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Assessment of the cross-protective capability of recombinant capsid proteins derived from pig, rat, and avian hepatitis E viruses (HEV) against challenge with a genotype 3 HEV in pigs

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Abstract

Hepatitis E virus (HEV), the causative agent of hepatitis E, is primarily transmitted via the fecal-oral route through contaminated water supplies, although many sporadic cases of hepatitis E are transmitted zoonotically via direct contact with infected animals or consumption of contaminated animal meats. Genotypes 3 and 4 HEV are zoonotic and infect humans and other animal species, whereas genotypes 1 and 2 HEV are restricted to humans. There exists a single serotype of HEV, although the cross-protective ability among the animal HEV strains is unknown. Thus, in this study we expressed and characterized N-terminal truncated ORF2 capsid antigens derived from swine, rat, and avian HEV strains and evaluated their cross-protective ability in a pig challenge model. Thirty, specific-pathogen-free, pigs were divided into 5 groups of 6 pigs each, and each group of pigs were vaccinated with 200 μg of swine HEV, rat HEV, or avian HEV ORF2 antigen or PBS buffer (2 groups) as positive and negative control groups. After a booster dose immunization at 2 weeks post-vaccination, the vaccinated animals all seroconverted to IgG anti-HEV. At 4 weeks post-vaccination, the animals were intravenously challenged with a genotype 3 mammalian HEV, and necropsied at 4 weeks post-challenge. Viremia, fecal virus shedding, and liver histological lesions were compared to assess the protective and cross-protective abilities of these antigens against HEV challenge in pigs. The results indicated that pigs vaccinated with truncated recombinant capsid antigens derived from three animal strains of HEV induced a strong IgG anti-HEV response in vaccinated pigs, but these antigens confer only partial cross-protection against a genotype 3 mammalian HEV. The results have important implications for the efficacy of current vaccines and for future vaccine development, especially against the novel zoonotic animal strains of HEV.

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Keywords

Hepatitis E virus (HEV); Capsid protein; Vaccine; Pigs; Cross-Protection

Introduction

Hepatitis E virus (HEV), the causative agent of acute hepatitis E in humans, is an important pathogen worldwide [1–4]. The mortality rate can reach up to 28% in HEV-infected pregnant women, although it is less than 1% in the general populations [5]. Large outbreaks of acute hepatitis E due to contaminated water sources occur in countries where modern sanitation practices are not commonly implemented [3, 6]. While less common, zoonotic transmissions leading to sporadic or cluster cases of acute hepatitis E are a growing concern in both industrialized and developing countries [2]. Thus far, at least four recognized and two putative genotypes of mammalian HEV have been identified worldwide. The genotypes 1 and 2 HEV infect only humans, while genotypes 3 and 4 HEV have an expanded host range and are zoonotic [2–4]. The putative genotypes 5 and 6 were recently identified from wild boars in Japan [10] and rats in Germany [21], respectively.

Meng et al (1997) identified the first animal strain of HEV in pigs designated as swine HEV, while another animal strain of HEV, avian HEV, was isolated from chickens in 2001 [7, 8]. HEV infection in pigs appears to be asymptomatic [7]. All swine HEV strains identified worldwide thus far belong to either genotype 3 or 4 [2, 3, 9] except for a putative new genotype recently isolated from a wild boar in Japan [10]. Genotypes 3 and 4 HEV are able to infect across species barriers [2–4, 11]. Additionally, a variant strain of genotype 3 HEV isolated from rabbits was capable of infecting pigs [12]. Confirmed zoonotic human infections arise primarily from consumption of contaminated pig liver, pork products, and deer meats [13, 14] as these products contain infectious HEV [15].

Avian HEV is enzootic within chicken flocks and is identified as the cause of hepatitis-splenomegaly syndrome (HS syndrome) [16]. Thus far at least three genotypes of avian HEV have been identified from chickens worldwide including the United States, Australia, Spain, and China [17–20]. Avian HEV only shares 50–60% nucleotide sequence identity with swine or human HEV strains and likely belongs to a separate genus [8, 17, 18]. Besides pigs, humans, and chickens, HEV has also been genetically identified from rats [21, 22], mongoose [3], deer [14, 23], rabbits [24, 25], and even fish [26]. All mammalian HEV genotypes identified thus far belong to a single serotype.

