Porcine Torsade Virus Vaccines and Diagnosis

Huang YW, Ni YY, Dryman BA, and Meng XJ. ORF1 protein [Torque teno sus virus 1 b]. GenBank Acc. No. ADD46854. Updated Nov. 17, 2010.*


Anderson, et al., “Failure to genotype herpes simplex virus by real-time PCR assay and melting curve analysis due to sequence variation within probe binding sites”. Journal of Clinical Microbiology. 2003, pp. 2135-2137 vol. 41, American Society for Microbiology.


(Continued)

Primary Examiner — Benjamin P Blumel
Assistant Examiner — Rachel Gill
Attorney, Agent, or Firm — Blank Rome LLP

ABSTRACT

The present invention provides four purified preparation containing a polynucleic acid molecule encoding porcine Torsade teno virus (PTTV) genotypes or subtypes PTTV1a-VA, PTTV1b-VA, and PTTV2c-VA. The present invention also provides infectious DNA clones, biologically functional plasmid or viral vector containing the infectious nucleic acid genome molecule of the same. The present invention further provides live, attenuated, vector-expressed and purified recombinant capsid subunit or killed viral vaccines for protection against PTVT infection. The present invention additionally provides subunit vaccines comprising PTV specific gene products, especially ORF1 capsid gene product for protection against PTVT infection. Further, the present invention provides methods for diagnosing PTVT infection via polymerase chain reaction (PCR) using specific primer for PTTV1, PTTV2, and individual PTTV1 genotypes. Finally, the present invention provides methods for diagnosing PTVT infection via immunological methods, e.g., enzyme-linked immunosorbent assay (ELISA) and Western blot using PTV specific antigens for detecting serum PTV specific antibodies.

5 Claims, 31 Drawing Sheets
(56) References Cited

OTHER PUBLICATIONS


References Cited

OTHER PUBLICATIONS


Genbank; GU456384.1.

Genbank; GU456385.1.

Genbank; GU456386.1.


* cited by examiner
FIG. 2

- GENUS (36-55%)
- SPECIES (55-67%)
- TYPES (67-85%)
- SUBTYPES (85-95%)
- VARIANTS (>95%)

PERCENTAGE IDENTITY

FREQUENCY
FULL-LENGTH GENOME

TYPE 1a
Sd-TTV31(AB076001)

PTTV1a-VA

PTTV1b-VA

TYPE 1b
TTV-1p(AY823990)

SPECIES 1

SPECIES 2

100 CHANGES

SUBTYPE 2c
PTTV2c-VA

SUBTYPE 2a
TTV-2p(AY823991)

PTTV2b-VA

FIG. 3A
FIG. 3B

FIG. 3C
FIG. 3D

FIG. 3E
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<td>PTV-2c-VA</td>
<td>LMLLKKQHLVLSQKDCPRNKRKPVTLKIRPPPLTSQWRLSRELAKMLPLVRLGSL1DSELREDLEGNAFYSVLYGASEKSHGKSWNSQMKYYWLY 263</td>
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<td>PTTV2b-VA</td>
<td>DTGVGNAVYVILLOKQKVSDNPGDMATQFVTG--SGQHPDADSHHEMNQEGWYQLFFGQSEQDIKEAHEPDQIVREYRDPKSKKLQIGVIGNASSNF 361</td>
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FIG. 4B
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<td>MP--EHWEAWEATKGWHDLCRCGNYQDHWLLADGDAALAAVDAIER--DAMGDDATTATGRVTIGDDGW</td>
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<td>MP--EHWEAWEATKGWHDLCRCGNYQDHWLLADGDAALAAVDAIER--DAMGDDATTATGRVTIGDDGW</td>
<td>M------EERWLTAVYCAHLFCSDKPKKHLEKCLTD--AIADAEDDRHGDTGGGATFDDIGIDALLAAAAQR</td>
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<td>TTV-1p</td>
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<td>M------EERWLTAVYCAHLFCSDKPKKHLEKCLTD--AIADAEDDRHGDTGGGATFDDIGIDALLAAAAQR</td>
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**FIG. 5**
**FIG. 7A**

Diagram showing temperature vs. -d(RFU)/dT with lines for Primer Cross-Dimerization and PTTV1/2 Standard.

**FIG. 7B**

Diagram showing temperature vs. -d(RFU)/dT with lines for Sample #5 and specific RFU values.
FIG. 7C

FIG. 7D
FIG. 7E
FIG. 9A

HYDROPHILICITY

aa 1-49

DOMAINE: aa 322-349

DOMAINE II: aa 536-625

TTTV2p

TTTV2\#472142

PTTV2b-VA

PTTV2c-VA

aa 322-349

aa 536-625
CONVENTIONAL PIGS FROM VIRGINIA

FIG. 11A

CD/CD PIGS

FIG. 11B

GNOTOBIOTIC PIGS

FIG. 11C
CONVENTIONAL PIGS FROM WISCONSIN

PORCINE SERUM FROM A COMPANY

FIG. 12
FIG. 13
ARRIVAL TWO MONTHS AFTER ARRIVAL

FIG. 14A

FIG. 14B
FIG. 15A

FIG. 15B

FIG. 15C
FIG. 16

CONVENTIONAL PIGS FROM WISCONSIN

KDa  M  1  2  3  4  5
188
98
62
49
38
28
14
6
3
FIG. 17A

FIG. 17B

FIG. 17C

FIG. 17D

FIG. 17E

FIG. 17F
FIG. 19A

FIG. 19B
FIG. 21
FIG. 23A

FIG. 23B
Porcine Torque Teno Virus Vaccines and Diagnosis

REFERENCE TO RELATED APPLICATION

This patent application claims the benefit of U.S. Provisional Patent Application No. 61/235,833, filed on Aug. 21, 2009, and U.S. Provisional Patent Application 61/316,519, filed on Mar. 23, 2010, whose disclosures are hereby incorporated by reference in their entirety into the present disclosure.

FIELD OF INVENTION

The present invention relates to vaccines for protecting against porcine Torque teno virus (TTV) infection, and infectious DNA clones of porcine TTV (PTTV) and their uses thereof. The present invention also relates to diagnosis of porcine Torque teno virus (PTTV) infection, particularly diagnosis of species- or type-specific PTTV infection, and simultaneous infection of multiple strains from different genotypes.

BACKGROUND OF THE INVENTION


Biochem Biophys Res Commun 241(1), 92-7.). Since then, a large number of patients with post-transfusion non-A-E hepatitis in 1997 incorporated by reference in their entirety into the present disclosure.

transfusion hepatitis of unknown etiology. Biochem Biophys Res Commun 241(1), 92-7.). Since then, a large number of patients with post-transfusion non-A-E hepatitis in 1997 incorporated by reference in their entirety into the present disclosure.

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human TTV is not considered to be directly associated with the disease. Subsequent studies were not able to produce respective TTV infectious DNA clones into cultured cells near full-length genomic sequences of PTTV are reported. Proteinsynthesis by TT Viruses. Curr Top Microbiol Immunol. 2008, 331, 53-64; Mueller et al., 2008, supra; Qi et al., 2005, supra). Accordingly, it is likely that, when more data regarding the animal TTV become available, the presumed genome structure of animal TTV will need to be modified.

Although TTV was first identified in a cryptogenic hepatitis patient, subsequent studies were not able to produce evidence of a significant role of TTV in the pathogenesis of hepatitis or other diseases (Hino and Miyata, 2007, supra; Muggi, F., and Bendinelli, M. (2009). Immunobiology of the Torque teno viruses and other anelloviruses. Curr Top Microbiol Immunol. 331, 65-90; Okamoto, 2009a, supra). While human TTV is not considered to be directly associated with a disease, porcine TTV (PTTV) was recently shown to partially contribute to the experimental induction of porcine dermatitis and nephropathy syndrome (PDNS) combined with porcine reproductive and respiratory syndrome virus (PRRSV) infection (Krakowka, S., Hartunian, C., Hamberg, A., Shoup, D., Rings, M., Zhang, Y., Allan, G., and Ellis, J. A. (2008). Evaluation of induction of porcine dermatitis and nephropathy syndrome in gnotobiotic pigs with negative results for porcine circovirus type 2. Am J Vet Res 69, 1615-22), whereas pigs inoculated with both PTTV1 and porcine circovirus type 2 (PCV2) developed acute postweaning multisystemic wasting syndrome (PMWS) (Ellis et al., 2008, supra). Although PCV2 is considered as the primary causative agent for clinical PMWS or PCV-associated diseases (PCVAD), a higher prevalence of PTTV2 infection in PMWS-affected pigs with low or no PCV2 than that in non-PMWS-affected pigs was observed in Spain (Kekarainen et al., 2006, supra). The data collectively suggest that porcine TTVs may serve as co-factors in triggering or exacerbating diseases in pigs.

Porcine TTV has been detected in porcine serum, fecal, saliva, semen and tissue samples of infected pigs, indicating its diverse transmission routes including both horizontal and vertical transmissions (Kekarainen et al., 2007, supra; Pozzuto, T., Mueller, B., Meelman, B., Ringler, S. S., McIntosh, K. A., Ellis, J. A., Mankertz, A. and Krakowka, S., 2009). In utero transmission of porcine torque teno viruses. Vet Microbiol 137, 375-9; Sibila, M., Martinez-Guino, L., Huerta, E., Llorens, A., Mora, M., Grau-Roma, L., Kekarainen, T. and Segales, J., 2009. Swine torque teno virus (TTV) infection and excretion dynamics in conventional pig farms. Vet Microbiol 139, 213-8). However, current detection of porcine TTV infection was mainly based upon conventional PCR assays. Thus far, neither serological assay nor viral culture system has been established. In particular, nested PCR amplifications of the conserved regions in the UTR, of PTTV1 and PTTV2, respectively, developed by a Spanish group, have become widely used (Kekarainen et al., 2006, supra). Since the amount of virus is likely associated with the severity of clinical diseases, as demonstrated for PCV2-induced PCVAD (Opriessnig, T., Meng, X. J. and Halbur, P. G., 2007. Porcine circovirus type 2 associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. J Vet Diagn Invest 19, 591-615), it will be important to determine the viral load of porcine TTV by quantitative real-time PCR or the presence of TTV DNA by conventional PCR. In addition, real-time PCR is more reliable, rapid and less expensive than conventional PCR. Recently, two TaqMan probe-based real-time PCR assays were described for detection and quantification of two porcine TTV species (Brassard, J., Gagne, M. J., Houde, A., Potiras, E. and Ward, R, 2009. Development of a real-time TaqMan PCR assay for the detection of porcine and bovine Torque teno virus. J Appl Microbiol. Nov. 14, 2009. Epub ahead of print; Gille, A., Pesch, S., Eisking, W. S., Keller, C. and Ollinger, V. F., 2009. Porcine Torque teno virus: Deter-

United States, Canada, Spain, China, Korea and Thailand (McKeown, N. E., Fenaux, M., Halbur, P. G., and Meng, X. J. (2004). Molecular characterization of porcine TTV, an orphan virus, in pigs from six different countries. Vet Microbiol 104(1-2), 113-7). Whether porcine TTVs play a significant role in pathogenesis of specific swine diseases is still debatable. In a gnotobiotic pig model, it was shown that PTTV1 infection alone did not develop any clinical diseases but induced mild histological lesions (Krakowka, S., and Ellis, J. A., 2008. Evaluation of the effects of porcine genogroup 1 torque teno virus in gnotobiotic swine. Am J Vet Res 69, 1623-9). Gnotobiotic pigs that were experimentally inoculated with both PTTV1 and porcine reproductive and respiratory syndrome virus (PRRSV) developed clinical porcine dermatitis and nephropathy syndrome (PDNS) (Krakowka, S., Hartunian, C., Hamberg, A., Shoup, D., Rings, M., Zhang, Y., Allan, G. and Ellis, J. A., 2008. Evaluation of induction of porcine dermatitis and nephropathy syndrome in gnotobiotic pigs with negative results for porcine circovirus type 2. Am J Vet Res 69, 1615-22), whereas pigs inoculated with both PTTV1 and porcine circovirus type 2 (PCV2) developed acute postweaning multisystemic wasting syndrome (PMWS) (Ellis et al., 2008, supra). Although PCV2 is considered as the primary causative agent for clinical PMWS or PCV-associated diseases (PCVAD), a higher prevalence of PTTV2 infection in PMWS-affected pigs with low or no PCV2 than that in non-PMWS-affected pigs was observed in Spain (Kekarainen et al., 2006, supra). The data collectively suggest that porcine TTVs may serve as co-factors in triggering or exacerbating diseases in pigs.
The present invention provides an infectious nucleic acid molecule ("infectious DNA clone") of porcine Torque teno virus (PTTV) comprising a nucleic acid molecule encoding an infectious PTTV which contains at least one copy of genomic sequence having at least 80% homology to a genomic sequence selected from the group consisting of genotypes of PTTVla-VA, PTTVlb-VA, PTTV2b-VA, and PTTV2c-VA.

According to one aspect of the present invention, the infectious DNA clones of PTTV of set forth in claim 1, wherein the genomic sequence is selected from sequences set forth in SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.

The present invention provides a biologically functional plasmid or viral vector containing the infectious PTTV genomes.

The present invention provides a suitable host cell transfected with the infectious clone DNA plasmid or viral vector.

The present invention provides an infectious PTTV produced by cells transfected with the PTTV infectious DNA clones.

The present invention also provides a viral vaccine comprising a nontoxic, physiologically acceptable carrier and an immunogenic amount of a member selected from the group consisting of (a) a nucleic acid molecule containing at least one copy of genomic sequence having at least 80% homology to a genomic sequence selected from the group consisting of PTTV genotypes or subtypes PTTVla-VA, PTTV1b-VA, PTTV2b-VA, and PTTV2c-VA, or its complementary strand, (b) a biologically functional plasmid or viral vector containing a nucleic acid molecule containing at least one copy of genomic sequence having at least 80% homology to a genomic sequence selected from the group consisting of PTTV genotypes or subtypes PTTVla-VA, PTTV1b-VA, PTTV2b-VA, and PTTV2c-VA, or its complementary strand, and (c) an avirulent, infectious nonpathogenic PTTV which contains at least one copy of genomic sequence having at least 80% homology to a genomic sequence selected from the group consisting of PTTV genotypes or subtypes PTTVla-VA, PTTV1b-VA, PTTV2b-VA, and PTTV2c-VA, or its complementary strand.

According to one aspect of the present invention, the vaccine contains live PTTV virus derived from the PTTV infectious clones. According to another aspect of the present invention, the vaccine contains killed PTTV virus derived from the PTTV infectious clones.

The present invention provides purified recombinant proteins expressed from the ORF1 capsid genes of PTTV genotypes or subtypes PTTVla-VA, PTTV1b-VA, and PTTV2c-VA in bacterial expression system, and the use of these recombinant capsid proteins as subunit vaccines against PTTV infections. In one embodiment of the present invention, the recombinant capsid proteins for the use as subunit vaccines are expressed in baculovirus expression system and other expression vector systems.

According to a further aspect of the present invention, the vaccine contains killed PTTV virus derived from the PTTV infectious clones.

The present invention provides purified recombinant proteins expressed from the ORF1 capsid genes of PTTV genotypes or subtypes PTTVla-VA, PTTV1b-VA, and PTTV2c-VA in bacterial expression system, and the use of these recombinant capsid proteins as subunit vaccines against PTTV infections. In one embodiment of the present invention, the recombinant capsid proteins for the use as subunit vaccines are expressed in baculovirus expression system and other expression vector systems.

According to a further aspect of the present invention, the vaccine contains killed PTTV virus derived from the PTTV infectious clones. According to another aspect of the present invention, the vaccine contains killed PTTV virus derived from the PTTV infectious clones.

The present invention further provides a method of immunizing a pig against PTTV viral infection, comprising administering to a pig an immunologically effective amount of the viral vaccine.

According to one aspect of the present invention, the method comprising administering the recombinant subunit capsid protein, the infectious nucleic acid molecule or live PTTV virus to the pig.

According to another aspect of the present invention, the method comprising administering the vaccine parenterally, intranasally, intradermally, or transdermally to the pig. According to a further aspect of the present invention, the method comprising administering the vaccine intralymphoidly or intramuscularly to the pig.
The present invention also provides an isolated polynucleotide consisting of the sequence of the nucleotide sequence of PTTV1a-VA set forth in SEQ ID NO:9.

The present invention also provides an isolated polynucleotide consisting of the sequence of the nucleotide sequence of PTTV1b-VA set forth in SEQ ID NO:10.

The present invention also provides an isolated polynucleotide consisting of the sequence of the nucleotide sequence of PTTV2b-VA set forth in SEQ ID NO:11.

The present invention also provides an isolated polynucleotide consisting of the sequence of the nucleotide sequence of PTTV2c-VA set forth in SEQ ID NO:12.

The present invention further provides a subunit vaccine comprising an immunogenic fragment of a polypeptide sequence or a complete protein translated according to a polynucleotide sequence selected from the group consisting of ORF1, ORF2, ORF1/1, and ORF2/2 of PTTV genotypes or subtypes PTTV1a-VA, PTTV1b-VA, PTTV2b-VA, and PTTV2c-VA, particularly the ORF1 encoding the capsid protein.

According to one aspect of the present invention, the polynucleotide sequence is selected from the group consisting of ORF1 of PTTV genotypes or subtypes PTTV1a-VA, PTTV1b-VA, PTTV2b-VA, and PTTV2c-VA.

According to another aspect of the present invention, the polynucleotide sequence is ORF1 of PTTV genotype PTTV1a-VA. According to a further aspect of the present invention, the polynucleotide sequence is ORF1 of PTTV genotype PTTV1b-VA. According to yet another aspect of the present invention, the polynucleotide sequence is ORF1 of PTTV subtype PTTV2c-VA.

According to one aspect of the present invention, the polypeptide sequence is selected from the group consisting of sequence set forth in SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28.

According to another aspect of the present invention, the polypeptide sequence is set forth in SEQ ID NO:13. According to another aspect of the present invention, the polypeptide sequence is set forth in SEQ ID NO:14. According to another aspect of the present invention, the polypeptide sequence is set forth in SEQ ID NO:16. In one specific embodiment of the present invention, the polypeptide sequence is C-terminal region (aa 310-625) of SEQ ID NO:16. According to another aspect of the present invention, the polypeptide sequence is set forth in SEQ ID NO:20.

According to one aspect of the present invention, the vaccine further contains an adjuvant.

According to another aspect of the present invention, the method comprises administering the vaccine parenterally, intramuscularly, intradermally, or transdermally to the pig.

According to a further aspect of the present invention, the method comprises administering the vaccine intralymphoidly or intramuscularly to the pig.

The present invention additionally provides a method for diagnosing PTTV1 infection and quantification of PTTV1 load, comprising extracting DNA from a sample suspected of PTTV1 infection, performing polymerase chain reaction (PCR) using primers comprising the sequences set forth in SEQ ID NO:29 and SEQ ID NO:30, and detecting PTTV1 specific amplification. According to one aspect of the present invention, the polymerase chain reaction is a SYBR green real-time PCR.

The present invention further provides a method for diagnosing PTTV2 infection and quantification of PTTV2 load, comprising extracting DNA from a sample suspected of PTTV2 infection, performing polymerase chain reaction (PCR) using primers comprising the sequences set forth in SEQ ID NO:31 and SEQ ID NO:32, and detecting PTTV2 specific amplification. According to one aspect of the present invention, the polymerase chain reaction is a SYBR green real-time PCR.

The present invention further provides a method for simultaneously detecting and diagnosing PTTV1 and PTTV2 infection, comprising extracting DNA from a sample suspected of PTTV1 infection, performing a first polymerase chain reaction (PCR) using primers comprising the sequences set forth in SEQ ID NO:33 and SEQ ID NO:34, performing a second PCR using primers comprising the sequences set forth in SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, and SEQ ID NO:38, and detecting PTTV1a and PTTV1b specific amplification.

The present invention provides a method for diagnosing PTTV infection, comprising immobilizing an immunogenic fragment of a polypeptide sequence translated according to a polynucleotide sequence selected from the group consisting of ORF1, ORF2, ORF1/1, and ORF2/2 of PTTV genotypes or subtypes PTTV1a-VA, PTTV1b-VA, PTTV2b-VA, and PTTV2c-VA; contacting a serum sample from a pig suspected of PTTV infection with the immobilized immunogenic fragment, and detecting captured antibody specific to the immunogenic fragment.

According to one aspect of the present invention, the polynucleotide sequence is selected from the group consisting of ORF1 of PTTV genotypes or subtypes PTTV1a-VA, PTTV2b-VA, and PTTV2c-VA.

