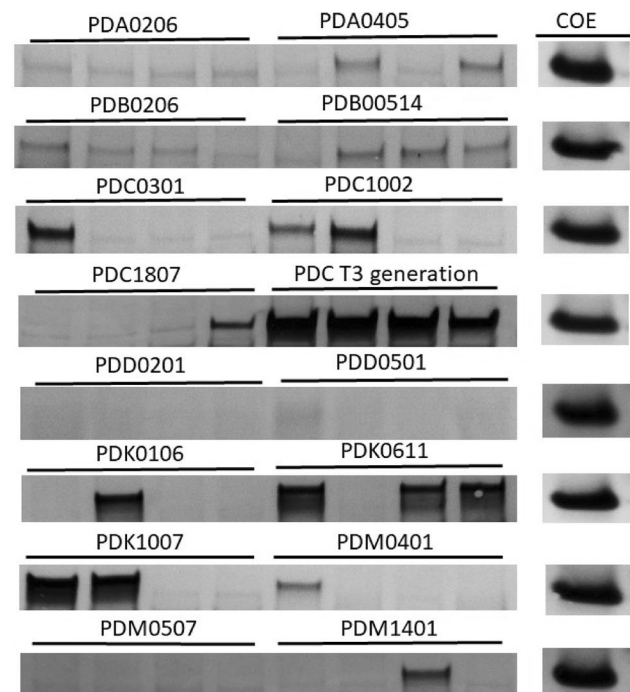


Fig. 2 Western blot of S1 expression in representative seed. Four representative individual seeds for the indicated plant were pulverized and extracted in 1 X PBS + 1%SDS. The protein was transferred to PVDF membrane and incubated with polyclonal rabbit antibody raised to commercially synthesized COE portion of the spike protein (shown as a positive control at the right). Note that in all cases except PDC T3 generation, T1 generation seed crossed to untransformed maize is shown and half of the seed are expected to be positive. PDC T3 has been self-crossed leading to expression in all seeds. The predicted sizes for PDC, PDK, PDM and the COE are 81.2 kDa, 97.6 kDa, 87.4 kDa, and 16.1 kDa respectively. The expected segregation pattern with a robust band in approximately half the seed in T1 generation plants and not in untransformed maize (not shown) is indicative of specificity, while other non-specific bands were observed in all seed. However, the bands for maize-produced S1 run at a higher apparent molecular weight than predicted, possibly due to post-translational modifications such as glycosylation

PDK the plant transcription unit was duplicated in the final construct. Constructs targeting expression to the cell wall (PDA), to the vacuole (PDB), or under control of a promoter targeting expression to the endosperm portion of the kernel (PDD) showed minimal expression relative to PDC, PDK, and PDM. Early generation heterozygous plants from construct PDC were used in the animal study. However, to increase the potential yield, a back-cross program was simultaneously undertaken for constructs PDC, PDK and PDM. Homozygous lines have been identified (Fig. 3) and this should at least double the yield for future use.

Animal study

PDC maize material was prepared as ground meal and fed to pigs as a prime and booster dose (Table 1). Three groups of four pigs each were either injected with an existing commercial vaccine, fed maize material containing PEDV S1, or fed control maize material. Quantitation of S1 was by visual comparison of the intensity of bands on western blots using 10 ng of commercially-synthesized COE peptide as standard. Although the different sizes of the recombinant protein and the COE standard may affect the accuracy of this estimate, each dose contained approximately 0.8 mg of S1.

The pigs were screened before immunization and found to have negligible levels of serum neutralizing antibody titer (SNA). SNA were only observed prior to the challenge in pigs administered the injected vaccine. After the challenge, the pigs administered orally delivered antigens and injected vaccines both showed a significant increase in SNA over the control group ($p < 0.05$, Fig. 4). In both cases the response was statistically different from the control group administered untransformed maize. There was not, however,

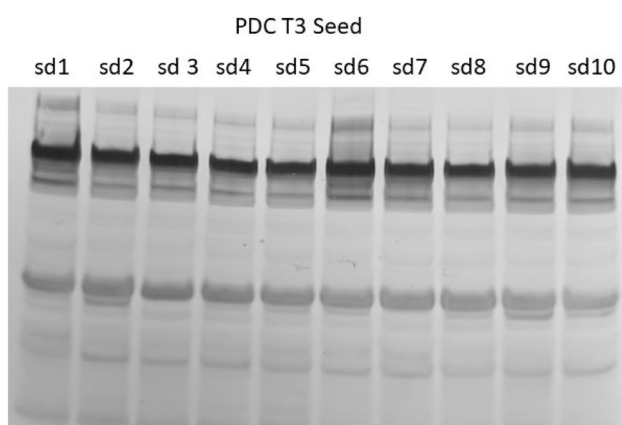


Fig. 3 PEDV S1 expression is maintained in homozygous plants after a back-cross program. Ten individual seed from plants harboring construct PDC after self-crossing for homozygosity were tested for S1 expression by western blot as described for Fig. 2. After initial analysis (Fig. 2), the recombinant COE standard is not included to maximize gel space when screening individual seed for homozygous lines