Recombinant ORF2 capsid proteins of HEV are capable of inducing neutralizing antibodies when used as candidate vaccines in humans [27, 28], non-human primates [29], and chickens [30]. These candidate vaccines contain immunogenic epitopes toward the C-terminus of the 660 amino acid capsid protein [31]. When the 111 N-terminal amino acids are absent, the truncated capsid protein forms virus-like particles (VLPs) [32] which enhance vaccine efficacy [32, 33]. HEV capsid antigens are capable of cross-reacting with antibodies from heterologous HEV strains [12, 34, 35] while convalescent sera from animals infected with any of the four recognized HEV genotypes were capable of neutralizing genotype 1 HEV *in vitro* [31]. The objective of this study was to determine if the recombinant capsid antigens derived from swine, rat, and avian HEV strains can induce cross-protection against a genotype 3 HEV challenge in a pig model.

Materials and Methods

Expression and purification of truncated recombinant capsid proteins derived from swine, rat, and avian HEV strains

The N-terminal truncated capsid proteins from swine, rat, and avian HEVs were expressed in a bacterial expression system to generate the immunogenic ORF2 capsid proteins lacking the 111 N-terminal amino acid residues [32, 36]. Briefly, the truncated ORF2 genes were amplified from respective plasmids containing avian, genotype 3 swine, and rat HEV ORF2 genes with strain-specific primers (Table 1). Each amplified PCR product was gel-purified, digested with restriction enzymes, and subsequently cloned into the pRSET-A bacterial expression vector (Invitrogen). The authenticity of each ORF2 gene insert was verified by sequencing. Each recombinant pRSET-A plasmid was transformed into BL21(DE3)pLysS (Novagen) *E. coli* cells and then grown in an auto-inducing expression system using Overnight Express Instant TB Medium (Novagen) essentially as described [12]. To facilitate the purification of the resulting recombinant capsid proteins, six histidine residues were incorporated into the proteins.

To purify the recombinant capsid proteins, bacterial cell pellets were lysed with BugBuster Protein Extraction Reagent (Novagen) mixed with 6M urea as a denaturant for the otherwise insoluble protein. Histidine-tagged proteins were extracted from the supernatant of cell lysates using HisPur nickel spin columns. Each protein solution underwent sequential dialysis steps against decreasing concentrations of urea using Slide-A-Lyzer dialysis cassettes (Thermo Scientific) with the final urea concentrations of 2M in Tris-HCL buffer. Each recombinant capsid protein was confirmed by Western blot using IRDye800 conjugated with antibody against poly-histidine residues, and visualized with an Odyssey Imager (LI-COR Biosciences) at 800nm. Additionally, Western blot was also performed using a hyperimmune antiserum from a pig immunized with a purified recombinant capsid antigen of a genotype 1 HEV as the primary detecting antibody [7]. The secondary antibody was IRDye800 conjugated with anti-swine antibody as previously described [12]. Each protein was diluted with the final elution buffer to a concentration of 200 μg/ml and stored at −80°C.

Challenge HEV virus stocks

The genotype 3 HEV (Meng strain) virus stock with a $10^{4.5}$ 50% pig infectious dose (PID_{50}) was used as the challenge virus. The generation and titration of the infectious virus stock was reported previously [37, 38].

Vaccination and challenge experimental design

Thirty, 5-week-old, specific-pathogen-free (SPF), pigs that were negative for IgG anti-HEV were separated into 5 groups of 6 pigs each and housed in a BSL-2 facility. Pigs in each group were vaccinated intramuscularly with 1 ml of PBS (later challenged with PBS as negative control), PBS (later challenged with HEV as positive control), swine HEV capsid antigen, rat HEV capsid antigen, or avian HEV capsid antigen. At two weeks post-vaccination (wpv), a booster dose of each antigen was administered to each pig. Each ml of vaccine contained approximately 200μg of respective antigen and was administered equally (0.5 ml) to muscles on each side of the neck. At 4 wpv when the vaccinated pigs all developed a high titer of IgG anti-HEV, all pigs with the exception of the negative control group were intravenously challenged with 1 ml of the prototype genotype 3 HEV (Meng strain) containing $10^{4.5}$ PID_{50} infectious virus. The negative control pigs were challenged with PBS buffer. All pigs were necropsied at 4 weeks post-challenge (wpc) at which time the microscopic lesions in the liver peak [39].