According to one embodiment of the present invention, the polynucleotide sequence is ORF1 of PTTV genotype PTTV1a-VA. According to another embodiment of the present invention, the polynucleotide sequence is ORF1 of PTTV genotype PTTV1b-VA. According to a further embodiment of the present invention, the polynucleotide sequence is ORF1 of PTTV subtype PTTV2c-VA.

According to another aspect of the present invention, the polypeptide sequence is selected from the group consisting of sequence set forth in SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22,
Upon deduced amino acid sequences of ORF2 among seven porcine TTV strains; nucleotide sequences; neighbor-joining method based upon the full-length genomic cloning and assemblies of four prototype U.S. strains of porcine TTV virus group 1 (top panel: species 1) and group 2 (bottom panel: species 2) strains: (PTTV1a-VA: SEQ ID NO: 9, Sd-TTV31–SEQ ID NO: 53, PTTV1b-VA: SEQ ID NO: 10, TTV-1p-SEQ ID: 56, TTV-2p-SEQ ID NO: 59, PTTV2b-VA: SEQ ID NO: 60, PTTV2c-VA: SEQ ID NO: 16).

According to one embodiment of the present invention, the polypeptide sequence is set forth in SEQ ID NO: 13. According to another aspect of the present invention, the polypeptide sequence is set forth in SEQ ID NO: 14. According to another embodiment of the present invention, the polypeptide sequence is set forth in SEQ ID NO: 15. According to a further embodiment of the present invention, the immunogenic fragment is C-terminal region (aa 310-625) of SEQ ID No:16. According to yet another embodiment of the present invention, the polypeptide sequence is set forth in SEQ ID NO: 20.

The present invention provides three standardized enzyme-linked immunosorbent assays (ELISA) to diagnose PTTV infections and detect antibodies in serum of pigs infected by PTTV genotypes PTTV1a-VA, PTTV1b-VA, and all known subtypes in PTTV species 2.

The ELISA diagnostic tests are based on the bacterial-expressed or baculovirus-expressed recombinant ORF1 capsid protein of PTTV genotypes PTTV1a-VA, PTTV1b-VA, and PTTV2c-VA.

According to another aspect of the present invention, the detecting captured antibody is via Western blot. According to yet another aspect of the present invention, the detecting captured antibody is via enzyme-linked immunosorbent assay (ELISA).

**BRIEF DESCRIPTION OF THE DRAWINGS**

The above-mentioned features of the invention will become more clearly understood from the following detailed description of the invention read together with the drawings in which:

**FIGS. 1A and 1B (top panel and bottom panel)** represent the schematic diagram of genomic structures, strategies for genomic cloning and assemblies of four prototype U.S. strains of porcine TTV virus group 1 (top panel: species 1) and group 2 (bottom panel: species 2) strains: (PTTV1a-VA: SEQ ID NO: 9, Sd-TTV31–SEQ ID NO: 53, PTTV1b-VA: SEQ ID NO: 10, TTV-1p-SEQ ID: 56, TTV-2p-SEQ ID NO: 59, PTTV2b-VA: SEQ ID NO: 60, PTTV2c-VA: SEQ ID NO: 16); 11C: pSC-PTTV2c (from the US PTTV isolate PTTV2c-VA; GenBank accession no. GU456384).

**FIG. 2** represents PASC (pairwise sequence comparisons) distribution of nucleotide sequence comparisons of 121 TTV strains available in GenBank database. The genus, species, types, subtypes and variants and their corresponding percentage of nucleotide sequence identities are displayed.

**FIG. 3A** illustrates a phylogenetic tree constructed by the neighbor-joining method based upon the full-length genomic nucleotide sequences.

**FIG. 3B** illustrates a phylogenetic trees constructed based upon deduced amino acid sequences of ORF1 among seven porcine TTV strains.

**FIG. 3C** illustrates a phylogenetic trees constructed based upon deduced amino acid sequences of ORF1/1 among seven porcine TTV strains.

**FIG. 3D** illustrates a phylogenetic trees constructed based upon deduced amino acid sequences of ORF2 among seven porcine TTV strains.

**FIG. 3E** illustrates a phylogenetic trees constructed based upon deduced amino acid sequences of ORF2/2 among seven porcine TTV strains.

**FIG. 4** represents an alignment of the full-length amino acid sequences of ORF1 among seven PTTV strains: (PTTV1a-VA–SEQ ID NO: 13, Sd-TTV31–SEQ ID NO: 54, PTTV1b-VA–SEQ ID NO: 14, TTV-1p–SEQ ID NO: 57, TTV-2p–SEQ ID NO: 60, PTTV2b-VA–SEQ ID NO: 15, and PPT2c-VA–SEQ ID NO: 16).

**FIG. 5** represents an alignment of the full-length amino acid sequences of ORF2 among seven PTTV strains: (PTTV1a-VA–SEQ ID NO: 17, Sd-TTV31–SEQ ID NO: 55, PTTV1b-VA–SEQ ID NO: 18, TTV-1p–SEQ ID NO: 58, TTV-2p–SEQ ID NO: 61, PTTV2b-VA–SEQ ID NO: 19, and PPT2c-VA–SEQ ID NO: 20).

**FIG. 6A** illustrates melting curves of PTTV1 real-time PCR products after 40 cycles of amplifications of respective standard template (indicated in blue) and 20 porcine serum samples;

**FIG. 6B** illustrates melting curves of PTTV2 real-time PCR products after 40 cycles of amplifications of respective standard template and 20 porcine serum samples; FIGS. 7A-7E illustrate melting curve analysis (MCA) of PTTV1/PTTV2 SYBR green-based duplex real-time PCR;

**FIG. 8** represents an alignment of nucleotide sequences with the N-terminal part of the putative ORF1 among seven PTTV strains (PTTV1a–VA–SEQ ID NO: 9, Sd-PTTV31–SEQ ID NO: 53, PTTV1b-VA–SEQ ID NO: 10, TTV-1p–SEQ ID NO: 56, TTV-2p–SEQ ID NO: 59, PTTV2b-VA–SEQ ID NO: 60, PTTV2c-VA–SEQ ID NO: 12).

**FIGS. 9A and 9B** represent hydrophility profiles and conserved regions of the four known porcine PTTV2 (PTTV-2p–SEQ ID NO: 60, PTTV2#472142–SEQ ID NO: 62, PTTV2b-VA–SEQ ID NO: 15, and PPT2c-VA–SEQ ID NO: 16);

**FIGS. 10A-10C** illustrate the expression and purification of recombinant PTTV2c ORF1 capsid protein; **FIGS. 11A-11C** show representative results of Western blot analyses of selected porcine serum samples;

**FIG. 12** illustrates the consistency of PTTV2c-ORF1-based Western blot and ELISA;

**FIG. 13** shows Box-and-Whisker-plots of PTTV2 serum antibody level by viral load in 138 pigs from different sources;

**FIG. 14A** illustrates a retrospective evaluation of the viral load of PTTV2;

**FIG. 14B** illustrates antibody level to PTTV2 ORF1 capsid protein in 10 pigs growing from arrival to two months after arrival;

**FIGS. 15A-15C** illustrate the expression and purification of PTTV1a and PTTV1b recombinant ORF1 capsid protein; and

**FIG. 16** shows examples of PTTV1a-ORF1-based Western blot analyses of selected porcine serum samples from a farm of Wisconsin.

**FIGS. 17A-17F** represent the schematic diagrams of construction of full-length genomic DNA clones of porcine TTVs. **17A**: pSC-PTTV1a (from the US PTTV isolate PTTV1a-VA; GenBank accession no. GU456383). **17B**: pSC-PTTV1b (from the US PTTV isolate PTTV1b-VA; GenBank accession no. GU456384). **17C**: pSC-PTTV2c (from the US PTTV isolate PTTV2c-VA; GenBank accession no. GU456386). **17D**: pSC-2PTTV2c-RR (tandem-dimerized genomes). **17E**: TTV2-#471942-full (from the Germany PTTV isolate TTV2-#471942; a gift from Dr. Andreas Gallei, not generated by the applicants). **17F**: pSC-B-amplkan vector (indicated in black). Grey arrows indicated the PTTV genomic copies;
FIGS. 18A and 18B represent the identification of porcine TTV full-length DNA clones by restriction digestion patterns. 18A: BamHI single digestion of pSC-PTTV1a, pSC-PTTV1b and pSC-PTTV2c clones and the backbone vector pSC-B-amp/kan (pSC-B). The 4.3-Kb fragments indicated the size of the backbone vector whereas the 2.8-Kb fragments indicated the inserted PTTV genomes (black arrowheads). 18B: Comparisons of the Hind III single digestion between pSC-PTTV2b and pSC-2PTTV2b-RR (left; derived from the clone TTV2-#471942-full) and All II single digestion between pSC-PTTV2c and pSC-2PTTV2c-RR (right). M: DNA markers; FIGS. 19A and 19B represent the immunofluorescence assay (IFA) results of transfection (19A) or transfected cell passaging (19B) of the concatemerized PTV2-#471942-full DNA in PK-15 cells using a PTTV2-specific anti-ORF1 polyclonal antibody (Ab). 19A: Results observed at 5 days post-transfection. 19B: Cells transfected with DNA clones were passaged and used for the IFA detection at 2 days post-passaging. Magnification = 200x. DAPI was used to stain the cell nucleus; FIGS. 20A and 20B represent the IFA results of transfection (20A) or transfected cell passaging (20B) of the concatemerized PTTV2c DNA in PK-15 cells using a PTTV2-specific anti-ORF1 Ab. 20A: Results observed at 5 days post-transfection. 20B: Cells transfected with the DNA clones were passaged and used for the IFA detection at 2 days post-passaging. Magnification = 200x. DAPI was used to stain the cell nucleus; FIG. 21 represents the IFA results of transfection of the concatemerized PTTV1a DNA in PK-15 cells at 3 days post-transfection using a PTTV1-specific anti-ORF1 Ab. Magnification = 200x. DAPI was used to stain the cell nucleus; FIGS. 22A and 22B represent the IFA results of transfection of the pSC-2PTTV2b-RR plasmid (22A) or pSC-2PTTV2c-RR plasmid (22B) in PK-15 cells at 3 days post-transfection. Magnification = 200x. DAPI was used to stain the cell nucleus; FIGS. 23A and 23B represent the determination of the in vivo infectivity of the two porcine TTV2 DNA clones, pSC-2PTTV2b-RR and pSC-2PTTV2c-RR, in conventional pigs, respectively. 23A: Changes of viremia or virus titers (copies/ml) as determined by PTTV2-specific real-time PCR. 23B: Serocconversion to IgG anti-porcine TTV2 ORF1 antibodies in pigs. Anti-PTTV2 antibody is plotted as the ELISA optical density (A405). The ELISA cutoff value, indicated by a dashed line in each panel, is 0.4.

**TABLE 1**

<table>
<thead>
<tr>
<th>Primer ID</th>
<th>Sequence (5' to 3')</th>
<th>Used for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTV1-nF (SEQ ID NO: 45)</td>
<td>TACACTCCCGGTTTCAGGACGCT</td>
<td>Detection of porcine TTV1</td>
</tr>
<tr>
<td>TTV1-nR (SEQ ID NO: 46)</td>
<td>ACTCGGCCATTCCGAACTCAC</td>
<td>Detection of porcine TTV1</td>
</tr>
<tr>
<td>TTV1-nF (SEQ ID NO: 47)</td>
<td>CAAATTTGCTTCGCTCGCCG</td>
<td>Detection of porcine TTV1</td>
</tr>
<tr>
<td>TTV1-nR (SEQ ID NO: 48)</td>
<td>TACCTTATATCCGCTTCTGGAAC</td>
<td>Detection of porcine TTV1</td>
</tr>
</tbody>
</table>
Unexpectedly, two groups of sequence data from each construct were identified, indicating that there exist two types of PTTVs in genogroup 1 and genogroup 2 from the same pig. In order to differentiate and assemble the four PTTV strains, sequence comparisons were performed together with the three known PTTV strains, Sd-TTV31, TTV-1p and TTV-2p (FIGS. 1B and 1C).

FIG. 1B illustrates differentiation and assembly of full-length genomic sequences of PTTVl strains PTTVla-VA and PTTVlb-VA with PCR fragments B and C that were subsequently cloned. The initiation codons of ORF1 and ORF2 in the fragment B as well as the termination codons of ORF1 in the fragment C are marked by “A” or “*”. The corresponding sequences of two known PTTVl strains, Sd-TTV31 and TTV-1p, are also shown. Conserved sequences are shaded, and dashes indicate nucleotide deletions. The unique nucleotides within the overlapping region (boxed with dashed-line) for each strain (a continuous “AG” nucleotides for PTTV2b-VA (SEQ ID NO: 21) and two single “A” and “G” nucleotides for PTTV2c-VA (SEQ ID NO: 12)) are shown, respectively. Differentiation of the two PTTV2 strains was easier. A unique continuous “AG” nucleotides located in the overlapping region of two PCR fragments was shared by two groups of sequence data from fragments E and F, respectively (FIG. 1C). The assembled full-length genomic sequence represented a PTTV2 strain and was designated as PTTV2b-VA (SEQ ID NO: 11). Similarly, the complete genomic sequence of a second strain designated as PTTV2c-VA (SEQ ID NO: 12) was assembled based upon two unique single “A” and “G” nucleotides shared in the overlapping region by another set of sequence data from fragments E and F, respectively (FIG. 1C). Phylogenetic analyses using four sequences from fragments E and F together with the two corresponding sequences from TT-2p also supported this assignment (data not shown).

The present invention provides four isolated porcine TTV virus genotypes or subtypes that are associated with viral
infections in pigs. This invention includes, but is not limited to, porcine TTV virus genotypes or subtypes PTTV1a-VA, PTTV1b-VA, PTTV2b-VA, and PTTV2c-VA, the virus genotypes or subtypes which have nucleotide sequences set forth in SEQ ID NO:9 (PTTV1a-VA), SEQ ID NO:10 (PTTV1b-VA), SEQ ID NO:11 (PTTV2b-VA), and SEQ ID NO:12 (PTTV2c-VA), their functional equivalent or complementary strand. It will be understood that the specific nucleotide sequence derived from any porcine TTV will have slight variations that exist naturally between individual viruses. These variations in sequences may be seen in deletions, substitutions, insertions, and the like.

The proposed genomic structure for each of the four PTTV strains was analyzed in detail and summarized in Table 2, together with the three known PTTV strains, Sd-TTV31, TTV-1p and TTV-2p. All the four U.S. strains of PTTV have a similar genomic size of 2,878 by (PTTV1a-VA SEQ ID NO:9), 2,875 by (PTTV1b-VA SEQ ID NO:10), 2,750 by (PTTV2b-VA SEQ ID NO:11), and 2,803 by (PTTV2c-VA SEQ ID NO:12), respectively. Both PTTV1a-VA (SEQ ID NO:9) and Sd-TTV31 have the same genomic length. The published sequences of the strains TTV-1p and TTV-2p all have many undetermined nucleotides in the GC-rich region of the UTR. After artificial filling of these nucleotides with the consensus sequences corresponding to PTTV1 and PTTV2, it was shown that the TTV-1p is more closely-related to PTTV1a-VA (SEQ ID NO:10), and that TTV-2p is more closely-related to PTTV2b-VA (SEQ ID NO:11) in, genomic length, respectively (data not shown).

The assembled genomic sequences of porcine TTV virus genotypes or subtypes PTTV1a-VA (SEQ ID NO:9), PTTV1b-VA (SEQ ID NO:10), PTTV2b-VA (SEQ ID NO:11), and PTTV2c-VA (SEQ ID NO:12) are submitted to Genbank® (Nucleic Acid Research, 2008 January:36 (Database issue):25-30) with accession numbers GU456383, GU456384, GU456385, and GU456386, respectively.

TABLE 2

Comparison of the genomic organization and ORFs of the seven porcine TTV strains

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>Porcine TTV species 1</th>
<th>Porcine TTV species 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>PTTV1a-VA</td>
<td>TTV-1p</td>
</tr>
<tr>
<td>Country</td>
<td>USA</td>
<td>Brazil</td>
</tr>
<tr>
<td>Full-length (nt)</td>
<td>2878</td>
<td>2875</td>
</tr>
<tr>
<td>GenBank accession #</td>
<td>GU456383</td>
<td>AY823990</td>
</tr>
<tr>
<td>TATA box</td>
<td>288-291</td>
<td>233-236</td>
</tr>
<tr>
<td>Putative mRNA 5'-end ORF1 Size (aa)</td>
<td>635</td>
<td>624</td>
</tr>
<tr>
<td>Exon #</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Initiation</td>
<td>534</td>
<td>476</td>
</tr>
<tr>
<td>Termination</td>
<td>2441</td>
<td>2350</td>
</tr>
<tr>
<td>ORF2 Size (aa)</td>
<td>73</td>
<td>68</td>
</tr>
<tr>
<td>Exon #</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Initiation</td>
<td>430</td>
<td>393</td>
</tr>
<tr>
<td>Termination</td>
<td>651</td>
<td>599</td>
</tr>
<tr>
<td>ORF1/1 Size (aa)</td>
<td>174</td>
<td>178</td>
</tr>
<tr>
<td>Exon #</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Initiation</td>
<td>534</td>
<td>476</td>
</tr>
<tr>
<td>Splicing</td>
<td>647/648</td>
<td>595/596</td>
</tr>
<tr>
<td>Termination</td>
<td>2441</td>
<td>2350</td>
</tr>
<tr>
<td>ORF2/2 (ORF3) Size (aa)</td>
<td>224</td>
<td>199</td>
</tr>
<tr>
<td>Exon #</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Initiation</td>
<td>430</td>
<td>393</td>
</tr>
<tr>
<td>Splicing</td>
<td>647/648</td>
<td>595/596</td>
</tr>
<tr>
<td>Termination</td>
<td>2487</td>
<td>2330</td>
</tr>
<tr>
<td>Polyadenylation signal (AATAAA)</td>
<td>25458-25463</td>
<td>2473-24785</td>
</tr>
</tbody>
</table>

The numbers (except sizes of the full-length genome, ORFs and the exon numbers) indicate the nucleotide (nt) positions on the genome of respective MTV strains. Two recent studies have identified the transcribed viral mRNAs and the expression of at least six viral proteins during human TTV replication (Mueller et al., 2008, supra; Qiu et al., 2005, supra), which is more than the predicted number of ORFs encoded by human TTV (Okamoto, H., Nishizawa, T., Tawara, A., Takahashi, M., Kishimoto, J., Sai, T., and Sugai, Y. (2000b). TT virus mRNAs detected in the bone marrow cells from an infected individual. Biochem Biophys Res Commun 279(2), 700-7), therefore we included the new human TTV genomic information for comparison with the PTTV sequences. The 5’-ends of the mRNA transcripts of human TTV strain P/1C1 were mapped to an “A” that is 25 at downstream of the TATA-box (Mueller et al., 2008, supra). This starting point, its adjacent sequence (CAGATGCGCTG AGTTTATGCGCGC (SEQ ID NO:39); the starting point was
underlined) and the distance to the upstream TATA-box (24 nt; Table 2) are very conserved in all seven PTTV strains, suggesting that PTTV and human TTV may utilize a common 5'-end of mRNA for translation.

Five additional completely-conserved regions were identified in the vicinity of the TATA-box among all seven PTTV strains. Two regions of 11 nt each (AGTCCTCATTT (SEQ ID NO:40) and AACCAATCAGA (SEQ ID NO:41)) are located in the upstream of the TATA-box, whereas the remaining three regions (CTGGGCGGGTGCCGGAG of 17 nt (SEQ ID NO:42); CGGAGTCAGGGGC of 14 nt (SEQ ID NO:43); TATCGGGCAGG of 11 nt (SEQ ID NO:44)) are located between the proposed 5'-end of mRNA and the initiation codon of ORF2. These conserved PTTV-specific sequences may contain the common elements regulating the viral gene expression.

Previously, three ORFs (ORFs 1-3) were proposed in the genome of the three known PTTV strains, respectively (Neil et al., 2005, supra; Okamoto et al., 2002, supra). The four prototype U.S. strains of PTTV identified in this study possess this structure. The corresponding ORF3 in human TTV has been renamed as ORF2/2 since it initiates at the same ATG in ORF2 and remains in the same ORF (extending replication in cultured cells (Prasetyo, A. A., Kamahora, T., et al., 2008, supra)) after the splicing (FIG. 1A) (Mueller et al., 2008, supra; Qiu et al., 2005, supra). We follow the nomenclature of human TTV for revising PTTV classification in this study.

TTV ORF1/1 is a newly identified viral protein that is encoded by two exons in ORF1 (Qiu et al., 2005, supra). ORF1/1 share the identical N- and C-terminal part with ORF1. The PTTV ORF1/1 counterpart was readily identified in all seven PTTV strains (FIG. 1A and Table 2).