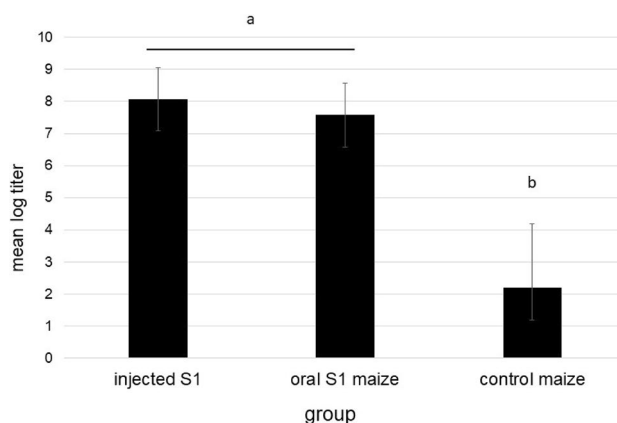


Fig. 4 Pig serum neutralizing antibody response. Serum neutralizing antibody response from the indicated groups ($n = 4$) were assessed by fluorescent focus neutralization assay at 15 days post-challenge (dpc), as described in the methods section. At 15 dpc treatment groups containing S1 (one and two, a) showed significant differences with the control group (three, b) with p -values for each comparison substantially below 0.05. There was no significant difference in the titers between treatment groups one (injected S1) and two (orally-delivered maize S1)

a statistically significant difference between the two vaccinated groups. Symptoms were monitored daily but only a few pigs showed symptoms of minor diarrhea, with no correlation to any group. This is most likely because the age of the pigs (~2 months) had passed the most susceptible period, as newborn pigs are most susceptible to PEDV.

Discussion

We have demonstrated relatively high levels of accumulation in maize grain of the PEDV spike protein S1 with an ER-targeting signal. Current estimates of expression are at least 20 mg/kg seed. Although limited availability of suitable reagents have precluded development of an ELISA at this time, in future work we will continue refine the accuracy of this estimate. Typically, at least a tenfold increase in expression can be achieved through a program of traditional back-crossing into elite germplasm and selecting the highest-expressing ears at each generation (Hood et al. 2012). Thus we can predict long-term yields of the antigen to be at least 200 mg/kg and most likely higher. With average grain production at 200 mg antigen/kg seed and a dose of 20 mg, one acre can produce 0.8 kg antigen or 40,000 doses. Therefore a typical small farm of 160 acres can produce over 6 million doses. This level of scale-up can allow for the cost-efficient production of a subunit vaccine, especially as this antigen has been very difficult to express in other systems. More critically, however, we have also shown that after the virus challenge, maize-produced and orally delivered S1 was

able to elicit serum neutralizing antibodies in pigs at levels similar to those elicited by the commercial injected vaccine post-challenge. Prior to the virus challenge, little serum neutralizing antibody response was detected in the group fed maize-produced S1 or control maize. This observation is similar to earlier results with TGEV that showed significant titers only after the viral challenge. Presumably, this is due to a response below the limits of detection after the first boost of the vaccine. The maximum SNA titer 15 days after challenge and 8 weeks after primary immunization was 192 to 271, or 7.6 to 8.0 converted to a base-2 log scale. Several variables make direct comparison difficult, but in one study by Oh et al. (2014), injection of recombinant S1 protein led to SNA levels of approximately 8 to 32 on a log₂ scale at three and six weeks after primary immunization, respectively. In the study by Makadiya et al. maximum titers of approximately 90 (unconverted titer) are reported after injection of purified recombinant protein (Makadiya et al. 2016). In a more recent report (Choe et al. 2020), a similar neutralizing antibody titer of 7–8 on a log₂ scale was obtained on oral immunization with S1-containing microspheres. The work presented here indicates that the orally-delivered S1 produced in maize can elicit a response similar to other subunit vaccine delivery systems.

The relatively high serum response in this study is surprising because SNA are normally low compared to the response in mucosal tissues when candidates are orally delivered. However, these results are also encouraging, since the mucosal response is likely to be even more robust and may be more protective for this disease than the serum response. Future work will include development of reliable methods for detection of mucosal response.

Newborn piglets are most susceptible to PEDV and clinical symptoms in this study were minimal after the viral challenge in all groups due to the older age of the pigs. The ideal commercial vaccine will use vaccination of sows to allow for lactogenic immunity from colostrum to be passed to suckling piglets. Additional material is being generated that will allow more extensive studies addressing lactogenic immunity. We anticipate higher levels of antigen for the much larger dams. This can be achieved either by increasing the S1 concentrations using selection, as described earlier for other antigens (Hood et al. 2012) and shown in Fig. 2 (PDC T3 generation), for PEDV S1 or by feeding larger amounts of maize flour to the sows.

In addition, while construct PDC was the first for which adequate material was available for this initial animal study, a more robust immune response may be obtained using higher amounts of antigen from constructs PDK and PDM. These constructs harbor an extended region of the S1 domain that includes additional epitopes as a fusion with two carrier proteins. Fusion of the antigen to either the DC peptide or LTB both showed relatively good accumulation of

the S1 antigen. These carrier peptides can enhance immunogenicity in some cases, including reports for PEDV (Wang et al. 2017; Chang et al. 2018). Future studies with these constructs will address whether an increase in immunogenicity is observed with the extended S1 region and on addition of LTB or the DC peptide.

In conclusion, we have demonstrated high levels of expression of a recalcitrant antigen, PEDV spike protein, in transgenic maize. On oral feeding to pigs, this spike protein elicited a serum neutralizing antibody response that after challenge was comparable to that elicited by a commercial vaccine. After optimization, this system should result in a heat-stable oral vaccine for PEDV that can be conveniently administered in feed. Once the system is established for one PEDV strain it should be possible to prepare new maize lines expressing the S1 protein from different strains with different sequences as needed.

Acknowledgements We thank Todd Anderson, Madisen Baldwin, Justin Borba, Susan Christopher, Matthew Humkey, Audrey Lent, and Adam Tassinari for assistance with plant transformation and greenhouse plant care.

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