Sample collection

Serum samples and fecal swab materials were collected prior to vaccination and weekly thereafter from each pig. Following vaccination, the weekly serum samples were tested for IgG anti-HEV by an ELISA [7]. After challenge, the sera and 10% suspension of fecal samples were tested weekly for the presence of HEV RNA by a nested RT-PCR. Liver, bile, and small intestinal contents were collected at necropsy. Bile and 10% suspension of small intestinal contents were tested for HEV RNA while the liver samples were evaluated for histological hepatic lesion by a pathologist using a scoring system previously described [39].

RT-PCR to detect HEV RNA

Serum and fecal samples were tested by a genotype 3 HEV-specific nested RT-PCR as described previously [37] except that the strain-specific primers target the ORF1 gene. Briefly, total RNAs were extracted from 100 μl of 10% fecal suspension or serum samples using TriZol reagents (Life Technologies) and resuspended in 30 μl of sterile water. Reverse transcription reactions were performed at 42°C for 1 hr with 1 μl (10 μM) of the external reverse primer HEVORF1-REV1, 1 μl (200 units/μl) of Superscript II reverse transcriptase (Life Technologies), 1 μl of 0.1M dithiothreitol, 4 μl of 5× RT buffer, 0.5 μl (40 units/μl) of RNasin ribonuclease inhibitor (Promega), and 1 μl of 10 mM deoxynucleoside triphosphates. For the first round PCR, forward primer HEVORF1-FWD1 and reverse primer HEVORF1-REV1 (Table 1) were used to amplify a 471 bp product. For the second round nested PCR, forward primer HEVORF1-FWD2 and reverse primer HEVORF1-REV2 (Table 1) were utilized to amplify a 277 bp fragment using 5 μl of the first round PCR product as the template and AmpliTaq Gold DNA polymerase (Applied Biosystems). The cycling parameters included an initial denaturation/polymerase activation step at 95°C for 9 min, followed by 39 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 52°C, extension for 1 min at 72°C, and a final extension step at 72°C for 7min. The resulting PCR products were analyzed via electrophoresis on a 1% agarose gel.

Enzyme-linked immunosorbent assay (ELISA) to detect IgG anti-HEV

Pig serum samples were tested by an ELISA as previously described [7, 37, 40] except that the respective homologous antigen derived from swine, avian and rat HEVs was used in this study. The ELISA cutoff was set at 3 standard deviations above the mean OD values of the pre-vaccinated serum samples. All sera were tested in duplicate at a 1:100 dilution.

Statistical analyses

Histopathologic lesions were recorded as lesion scores (Table 2). The means of lesion scores were compared by an unpaired *t-test* (Excel, Microsoft Inc.). A *P*-value below 0.05 was considered significant.

RESULTS

Characterization of the truncated recombinant capsid antigens of swine, rat, and avian HEV expressed in *E. Coli*

The truncated ORF2 proteins derived from swine, avian and rat HEV were each expressed as insoluble inclusion bodies with an approximate predicted size of 58 kDa [41]. Following nickel affinity chromatography purification, the his-tag fused to each protein was properly identified on each of the recombinant fusion proteins using Western blot with IRDy800 (LI-COR Biosciences) conjugated antibodies to poly-histidine tags (Fig. 1A). Further Western blot analyses using anti-HEV ORF2 hyperimmune swine antiserum raised against a genotype 1 human HEV confirmed the expression of swine, rat, and avian ORF2 proteins (Fig. 1B). The antiserum raised against the genotype 1 human HEV cross-reacted to the

truncated recombinant capsid antigens from genotype 3 swine HEV, avian HEV and rat HEV (Fig. 1B), further confirming the existence of a single HEV serotype.

The truncated recombinant capsid antigens derived from swine, rat, or avian HEV induced strong IgG anti-HEV antibody responses in vaccinated pigs

Pigs vaccinated intramuscularly with swine, rat, or avian HEV ORF2 capsid antigens all developed strong IgG anti-HEV antibody responses as detected with the respective antigen-specific ELISA (Fig 2B, 2C, 2D). There was no detectable difference in the level or pattern of antibody responses among the vaccinated groups, indicating that the capsid antigens derived from different animal HEV sources are capable of inducing antibody production in pigs. After a booster dose at two weeks after the initial vaccination, all pigs in each group seroconverted to IgG anti-HEV. The control pigs that were vaccinated with PBS were seronegative (Fig 2A).