The ORF1 and ORF2 are encoded by a ~2.8 kb viral mRNA whereas the ORF1/1 and ORF2/2 are encoded by a spliced viral mRNA with ~1.2 kb in human TTV (Mueller et al., 2008, supra; Qiu et al., 2005, supra). Since these four ORFs were also deduced in PTTV genomes, and since the sequences and positions of the putative splice donor and acceptor sites in the seven PTTV strains are very conserved (Table 2), it is speculated that porcine TTV probably also encodes the two corresponding mRNAs.

Most of the human TTV strains share a genetic similarity with the CAV, encoding a TTV apoptosis-inducing protein (TAIP) in which its CAV counterpart was named apoptin (de Smidt, M. H., and Noteborn, M. H. (2009). Apoptosis-inducing proteins in chicken anemia virus and TT virus. Curr Top Microbial Immunol 331, 131-49). The ORF of TAIP is embedded within the ORF2. However, the corresponding TAIP does not exist in porcine TTV. A recent study showed that the expression of apoptin or TAIP was required for CAV replication in cultured cells (Prasetyo, A. A., Kamahora, T., Kuroishi, A., Murakami, K., and Hino, S. (2009). Replication of chicken anemia virus (CAV) requires apoptin and is complemented by VP3 of human torque teno virus (TTV). Virology 385(1), 85-92).

Pairwise sequence comparisons (PASC) is a useful method that plots the frequency distribution of pairwise nucleotide sequence identity percentages from all available genomic sequence of viruses in the same family (Bao, Y., Kapustin, Y., and Tatusova, T. (2008). Virus Classification by Pairwise Sequence Comparison (PASC). In “Encyclopedia of Virology, 5 vols.” (B. W. J. Mahy, and M. H. V. Regenmortel, Eds.), Vol. 5, pp. 342-8. Elsevier, Oxford). The different peaks generated by the PASC program usually represent groups of virus genera, species, types, subtypes and strains (FIG. 2). In this study, we performed PASC analyses of TTV using 121 full-length genomic sequences of human and animal TTV-related strains available in GenBank database (FIG. 2). Assuming that TTV members are classified into a separate family, Anelloviridae, the two major peaks, at 36-55% and 55-67% nucleotide sequence identities, represent groups of genera and species, respectively (FIG. 2). Accordingly, a TTV type is defined as a group of TTV having 67-85% nucleotide sequence identity whereas a TTV subtype may be defined as a group of TTV sequences sharing 85-95% nucleotide sequence identity. TTV strains sharing more than 95% nucleotide sequence identity may be further classified into variants (FIG. 2). A similar classification has been proposed using sequences of 103 TTV isolates by Jelcic et al (Jelcic, I., Hotz-Wagenblatt, A., Hunziker, A., Zur Hausen, H., and de Villiers, E. M. (2004). Isolation of multiple TT virus genotypes from spleen biopsy tissue from a Hodgkin’s disease patient: genome reorganization and diversity in the hypervariable region. J Virol 78(14), 7498-507).

This proposed criteria of TTV classification were applied to phylogenetic analyses of the genomic sequences of the 4 prototype U.S. strains of PTTV and the 3 other known PTTV strains. Pairwise comparison of full-length nucleotide sequences among these strains showed that the four PTTV1 strains have 54.0-56.4% nucleotide sequence identity compared to the three PTTV2 strains (Table 3). Therefore, the previously designated “genogroup” of PTTV in the literature will probably be more appropriate to designate as “species”, and PTTV1 and PTTV2 probably should represent porcine TTV species 1 and species 2, respectively. PTTV species 1 consists of two types of viruses designated as type 1a (including Sd-TTV31 and PTTV1-VA (SEQ ID NO:9)) and type 1b (including TTV-1p and PTTV1b-VA (SEQ ID NO:10)), respectively, since the nucleotide sequence identity between these two types of viruses is between 69.8-70.7% (Table 3). Sd-TTV31 and TTV1a-VA (SEQ ID NO:9) are recognized as variant strains of the same species due to their higher sequence identity (95.1%). However, the two type 1b strains, TTV-1p and PTTV1b-VA (SEQ ID NO:10), may belong to two different subtypes (nucleotide sequence identity: 86.4%). For PTTV species 2, three strains are likely to be classified into separate subtypes (TTV-2p for subtype 2a, PTTV2b-VA (SEQ ID NO:11) for subtype 2b, and PTTV2c-VA (SEQ ID NO:12) for subtype 2c, respectively) based upon their 86.5-90.9% nucleotide sequence identity. This proposed new classification system for PTTV was clearly evident in the phylogenetic tree (FIG. 3A). Phylogenetic trees constructed based upon the deduced amino acid sequences of ORF1, ORF1/1, ORF2 and ORF2/2 of PTTV were also consistent with this proposed classification (FIGS. 3B to 3E).
The data were generated by using the PASC program, and the values indicate % nucleotide sequence identities.

Unique mutations and deletions and/or insertions are scattered throughout the genomes between PTTV species, types and subtypes. For example, the location of ORF1 initiation and termination codons and the ORF2 initiation codons between PTTV type 1a and 1b, which was shown in FIG. 1B as mentioned above, are different. The two PTTV1b strains also have a 2-codon deletion after the ORF2 initiation compared to PTTV1a (FIG. 1B).

Remarkably, both TTV-2p and PTTV2b-VA have a large 52-nt deletion, which is 39 nt upstream of the first 11-nt conserved sequence (AGTCCTCATTT (SEQ ID NO:40)) in the UTR, compared to PTTV2c-VA. Due to this deletion, the genomic size of PTTV2b-VA (probably TTV-2p as well) was significantly smaller than that of PTTV2c-VA (Table 2). A number of “subviral” human TTV clones have been isolated from serum samples that are considered as full-length TTV genomes since the ORFs in a majority of these subviral molecules usually remain intact (de Villiers et al., 2009; Leppik et al., 2007). They have variable lengths in the UTR that are completely or partially deleted. The situation of TTV-2p and PTTV2b-VA appears to resemble that of the human TTV subviral molecules, implying that subtypes PTTV2a and PTTV2b might be the subviral molecules derived from subtype PTTV2c. Of note, the 3'-terminal sequence of a nested-PCR primer TTV2-nF (Table 1) that is commonly used for detection of the PTTV2 from field samples by other groups (Ellis et al., 2008, supra; Kekarainen et al., 2007, supra; Kekarainen et al., 2006, supra; Krakowka et al., 2008, supra) is located at both sides of the deletion. Therefore, the current nested-PCR assay for PTTV2 detection is likely not sufficient to identify the genetically diverse strains of PTTV2c subtype.

The source of the isolated virus strain is serum, fecal, saliva, semen and tissue samples of pigs having the porcine TTV viral infection. However, it is contemplated that recombinant DNA technology can be used to duplicate and chemically synthesize the nucleotide sequence. Therefore, the scope of the present invention encompasses the isolated polynucleotide which hybridizes to and which is at least 67% complementary to the nucleotide sequence set forth in SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, preferably 85% complementary, or more preferably 95% complementary; or an immunogenic fragment selected from the group consisting of an amino acid sequence of ORF1 protein set forth in SEQ ID NO:13 (PTTV1a-VA), SEQ ID NO:14 (PTTV1b-VA), SEQ ID NO:15 (PTTV2b-VA), SEQ ID NO:16 (PTTV2c-VA), an amino acid sequence of ORF2 protein set forth in SEQ ID NO:17 (PTTV1a-VA), SEQ ID NO:18 (PTTV1b-VA), SEQ ID NO:19 (PTTV2b-VA), SEQ ID NO:20 (PTTV2c-VA), an amino acid sequence of ORF1/1 protein set forth in SEQ ID NO:21 (PTTV1a-VA), SEQ ID NO:22 (PTTV1b-VA), SEQ ID NO:23 (PTTV2b-VA), SEQ ID NO:24 (PTTV2c-VA), an amino acid sequence of ORF2/2 protein set forth in SEQ ID NO:25 (PTTV1a-VA), SEQ ID NO:26 (PTTV1b-VA), SEQ ID NO:27 (PTTV2b-VA), SEQ ID NO:28 (PTTV2c-VA). The immunogenic or antigenic coding regions or fragments can be determined by techniques known in the art and then used to make monoclonal or polyclonal antibodies for immunoreactivity screening or other diagnostic purposes. The invention further encompasses the purified, immunogenic protein encoded by the isolated polynucleotides. Desirably, the protein may be an isolated or recombinant ORF1 protein or an ORF2 protein of at least one of the above isolated porcine TTV subtypes, more desirably ORF1 protein.

The ORF1 of porcine TTV is believed to encode a structural and replication-associated protein (Maggi, F., and Bendinelli, M. (2009). Immunobiology of the Torque teno viruses and other anelloviruses. *Curr Top Microbiol Immunol* 331, 65-90). The ORF1-encoding products of seven PTTV strains have 624-635 aa in length and possess a high number of arginine residues at the N-terminus that are thought to have the DNA-binding activity (FIG. 4). In FIG. 4, conserved sequences are shaded. Dashes indicate amino acid deletions. The RCR motifs are boxed with solid lines. Three HVRs (PTTV1-HVRs 1, 2 and 3) of PTTV1 strains and two HVRs (PTTV2-HVRs 1 and 2) of PTTV2 strains are boxed with dashed lines. The connection boundaries of ORF1/1 are indicated by arrows. The predicted rolling-circle replication (RCR) motifs (Ilyina, T. V., and Koonin, E. V. (1992). Con-
observed sequence motifs in the initiator proteins for rolling circle DNA replication encoded by diverse replicons from eubacteria, eucaryotes and archaeabacteria. Nucleic Acids Res 20(13), 3279-85) are presented at different positions in different PTTV types and subtypes that may be type- or subtype-specific. RCR motif-II (YxxK) is conserved in the PTTV type 1a (aa position 14-17 of PTTV1a-VA SEQ ID NO:13) and type 1b strains (aa position 379-382 of PTTV1b-VA SEQ ID NO:14), respectively, whereas the same conserved motif identified in all three PTTV2 strains is located at aa position 482-485 of PTTV2b-VA SEQ ID NO:15 (FIG. 4). Both PTTV2b-VA SEQ ID NO:15 and PTTV2c-VA SEQ ID NO:16 also have a conserved RCR motif-II (HxxQ) at aa position 331-333 of PTTV2b-VA that is absent in TTV-2p (FIG. 4).

The ORF1 proteins of PTTV strains between species 1 and 2 share very low aa identity with only 22.4 to 25.8%, which makes it difficult to identify significantly conserved aa sequences between the two species (FIG. 4). In PTTV species 1, the aa identity of ORF1 between type 1a and 1b strains are 50.3-52.7%. Three major hypervariable regions (HVR), PTTV1-HVRI to 3, with a relatively high number of aa substitutions, were identified among the four PTTV1 strains, whereas two HVRS (PTTV2-HVRI and 2) were observed among the three PTTV2 strains (FIG. 4): The three PTTV2 strains have an approximately 20-aa deletion in the corresponding PTTV1-HVRI region. Moreover, the two HVRS of PTTV2 are within the corresponding PTTV1-HVRI3 region (FIG. 4). These HVRS are located only in the ORF1 but not in the truncated ORF1/1. They likely play a role in evading the host immune surveillance and helping PTTV to establish a persistent infection, as suggested by studies of human TTV.

The aa sequences of ORF2 differed considerably between the four PTTV1 (PTTV1a-VA SEQ ID NO:17, PTTV1b-VA SEQ ID NO:18) and three PTTV2 (PTTV2b-VA SEQ ID NO:19; PTTV2c-VA SEQ ID NO:20) strains (FIG. 5). However, they share a conserved protein-tyrosine phosphatase (PTPase)-like motif (Wx5Hx3Cx6x5H) at the N-terminus (FIG. 4). This motif is also conserved among all human TTV, TTMV and TTMDV strains as well as CAV. The TTMV or CAV ORF2 protein also exhibited a serine/threonine phosphatase (ST PPase) activity (Peters, M. A., Jackson, D. C., Crabb, B. S.,Tivendale, K. A., and Browning, G. F. (2002). Chicken anemia virus VP2 is a novel dual specificity protein phosphatase. J Biol Chem 277(42), 39566-73). The dual specificity of the ORF2 protein is thought to regulate host gene transcription, signal transduction and cytokine responses during viral replication. Recently, mutagenesis analyses of two conserved basic aa residues before the last histidine residue of the motif in CAV revealed that the two residues affect virus replication, cytopathology in vitro and attenuation in vivo (Peters, M. A., Crabb, B. S., Washington, E.A., and Browning, G. F. (2006). Site-directed mutagenesis of the VP2 gene of Chicken anemia virus affects virus replication, cytopathology and host-cell MHC class I expression. J Gen Virol 87(Pt 4), 823-31; Peters, M. A., Crabb, B. S., Tivendale, K. A., and Browning, G. F. (2007). Attenuation of chicken anemia virus by site-directed mutagenesis of VP2. J Gen Virol 88(Pt 8), 2168-75). The two basic aa residues ("KK") are conserved in the three PTTV2 strains. However, only the first basic residue ("R") is retained in the two PTTV1a strains whereas both basic residues are substituted in the PTTV1b strains (FIG. 5). In FIG. 8, dashes indicate amino acid deletions. The five conserved amino acids within the common motif Wx5Hx3Cx6H (underlined) identified in TTV, TTMV and CAV are shaded. The positions of the two basic aa residues before the last histidine of the motif, which have been shown to affect virus replication, cytopathology or in vivo attenuation of CAV, are indicated by "**".

In summary, the present invention has determined the full-genome genomic sequences of four porcine TTV strains representing different genotypes or subtypes in a serum sample of a single boar in Virginia. The finding from this study clearly indicates that, similar to human TTV, multiple PTTV infections with distinct genotypes or subtypes exist and probably are common in pigs. We have also provided new information regarding the genomic organization, the degree of variability and the characteristics of conserved nucleotide and amino acid motifs of PTTV, which will improve the current PCR detection assay, aid in developing reagents for serological diagnostics and help initiate the structural and functional study of PTTV. A new classification of PTTV is also proposed in this study based upon the phylogenetic and genetic analyses of the genomic sequences of seven known PTTV strains. The present invention also provides methods for diagnostics of porcine TTV infection by detecting viral DNA in samples of porcine TTV infected pigs or other mammals. One preferred embodiment of the present invention involves methods for detecting porcine TTV nucleic acid sequences in a porcine or other mammalian species using oligonucleotide primers for polymerase chain reaction (PCR) to further aid in the diagnosis of viral infection or disease. The diagnostic tests, which are useful in detecting the presence or absence of the porcine TTV viral nucleic acid sequence in the porcine or other mammalian species, comprise isolating viral DNA from samples of porcine TTV infected pigs, or pigs suspected of infection of TTV, and performing SYBR green real-time quantitative PCR using PTTV1-specific (SEQ ID NO:29/ SEQ ID NO:30) or PTTV2-specific (SEQ ID NO:31/SEQ ID NO:32) primers.

In another embodiment of the present invention, the diagnostic method may be adapted to simultaneously detect PTTV1 and PTTV2 by using PTTV1/PTTV2 duplex real-time PCR. More specifically, the method comprises isolating viral DNA from samples of porcine TTV infected pigs or pigs suspected of infection of TTV, performing real-time PCR using both PTTV1-specific (SEQ ID NO:29/SEQ ID NO:30) or PVVT2-specific (SEQ ID NO:31/SEQ ID NO:32) primers in the same real-time PCR reaction. Since the Tm value between PTTV1 and PTTV2 can be distinguished by MCA, the presence of PTTV1 and PTTV2 DNA can be simultaneously detected.

In a further embodiment of the present invention, the diagnostic method may employ duplex nested PCR. The method comprises isolating viral DNA from samples of porcine TTV infected pigs or pigs suspected of infection of TTV, performing a first round of PCR using one pair of primers Plab-mF (SEQ ID NO:33)/Plab-mR (SEQ ID NO:34), and performing a second round of PCR using a mixture of two pairs of primers, Pl1-nf (SEQ ID NO:35)/Pl1-nr (SEQ ID NO:36) and Plb-nf (SEQ ID NO:37)/Plb-nr (SEQ ID NO:38) for detection of PTTV1b, and visualizing the PCR products. The above diagnostics methods maybe optimized by one skilled in the art according to well known methods in the art.

Accordingly, an embodiment of the present invention develops two novel singleplex SYBR green real-time PCR assays to quantify the viral loads of two porcine TTV species, respectively. PTTV1- and PTTV2-specific primers were designed to target the extremely conserved regions across six PTTV1 and four PTTV2 full-length genomes available to date, respectively. Another embodiment of the present invention combines the two singleplex assays into a duplex real-
time PCR assay followed by MCA of the viral amplicons that can be identified by their distinct melting temperatures for simultaneous detection of the two porcine TTV species, PTTV1a and PTTV1b. In a third embodiment, a duplex nested PCR assay for simultaneous amplification of the viral DNAs from two types of PTTV in the first round PCR and differential detection of types 1a and 1b in the second round PCR was developed for the identification of two types of porcine TTV species, PTTV1a and PTTV1b, in a single sample. These assays represent simple and practical tools for diagnoses of species- or type-specific porcine TTVs.

Potential primers sequences were identified by multiple sequence alignments of 10 available porcine TTV full-length genomes. PTTV1-specific primers TTV1F (SEQ ID NO:29) and TTV1R (SEQ ID NO:30) were designed based upon two conserved genomic regions immediately before the putative ORF2 across six PTTV1 genomes, whereas PTTV2-specific primers TTV2F4 (SEQ ID NO:31) and TTV2R4 (SEQ ID NO:32) were designed based upon two conserved genomic regions immediately after the putative ORF2/1 across four PTTV1 genomes (Table 4). Primers showed no potentials for self- and cross-dimerization. The expected amplicon sizes were a 118-bp fragment from the PTTV1 primers corresponding to the PTTV1-VA genome and a 200-bp fragment from the PTTV2 primers corresponding to the PTTV2c-VA genome, respectively.

According to another specific embodiment of the present invention, SYBR green duplex real-time PCR is utilized for the simultaneous detection of PTTV1 and PTTV2 DNA. The 7-degree difference of Tm value between PTTV1 (87.0° C.) and PTTV2 (80.0° C.) made it feasible to distinguish them from one another by the MCA. Therefore, two singleplex assays can be coupled into a duplex real-time PCR assay for the simultaneous detection of PTTV1 and PTTV2. A positive sample was one that had a symmetrical melt peak within the known TW, for that product. This new assay was first validated by using a 10-fold dilution of PTTV1 and PTTV2 standards mixture. The non-template negative control using sterile water as the template showed a non-specific amplification caused by cross-dimerization between the PTTV1 and PTTV2 primers not seen in the singleplex assays (FIG. 7a). This produced a distinct melt peak between 72.00 C. and 76.00 C. FIG. 7A shows melt peaks of PTTV1 standard (red; Tm:87.0° C.), PTTV2 standard (green; Tm:80.0° C.) and non-template negative control (caused by primer cross-dimerization; black). FIGS. 7B-7E show melt peaks of representative serum samples with distinct viral loads of PTTV1 and PTTV2. FIG. 7B shows boar serum sample no. 5: relatively high viral loads of both PTTV1 and PTTV2, but

<table>
<thead>
<tr>
<th>Primer ID</th>
<th>Sequence (5' to 3')</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTV1F</td>
<td>TCCGAATGGCTGAGTTTATGC</td>
<td>PTTV1-specific real-time PCR</td>
</tr>
<tr>
<td>TTV1R</td>
<td>TCCGCTCAGCTGCTCCT</td>
<td>PTTV1-specific real-time PCR</td>
</tr>
<tr>
<td>TTV2F4</td>
<td>GGTGGTAAAGAGGATGAA</td>
<td>PTTV2-specific real-time PCR</td>
</tr>
<tr>
<td>TTV2R4</td>
<td>AATAGATTGGACACAGAGAG</td>
<td>PTTV2-specific real-time PCR</td>
</tr>
<tr>
<td>Plab-1F</td>
<td>TATCGGGCAGGAGCAGCT</td>
<td>Duplex nested PCR</td>
</tr>
<tr>
<td>Plab-1R</td>
<td>TAGGGGCGCGCTCTACGT</td>
<td>Duplex nested PCR</td>
</tr>
<tr>
<td>Plab-2F</td>
<td>CCTACATGAAGGAGAAAGACT</td>
<td>Duplex nested PCR</td>
</tr>
<tr>
<td>Plab-2R</td>
<td>CCAGCTGCTCAAGGCTG</td>
<td>Duplex nested PCR</td>
</tr>
</tbody>
</table>

According to one specific embodiment of the present invention, SYBR green simplex real-time PCR using PTTV1- and PTTV2-specific primers can be used specifically to detect porcine TTV1 and TTV2 DNA, respectively. For PTTV1, a standard curve was established over a range of target DNA concentrations per 25 µl. The linear range was shown to span 4.4x10^7 to 4.4x10^8 copies. The minimum detection limit (44 copies) corresponded to a threshold cycle (Ct) of 37.57. For PTTV2, standard curve was also generated and used to detect DNA concentration ranging from 8.6x10^0 to 8.6x10^8 copies per 25 µl reaction. The corresponding Ct of minimum detection limit (8.6 copies) was 36.53. According to another specific embodiment of the present invention, SYBR green simplex real-time PCR using PTTV1- and PTTV2-specific primers can be used specifically to detect porcine TTV1 and TTV2 DNA, respectively. For PTTV1, a standard curve was established over a range of target DNA concentrations per 25 µl. The linear range was shown to span 4.4x10^7 to 4.4x10^8 copies. The minimum detection limit (44 copies) corresponded to a threshold cycle (Ct) of 37.57. For PTTV2, standard curve was also generated and used to detect DNA concentration ranging from 8.6x10^0 to 8.6x10^8 copies per 25 µl reaction. The corresponding Ct of minimum detection limit (8.6 copies) was 36.53.
In one example, when the duplex real-time assay was applied to the 20 serum samples of adult boars, samples with relatively high viral loads of both PTTV1 and PTTV2 displayed two distinct melt curves corresponding to PTTV1 and PTTV2 without a non-specific melt peak (FIGS. 7D & 7C), whereas samples with low viral load of either PTTV1 or PTTV2 showed virus-specific as well as non-specific melt curves (FIGS. 7D & 7E). Although the two melt peaks in sample #14 were very small, they were considered positive since they displayed a visually distinct and symmetrical rise and fall at the appropriate T_m of PTTV1 and PTTV2 (FIG. 7D). In contrast, sample #10 was considered only PTTV1 positive because a symmetrical PTTV2 melt peak was not evidently present (FIG. 7E). These results were consistent with that of the two singleplex assays (Table 5). Moreover, the size and shape of melt peaks qualitatively reflected the corresponding viral load in the detected sample.