Hepatic histological lesion scores at 4 weeks post-challenge (wpc)

Sections of liver samples from each pig were collected at necropsy at 4 wpc and processed for routine histological examination for the presence or absence of lymphoplasmacytic hepatitis (Table 2) by a pathologist as described previously [39]. Only mild lymphoplasmacytic hepatitis was observed (Table 2), which is consistent with previous studies [39], as genotype 3 HEV infection is generally subclinical in pigs [7]. In general, the pigs vaccinated with avian, swine or rat HEV antigens all had lower average mean histological scores when compared to the unvaccinated and challenged pigs (Table 2). Interestingly, the group with the lowest average hepatic lesion score was vaccinated with the avian HEV ORF2 antigen.

Detection of HEV RNA in serum and feces with RT-PCR

After challenge, the detection of HEV RNA was performed using a nested RT-PCR with primers that target the ORF1 gene regions. Positive and negative control sera or fecal suspension of a genotype 3 HEV were included in all steps of the RNA extraction, reverse transcription and nested PCR processes to ensure proper performance of the RT-PCR. HEV viremia was not detected in any of the challenged pigs, which is consistent with the nature of genotype 3 HEV infection in pigs (Table 3). All 6 unvaccinated pigs shed virus in feces after challenge, and the virus shedding lasted 1–2 weeks (Table 3). In contrast, pigs vaccinated with swine HEV or avian HEV had only 3 pigs within each group that shed virus in feces, although the pigs vaccinated with rat HEV antigen all had fecal virus shedding. At necropsy, HEV RNA was not detected in the bile or small intestinal contents of any pig. As expected, the negative control pigs challenged with PBS buffer had no viremia or fecal virus shedding at any time.

DISCUSSION

The current licensed and experimental HEV vaccines are all based on the recombinant capsid protein derived from a single strain of HEV. The identification of numerous genetically distinct animal strains of HEV and the demonstrated cross-species infection by these animal strains raise a question as to whether a vaccine based on a single HEV strain will confer sufficient protection against strains from different genotypes especially those animal strains with zoonotic potential. To our knowledge, this is the first study evaluating the cross-protection efficacy of recombinant capsid antigens derived from various animal HEV strains as candidate vaccines against HEV challenge in a pig model.

Vaccine trials of HEV ORF2 antigens have been performed in chickens [30], non-human primates [36, 41], and humans [27, 28]. A recombinant truncated capsid protein expressed in

baculovirus induced complete protection in rhesus monkeys against both hepatitis and infection after challenge with genotype 1 or 3 HEV. Monkeys were also completely protected from hepatitis and partially protected from infection after challenge with a genotype 2 HEV [42], indicating effective cross-protection among the four recognized genotypes of mammalian HEV [43]. In a comprehensive human vaccine trial with over 100,000 participants, a bacterially-expressed recombinant HEV ORF2 protein induced complete protection for at least 1 year [27], and this vaccine has now been approved and licensed in China. It has also been shown that previous infection with a strain of HEV likely confers protection against subsequent infections from different strains [44]. We previously demonstrated that prior infection of pigs with a genotype 3 swine HEV protected pigs against subsequent infections with genotypes 3 and 4 human HEV and partially protected against homologous genotype 3 swine HEV re-infection [37].

Since HEV infection in pigs is subclinical, the incidence and duration of viremia and fecal virus shedding as well as microscopic liver lesions are typically used as parameters to evaluate protection against infection. In this present study, all 6 unvaccinated pigs had fecal virus shedding after challenge lasting two consecutive weeks in 50% of the pigs. In contrast, in avian HEV and swine HEV antigen-vaccinated pigs, only three pigs in each group had fecal virus shedding with only 1 pig lasting 2 weeks, indicating that the avian HEV and swine HEV capsid antigens induce certain levels of protection against the genotype 3 HEV challenge (Table 3). Although the protection was not complete in all pigs, as seen similarly in non-human primate and chicken HEV capsid vaccine studies, the results from this study did indicate a cross-protection of the avian HEV capsid antigen against genotype 3 mammalian HEV. Although the avian HEV shares only 50–60% sequence identity with mammalian HEV strains [2, 3, 17], the avian HEV may be more antigenically related to the mammalian HEV. In fact, the capsid protein of avian HEV has been shown to share common antigenic epitopes with the human HEVs [45, 46]. In pigs vaccinated with the rat HEV capsid antigen, all challenged pigs had fecal virus shedding similar to the unvaccinated challenged control pigs, suggesting that the rat HEV antigen does not induce sufficient level of protection against genotype 3 HEV. This is not surprising as the rat HEV is genetically very divergent from human HEVs, and in fact the rat HEV belongs to a unique new genotype [21, 22]. It is possible that the rat HEV may not share common neutralizing epitopes in the capsid with the genotype 3 HEV.