According to another aspect of the present invention, duplex nested PCR is used for differential detection of two porcine TTV types, PTTV1a and PTTV1b. The inventor of the present invention demonstrated the existence of two distinct genotypes, tentatively named PTTV1a and PTTV1b, in porcine TTV species 1. To further determine whether the co-infection of PTTV1a and PTTV1b is common in pigs, a novel duplex nested PCR assay to quickly distinguish between the two was developed. Alignment of porcine TTV genomic DNA sequences identified a conserved genomic region located at the N-terminal part of the putative ORF1 encoding the viral capsid protein (FIG. 8). This region also contains the entire ORF2 and the partial UTR in the upstream. Primers P1ab-mF (SEQ ID NO:33)/P1ab-mR (SEQ ID NO:34) were designed to simultaneously amplify both PTTV1a and PTTV1b DNAs in the first-round PCR. A mixture of PTTV1a-specific primers P1a-nf (SEQ ID NO:35)/P1a-nR (SEQ ID NO:36) and PTTV1b-specific primers P1b-nf (SEQ ID NO:37)/P1b-nR (SEQ ID NO:38) was used to differentially amplify each genotype in the second-round PCR. The final PCR products of PTTV1a and PTTV1b were 162 by and 96 by in sizes, respectively, which could be easily distinguished by gel electrophoresis on a 1% agarose gel stained with ethidium bromide. This assay was not expected to detect PTTV2 DNA due to the specificity of primers (FIG. 8). In FIG. 8, conserved sequences were indicated by dots and shaded. Dashes indicated nucleotide deletions. The locations and directions of three pairs of primers used for duplex nested PCR were marked by arrows.

In one example, the 20 serum samples from adult boars that were subjected to the duplex nested PCR assay were all found to be positive for both PTTV1a and PTTV1b, as determined by visualizing two bands of the expected sizes and subsequent sequencing confirmation of PCR products (data not shown). No PCR products were amplified in the 19 semen samples, which was consistent with the results of PTTV1 conventional nested PCR and real-time PCR assays described above.

Infection of pigs with the two species of porcine TTV has been found back to 1985 in Spanish pig farms according to a retrospective investigation (Segales et al., 2009, supra). However, whether porcine TTVs are associated with any particular pig diseases remains elusive. Since both of porcine TTV species have a high prevalence in domestic pigs, determination of TTV viral loads is presumably more important than assessing the presence of TTV DNA. The level of viral loads in serum and semen samples has been indicated as an important marker for PCVAD in PCV2 infection (Opriessnig et al., 2007, supra). Therefore, establishment of quantitative PTTV-specific real-time PCR assays would help identify potential disease conditions associated with porcine TTVs.
could replace the conventional nested PCR for detection purpose. In comparison with real-time PCR, the conventional nested PCR assay for porcine TTVs detection is time-consuming (requiring total 4 rounds of PCR), laborious and prone to sample contamination occurring during multiple rounds of PCR processing. Due to the difference of $T_m$ value between PTTV1 and PTTV2 species, an MCA following duplex PCR amplification is able to ensure distinct reaction specificity. Another advantage of this duplex real-time assay is that inclusion of PTTV1 and PTTV2 standards is dispensable when performing the described protocol, which makes it easier for much wider use in any diagnostic labs equipped with an automated real-time PCR instrument.

Multiple infection of porcine TTVs with distinct genotypes or subtypes of the same species has been demonstrated (Gallego et al., 2009, supra). In particular, our previous study showed that porcine TTV species PTTV1a and PTTV2a, each consists of two distinct types, PTTV1a (including strains SD-TTV31 and PTTV1a-VA) and PTTV1b (including strains TTV-1p and PTTV1b-VA). The two newly published PTTV1 isolates with full-length genomes, sWJTHY-TT27 (GQ120664) from Canada and FTV1 #471819 (GU188045) from Germany, were both classified into type 1b based upon the phylogenetic analysis (data not shown). The duplex nested PCR described in this study confirmed that dual infection of two PTTV1 genotypes frequently occurred in pigs. This novel assay is the first diagnostic PCR approach developed to distinguish between PTTV1a and 1b so far. Since it is currently not known whether one or both of PTTV1a and PTTV1b infection represents a relevant factor associated with our differential PCR assay should be of great value for future potential disease associations of these two PTV types.

According to another aspect of the invention, porcine TTV ORF proteins were expressed and used in immunodetection assays to detect the presence of porcine TTV specific antibodies. In one embodiment of the present invention, three truncated and Histidine-tagged ORF1 proteins of PTTV1a, PTTV1b and PTTV2, were expressed and purified in Escherichia coli (E. coli), respectively. Furthermore, both serum Western blot and ELISA assays based on these recombinant antigens were developed and validated using porcine serum samples from different sources. In particular, serological testing using the PTTV1a-, PTTV1b- and PTTV2-specific ELISA provides an accurate and simple tool for revealing the association of porcine TTV infection with diseases.

According to a further aspect of the invention, porcine TTV ORF proteins were expressed and purified as recombinant ORF1 capsid protein in E. coli expression system (Fig. 10, Fig. 15). Three truncated and His-tagged ORF1 capsid proteins of PTTV1a, PTTV1b and PTTV2, were expressed and purified in Escherichia coli (E. coli), respectively, and served as recombinant capsid subunit vaccines against PTTV infection.

Four porcine TTV2 strains, TTV-2p, TTV2/472142, PTTV2b-VA and PTTV2c-VA, had available complete genomic sequences to date. Although they are phylogenetically classified into three putative subtypes, a comparative analysis of hydrophilicity profiles of the ORF1 encoding amino acids from four PTTV2 showed that they shared three hydrophilic regions, an arginine-rich region from aa 1-49 at the N-terminal and two particular domains (I and II) located at the middle and C-terminal part, respectively (Fig. 9A). The C-terminal region used for truncated PTTV2c-VA ORF1 expression and the corresponding regions shared in other three PTTV2 strains were indicated by a dashed box. Alignments of amino acid sequence demonstrated high levels of sequence conservation of domains I (aa 522-549) and II (aa 536-565) across the four PTTV2 strains (Fig. 9B).

Since hydrophilic domains are believed to be important for the antigenicity of many proteins, the C-terminal region (aa 510-525) of the PTTV2c-VA ORF1SEQ ID NO:16 containing the two domains was chosen for protein expression, which would be used as antigen for PTTV2-specific antibody detection in porcine serum. According to one aspect of the invention, expression of the truncated PTTV2c ORF1 was sufficient for detection of all PTTV2 subtypes (2a, 2b and 2c; also see Fig. 3A).

According to one embodiment of the present invention, the C-terminal part of the PTTV2c ORF1 gene fused with 8×His tags was constructed and expressed in E. coli. The recombinant protein was insoluble and expressed within the bacterial inclusion bodies. Fig. 10A shows SDS-PAGE of unpurified 2c-ORF1 products. Fig. 10B shows SDS-PAGE of purified 2c-ORF1 products. Fig. 10C shows Western blot analysis of purified 2c-ORF1 products using an anti-His-tagged mAb.

White arrowheads indicated the ORF1 protein with the expected size and its truncated product whereas black arrowheads show the putative dimers of the expected and truncated proteins. M: protein markers. In Fig. 10A, two significant polypeptides (white arrowheads) were produced in the 2c-ORF1 unpurified sample in comparison with the control sample. The band of ~40 KDa was consistent with the expected size of 2c-ORF1 whereas the ~30 KDa polypeptide was probably an N-terminally truncated product from the former. After purification with a nickel-affinity column, four polypeptides including the two described significant bands were showed in SDS-PAGE (Fig. 10B). They were also detected by western blot using an anti-His-tagged mAb (Fig. 10C). Two high-molecular-mass bands (black arrowheads) were the homodimers formed by the two polypeptides of ~40 KDa and ~30 KDa, respectively, based on the predicted sizes (~80 KDa and ~60 KDa). The results demonstrated that the purified C-terminal PTTV2c-ORF1 was successfully produced and could be used for porcine TTV2 antibody detection in porcine sera.
These negative samples were pooled and used as a negative reference in development of a PTTV2-specific ELISA. The remaining samples from this source were positive (FIG. 12, the four lines in the left). In addition, porcine sera from a commercial company used in cell culture (supposed to be OIE diseases-free) also displayed strong anti-PTTV2-ORF2 positivity (FIG. 12), which was used as a positive control for ELISA. The concentrations of purified 2c-ORF1 antigen, porcine sera and IgG conjugate were determined by check-board titration to present low background signal and give the highest difference of OD450 value between the positive and negative controls. The optimal antigen amount was 69 ng per well, and the optimal ELISA results were obtained by use of a 1:100 dilution of serum samples and a 1:4000 dilution of IgG conjugates. The ELISA cutoff values ranged from 0.25 to 0.5 in each trial. FIG. 4 shows a representative result reflecting the consistency of serum western blot and the developed ELISA.

138 conventional pig sera samples from 3 herds were chosen to analyze the correlation between PTTV2 viral load by real-time PCR and anti-PTTV2 IgG antibody level by ELISA. The results showed that pigs with undetectable or lower PTTV2 viral load (10^4 copies/ml) were more likely to have a lower serum PTTV2 antibody titer than pigs with middle values of PTTV2 viral load (FIG. 13).

In particular, sera from 10 pigs in the same herd were also analyzed by comparing the PTTV2 viral loads and anti-PTTV2 antibody levels of their sera from their arrival in the new facility to two months after arrival. Nine of the 10 pigs had decreased viral loads (three had no detectable virus) after 2 months whilst the anti-PTTV2 antibody titers increased in nine of 10 pigs (FIGS. 14A and 14B). The results suggested that the 10 pigs acquired PTTV2 infection at early stage, which induced humoral response and produced anti-ORF1 capsid IgG antibody progressively. The PTTV2-ORF1 IgG antibody was able to neutralize or even clear the virus, indicating the ORF1 indeed encode a viral capsid protein and may contain neutralizing epitopes against PTTV2.

According to one embodiment of the present invention, the C-terminal PTTV1a- and PTTV1b-ORF1 proteins were expressed and purified in E. coli system, respectively. SDS-PAGE and western blot analysis using an anti-His-tagged mAb showed that both 1a- and 1b-ORF products had two polypeptides, one with expected size (~40 KDa) and another as the putative homodimer (~80 KDa) (FIG. 15A-C). FIG. 15A shows SDS-PAGE of unpurified and purified 1a-ORF1 products. FIG. 15B shows SDS-PAGE of purified 1b-ORF1 and 1b-ORF1etr products. FIG. 15C shows Western blot analysis of purified 1a- and 1b-ORF1 products using an anti-His-tagged mAb. White arrowheads indicate the ORF1 protein with the expected size whereas black arrowheads show the putative dimer of the ORF1 proteins. Compared to 2c-ORF1 expression, no truncated polypeptide was observed. As a comparative control, expression of a C-terminal-truncated 1b-ORF1 region (1b-ORF1true) resulted in a lower-molecular-mass polypeptide compared to its C-terminal-non-truncated counterpart 1b-ORF1 (FIG. 15B).

According one embodiment of the present invention, the purified C-terminal PTTV1a- and PTTV1b-ORF1 proteins were used to develop genotype-specific serum Western Blots and ELISA as described for PTTV2 above. FIG. 16 shows negative (lanes 1-2) and positive (lanes 3-5) examples of serum Western blot using 1a-ORF1 as antigen. The same antigen amount (69 ng), dilution of sera (1:100) and dilution of IgG conjugate (1:4000) as PTTV2-ORF1 were used in PTTV1a- and PTTV1b-specific ELISA (data not shown).

Additionally, the present invention provides a useful diagnostic reagent for detecting the porcine TTV infection which comprise a monoclonal or polyclonal antibody purified from a natural host such as, for example, by inoculating a pig with the porcine TTV or the immunogenic composition of the invention in an effective immunogenic quantity to produce a viral infection and recovering the antibody from the serum of the infected pig. Alternatively, the antibodies can be raised in experimental animals against the natural or synthetic polypeptides derived or expressed from the amino acid sequences or immunogenic fragments encoded by the nucleotide sequence of the isolated porcine TTV. For example, monoclonal antibodies can be produced from hybridoma cells which are obtained from mice such as, for example, Balb/c, immunized with a polypeptide antigen derived from the nucleotide sequence of the isolated porcine TTV. Selection of the hybridoma cells is made by growth in hypoxantine, thymidine and aminopterin in a standard cell culture medium like Dulbecco's modified Eagle's medium (DMEM) or minimal essential medium. The hybridoma cells which produce antibodies can be cloned according to procedures known in the art. Then, the discrete colonies which are formed can be transferred into separate wells of culture plates for cultivation in a suitable culture medium. Identification of antibody secreting cells is done by conventional screening methods with the appropriate antigen or immunogen. Cultivating the hybridoma cells in vivo or by obtaining ascites fluid in mice after injecting the hybridoma produces the desired monoclonal antibody via well-known techniques.

For another alternative method, porcine TTV capsid protein can be expressed in a baculovirus expression system or E. coli expression system according to procedures known in the art. The expressed recombinant porcine TTV capsid protein can be used as the antigen for diagnosis in an enzyme-linked immunoabsorbent Assay (ELISA). The ELISA assay based on the porcine recombinant capsid antigen, for example, can be used to detect antibodies to porcine TTV in porcine and mammalian species. Although the ELISA assay is preferred, other known diagnostic tests can be employed such as immunofluorescence assay (IFA), immunoperoxidase assay (IPA), etc.

Desirably, a commercial ELISA diagnostic assay in accordance with the present invention can be used to diagnose porcine TTV infection in pigs. The examples illustrate using purified ORF1 and ORF2 proteins of porcine TTV to develop an ELISA assay to detect anti-PTTV antibodies in pigs. Sera collected from pigs infected with porcine TTV, and negative sera from control pigs are used to validate the assay. PTTV2 specific, PTTV1a specific, and PTTV1b specific antibodies were demonstrated to specifically recognize PTTV ORF proteins. Further standardization of the test by techniques known to those skilled in the art may optimize the commercialization of a diagnostic assay for porcine TTV.

Another aspect of the present invention is the unique immunogenic composition comprising the isolated porcine TTV or an antigenic protein encoded by an isolated poly-nucleotide described hereinabove and its use for raising or producing antibodies. The composition contains a nontoxic, physiologically acceptable carrier and, Optionally, one or more adjuvants. Suitable carriers, such as, for example, water, saline, ethanol, ethylene glycol, glycerol, etc., are easily selected from conventional excipients and co-formulants may be added. Routine tests can be performed to ensure physical compatibility and stability of the final composition.

In accordance with the present invention, there are further provided infectious molecular and nucleic acid molecules of porcine Torque teno (TTV), live viruses produced from the
nucleic acid molecule and veterinary vaccines to protect pigs from porcine TTV viral infection or disease caused by porcine TTV co-infection with other viruses. The invention further provides immunogenic polypeptide expression products that may be used as vaccines.

The novel infectious DNA molecule of porcine TTV comprises a nucleic acid molecule encoding at least a portion of an infectious TTV genome. BamHI single digestion of the infectious DNA clone preferably contains at least one of ORF1, ORF2, ORF1/1, and ORF2/2 gene of the TTV1 or TTV2. Multiple copies of the TTV1-VA (SEQ ID NO:9), TTV1-VA (SEQ ID NO:10), TTV2-VA (SEQ ID NO:11), or TTV2-VA (SEQ ID NO:12) genome may be inserted into a single DNA molecule to construct tandem infectious TTV clones.

The cloned genomic DNA of TTV, particularly TTV1-VA, TTV1-VA, TTV2-VA, and tandem TTV2-RR, TTV2-RR, described herein is shown to be in vitro or in vivo infectious when transfected into PK-15 cells and given to pigs. This new, readily reproducible pathogenic agent lends itself to the development of a suitable vaccination program to prevent TTV infection in pigs.

According to a further embodiment of the present invention, three one-genome-copy TTV DNA clones were derived from the prototype US isolates TTV1-VA, TTV1-VA, and TTV2-VA by fusion PCR, respectively. Each of the full-length genomic DNA was inserted into a cloning vector pSC-B-amp/kan by blunt-end ligation. The restriction site BamHI is the unique site on the three TTV genomes, which was engineered at both ends of the three genomes to facilitate the generation of concatenates and thus mimic the TTV circular genome. BamHI single digests of the selected plasmid DNA of each clone clearly resulted in two different fragments of 4.3-Kb and 2.8-Kb in size (FIG. 18A). The 4.3-Kb fragments represented the backbone vector whereas the 2.8-Kb fragments represented the inserted TTV genomic DNA. The empty vector pSC-B-amp/kan digested with the same enzyme only showed a 4.3-Kb band (FIG. 18A). The resulting TTV clones were designated pSC-PTTV1a, pSC-PTTV1b and pSC-PTTV2c, respectively (FIG. 17A-C).

Furthermore, two copies of the full-length TTV2-VA genome derived from the clone pSC-PTTV2c were ligated in tandem into the pSC-B-amp/kan vector to generate the clone pSC-2PTTV2-RR (FIG. 17D). Comparison of the Afl II single digestion patterns between pSC-PTTV2c and pSC-2PTTV2-RR showed that the latter plasmid had an additional 2.8-Kb fragment representing the second copy of TTV2 genome (FIG. 18A, right panel). Subsequently, we utilized the same cloning strategy to produce a tandem-dimerized TTV2 genome clone derived from the Germany TTV clone TTV2-#471942-full. An additional 2.8-Kb fragment representing the second copy of TTV2 genome was present in this construct, designated pSC-2PTTV2-RR (FIG. 17F), which was digested with the Hind III alone when compared to its one-genome-copy counterpart (FIG. 18B, left panel), confirming the successful construction.

The replication competencies of the constructed TTV infectious clones were tested in vitro transfection of PK-15 cells. IFI using the commercially generated rabbit polyclonal antibodies against TTV2 ORF1 confirmed that both the concatamers of clones TTV2-#471942-full and pSC-PTTV2c were replication competent, respectively (FIG. 19A and FIG. 20A). Passage of the transfected cells did not eliminate or reduce the fluorescent signals (FIG. 19B and FIG. 20B), suggesting that the expression of ORF1 proteins was resulted from the TTV2 concatamers that mimicked the natural TTV2b or TTV2c circular molecules. No fluorescent signals was observed in mock-transfected cells or DNA-transfected cells using pre-immune rabbit serum as the antibody for IFA detection (data not shown). The concatamers of the clone pSC-PTTV1a also showed to be replication-competent using an anti-PTTV1 ORF1 antibody (FIG. 21). The positive fluorescent signals were located in the nucleus of transfected or passaged cells, indicating that porcine TTVs likely replicate in the cell nucleus. It is not unexpected because porcine circovirus (PCV) has a similar expression pattern in vitro.

Direct transfection of the tandem-dimerized clone pSC-2PTTV2b-RR or pSC-2PTTV2c-RR in PK-15 cells results in viral replication and produces the ORF1 capsid antigen. IFA using antibodies against TTV2 ORF1 confirmed that both clones were also replication-competent and the positive ORF1 antigens were localized in the nuclei (FIGS. 22A and B).

According to one embodiment of the present invention, infectious clones of porcine TTV can be used to inoculate pigs, which will then elicit an immune response of the host animal and stimulate production of neutralizing antibodies. In one particular embodiment of the present invention, the two tandem-dimerized TTV2 clones were infectious when injected into the lymph nodes and muscles of conventional pigs.

To test the in vivo infectivity of TTV2 molecular clones, conventional pigs were inoculated with the clone pSC-2TTV2b-RR or pSC-2TTV2c-RR. Serum samples were collected from animals at 0, 7, 14, 21 and 28 days post-inoculation (DPI). TTV2 DNA was detected in pSC-2TTV2c-RR-inoculated pigs beginning at 7 DPI (#92), 14 DPI (#188 and #191) and 21 DPI (#180), respectively (FIG. 23A, left panel). TTV2 viremia appeared late for pigs inoculated with the clone pSC-2TTV2b-RR: two began at 14 DPI (#189 and #192), one at 21 DPI (#181) and one at 28 DPI (#193) (FIG. 23A, left panel). The viral loads increased during the course in all inoculated pigs that had the highest viral loads at 28 DPI before necropsy, as determined by TTV2-specific real-time PCR (FIG. 23A). The real-time PCR products amplified from selected pigs were sequenced and found to have identical sequences to the corresponding regions of pSC-2TTV2b-RR or pSC-2TTV2c-RR (data not shown).

All inoculated pigs were negative for TTV2 ORF1 antibodies at 0 and 7 DPI. At 14 DPI, all the four pSC-2TTV2b-RR-inoculated pigs seroconverted to anti-PTTV2 ORF1 IgG, whereas pigs in pSC-2TTV2c-RR-inoculated group seroconverted at 14 (#92 and #180), 21 (#181) and 28 (#188) DPI, respectively (FIG. 23B). The results indicated that active porcine TTV2b or TTV2c infection had occurred.

Vaccines of the infectious viral and infectious molecular DNA clones, and methods of using them, are also included within the scope of the present invention. Inoculated pigs are protected from viral infection and associated diseases caused by TTV2 infection or co-infection. The novel method protects pigs in need of protection against viral infection by administering to the pig an immunologically effective amount of a vaccine according to the invention, such as, for example, a vaccine comprising an immunogenic amount of the infectious TTV DNA, a plasmid or viral vector containing the infectious DNA clone of TTV, the recombinant TTV DNA, the polypeptide expression products, the bacteria-expressed or baculovirus-expressed purified recombinant ORF1 capsid protein, etc. Other antigens such as PRRSV, PPV, other infectious swine agents and immune stimulants may be given
The vaccines comprise, for example, the infectious viral and molecular DNA clones, the cloned PTTV infectious DNA genome in suitable plasmids or vectors such as, for example, the pSC-B vector, an avirulent, live virus, an inactivated virus, expressed recombinant capsid subunit vaccine, etc. in combination with a nontoxic, physiologically acceptable carrier and, optionally, one or more adjuvants. The vaccine may also comprise the infectious TTV2 molecular DNA clone described herein. The infectious PTTV DNA, the plasmid DNA containing the infectious viral genome and the live virus are preferred with the live virus being most preferred. The avirulent, live viral vaccine of the present invention provides an advantage over traditional viral vaccines that use either attenuated, live viruses which run the risk of reverting back to the virulent state or killed cell culture propagated whole virus which may not induce sufficient antibody immune response for protection against the viral disease. Vaccines and methods of using them are also included within the scope of the present invention. Inoculated mammalian species are protected from serious viral infection, may also provide protection for disease related to co-infection of PTTV, such as porcine dermatitis and nephropathy syndrome (PDNS), postweaning multisystemic wasting syndrome (PMWS), and other related illness. The vaccines comprise, for example, an inactivated or attenuated porcine TTV virus, a nontoxic, physiologically acceptable carrier and, optionally, one or more adjuvants.

The adjuvant, which may be administered in conjunction with the vaccine of the present invention, is a substance that increases the immunological response of the pig to the vaccine. The adjuvant may be administered at the same time and at the same site as the vaccine, or at a different time, for example, as a booster. Adjuvants also may advantageously be administered to the pig in a manner or at a site different from the manner or site in which the vaccine is administered. Suitable adjuvants include, but are not limited to, aluminum hydroxide (alum), immunostimulating complexes (ISCOMs), non-ionic block polymers or copolymers, cytokines (like IL-1, IL-2, IL-7, IFN-α, IFN-β, IFN-γ, etc.), saponins, monophosphoryl lipid A (MLA), muramyl dipeptides (MDP) and the like. Other suitable adjuvants include, for example, aluminum potassium sulfate, heat-labile or heat-stable enterotoxin isolated from Escherichia coli, cholera toxin or the B subunit thereof, diphtheria toxin, tetanus toxin, pertussis toxin, Freund's incomplete or complete adjuvant, etc. Toxin-based adjuvants, such as diphtheria toxin, tetanus toxin and pertussis toxin may be inactivated prior to use, for example, by treatment with formaldehyde.

The vaccines may further contain additional antigens to promote the immunological activity of the infectious PTTV DNA clones such as, for example, porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), other infectious swine agents and immune stimulants.

The new vaccines of this invention are not restricted to any particular type or method of preparation. The cloned viral vaccines include, but are not limited to, infectious DNA vaccines (i.e., using plasmids, vectors or other conventional carriers to directly inject DNA into pigs), live vaccines, modified live vaccines, inactivated vaccines, subunit vaccines, attenuated vaccines, genetically engineered vaccines, etc. These vaccines are prepared by standard methods known in the art.

As a further benefit, the preferred live virus of the present invention provides a genetically stable vaccine that is easier to make, store and deliver than other types of attenuated vaccines.

Another preferred vaccine of the present invention utilizes suitable plasmids for delivering the nonpathogenic DNA clone to pigs. In contrast to the traditional vaccine that uses live or killed cell culture propagated whole virus, this invention provides for the direct inoculation of pigs with the plasmid DNA containing the infectious viral genome.

Additional genetically engineered vaccines, which are desirable in the present invention, are produced by techniques known in the art. Such techniques involve, but are not limited to, further manipulation of recombinant DNA, modification of or substitutions to the amino acid sequences of the recombinant proteins and the like.

Genetically engineered vaccines based on recombinant DNA technology are made, for instance, by identifying alternative portions of the viral gene encoding proteins responsible for inducing a stronger immune or protective response in pigs (e.g., proteins derived from ORF1, ORF1/1, ORF2, ORF2/2, etc.). Such identified genes or immuno-dominant fragments can be cloned into standard protein expression vectors, such as the baculovirus vector, and used to infect appropriate host cells (see, for example, O'Reilly et al., "Baculovirus Expression Vectors: A Lab Manual," Freeman & Co., 1992). The host cells are cultured, thus expressing the desired vaccine proteins, which can be purified to the desired extent and formulated into a suitable vaccine product. The recombinant subunit vaccines are based on bacteria-expressed (FIG. 10, FIG. 15) or baculovirus-expressed ORF1 capsid proteins of PTTV1a, PTTV1b and PTTV2.

If the clones retain any undesirable natural abilities of causing disease, it is also possible to pinpoint the nucleotide sequences in the viral genome responsible for any residual virulence, and genetically engineer the virus avirulent through, for example, site-directed mutagenesis. Site-directed mutagenesis is able to add, delete or change one or more nucleotides (see, for instance, Zoller et al., DNA 3:479-488, 1984). An oligonucleotide is synthesized containing the desired mutation and annealed to a portion of single stranded viral DNA. The hybrid molecule, which results from that procedure, is employed to transform bacteria. Then double-stranded DNA, which is isolated containing the appropriate mutation, is used to produce full-length DNA by ligation to a restriction fragment of the latter that is subsequently transfected into a suitable cell culture. Ligation of the genome into the suitable vector for transfer may be accomplished through any standard technique known to those of ordinary skill in the art. Transformation of the vector into host cells for the production of viral progeny may be done using any of the conventional methods such as calcium-phosphate or DEAE-dextran mediated transfection, electroporation, protoplast fusion and other well-known techniques (e.g., Sambrook et al., "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, 1989). The cloned virus then exhibits the desired mutation. Alternatively, two oligonucleotides can be synthesized which contain the appropriate mutation. These may be annealed to form double-stranded DNA that can be inserted in the viral DNA to produce full-length DNA.

An immunologically effective amount of the vaccines of the present invention is administered to a pig in need of protection against viral infection. The immunologically effective amount or the immunogenic amount that inoculates the pig can be easily determined or readily titrated by routine testing. An effective amount is one in which a sufficient immunological response to the vaccine is attained to protect the pig exposed to the PTTV virus. Preferably, the pig is protected to an extent in which one to all of the adverse physiological symptoms or effects of the viral disease are significantly reduced, ameliorated or totally prevented.
The vaccine can be administered in a single dose or in repeated doses. Dosages may range, for example, from about 1 microgram to about 1,000 micrograms of the plasmid DNA containing the infectious chimeric DNA genome (dependent upon the concentration of the immuno-active component of the vaccine), preferably 100 to 200 micrograms of the porcine TTV DNA clone, but should not contain an amount of virus-based antigen sufficient to result in an adverse reaction or physiological symptoms of viral infection. Methods are known in the art for determining or titrating suitable dosages of active antigenic agent to find minimal effective dosages based on the weight of the pig, concentration of the antigen and other typical factors. Preferably, the infectious viral DNA clone is used as a vaccine, or a live infectious virus can be generated in vitro and then the live virus is used as a vaccine. In that case, from about 50 to about 10,000, of the 50% tissue culture infective dose (TCID 50) of live virus, for example, can be given to a pig.

The new vaccines of this invention are not restricted to any particular type or method of preparation. The vaccines include, but are not limited to, modified live vaccines, inactivated vaccines, subunit vaccines, attenuated vaccines, genetically engineered vaccines, etc.

The advantages of live vaccines are that all possible immune responses are activated in the recipient of the vaccine, including systemic, local, humoral and cell-mediated immune responses. The disadvantages of live virus vaccines, which may outweigh the advantages, lie in the potential for contamination with live adventitious viral agents or the risk that the virus may revert to virulence in the field.

To prepare inactivated virus vaccines, for instance, the virus propagation and virus production can occur in cultured porcine cell lines such as, without limitation PK-15 cells. Serial virus inactivation is then optimized by protocols generally known to those of ordinary skill in the art or, preferably, by the methods described herein.

Inactivated virus vaccines may be prepared by treating the porcine TTV with inactivating agents such as formalin or hydrophobic solvents, acids, etc., by irradiation with ultraviolet light or X-rays, by heating, etc. Inactivation is conducted in a manner understood in the art. For example, in chemical inactivation, a suitable virus sample or serum sample containing the virus is treated for a sufficient length of time with a sufficient amount or concentration of inactivating agent at a sufficiently high (or low, depending on the inactivating agent) temperature or pH to inactivate the virus. Inactivation by heating is conducted at a temperature and for a length of time sufficient to inactivate the virus. Inactivation by irradiation is conducted using a wavelength of light or other energy source for a length of time sufficient to inactivate the virus. The virus is considered inactivated if it is unable to infect a cell susceptible to infection.

The preparation of subunit vaccines typically differs from the preparation of a modified live vaccine or an inactivated vaccine. Prior to preparation of a subunit vaccine, the protective or antigenic components of the vaccine must be identified. In the present invention, antigenic components of PTTV were identified as the ORF1 capsid proteins of PTTV1a, PTTV1b and PTTV2, which were expressed and purified in Escherichia coli (E. coli) in this invention, and other expression system, such as baculovirus expression system, for use as subunit recombinant capsid vaccines. Such protective or antigenic components include certain amino acid segments or fragments of the viral capsid proteins which raise a particularly strong protective or immunological response in pigs; single or multiple viral capsid proteins themselves, oligomers thereof, and higher-order associations of the viral capsid proteins which form virus substructures or identifiable parts of such substructures; oligosaccharides, glycolipids or glycoproteins present on or near the surface of the virus or in viral substructures such as the lipoproteins or lipid groups associated with the virus, etc. Preferably, the ORF1 protein is employed as the antigenic component of the subunit vaccine. Other proteins may also be used such as those encoded by the nucleotide sequence in the ORF2, ORF1/1, and ORF2/2 genes. These immunogenic components are readily identified by methods known in the art. Once identified, the protective or antigenic portions of the virus (i.e., the “subunit”) are subsequently purified and/or cloned by procedures known in the art. The subunit vaccine provides an advantage over other vaccines based on the live virus since the subunit, such as highly purified subunits of the virus, is less toxic than the whole virus.

If the subunit vaccine is produced through recombinant genetic techniques, expression of the cloned subunit such as the ORF1, ORF2, ORF1/1, and ORF2/2 genes, for example, may be expressed by the method provided above, and may also be optimized by methods known to those in the art (see, for example, Maniatis et al., “Molecular Cloning: A Laboratory Manual,” Cold Spring Harbor Laboratory, Cold Spring Harbor, Mass. (1989)). On the other hand, if the subunit being employed represents an intact structural feature of the virus, such as an entire capsid protein, the procedure for its isolation from the virus must then be optimized. In either case, after optimization of the inactivation protocol, the subunit purification protocol may be optimized prior to manufacture.

To prepare attenuated vaccines, the live, pathogenic virus is first attenuated (rendered nonpathogenic or harmless) by methods known in the art or, preferably, as described herein. For instance, attenuated viruses may be prepared by the technique of the present invention which involves the novel serial passage through embryonated pig eggs. Attenuated viruses can be found in nature and may have naturally-occurring gene deletions or, alternatively, the pathogenic viruses can be attenuated by making gene deletions or producing gene mutations. The attenuated and inactivated virus vaccines comprises the preferred vaccines of the present invention.

Genetically engineered vaccines, which are also desirable in the present invention, are produced by techniques known in the art. Such techniques involve, but are not limited to, the use of RNA, recombinant DNA, recombinant proteins, live viruses and the like.

For instance, after purification, the wild-type virus may be isolated from suitable clinical, biological samples such as serum, fecal, saliva, semen and tissue samples by methods known in the art, preferably by the method taught herein using infected pigs or infected suitable cell lines. The DNA is extracted from the biologically pure virus or infectious agent by methods known in the art, and purified by methods known in the art, preferably by ultracentrifugation in a CsCl gradient. The cDNA of viral genome is cloned into a suitable host by methods known in the art (see Maniatis et al., id.), and the virus genome is then analyzed to determine essential regions of the genome for producing antigenic portions of the virus. Thereafter, the procedure is generally the same as that for the modified live vaccine, an inactivated vaccine or a subunit vaccine.

Genetically engineered vaccines based on recombinant DNA technology are made, for instance, by identifying the portion of the viral gene which encodes for proteins responsible for inducing a stronger immune or protective response in pigs (e.g., proteins derived from ORF1, ORF2, ORF1/1, and ORF2/2, etc.). Such identified genes or immuno-dominant fragments can be cloned into standard protein expression.
vectors, such as the baculovirus vector, and used to infect appropriate host cells (see, for example, O’Reilly et al., “Baculovirus Expression Vectors: A Lab Manual” Freeman & Co. (1992)). The host cells are cultured, thus expressing the desired vaccine proteins, which can be purified to the desired extent and formulated into a suitable vaccine product.

Genetically engineered proteins, useful in vaccines, for instance, may be expressed in insect cells, yeast cells or mammalian cells. The genetically engineered proteins, which may be purified or isolated by conventional methods, can be directly inoculated into a porcine or mammalian species to confer protection against porcine TTV.

An insect cell line (like s19, s21, or HIGH-FIVE) can be transformed with a transfer vector containing polynucleic acids obtained from the virus or copied from the viral genome which encodes one or more of the immuno-dominant proteins of the virus. The host cell line may be co-transfected with the linearized baculovirus DNA and a plasmid containing the desired polynucleotides. The host cell line may be co-transfected with the linearized baculovirus DNA and a plasmid in order to make a recombinant baculovirus.

Alternatively, DNA from the isolated porcine TTV which encode one or more capsid proteins can be inserted into live vectors, such as a poxvirus or an adenovirus and used as a vaccine.

An immunologically effective amount of the vaccine of the present invention is administered to a porcine or mammalian species in need of protection against said infection or syndrome. The “immunologically effective amount” can be easily determined or readily titrated by routine testing. An effective amount is one in which a sufficient immunological response to the vaccine is attained to protect the pig or other mammal exposed to the porcine TTV virus, or porcine TTV co-infection, which may cause porcine dermatitis and nephropathy syndrome (PDNS), postweaning multisystemic wasting syndrome (PMWS) or related illness. Preferably, the pig or other mammalian species is protected to an extent in which one to all of the adverse physiological symptoms or effects of the viral disease are found to be significantly reduced, ameliorated or totally prevented.

The vaccine can be administered in a single dose or in repeated doses. Dosages may contain, for example, from 1 to 1,000 micrograms of virus-based antigen (dependent upon the concentration of the immuno-active component of the vaccine), but should not contain an amount of virus-based antigen sufficient to result in an adverse reaction or physiological symptoms of viral infection. Methods are known in the art for determining or titrating suitable dosages of active antigenic agent based on the weight of the bird or mammal, concentration of the antigen and other typical factors.

The vaccine can be administered to pigs. Also, the vaccine can be given to humans such as pig farmers who are at high risk of being infected by the viral agent. It is contemplated that a vaccine based on the porcine TTV can be designed to provide broad protection against both porcine and human TTV. In other words, the vaccine based on the porcine TTV can be preferentially designed to protect against human TTV infection through the so-called “Jennerian approach” (i.e., cowpox virus vaccine can be used against human smallpox by Edward Jenner). Desirably, the vaccine is administered directly to a porcine or other mammalian species not yet exposed to the TTV virus. The vaccine can conveniently be administered orally, intrabuccally, intranasally, transdermally, parenterally, etc. The parenteral route of administration includes, but is not limited to, intramuscular, intravenous, intraperitoneal and subcutaneous routes.

When administered as a liquid, the present vaccine may be prepared in the form of an aqueous solution, a syrup, an elixir, a tincture and the like. Such formulations are known in the art and are typically prepared by dissolution of the antigen and other typical additives in the appropriate carrier or solvent systems. Suitable carriers or solvents include, but are not limited to, water, saline, alcohol, ethylene glycol, glycerol, etc. Typical additives are, for example, certified dyes, flavors, sweeteners and antimicrobial preservatives such as thimerosal (sodium ethylmercurithiosalicylate). Such solutions may be stabilized, for example, by addition of partially hydrolyzed gelatin, sorbitol or cell culture medium, and may be buffered by conventional methods using reagents known in the art, such as sodium hydrogen phosphate, sodium dihydrogen phosphate, potassium hydrogen phosphate, potassium dihydrogen phosphate, a mixture thereof, and the like.

Liquid formulations also may include suspensions and emulsions which contain suspending or emulsifying agents in combination with other standard co-formulants. These types of liquid formulations may be prepared by conventional methods. Suspensions, for example, may be prepared using a colloid mill. Emulsions, for example, may be prepared using a homogenizer.

Parenteral formulations, designed for injection into body fluid systems, require proper isotonicity and pH buffering to the corresponding levels of mammalian body fluids. Isotonicity can be appropriately adjusted with sodium chloride and other salts as needed. Suitable solvents, such as ethanol or propylene glycol, can be used to increase the solubility of the ingredients in the formulation and the stability of the liquid preparation. Further additives which may be employed in the present vaccine include, but are not limited to, dextrose, conventional antioxidants and conventional chelating agents such as ethylenediamine tetraacetic acid (EDTA). Parenteral dosage forms must also be sterilized prior to use.

The following examples demonstrate certain aspects of the present invention. However, it is to be understood that these examples are for illustration only and do not purport to be wholly definitive as to conditions and scope of this invention. It should be appreciated that when typical reaction conditions (e.g., temperature, reaction times, etc.) have been given, the conditions both above and below the specified ranges can also be used, though generally less conveniently. The examples are conducted at room temperature (about 25°C. to about 28°C.) and at atmospheric pressure. All parts and percents referred to herein are on a weight basis and all temperatures are expressed in degrees centigrade unless otherwise specified.