In general, histological hepatic lesions characterized by lymphoplasmacytic inflammation are mild in pigs infected with swine HEV. At necropsy, the mean score of liver lesions in the unvaccinated/challenged group was higher than in all three vaccinated/challenged groups, suggesting a certain level of cross-protection of the three animal HEV antigens against genotype 3 HEV, which is consistent with the fecal virus shedding data. Interestingly, the pigs vaccinated with the avian HEV antigen had no detectable histological liver lesions, further indicating that avian HEV capsid antigen confers cross-protection against genotype 3 mammalian HEV. The inability of propagating HEV in a cell culture system to produce a higher titer challenge virus stock and the subclinical infection nature of genotype 3 HEV in pigs limited our ability to conduct a more robust challenge study to fully assess the cross-protective ability of these animal HEV-derived antigens. We are also limited by the fact that samples were collected weekly whereas daily samples would probably provide a better picture regarding the duration of fecal virus shedding. The lack of viremia in even the unvaccinated/challenged pigs is not unexpected since viremia is often absent or transient in HEV-infected pigs [12, 37, 40, 47]. Nevertheless the combined histological evaluation and fecal virus shedding data suggest that the capsid antigens derived from avian, swine and rat HEV confer at least partial cross-protection against genotype 3 HEV challenge in pigs.

The results from the Western blot analysis revealed that the truncated capsid antigens derived from swine, rat, and avian HEV cross-reacted antigenically with anti-HEV antiserum raised against a genotype 1 human HEV [7]. Similarly, we previously showed that the rabbit HEV antiserum cross-reacted with swine, human, rat and avian HEV ORF2 protein, and conversely the rabbit HEV ORF2 antigen cross-reacted with genotype 1 human, genotype 3 swine, avian and rat HEV antisera [12]. Taken together, these results demonstrated antigenic cross-reactivity among animal strains of HEV but also significant antigenic variability which may explain the partial cross-protection seen in this study.

In conclusion, vaccination of pigs with recombinant capsid antigens derived from three animal strains of HEV induced strong IgG anti-HEV antibody response in vaccinated pigs, which is consistent with other studies that showed a robust seroconversion to IgG anti-HEV after vaccination with HEV ORF2 antigens [29, 30, 41]. However, the recombinant antigens from the 3 animal strains of HEV appear to confer only partial cross-protection against a genotype 3 mammalian HEV. Additional studies are warranted to fully evaluate if the current experimental vaccines based on a single HEV strain will confer efficient protection against all known mammalian genotypes of HEV, especially the novel zoonotic animal strains of HEV. Furthermore, it will be interesting in the future to determine if addition of an adjuvant will further enhance the protection of the ORF2 antigen-based vaccine.

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Highlights

- Capsid antigens from rat, swine and chicken HEVs cross-reacted with human HEV
- Capsid antigens derived from animal HEVs induced a strong IgG anti-HEV response
- Capsid antigens from animal HEVs only partially protect against a genotype 3 HEV
- The results have important implications for the efficacy of current HEV vaccines