**EXAMPLE 1**

Viral DNA Extraction, Nested PCR and Genomic PCR

Convenient serum and semen samples from 20 conventional adult boars from a Virginia pig farm were used in the study. Total DNA was isolated from 20 serum and 19 semen samples using QIAamp DNA mini kit (Qingen). To screen for the positive PTTV-containing samples, nested PCR amplifications of the conserved regions in the UTR of PTTV1 and PTTV2 were initially performed by using AmpliTag Gold polymerase (Applied Biosystems). The two primer pairs used to amplify the fragment A of PTTV1 were TTV1-mF (SEQ ID NO:45)/TTV1-mR (SEQ ID NO:46) (for the first-round PCR) and TTV1-mF (SEQ ID NO:47)/TTV1-mR (SEQ ID NO:48) (for the second-round PCR), whereas the two primer pairs used to amplify the fragment D of PTTV2 were TTV2-mF (SEQ ID NO:49)/TTV2-mR (SEQ ID NO:50) (for the...
first-round PCR) and TTV2-nF (SEQ ID NO:51)/TTV2-nR (SEQ ID NO:52) (for the second-round PCR; FIG. 1A and Table 1).

In order to amplify the full-length genomic sequences of both PTTV1 and PTTV2, we first performed an inverse genomic PCR using a pair of conserved gene-specific primers TTV1-IF (SEQ ID NO:1)/TTV (SEQ ID NO:4) located in region A for PTTV1 and another pair of gene-specific primers TTV2-IF (SEQ ID NO:5)/TTV2-IR (SEQ ID NO:8) located in region D for PTTV2, respectively, with Herculase II Fusion DNA Polymerase (Stratagene) according to the manufacturer’s instructions. No PCR products with expected sizes were detected. Subsequently we designed new sets of primers to amplify two regions covering the complete PTTV1 and PTTV2 genomes in the second-round PCR, respectively (FIG. 1A). The primer pairs used to amplify fragments B and C of PTTV1 were TTV1-IF (SEQ ID NO:1)/TTV1-2340R (SEQ ID NO:2) and TTV1-2311F (SEQ ID NO:3)/TTV1-IR (SEQ ID NO:4), respectively, whereas the primer pairs used to amplify fragments E and F of PTTV2 were TTV2-IF (SEQ ID NO:5)/TTV2-2316R (SEQ ID NO:6) and TTV2-GCF (SEQ ID NO:7)/TTV2-IR (SEQ ID NO:8), respectively (FIG. 1A and Table 1). Fragments C and F contain the GC-rich regions of PTTV1 and PTTV2, respectively. The amplified PCR products were individually excised, purified, and subsequently cloned into a pSC-B-amp/kan vector (Stratagene) by Stratagene Cloning Blunt PCR cloning strategy according to the manufacturer’s instructions (Stratagene) followed by DNA sequencing.

EXAMPLE 2
Screening for Porcine TTV Positive Samples Collected from Boars in a Farm from Virginia

Porcine TTV DNA was previously detected from pigs in different geographic regions by nested-PCR based on the UTR sequence of a Japanese PTTV1 strain Sd-TTV31 (McKeown et al., 2004, supra). With the recent identification of PTTV2, two different sets of nested-PCR primers have been used to amplify region A of PTTV1 and region D of PTTV2, respectively (FIG. 1A) (Ellis et al., 2008, supra; Kekarainen, T., Sibila, M., and Segales, J. (2006). Prevalence of PTTV2, two different sets of nested-PCR primers have been used to guide real-time PCR primer selections using the Beacon Designer program (PREMIER Biosoft International, Palo Alto, Calif.). Primers used for the duplex nested PCR of PTTV1 were designed by the Lasergene package (DNASTAR Inc., Madison, Wis.). Full-length genomic sequences of ten porcine TTV strains and their corresponding GenBank accession numbers used for the alignment and comparison are Sd-TTV31 (AB076001), TTV1-1p (AY823990) and TTV2-2p (AY823991). Pairwise sequence comparisons (PASC) were performed using 121 full-length genomic sequences of human and animal TTV-related strains available in GenBank with an online program PASC (http://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?textpage:overview) (Bao et al., 2008).

Phylogenetic trees were constructed by the neighbor-joining method in the PAUP 4.0 program (David Swofford, Smithsonian Institute, Washington, D.C.; distributed by Sinauer Associate Inc.) based upon the full-length genomic sequences and the deduced amino acid sequences of 4 ORFs of seven PTTV strains. The data were obtained from 1000 re-sampling.

EXAMPLE 4
Design of PCR Primers for Diagnosing Porcine PTTV Infection

Analyses and alignment of DNA sequences were performed using Lasergene package (DNASTAR Inc., Madison, Wis.). Full-length genomic sequences of ten porcine TTV strains and their corresponding GenBank accession numbers used for the alignment were as follows. Species PTTV1: Sd-TTV31 (AB076001), PTTV1a-VA (GU456383), TTV1-1p (AY823990), PTTV1b-VA (GU456384), swSTHY-TT27 (GQ120664) and TTV1 #471819 (GU188045). Species PTTV2: PTTV2b-VA (GU456385), PTTV2c-VA (GU456386), TTV2-2p (AY823991) and TTV2 #472142 (GU188046). The conserved sequences among the 6 PTTV1 and 4 PTTV2 genomes were identified, respectively, and subsequently used to guide real-time PCR primer selections using the Beacon Designer program (PREMIER Biosoft International, Palo Alto, Calif.). Primers used for the duplex nested PCR of PTTV1 were designed by the Lasergene package.
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EXAMPLE 6

Extraction of Viral DNA for PCR Assays

Total DNA was isolated from 20 serum and 19 semen samples collected from 20 conventional adult boars (with no clinical syndromes) from a Virginia pig farm using QiAamp DNA mini kit (Qiagen) as described previously (Huang et al., 2010). A sample volume of 400 µl for sera and semen was used to extract DNA with a final eluate of 50 µl sterile water. All extracted DNA samples were stored at —20°C until real-time PCR testing. Detection of porcine TTVs in these samples by conventional nested PCR had been described previously (Huang et al., 2010). Total DNA extracted from a goat serum sample with the same procedure was used as the negative control.

EXAMPLE 7

SYBR Green Real-Time Quantitative PCR Assays

PTTV1- and PTTV2-specific real-time PCR were performed, respectively, using SensiMix SYBR & Fluorescein kit (Quintace Ltd) and the MyiQ iCYCLER Real Time PCR instrument (BIO-RAD Laboratories). Each 25-µl reaction contained 12.5 µl of SYBR green Master Mix, 4 µl of extracted DNA, 0.5 µl of each primer (10 nM) and 7.5 µl of sterile water. The PCR condition for PTTV1 was 10 min at 95°C followed by 40 cycles of amplification (15 sec at 95°C, 30 sec at 59.4°C, 10 sec at 72°C). This was immediately followed by a melting point analysis obtained by gradually increasing the temperature form 55°C to 95°C with the fluorescence signal being measured every 0.5°C. The PCR condition for PTTV2 was the same as PTTV1 except that the annealing temperature was 56°C. PTTV1 and PTTV2 standard templates were included as positive controls in every run. Amplification and data analysis were carried out using MyiQ System software (BIO-RAD Laboratories). All samples were run in duplicate on the same plate.

EXAMPLE 8

Specificity and Sensitivity of Two Singleplex Assays

The optimal annealing temperatures for amplification of PTTV1- and PTTV2-specific assays were 59.4°C and 56°C, respectively, as determined by a 10-fold dilution of amplifications using a gradient of annealing temperatures. Amplification of the 118-bp product using primers TTV1F/TTV1R was obtained only with PTTV1 template whereas amplification of the 200-bp product with PTTV2 template was only observed when primers TTV4/TTV4R were used. Neither assay yielded any cross-amplification from the other, confirming the specificity of the primers and targets (data not shown).

EXAMPLE 9

Quantification of Porcine TTV1 and TTV2 in Boar Serum and Semen Samples

Viral load was expressed as copy numbers of PTTV1 or PTTV2 genomes per ml of original boar serum samples. PTTV1 DNA were detected in all 20 serum samples ranging from 1.91×10² to 3.25×10⁵ copies/ml whereas PTTV2 DNA were detected in 19 serum samples (except #10) ranging from 3.59×10⁵ to 1.39×10⁶ copies/ml. The result was consistent to our previous study by using conventional nested PCR (Table 5). None of the semen samples were PTTV1-positive whereas three semen samples were PTTV2-positive with very low viral loads (230, 244 and 357 copies/ml, respectively).

<table>
<thead>
<tr>
<th>Samples</th>
<th>PTTV1 real-time PCR</th>
<th>PTTV1 nested PCR</th>
<th>PTTV2 real-time PCR</th>
<th>PTTV2 nested PCR</th>
<th>PTTV1/PTTV2 duplex real-time PCR</th>
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<td>—</td>
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EXAMPLE 10

PTTV1/PTTV2 Duplex Real-Time PCR Assay

PTTV1/PTTV2 duplex real-time PCR assay was performed in a 25-µl PCR system containing 12.5 µl of SYBR green Master Mix, 0.5 µl of each PTTV1 primers, 0.5 µl of each PTTV2 primers, 4 µl of DNA and 6.5 µl of sterile water. The duplex PCR condition and melting point analysis were the same as PTTV1 except that the annealing temperature was 58°C. The melting peaks were analyzed to distinguish the PTTV1- and PTTV2-specific amplicons.

EXAMPLE 11

Duplex Nested PCR

The first-round PCR was performed with a Platinum PCR HiFi Supermix (Invitrogen) using 4 µl of extracted DNA in a total volume of 50 µl. The PCR condition was 30 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec with an initial
denaturation of the template DNA at 94°C for 2 min. A 4-μl aliquot of the first-round PCR product was used for the second-round PCR with the same PCR reagents and condition. One pair of primers P1ab-mF/P1ab-mR was used in the first-round PCR whereas a mixture of two pairs of primers, P1anF/P1an-R for detection of PTTV1a, and P1b-nF/P1b-nR for detection of PTTV1b, were used in the second-round PCR (Table 1). The amplification products were visualized by gel electrophoresis on a 1% agarose gel stained with ethidium bromide and two bands specific for each type were differentiated by UV light.

EXAMPLE 12

Construction of PTTV1 and PTTV2 ORF Expression Plasmids

The C-terminal parts of ORF1 of PTTV1a, PTTV1b and PTTV2c were amplified from the respective full-length DNA clones (pSC-PTTV1a, pSC-PTTV1b and pSC-PTTV2c; described elsewhere). The amplified fragments were expected to encode protein products with 319 aa for PTTV1a (ORF1 aa positions 317-635 (SEQ ID NO:13); GenBank accession no. GU456383), 318 aa for PTTV1b (ORF1 aa positions 322-639 (SEQ ID NO:14); GenBank accession no. GU456384), and 316 aa for PTTV2c (ORF1 aa positions 310-625 (SEQ ID NO:16); GenBank accession no. GU456386), respectively. A C-terminal truncated fragment of PTTV1b encoding 248 aa (ORF1 aa positions 322-569 (SEQ ID NO:14)) was also amplified and used as a comparison control for SDS-PAGE analysis. All the plasmids were constructed by cloning of the PCR products into an E. coli baculovirion/mammalian cells triple expression vector pTriEx1.1-Neo (Novagen) between the Ncol and Xhol positions (322-639 (SEQ ID NO:14); GenBank accession no. GU456383), were confirmed by DNA sequencing.

The four recombinant plasmids were designated pTriPTTV1a-ORF1, pTriPTTV1b-ORF1, pTriPTTV1b-ORF1true and pTriPTTV2c-ORF1. All cloned sequences were confirmed by DNA sequencing.

EXAMPLE 13

Expression of Recombinant PTTV1 and PTTV2 Proteins

The four expression plasmids were transformed into Rosetta 2 (DE3) pLacI competent cells (Novagen), respectively, and the bacteria were plated on LB agar plates containing 100 μg/ml ampicillin overnight at 37°C. A single transformation colony for each construct was used to inoculate 3 ml of LB medium containing 100 μg/ml of ampicillin (LB/amp), and grown 6-8 hours at 37 °C. The turbid 3 ml culture for each construct was then used to make bacterial stocks by adding 25% filter sterilized glycerol, and freezing the culture down at –80°C. Prior to purification, 10 μl of the frozen bacterial stock for each construct was used to inoculate a 3 ml starter culture of LB/amp, and grown for 6-8 hours at 37°C. A 100-ml of Overnight Express TB Media (Novagen) was inoculated with the starter culture to induce protein expression, and was grown 16-18 hours at 37°C. After incubation, the autoinduction culture underwent centrifugation at 3400 rpm for 15 minutes at 4°C. The resulting supernatant for each construct was discarded, and each of the bacterial pellets was treated with BugBuster and RNaseoyme according to the manufacturer’s protocol (Novagen), and Benzonase Nuclease (Novagen) was added for degradation of DNA and RNA. Each of the inclusion body pellets was subsequently resuspended with 840 μl of lysis buffer (6M Guanidine Hydrochloride, 0.1M sodium phosphate, 0.01M Tris-Chloride, 0.01M imidazole, pH 8.0), and frozen at –80°C for at least 30 minutes. It was then thawed, diluted with an additional 2.5 ml of lysis buffer and gently rotated for 30 minutes at room temperature. The lysate supernatants were collected by centrifugation at 15,000xg for 30 minutes at room temperature. A 50%-Ni-NTA His-bind slurry (Novagen) was added to each of the decanted supernatants, and the mixtures were shaken for 60 minutes at room temperature to promote his-tag binding. The lysate/resin mixtures were loaded into an empty chromatography column. After the initial flow-through, a 7-ml of lysis buffer was added to the column and allowed to flow through. Each column was then washed 2 times with 7 ml of wash buffer (8M Urea, 0.1M Sodium Phosphate, 0.15M Sodium Chloride, 0.02M imidazole, pH 8.0). Elution of the target protein was achieved by adding 4 separate 1 ml aliquots of elution buffer (8M Urea, 0.05M Sodium Phosphate, 1M Sodium Chloride, 0.5M Imidazole, pH 8.0) to the column. The four elution fractions were analyzed by SDS Page and Coomassie Blue Staining.

The elutions containing significant concentrations of the target protein were injected into a 0.5 ml-3 ml dialysis cassette with a 20,000 molecular weight cut-off (Pierce). A series of 4 dialysis buffers were used for dialysis; dialysis buffer 1 (6M Urea, 0.05M Sodium Phosphate, 0.8M Sodium Chloride, 0.3M Imidazole, pH 8.0), dialysis buffer 2 (4M Urea, 0.035M Sodium Phosphate, 0.533M Sodium Chloride, 0.2M Imidazole, pH 8.0), dialysis buffer 3 (2.67M Urea, 0.022M Sodium Phosphate, 0.356M Sodium Chloride, 0.133M Imidazole, pH 8.0) and dialysis buffer 4 (1.5M Urea, 0.0148 Sodium Phosphate, 0.237M Sodium Chloride, 0.089M Imidazole, pH 8.0). The dialysis cassette was sequentially submersed and rotated in each dialysis buffer for over 6 hours or 4°C. When dialysis was complete, the recombinant His-tagged fusion proteins were each removed from the cassettes, quantified using a NanoDrop and frozen at –80°C.

EXAMPLE 14

Purification and Dialysis of Recombinant Proteins

The recombinant proteins were insoluble and expressed within the bacterial inclusion bodies. Each of the bacterial pellets was treated with BugBuster and RNaseoyme according to the manufacturer’s protocol (Novagen), and Benzonase Nuclease (Novagen) was added for degradation of DNA and RNA. Each of the inclusion body pellets was subsequently resuspended with 840 μl of lysis buffer (6M Guanidine Hydrochloride, 0.1M sodium phosphate, 0.01M Tris-Chloride, 0.01M imidazole, pH 8.0), and frozen at –80°C for at least 30 minutes. It was then thawed, diluted with an additional 2.5 ml of lysis buffer and gently rotated for 30 minutes at room temperature. The lysate supernatants were collected by centrifugation at 15,000xg for 30 minutes at room temperature. A 50%-Ni-NTA His-bind slurry (Novagen) was added to each of the decanted supernatants, and the mixtures were shaken for 60 minutes at room temperature to promote his-tag binding. The lysate/resin mixtures were loaded into an empty chromatography column. After the initial flow-through, a 7-ml of lysis buffer was added to the column and allowed to flow through. Each column was then washed 2 times with 7 ml of wash buffer (8M Urea, 0.1M Sodium Phosphate, 0.15M Sodium Chloride, 0.02M imidazole, pH 8.0). Elution of the target protein was achieved by adding 4 separate 1 ml aliquots of elution buffer (8M Urea, 0.05M Sodium Phosphate, 1M Sodium Chloride, 0.5M Imidazole, pH 8.0) to the column. The four elution fractions were analyzed by SDS Page and Coomassie Blue Staining.

The elutions containing significant concentrations of the target protein were injected into a 0.5 ml-3 ml dialysis cassette with a 20,000 molecular weight cut-off (Pierce). A series of 4 dialysis buffers were used for dialysis; dialysis buffer 1 (6M Urea, 0.05M Sodium Phosphate, 0.8M Sodium Chloride, 0.3M Imidazole, pH 8.0), dialysis buffer 2 (4M Urea, 0.035M Sodium Phosphate, 0.533M Sodium Chloride, 0.2M Imidazole, pH 8.0), dialysis buffer 3 (2.67M Urea, 0.022M Sodium Phosphate, 0.356M Sodium Chloride, 0.133M Imidazole, pH 8.0) and dialysis buffer 4 (1.5M Urea, 0.0148 Sodium Phosphate, 0.237M Sodium Chloride, 0.089M Imidazole, pH 8.0). The dialysis cassette was sequentially submersed and rotated in each dialysis buffer for over 6 hours or 4°C. When dialysis was complete, the recombinant His-tagged fusion proteins were each removed from the cassettes, quantified using a NanoDrop and frozen at –80°C.

EXAMPLE 15

SDS-PAGE and Anti-His-Tagged Western Blot

A western blot was developed to detect purified recombinant proteins by using an anti-6XHis-tagged monoclonal antibody (Rockland). Equal volumes of each of the purified truncated ORF1 proteins and LDS/10% β-ME were mixed, and boiled at 95°C for 10 minutes. A 10-μl of the boiled sample was added to each appropriate well of a 4-12% Bis-Tris Polyacrylamide Gel (Invitrogen), and was run at 200 volts for 43 minutes in 1xMES running buffer (Invitrogen). The proteins were transferred to a PVDF membrane (Bio-Rad) using a Trans blot semi dry transfer apparatus and 1x transfer buffer (Invitrogen). Once transfer was complete, the PVDF membrane was incubated in Odyssey blocking buffer (Li-Cor) at room temperature for 1 hour. The anti-6XHis-tagged MAb was diluted at 1:1000 in Odyssey blocking buffer/0.2% tween 20, and transferred to the membrane after the previous Odyssey blocking buffer was removed. The MAb was left on a rocker to incubate with the membrane for either 2 hours at room temperature or 4°C overnight, and then the membrane was washed 3 times with tris buffered saline/0.05% tween 20 (TBS-T, Sigma). A Goat anti-rabbit IgG IRDye 800 (Li-Cor) antibody was diluted at 1:5000 in Odyssey blocking buffer/0.2% tween 20/0.1 SDS. It was transferred to the freshly
washed PVDF membrane, and allowed to incubate for 1 hour at room temperature while gently rocking. The membrane was washed 3 times with TBS-T, 1 time with TBS and imaged with the Li-Cor Odyssey.

EXAMPLE 16

Serum Western Blot

A serum western blot was developed, and used to identify positive and negative serum controls for ELISA development. After SDS-PAGE as described above, the proteins were transferred to a PVDF membrane that was subsequently incubated in Odyssey blocking buffer (Li-Cor) at room temperature for 1 hour. A selected serum sample was diluted at 1:100 in Odyssey blocking buffer/0.2% tween 20, and transferred to the membrane after the previous Odyssey blocking buffer was removed. The serum sample was left on a rocker to incubate with the membrane for 2 hours at room temperature, and then the membrane was washed 3 times with tris buffered saline/0.05% tween 20 (TBS-T, Sigma). A goat anti-swine IgG IRDye 800 antibody (Rockland) was diluted at 1:2500 in Odyssey blocking buffer/0.2% tween 200% SDS. It was transferred to the freshly washed PVDF membrane, and allowed to incubate for 1 hour at room temperature while gently rocking. The membrane was washed 3 times with TBS-T, 1 time with TBS and imaged with the Li-Cor Odyssey.