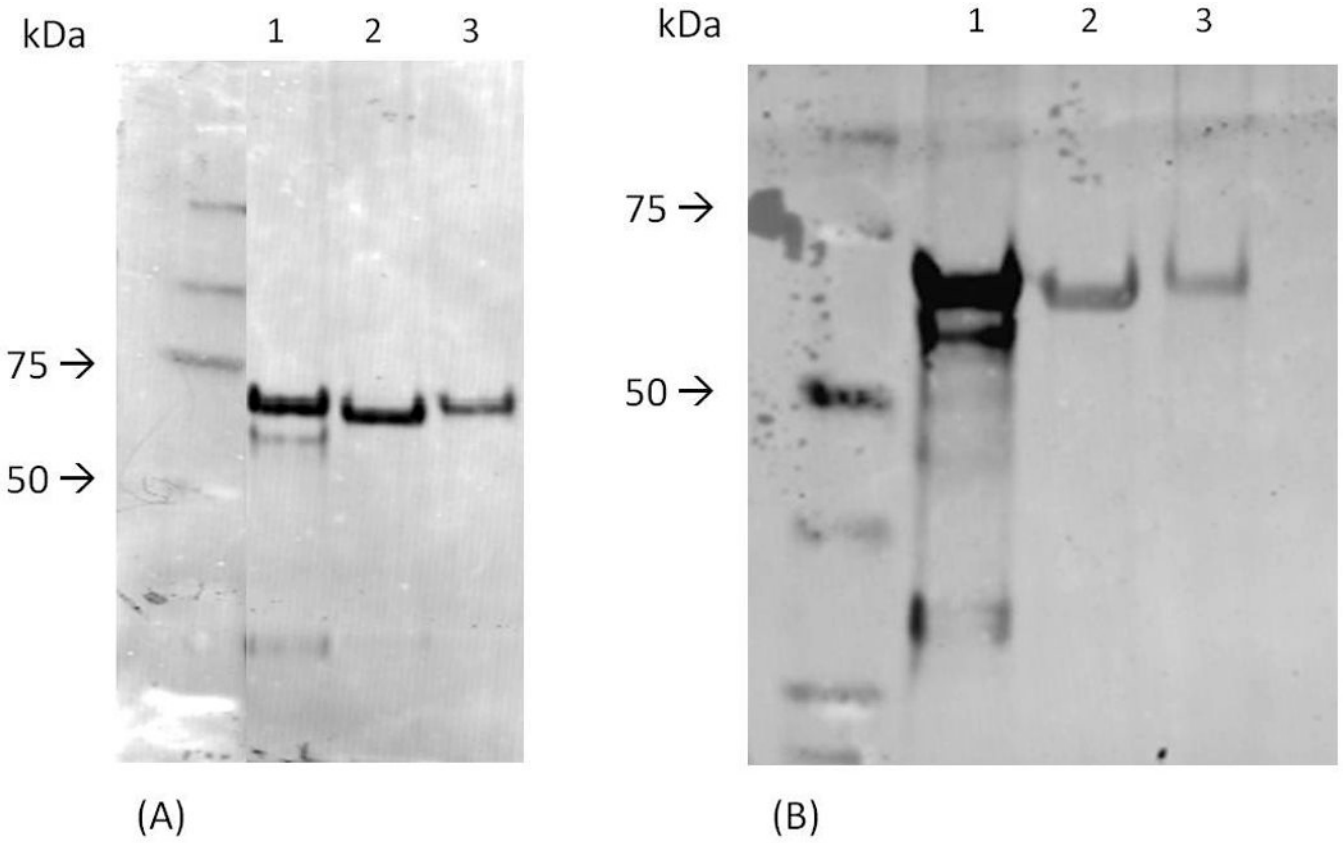

Figure 1. Western blot analyses of truncated bacterial-expressed recombinant swine, rat, and avian HEV ORF2 capsid antigens

Each lane was loaded with truncated recombinant capsid proteins derived from swine HEV (1), rat HEV (2), or avian HEV (3) HEV. **Panel A:** A PVDF membrane containing the separated proteins was incubated with IRDye800 conjugated antibody against 6-His residue tags to detect the respective recombinant fusion capsid proteins. **Panel B:** A PVDF membrane containing the separate proteins was first incubated with a anti-HEV ORF2 hyperimmune swine antiserum raised against the genotype 1 human HEV followed by incubation withIRDye800 conjugated antibody against swine IgG. Both membranes were read at wavelength 800 nm using an Odyssey Imager (LI-COR biosciences).