EXAMPLE 17

Indirect PTTV1a-, PTTV1b- and PTTV2-Specific ELISA

The optimal concentrations of the antigens used to coat the plates and dilutions of antisera and conjugates were determined by checkboard titration. The ELISA was initiated by diluting each of the purified recombinant His-tagged fusion proteins (PTTV1a, PTTV1b and PTTV2c, respectively) to 680 ng/ml in 1x Carbonate Coating Buffer (CCB) at a pH of 9.6, and coating medium binding ELISA plates (Greiner) with 100 µl/well. The plates were covered, and allowed to incubate at 37°C for 2 hours. After coating, the diluted proteins were removed, and each well was washed 3 times with 300 µl of 1xTBS-T. Protein Free Blocking Buffer (Pierce) was then added at a volume of 300 µl/well, and the plates were allowed to incubate at 37°C for 1 hour. Meanwhile, in a 96-well dilution block, the serum samples were diluted at 1:100 in 150 µl of protein free blocking buffer. The block was then removed, and 100 µl of each diluted serum sample was transferred to each corresponding well on the ELISA plates. The plates were allowed to incubate at 37°C for 2 hours, after which each well was washed 3 times with 300 µl of TBS-T. Next, the HRP-conjugated anti-swine IgG antibody was added at 1:4000 in 12 ml of protein free block, and 100 µl was added to each well of the plates. This was incubated at 37°C for 1 hour, and then each well was washed 3 times with 300 µl of TBS-T. In order to develop the ELISA, 100 µl of Sure Blue Reserve 1-Component (KPL) was added to each well of the plates. After 20 minutes, 100 µl of 1N HCL was added to each well to stop development. The plates were then read at 450 nm.

EXAMPLE 18

Data Analyses

Porcine sera used in cell culture research from a commercial company (manufactured in New Zealand and considered free from all OIE diseases) were used as a positive control for the three ELISA protocols because the sera were all PTTV1a-, PTTV1b- and PTTV2-positive as detected by serum western blot and displayed high OD values (>2.0). We initially used pooled gnotobiotic pig sera as a negative control as they were negative in western blot detection. Subsequently, in comparison of the negative gnotobiotic pig sera, we screened some porcine sera collected from a conventional pig farm in Wisconsin. They were also negative in western blot detection and their OD values corresponded to that of negative gnotobiotic pig sera. These conventional porcine sera were pooled and used as a negative control. The cutoff value for each ELISA was calculated as the mean OD value of the negative control group (n=4) plus 3 times of the standard deviation.

EXAMPLE 19

Construction of Full-Length Genomic DNA Clones of Porcine TTV1a, 1c and 2c

PCR fragments B and C from the US isolate PTV1a-VA (GenBank accession no. GU456383) were re-amplified from the constructs described previously, and were subsequently assembled into a full-length genomic DNA with a BamH I site at the both ends of the genome by overlapping PCR using the Herculase II Fusion DNA Polymerase (Stratagene) on the vector psc-B-amp/kan (Stratagene). The resulting construct was designated pSC-PTTV1a (FIG. 17A). Using the same strategy, the clone pSC-PTTV1b (FIG. 17B) originated from the US isolate PTV1b-VA (GenBank accession no. GU456384) and the clone pSC-PTTV2c (FIG. 17C) originated from the US isolate PTV2c-VA (GenBank accession no. GU456386) were constructed with the same restriction sites (BamH I) on the same backbone vector. Plasmid TTV2-471942 was a gift from Dr. Andreas Gallei (BIVI, Germany). TTV2-471942 was classified into the porcine TTV subtype 2b together with the US isolate PTV1b-VA based upon the phylogenetic analysis (data not shown).

EXAMPLE 20

Construction of Tandem-Dimerized DNA Clones of Porcine TTV2b and 2c

The full-length TTV2c genome was excised from the clone pSC-PTTV2c by BamH I digestion, purified and ligated to form concatamers. Ligated concatamers were cloned into the BamH I-pre-digested pSC-B-amp/kan vector to produce a tandem-dimerized DNA clone, pSC-2PTTV2c-RR (FIG. 1D). Similarly, a tandem-dimerized DNA clone, pSC-2PTTV2b-RR, was generated from the clone TTV2-471942-full using EcoRV restriction sites (FIG. 1F).

EXAMPLE 21

Generation of PTTV1a-, PTTV1b- and PTTV2-Specific Anti-ORF1 Polyclonal Antibodies

The ORF1-encoding product is the putative capsid protein of TTV. To generate PTTV1a-, PTTV1b- and PTTV2-specific anti-ORF1 polyclonal antibodies to detect the expression of PTTV ORF1 proteins and to determine the infectivity of PTTV DNA clones, the three ORF1 proteins from PTTV1a, PTTV1b and PTTV2c were expressed in E. coli, purified and were subsequently used to immunize New Zealand white rabbits, respectively, as a custom antibody production service at Rockland Immunocchemicals (Gilberts-
EXAMPLE 22

In Vitro Transfection of PTTV Infectious Clones

PK-15 cells were seeded at 2x10^5 cells per well onto a 6-well plate and grown until 60%-70% confluence before transfection. The DNA clones pSC-2PTTV2b-RR and pSC-2PTTV2c-RR were directly transfected into PK-15 cells, respectively, using Lipofectamine LTX (Invitrogen) according to the manufacturer’s protocol. For clones pSC-PTTV1a, pSC-PTTV2c and TTV2-#471942-full, their ligated concatemers, produced as described above, were used for transfection, respectively. Cells were cultured for 3 to 5 days, and were then applied to an immunofluorescence assay (IFA) to detect the expression of ORF1 of porcine TTVs. Alternatively, transfected cells were passaged into new 6-well plates and continued to culture for 3 days before the IFA detection.

EXAMPLE 23

Immunofluorescence Assay (IFA)

Transfected or passaged cells were washed 2 times with PBS and fixed with acetone. Five hundred microliters of the antibodies, specific to PTTV1a or PTTV2 at 1:500 dilution in PBS, was added over the cells and incubated for 1 hour at room temperature. Cells were washed 3 times with PBS and 500 μl Texas red- or Alexa Fluor 488-labeled goat anti-rabbit IgG (Invitrogen) at 1:200 dilution was then added. After 1-hour incubation at room temperature and washed with PBS, the cells were stained with 500 μl DAPI (KPL, Inc.) at 1:1000 dilution and visualized under a fluorescence microscope.

EXAMPLE 24

In Vivo Inoculation of Conventional Pigs with the Tandem-Dimerized Porcine TTV2 Clones

A pig inoculation study was performed to determine the infectivities of the two tandem-dimerized porcine TTV2 clones: pSC-2TTV2b-RR and pSC-2TTV2c-RR. Briefly, eight 4-week-old conventional pigs that were seronegative and viral DNA negative for porcine TTV2 were randomly assigned into two groups of four each. Each group of pigs was housed separately and maintained under conditions that met all requirements of the Institutional Committee on Animal Care and Use. All pigs in each group were injected by a combination of both the intra-lymph node route and intramuscular route. The four pigs (nos. 181, 189, 192 and 193) were each injected with 200 μg of the pSC-2TTV2b-RR plasmid DNA whereas another four pigs (nos. 92, 180, 188 and 191) were each inoculated with 200 μg of the pSC-2TTV2c-RR clone. Pigs were monitored daily for clinical signs of disease for a total of 28 days. All pigs were necropsied at 28 days postinoculation.

While the present invention has been illustrated by description of several embodiments and while the illustrative embodiments have been described in detail, it is not the intention of the applicants to restrict or in any way limit the scope of the appended claims to such detail. Additional modifications will readily appear to those skilled in the art. The invention in its broader aspects is therefore not limited to the specific details, representative apparatus and methods, and illustrative examples shown and described. Accordingly, departures may be made from such details without departing from the spirit or scope of applicants’ general inventive concept.

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<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 2
ggtcatcaga cgatccatct ccctcag

<210> SEQ ID NO 3
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 3
cctctgaggg agatggatcg tctgatga
<400> SEQUENCE: 4

```
ttgagctccc gaccaatcag aattgact
```

<210> SEQ ID NO 5
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 5

```
ttggcagga gctcgtgaga gc
```

<210> SEQ ID NO 6
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 6

```
aggtgcttga ggagtcggct ctg
```

<210> SEQ ID NO 7
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 7

```
tacccaggcg gttgagactc cagctct
```

<210> SEQ ID NO 8
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 8

```
cctcacgcag agcagttggt cctctca
```

<210> SEQ ID NO 9
<211> LENGTH: 2878
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 9