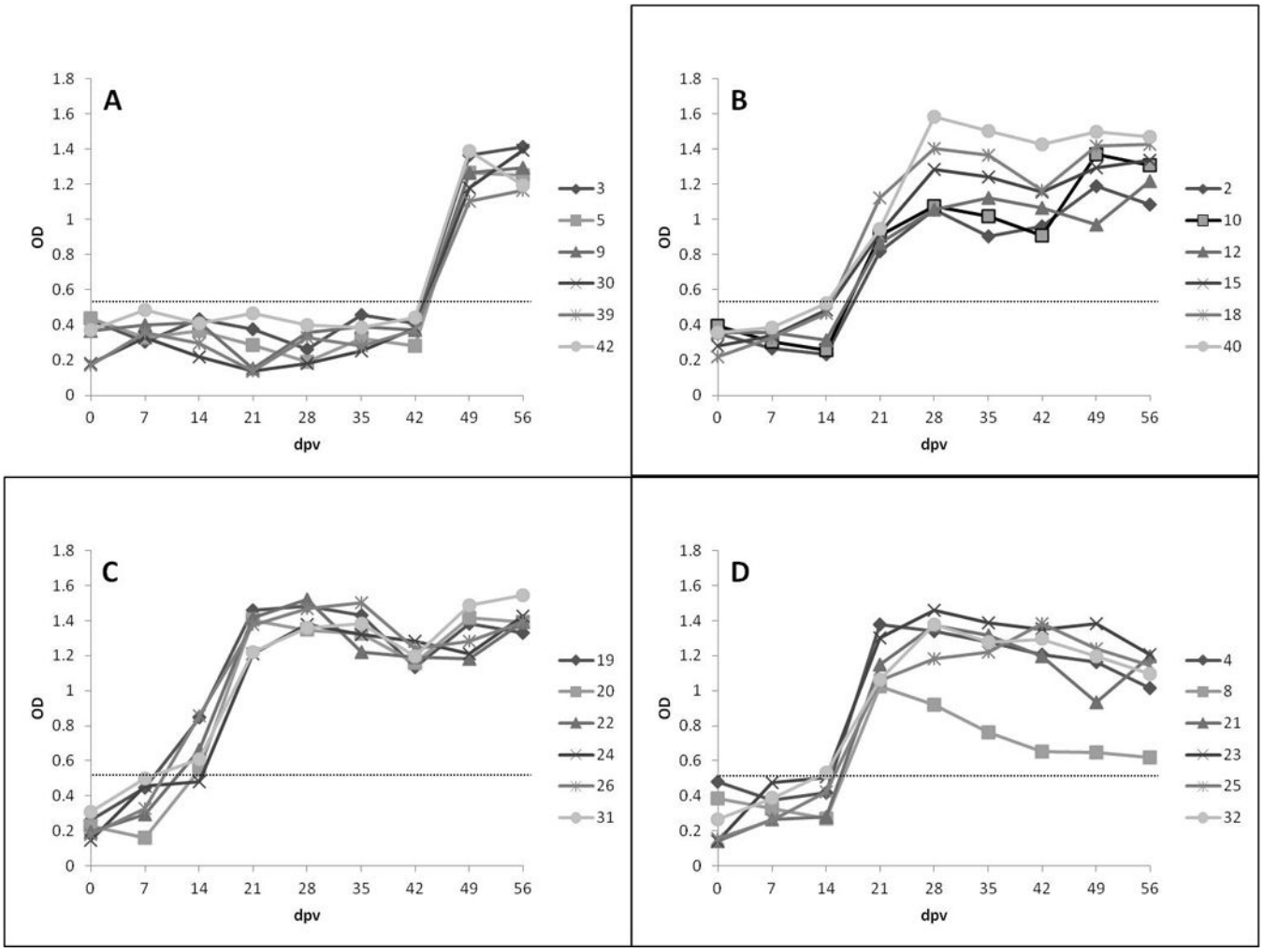

Figure 2. IgG anti-HEV antibody responses in pigs vaccinated with various recombinant HEV antigens as tested by ELISA

A: PBS buffer; B: swine HEV ORF2 antigen; C: rat HEV ORF2 antigen; D: avian HEV ORF2 antigen. Pigs were vaccinated on day 0 and a booster dose was given on day 14.

Table 1

Oligonucleotides used for amplification of truncated ORF2 proteins and for the detection of HEV RNA

Primer ID	Sequence (5'--3')	Purpose
SwineORF2-F[a]	5'-AAGGATCCATGGCTGTATCACCGGCCCCCGAC-3'	Amplification of swine
SwineORF2-R[a]	5'-AACTCGAGTTAAGACTCCCGGGTTTTACC-3'	HEV ORF2 gene
SPK240-F[b]	5'-CATCATGGATCCGCACAGGCACCGGC-3'	Amplification of rat
SPK241-R[b]	5'-CATCATAGATCTTCAGACACTATCGGCGGC-3'	HEV ORF2 gene
SPK239-F[b]	5'-CATCATGGATCCGACGTTGTCACCGCGG-3'	Amplification of avian
SPK238-R[b]	5'-CATCATAGATCTTTAGGGTGGTGAGGGGAATG-3'	HEV ORF2 gene
HEVORF1-FWD1	5'-TCGTCTCCTCTACACCTATC-3'	RT-PCR
HEVORF1-REV1	5'-CGATCTCTAACGCTAGGTTG-3'	RT-PCR
HEVORF1-FWD2	5'-CGACTGAGTTCATTATGCGTG-3'	RT-PCR
HEVORF1-REV2	5'-CCTCAGTTATAGTAAGCGCC-3'	RT-PCR

[a] Built-in BamHI and XhoI restriction sites underlined to facilitate cloning.

[b] Built-in BamHI and BglII restriction sites

Table 2

Hepatic histological lesion scores at 4 weeks post-challenge in vaccinated and control pigs challenged with a genotype 3 HEV

Vaccine group	Pig ID#	Challenge inocula	Liver histological lesion score [a]	Mean histological liver lesion Score
PBS	11	PBS	0	0.17
	13		1	
	14		0	
	16		0	
	17		0	
	29		0	
Swine HEV	2	Genotype 3	0	0.33
ORF2	10	HEV	0	
	12		1	
	15		0	
	18		0	
	40		1	
Rat HEV	19	Genotype 3	0	0.33
ORF2	20	HEV	0	
	22		1	
	24		0	
	26		0	
	31		1	
Avian HEV	4	Genotype 3	0	0.00 *
ORF2	8	HEV	0	
	21		0	
	23		0	
	25		0	
	32		0	
PBS	3	Genotype 3	0	0.67 *
	5	HEV	1	
	9		0	
	30		1	
	39		1	
	42		1	

[a] Lymphoplasmacytic scores: 0 = no inflammation, 1=1 to 2 lymphoplasmacytic infiltrates/10 hepatic lobules, 2 = 3 to 5 focal infiltrates/10 hepatic lobules, 3 = 6 to 10 focal infiltrates/10 hepatic lobules, and 4 = >10 focal infiltrates/10 hepatic lobules [39].

* statistical significance ($P < .05$)

Table 3

Fecal virus shedding and viremia in vaccinated pigs after challenge with a genotype 3 HEV as detected with nested RT-PCR

Vaccine antigen	Pig ID#	Virus Inocula	Positive (+) or negative (−) HEV RNA detected in serum/feces samples at indicated week post-challenge				
			0	1	2	3	4
PBS	11	PBS	−/−	−/−	−/−	−/−	−/−
	13		−/−	−/−	−/−	−/−	−/−
	14		−/−	−/−	−/−	−/−	−/−
	16		−/−	−/−	−/−	−/−	−/−
	17		−/−	−/−	−/−	−/−	−/−
	29		−/−	−/−	−/−	−/−	−/−
Swine HEV	2	Genotype 3	−/−	−/−	−/−	−/−	−/−
ORF2	10	HEV	−/−	−/+	−/+	−/−	−/−
	12		−/−	−/−	−/−	−/−	−/−
	15		−/−	−/+	−/−	−/−	−/−
	18		−/−	−/+	−/−	−/−	−/−
	40		−/−	−/−	−/−	−/−	−/−
Rat HEV	19	Genotype 3	−/−	−/−	−/+	−/−	−/−
ORF2	20	HEV	−/−	−/+	−/−	−/−	−/−
	22		−/−	−/+	−/+	−/−	−/−
	24		−/−	−/+	−/+	−/−	−/−
	26		−/−	−/−	−/+	−/−	−/−
	31		−/−	−/−	−/+	−/−	−/−
Avian HEV	4	Genotype 3	−/−	−/−	−/−	−/−	−/−
ORF2	8	HEV	−/−	−/−	−/−	−/−	−/−
	21		−/−	−/−	−/−	−/−	−/−
	23		−/−	−/+	−/−	−/−	−/−
	25		−/−	−/+	−/−	−/−	−/−
	32		−/−	−/+	−/+	−/−	−/−
PBS	3	Genotype 3	−/−	−/−	−/+	−/−	−/−
	5	HEV	−/−	−/−	−/+	−/−	−/−
	9		−/−	−/+	−/+	−/−	−/−

Vaccine antigen	Pig ID#	Virus Inocula	Positive (+) or negative (−) HEV RNA detected in serum/feces samples at indicated week post-challenge				
			0	1	2	3	4
	30		−/−	−/+	−/+	−/−	−/−
	39		−/−	−/+	−/+	−/−	−/−
	42		−/−	−/+	−/−	−/−	−/−