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aggcggacct gattgaagac tgaaaaccgt taaattcaaa tttgaaaatg gcgggcaaaa 
tggcggaagg ggggcggagt ttatgcaaat taattatatc aaagtagggag gacgctgatt 
ttagctgagtgttcagacag aacatctcag cggagtaagc tggcagctgtt cctccgtaga 
```

[...remaining sequences]
acagggacct gattgaagac tgacacagct tacattccaa tttgaaatg ggccecaac 120
atggcggcgg ggggcggagt ttatgcaaat taatttatgc aaagtaggag gagtcctatt 180
ttaattttag caaagttaga gggacgactt ctagtggttc gggagcttac ctggctcat 240
gcataggggt tcacactaact gattgaaggc gtttcgttta aagcgaaatg aagaagttc 300
ggctggaag gattgacgtt atgcgcaggc ggggctacag ggggagagc agctgacggc 360
agggctatag cggagaagac ctagttggaa gtttcgcaag ggtgaggcac 420
tctgcaagtc cgggctgtta aactgtactc gcattacctg gctactgaga 480
cgcgttcttg gcgcgcgtgc taagctctat agaagagagc gtttctgtgt gagaagagct 540
tcattacaag acgacgaggc tttaatttgt caaagattgg ggagcattgt ctgattggtc 600
tgcgtctact ctgagtcagc acgtgacaga gcgccttacc gacaaaaagc tttaaccca 660
aaagtaagtc gcggggggtt gattgaaggc ttgtggccaa atttctcact gcatttctaa 720
cggctacag ccacactaat acgcacatcc ggtgacagag ggggctatcc aggagctagc 780
aaagactagt ggggagagac ctagtttcgt cggagcttca gggggttgg 840
gagttcagtt cgggaggctc atacaggatt gggagctttg gcgagttggg 900
ggaagagttt gtctggtgta ctgtggtgta gtttcgtgta atgtttcgct 960
cggacataag actactgcctt tttggtgagat agagactata cagctactta 1020
atcaaaagac atoacgccgag aagtttatg atgctgacaga aagcagagac aagctataa 1080
gctgggata ggcgcaacag ggcggccgaa ggggagagc gggggtgttt ctagtttatt 1140
agggggtttc gcagtttctt ggcgcgcaag ggggctggtt gggagcgatg gggagctgaa 1200
tagtgcaaat ataccctatc gatgctttcc ggcgttctca aaggttttcc 1260
gcccagatga cagggattat gtttttctgt gcttgttactg gggagctaatg ggggtttgaa 1320
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gtgccaaag attttctgta gggagctaatg gggagctaatg gggagctaatg 1440
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tgctgggaaat gggagctaatg gggagctaatg gggagctaatg gggagctaatg 1560
acgacaggac cagcaggtcg gcgcgctgcc gggagctaatg gggagctaatg gggagctaatg 1620
tgactctcta gttgagctcc gccttcatag gggagctaatg gggagctaatg gggagctaatg 1680
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gcaaaatgtt gctgcaccaac gtcctccact gcacagtttt tggagacatc cagcagattt 1920
ggacactttg ggtttttcag gggacactaat gttttttttc ggcgttctca ggcgcgcctt 1980
aacaactagt taccaccaac gggagctaatg gggagctaatg gggagctaatg gggagctaatg 2040
ggcacagtttt ggcggctgtt ccacagctgc gtcctttaac ggttcgttgc gttttttttc 2100
acacacccaa cttgggcggag gggagctaatg gggagctaatg gggagctaatg gggagctaatg 2160
gttcgtttgat ctcctacaag cttacttttt cttttttttt gggagctaatg gggagctaatg 2220
gtttcgccaac tccacttcag cccaggtgca gttttttttt gttttttttt gttttttttt 2280
ctttctcaac gggagccgga aacagacgaa gggagctaatg gggagctaatg gggagctaatg 2340
agcatctgc gtccttgcct gtttaaccag gcctgagcag gcgagctaatg gggagctaatg 2400
<210> SEQ ID NO 11
<211> LENGTH: 2750
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 11

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ataaaccaca aactattaca ggaaactgca ataaattaag aaataaatta cacataacca 120
ccctaacacg gaaaaccttg caaaaaaaggg gaaataaatc tcattggctg ggccagaagt 180
cctcattaga ataagaaaag aaccaatcag aaacacttcc tcttttagag tatataagta 240
agtgccccga gaataaggctg agtttatgcc gctggtggta gacacgaaca gagctgagtg 300
tcaacccgc ttggccggtg ccggagctcc tgagagcgga gtcaaggggc ctatcgggca 360
ggcggtaatc cagcggaact gggcccccct ccatggaaga aagatggctg acggtagcgt 420
actgcgcgca cggattattc tgcgactgta aaaacccgaa aaaacatctt gaaaaatgcc 480
ttacgacgc ttcgacgagg gcggacacgg agatggaggg accgaggttg gaaactggatc 540
gagacgctac tttcgatatc ggtatcgacg cgctcctcgc cgccgccgcc caaaggtaag 600
gagacggagg aggaaagctc cggtcattca atggttccct cctagccgga gaacctgcct 660
taatgacgg ggttttcatta ctgaaaaaag acgtgagtga cacatccagaa tcataacca 720
aatagagaac cagcgggtat ttagcgggtgg ggtatccagc atggttccct cctagccgga gaacctgcct 780
taatgacgg ggttttcatta ctgaaaaaag acgtgagtga cacatccagaa tcataacca 840
aatagagaac cagcgggtat ttagcgggtgg ggtatccagc atggttccct cctagccgga gaacctgcct 900
aatagagaac cagcgggtat ttagcgggtgg ggtatccagc atggttccct cctagccgga gaacctgcct 960
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taatgacgg ggttttcatta ctgaaaaaag acgtgagtga cacatccagaa tcataacca 1140
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aatagagaac cagcgggtat ttagcgggtgg ggtatccagc atggttccct cctagccgga gaacctgcct 1260
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aatagagaac cagcgggtat ttagcgggtgg ggtatccagc atggttccct cctagccgga gaacctgcct 1500
aatagagaac cagcgggtat ttagcgggtgg ggtatccagc atggttccct cctagccgga gaacctgcct 1560
aatagagaac cagcgggtat ttagcgggtgg ggtatccagc atggttccct cctagccgga gaacctgcct 1620
aatagagaac cagcgggtat ttagcgggtgg ggtatccagc atggttccct cctagccgga gaacctgcct 1680

<214> ORGANISM: Torque teno virus

55
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tgaatgcacca atgcgcttct tattttgacc aaggtgcttaa aaggaacaca cagcttttgc 1860
tacaaacgac ccaataatgtt taggtgtgta aactaagggac tacaacctag taagaaaata 1920
tggtttttaaaa tttoagttgg gaggacaggg cccgaagaaga tttaaaacaa cctcagagga 1980
tccacgcacc atcactgtccc cttggaacc cggggaaggc tgtaccacgc gggtcaaga 2040
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gggtaccacca ctcctttgggc aaaaaacaga gaaagagcca ttaactcagact ccaagcaag 2220
gacggttttacta ctagctacag gcaggtggatct cttcacaaga gtaagagacgc aagagcagaa 2280
gcaccacagc caaaccacic gcacgcttct cagcccaccccc caggggttgtg taaagagggat 2340
gaaaaacagct gtaagtagata atacagacactcgacgccg cttcactcga tgcacagga 2400
cacatggaaac attcatacagct actaccacta caaaccacac agaanagaact atcattgaaat 2460
gactttttttc accctgcttaa actctttctc tctgttaactt aagtcatcct gcgctgca 2520
<210> SEQ ID NO 12
<211> LENGTH: 2803
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 12
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atataaaaca aagtatacgg cgaaxatgcac ataatttag aataatgatt cacaataacc 120
cacaaccaca ggaaaccacac caaaccacca caaaccaccc caaaccacac 180
cagggaccct cgacaagaggg ggggtaaaatt ttcattttcg gcgcgtcgccg gcgctctccta 240
gaatataaa caaaccacac ccaaaactcgt gcttatttttag agattagccttg cgcaccgctc 300
gacgtgggg ccctttttcc ccacagcgagcg ccctttttcc ccagcgagcg ccctttttcc ccagcgagcg 360
cctggggtgg cctggtttgg ggttgggtgg gggggttttg gctttttttt ctggtttttt ctttggcctt 420
tccacgcacc ggggggcccc ccgctttttt gcgcggtttt gcgcggtttt gcgcggtttt gcgcggtttt 480
cactagcaggg ccgctttttt gcgcggtttt gcgcggtttt gcgcggtttt gcgcggtttt gcgcggtttt 540
tctgggggct gcgcggtttt gcgcggtttt gcgcggtttt gcgcggtttt gcgcggtttt gcgcggtttt 600
ggagagagag ccaaccttct cccgcttttt cggtttttct cccgcttttt cggtttttct cccgcttttt 660
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taagcacagaga actagcAAA atgcacattg tccagatag ggtagctcta atagacctct 1200
caagacaccttg tggtaagggc tgggaaatg ctttttacag cgtactggga tatgaagcta 1260
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cctacatct cttctttgaa cccggggcag ggttaacaccc cggcggcggc 2100
eeggaaaaaaa aacaagtttgg aaccttctttg aggattggtt cttctctttgaa 2160
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cttacccccc tttaaagttg ggggacagcc aaaaaatgc aggtagaaaat caggggagag 2640
cagccccccccc ccccccttcctt ccccccttttc ccccccccttt ccccccccttt 2700
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<210> SEQ ID NO 13
<211> LENGTH: 635
<212> TYPE: PRT
<400> ORGANISM: Torque teno virus

<400> SEQUENCE: 13
Met Arg Phe Arg Arg Arg Phe Gly Arg Arg Arg Arg Tyr Tyr Arg
1 5 10 15
Lys Arg Arg Gly Gly Trp Arg Arg Arg Phe Arg Ile Arg Arg Arg Arg
20 25 30
Pro Trp Arg Arg Arg Val Arg Arg Arg Trp Arg Arg Ser Val Phe Arg
35 40 45
Arg Gly Gly Arg Arg Ala Arg Pro Tyr Arg Ile Ser Ala Trp Asn Pro
50 55 60
-continued

Lys Val Leu Arg Asn Cys Arg Ile Thr Gly Trp Trp Trp Pro Val Ile Gln
65  70  75  80

Cys Met Asp Gly Met Glu Trp Ile Lys Tyr Lys Pro Met Asp Leu Arg
85  90  96

Val Glu Ala Asn Trp Ile Phe Asn Lys Glu Asp Ser Lys Ile Glu Thr
100 105 110

Glu Gln Met Gly Tyr Leu Met Gln Tyr Gly Gly Trp Ser Ser Gly
115 120 125

Val Ile Ser Leu Glu Gly Leu Phe Asn Glu Asn Arg Leu Trp Arg Asn
130 135 140

Ile Trp Ser Lys Ser Asn Asp Gly Met Asp Leu Val Arg Tyr Phe Gly
145 150 155 160

Cys Arg Ile Arg Leu Tyr Pro Thr Glu Asn Glu Asp Tyr Leu Phe Trp
165 170 175

Tyr Asp Thr Glu Phe Asp Glu Gln Gln Arg Met Leu Asp Glu Tyr
180 185 190

Thr Glu Pro Ser Val Met Leu Gln Ala Lys Ser Arg Leu Ile Val
195 200 205

Cys Lys Gln Lys Met Pro Ile Arg Arg Arg Val Lys Ser Ile Phe Ile
210 215 220

Pro Pro Pro Ala Glu Leu Thr Thr Glu Trp Lys Phe Glu Gln Glu Leu
225 230 235 240

Cys Gln Phe Pro Leu Phe Asn Trp Ala Cys Ile Cys Ile Asp Met Asp
245 250 255

Thr Pro Phe Asp Tyr Asn Gly Ala Trp Arg Asn Ala Trp Trp Leu Met
260 265 270

Arg Arg Leu Gln Asn Gly Met Glu Tyr Ile Glu Arg Trp Gly Arg
275 280 285

Ile Pro Met Thr Gly Asp Thr Glu Leu Pro Pro Ala Asp Asp Phe Lys
290 295 300

Ala Gly Gly Val Asn Lys Asn Phe Lys Pro Thr Gly Ile Gln Arg Ile
305 310 315 320

Tyr Pro Ile Val Ala Val Cys Leu Val Glu Gly Asn Arg Val Val
325 330 335

Lys Trp Ala Thr Val His Asn Gly Pro Ile Asp Arg Trp Arg Lys Lys
340 345 350

Gln Thr Gly Thr Leu Lys Leu Ser Asn Leu Arg Gly Leu Val Leu Arg
355 360 365

Val Cys Ser Glu Ser Glu Thr Tyr Lys Trp Thr Gly Ser Glu Phe
370 375 380

Thr Gly Ala Phe Glu Gln Gln Asp Trp Pro Val Gly Gly Thr Glu Tyr
385 390 395 400

Pro Leu Cys Thr Ile Lys Met Asp Pro Glu Tyr Glu Asn Pro Thr Val
405 410 415

Glu Val Trp Ser Trp Lys Ala Asn Ile Pro Thr Ser Gly Thr Leu Lys
420 425 430

Asp Tyr Phe Gly Leu Ser Thr Gly Glu Gln Trp Lys Asp Thr Asp Phe
435 440 445

Ala Arg Leu Gln Leu Pro Arg Ser Ser His Asn Val Asp Phe Gly His
450 455 460

Lys Ala Arg Phe Gly Pro Phe Cys Val Lys Lys Pro Pro Val Glu Phe
465 470 475 480
Asp Asp Thr Ala Pro Asn Pro Leu Asn Ile Trp Val Val Tyr Thr Thr Phe
Tyr Phe Gln Phe Gly Gly Met Tyr Gln Pro Pro Thr Gly Ile Gln Asp
Pro Cys Thr Ser Asn Pro Thr Pro Val Met Val Gly Ala Val
Thr His Pro Lys Tyr Ala Gly Gln Gly Gly Ile Thr Thr Gly Ile Gly
Asp Gln Gly Ile Thr Ala Ala Ser Ile Arg Ala Ile Ser Ala Ala Pro
Pro Asp Thr Tyr Thr Gln Ser Ala Phe Leu Lys Ala Pro Glu Thr Glu
Lys Glu Glu Glu Arg Glu Ser Glu Thr Ser Phe Thr Ser Ala Glu Ser
Ser Ser Glu Gly Asp Gly Ser Ser Asp Ala Gln Ala Glu Arg Arg Ala
Ala Arg Lys Arg Val Ile Lys Leu Leu Leu Lys Arg Leu Ala Asp Arg
Pro Val Asp Asn Lys Arg Arg Arg Phe Ser Glu

<210> SEQ ID NO: 14
<211> LENGTH: 639
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 14

Met Ala Pro Thr Arg Arg Trp Arg Arg Asp Gly Arg Arg Arg Arg
1 5 10 15
Arg Tyr Arg Lys Arg Arg Tyr Gly Trp Arg Arg Arg Tyr Arg Arg
20 25 30
Arg Pro Arg Tyr Arg Arg Trp Leu Val Arg Arg Arg Arg
35 40 45
Ser Val Tyr Arg Arg Gly Gly Arg Arg Ala Arg Tyr Arg Ile Ser
50 55 60
Ala Phe Asn Pro Lys Val Met Arg Val Val Ile Arg Gly Trp Trp
65 70 75 80
Pro Ile Leu Gln Cys Leu Lys Gly Gln Glu Ser Leu Arg Tyr Arg
85 90 95
Leu Gln Trp Asp Thr Glu Lys Gln Trp Arg Val Lys Asp Tyr Glu
100 105 110
Asp Asn Tyr Gly Tyr Leu Val Gln Tyr Gly Gly Trp Gly Ser Gly
115 120 125
Glu Val Thr Leu Glu Gly Leu Tyr Gln Glu His Leu Leu Leu Arg Asn
130 135 140
Ser Trp Ser Lys Gly Asn Asp Met Leu Val Arg Tyr Phe Gly
145 150 155 160
Cys Ile Val Tyr Leu Tyr Pro Leu Gln Asp Gln Asp Tyr Trp Phe Trp
165 170 175
Trp Asp Thr Asp Phe Lys Leu Tyr Ala Glu Ser Ile Lys Glu Tyr
180 185 190
Ser Gln Pro Ser Val Met Met Ala Lys Arg Thr Arg Leu Val Ile
195 200 205
Ala Arg Asp Arg Ala Pro His Arg Arg Arg Val Arg Lys Ile Phe Ile
210 215 220
Pro Pro Pro Ser Arg Asp Thr Thr Gln Trp Gln Phe Gln Thr Asp Phe
225 230 235 240
Cys Lys Arg Pro Leu Phe Thr Trp Ala Ala Gly Leu Ile Asp Met Gln
245 250 255
Lys Pro Phe Asp Ala Asn Gly Ala Phe Arg Asn Ala Trp Trp Leu Glu
260 265 270
Thr Arg Asn Asp Gln Gly Glu Met Lys Tyr Ile Glu Leu Trp Gly Arg
275 280 285
Val Pro Pro Gln Gly Asp Thr Glu Leu Pro Lys Gln Ser Glu Phe Lys
290 295 300
Lys Gly Asp Asn Asn Pro Asn Tyr Asn Ile Thr Glu Gly His Glu Lys
305 310 315 320
Asn Ile Tyr Pro Ile Ile Tyr Val Asp Gln Lys Asp Gln Lys Thr
325 330 335
Arg Lys Lys Tyr Cys Val Cys Tyr Asn Lys Thr Leu Asn Arg Trp Arg
340 345 350
Lys Ala Gln Ala Ser Thr Leu Ala Ile Gly Asp Leu Gln Gly Leu Val
355 360 365
Leu Arg Gln Leu Met Asn Gln Glu Met Thr Tyr Trp Lys Ser Gly
370 375 380
Glu Phe Ser Ser Pro Phe Leu Gln Arg Trp Lys Gly Thr Arg Leu Ile
385 390 395 400
Thr Ile Asp Ala Arg Lys Ala Asp Thr Glu Asn Pro Lys Val Ser Ser
405 410 415
Trp Glu Trp Gly Gln Asn Trp Asn Thr Ser Gly Thr Val Leu Gln Glu
420 425 430
Val Phe Asn Ile Ser Leu Asn Thr Gln Ile Arg Glu Asp Asp Phe
435 440 445
Ala Lys Leu Thr Leu Pro Lys Ser Pro His Asp Ile Asp Phe Gly His
450 455 460
His Ser Arg Phe Gly Pro Phe Cys Val Lys Asn Glu Pro Leu Glu Phe
465 470 475 480
Gln Leu Leu Pro Pro Thr Pro Thr Asn Leu Trp Phe Glu Tyr Lys Phe
485 490 495
Leu Phe Gln Phe Gly Gly Glu Tyr Glu Pro Pro Thr Gly Ile Arg Asp
500 505 510
Pro Cys Ile Asp Thr Pro Ala Tyr Pro Val Pro Gin Ser Gly Ser Val
515 520 525
Thr His Pro Lys Phe Ala Gly Lys Gly Gly Met Leu Thr Glu Thr Asp
530 535 540
Arg Trp Gly Ile Thr Ala Ala Ser Ser Arg Thr Leu Ser Ala Asp Thr
545 550 555 560
Pro Thr Glu Ala Ala Gin Ser Ala Leu Arg Gly Asp Ala Glu Lys
565 570 575
Lys Gly Glu Glu Thr Glu Glu Thr Ala Ser Ser Ser Ser Ile Thr Ser
580 585 590
Asp Gin Ser Thr Glu Gly Ser Asp Gin Ser Gin Glu Thr
595 600 605
Ile Arg Arg Arg Arg Thr Trp Lys Arg Leu Arg Asp Met Val Arg
610 615 620
Gln Gin Leu Asp Arg Met Asp His Lys Arg Gin Arg Leu His
<210> SEQ ID NO 15
<211> LENGTH: 625
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 15

Met Pro Tyr Arg Arg Tyr Arg Arg Arg Arg Arg Arg Arg Arg Pro Thr Arg Arg
1      5      10     15
Tyr Arg His Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
20     25     30
Ala Pro Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
35     40     45
Pro Val Ile Gln Trp Phe Pro Pro Ser Arg Arg Thr Cys Leu Ile Glu Glu
50     55     60
Gly Phe Trp Pro Leu Ser Tyr Gly His Trp Phe Arg Thr Cys Leu Pro Pro
65     70     75     80
Met Arg Arg Leu Asn Gly Leu Ile Phe Thr Gly Gly Gly Cys Arg Trp
85     90     95
Thr Gln Trp Ser Leu Gln Asn Leu Phe His Glu Leu Asn Trp Arg
100    105    110
Asn Ile Trp Thr Ala Ser Asn Val Gly Met Glu Phe Ala Arg Phe Leu
115    120    125
Arg Gly Lys Phe Tyr Phe Phe Arg Phe Pro Arg Arg Ser Tyr Ile Val
130    135    140
Thr Thr Asp Gln Asp Ile Pro Cys Leu Pro Leu Pro Tyr Gln Asn Leu
145    150    155    160
Gln Pro Leu Leu Met Leu Leu Lys Gln His Lys Leu Val Leu Ser
165    170    175
Gln Lys Asp Cys Asn Pro Asn Arg Lys Glu Pro Val Thr Leu Lys
180    185    190
Phe Arg Pro Pro Pro Lys Leu Thr Ser Gln Trp Arg Leu Ser Arg Glu
195    200    205
Leu Ser Lys Ile Pro Leu Ile Arg Leu Gly Ile Ser Leu Ile Asp Leu
210    215    220
Ser Glu Pro Trp Leu Glu Gly Trp Gly Asn Ala Phe Tyr Ser Val Leu
225    230    235    240
Gly Tyr Glu Ala Ser Lys His Ser Gly Arg Thr Ser Asn Trp Thr Gln
245    250    255
Met Lys Tyr Phe Trp Ile Tyr Asp Thr Gly Val Gly Asn Ala Val Tyr
260    265    270
Val Ile Leu Leu Lys Asp Val Ser Asp Asn Pro Gly Asp Met Ala
275    280    285
Thr Gln Phe Val Thr Gly Ser Gly Gln His Pro Asp Ala Ile Asp His
290    295    300
Ile Glu Met Val Asn Glu Gly Trp Pro Tyr Trp Leu Phe Phe Tyr Glu
305    310    315    320
Gln Ser Glu Gln Asp Ile Lys Leu Ala His Asp Gln Asp Leu Val
325    330    335
Arg Glu Tyr Ala Arg Asp Pro Lys Ser Lys Lys Leu Lys Ile Gly Val
340    345    350
Ile Gly Trp Ala Ser Ser Asn Tyr Thr Thr Ala Gly Ser Asn Gln Asn
355    360    365
Ser Val Leu Gln Thr Pro Glu Ala Ile Gln Gly Gly Tyr Val Ala Tyr
370    375    380

<217> -continued
Ala Gly Ser Arg Ile Pro Gly Ala Gly Ser Ile Thr Asn Leu Phe Gln
385  390  395  400
Met Gly Trp Pro Gly Asp Glu Asn Trp Pro Pro Thr Asn Glu Asp Gln
405  410  415
Thr Asn Phe Asn Trp Gly Leu Arg Gly Leu Cys Val Leu Arg Asp Asn
420  425  430
Met Lys Leu Gly Ala Gln Glu Leu Asp Glu Cys Thr Met Leu Ser
435  440  445
Leu Phe Gly Pro Phe Val Glu Lys Ala Asn Thr Ala Phe Ala Thr Asn
450  455  460
Asp Pro Lys Tyr Phe Arg Pro Glu Leu Lys Asp Tyr Asn Val Val Met
465  470  475  480
Lys Tyr Ala Phe Lys Phe Gln Trp Gly Gly His Gly Thr Glu Arg Phe
485  490  495
Lys Thr Thr Ile Gly Asp Pro Ser Thr Ile Pro Cys Pro Phe Glu Pro
500  505  510
Gly Glu Arg Tyr His His Gly Val Gln Asp Pro Ala Lys Val Gln Asn
515  520  525
Thr Val Leu Asn Pro Trp Arg Tyr Arg Cys Asp Gly Ile Val Arg Thr
530  535  540
Asp Thr Leu Lys Arg Leu Leu Glu Leu Pro Thr Glu Thr Glu Glu Thr
545  550  555  560
Glu Lys Ala Tyr Pro Leu Leu Gly Gln Thr Glu Lys Glu Pro Leu
565  570  575
Ser Asp Ser Asp Glu Glu Ser Val Ile Ser Ser Thr Ser Ser Gly Ser
580  585  590
Ser Glu Glu Glu Thr Glu Arg Arg Lys His His Lys Pro Ser Lys
595  600  605
Arg Arg Leu Leu Lys His Leu Glu Arg Val Val Lys Arg Met Lys Thr
610  615  620
Leu
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<210> SEQ ID NO 16
<211> LENGTH: 625
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 16

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Trp Arg His Arg Arg Arg Arg Arg Phe Phe Arg Tyr Arg Arg
20  25
Ala Pro Arg Arg Arg Arg Thr Lys Val Arg Arg Arg Arg Arg Lys Ala
35  40
Pro Val Ile Glu Trp Phe Pro Pro Ser Arg Arg Thr Cys Leu Ile Glu
50  55  60
Gly Phe Trp Pro Leu Ser Tyr Gly His Trp Phe Arg Thr Cys Leu Pro
65  70  75  80
Met Arg Arg Leu Asn Gly Leu Ile Phe Thr Gly Gly Gly Asp Trp
85  90  95
Thr Glu Trp Ser Leu Gln Asn Leu Tyr His Glu Lys Leu Asn Trp Arg
100 105 110
Asn Ile Trp Thr Ala Ser Asn Val Gly Met Glu Phe Ala Arg Phe Leu
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Arg Arg Leu Leu Lys His Leu Glu Arg Val Val Lys Arg Met Lys Thr 610 615 620
Leu 625

<210> SEQ ID NO 17
<211> LENGTH: 73
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 17
Met Lys Glu Lys Asp Tyr Trp Glu Glu Ala Trp Leu Thr Ser Cys Thr 1 5 10 15
Ser Ile His Asp His His Asn Cys Gly Ser Trp Arg Asp His Leu 20 25 30
Trp Thr Leu Cys Ala Leu Asp Ala Asp Leu Ala Ala Ala Ala Asp 35 40 45
Ile Ile Glu Arg Glu Ala Asp Gly Gly Glu Asp Phe Gly Phe Val 50 55 60
Asp Gly Asp Pro Gly Asp Ala Gly Gly 65 70

<210> SEQ ID NO 18
<211> LENGTH: 72
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 18
Met Pro Glu His Trp Glu Glu Ala Trp Leu Glu Ala Thr Lys Gly Trp 1 5 10 15
His Asp Leu Asp Cys Arg Cys Asp Glu Trp Glu Asp His Leu Trp Leu 20 25 30
Leu Leu Ala Asp Gly Asp Ala Ala Ala Ala Val Asp Ala Ile 35 40 45
Glu Arg Asp Ala Met Gly Gly Glu Val Thr Thr Ala Thr Asp Arg 50 55 60
Val Thr Ile Gly Asp Asp Gly Trp 65 70

<210> SEQ ID NO 19
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 19
Met Glu Glu Arg Trp Leu Thr Val Ala Tyr Cys Ala His Gly Leu Phe 1 5 10 15
Cys Asp Cys Lys Asn Pro Lys Lys His Leu Glu Lys Cys Leu Thr Asp 20 25 30
Ala Ile Ala Asp Ala Glu Gly Asp Arg His Gly Asp Gly Gly Thr Gly
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Gly Gly Ala Thr Phe Asp Ile Gly Ile Asp Ala Leu Leu Ala Ala
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Ala Ala Gln Arg
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<210> SEQ ID NO 20
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 20
Met Glu Glu Arg Trp Leu Thr Val Ala Tyr Cys Ala His Gly Leu Phe
1  5  10  15
Cys Gly Cys Lys Asp Pro Lys His Leu Glu Lys Cys Leu Thr Asp
20  25  30
Ala Ile Ala Asp Ala Glu Gly Asp Arg His Gly Asp Gly Gly Thr Gly
35  40  45
Gly Gly Ala Ser Phe Asp Ile Gly Ile Asp Ala Leu Leu Ala Ala
50  55  60
Ala Ala Gln Arg
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<210> SEQ ID NO 21
<211> LENGTH: 174
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 21
Met Arg Phe Arg Arg Arg Arg Phe Gly Arg Arg Arg Arg Tyr Tyr Arg
1  5  10  15
Lys Arg Arg Gly Gly Trp Arg Arg Arg Phe Arg Ile Arg Arg Arg Arg
20  25  30
Pro Trp Arg Arg Phe Gly Gly Met Tyr Gln Pro Pro Thr Gly
35  40  45
Ile Gln Asp Pro Cys Thr Ser Asn Pro Thr Tyr Pro Val Arg Met Val
50  55  60
Gly Ala Val Thr His Pro Lys Tyr Ala Gly Glu Gly Ile Thr Thr
65  70  75  80
Gln Ile Gly Asp Gin Gly Ile Thr Ala Ala Ser Ile Arg Ala Ile Ser
85  90  95
Ala Ala Pro Pro Asp Thr Tyr Thr Gin Ser Ala Phe Leu Lys Ala Pro
100 105 110
Glu Thr Glu Lys Glu Glu Glu Arg Glu Ser Glu Thr Ser Phe Ser Ser
115 120 125
Ala Ala Ser Ser Glu Gly Asp Ser Arg Ser Arg Gin Ala Glu
130 135 140
Arg Arg Ala Ala Arg Lys Arg Val Ile Lys Leu Leu Leu Lys Leu Arg
145 150 155 160
Ala Asp Arg Pro Val Asp Asn Lys Arg Arg Arg Phe Ser Glu
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<211> LENGTH: 192
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus
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<210> SEQ ID NO 24
<211> LENGTH: 178
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 24
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1  5  10  15
Trp Arg His Arg Arg Trp Arg Arg Phe Phe Arg Tyr Arg Tyr Arg Arg Arg
20 25  30
Ala Pro Arg Arg Arg Thr Lys Trp Gly Gly His Gly Thr Glu Arg
35 40  45
Phe Lys Thr Thr Ile Gly Asp Pro Ser Thr Ile Pro Cys Pro Phe Glu
50 55  60
Pro Gly Glu Arg Tyr His His Gly Val Gln Asp Pro Ala Lys Val Gln
65 70  75  80
Asn Thr Val Leu Asn Pro Trp Asp Tyr Asp Cys Asp Gly Ile Val Arg
85 90  95
Thr Asp Thr Leu Lys Arg Leu Leu Glu Leu Pro Thr Glu Thr Glu Glu
100 105 110
Thr Glu Lys Ala Tyr Pro Leu Leu Gly Glu Thr Glu Lys Glu Pro
115 120 125
Leu Ser Asp Ser Asp Glu Glu Ser Val Ile Ser Ser Thr Ser Ser Gly
130 135 140
Ser Ser Gln Glu Glu Thr Gln Arg Arg Gln His Lys Pro Ser
145 150 155 160
Lys Arg Arg Leu Leu Lys His Leu Gln Arg Val Val Lys Arg Met Lys
165 170 175
Thr Leu

<210> SEQ ID NO 25
<211> LENGTH: 224
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 25
Met Lys Glu Lys Asp Tyr Trp Glu Glu Ala Trp Leu Thr Ser Cys Thr
1  5  10  15
Ser Ile His Asp His His Cys Asp Cys Gly Ser Trp Arg Asp His Leu
20 25  30
Trp Thr Leu Cys Ala Leu Asp Ala Asp Leu Ala Ala Ala Asp
35 40  45
Ile Ile Glu Arg Glu Ala Asp Gly Glu Gly Glu Asp Phe Gly Phe Val
50 55  60
Asp Gly Asp Pro Gly Asp Ala Gly Gly Ser Ala Ala Cys Thr Ser Leu
65 70  75  80
Pro Pro Glu Ser Lys Ile Pro Ala Leu Leu Thr Arg Pro Ile Leu Ser
85 90  95
Glu Trp Ser Glu Gln Leu His Thr Pro Asn Thr Pro Gly Lys Ala Glu
100 105 110
Ser Arg Pro Lys Leu Glu Ile Lys Val Ser Pro Leu Pro Leu Ser Val
115 120 125
Pro Ser Val Gln Leu His Gln Ile Pro Thr Arg Ser Arg Arg Ser
130 135 140
Lys Pro Arg Lys Pro Arg Lys Lys Arg Lys Glu Arg Val Arg Pro Val
145 150 155 160
Ser Arg Val Pro Lys Ala Leu Leu Arg Glu Met Asp Arg Leu Met Thr
165 170 175
Lys Glu Arg Asp Ala Leu Pro Glu Ser Glu Ser Ser Ser Tyr Phe Ser
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Ser Asp Ser Leu Thr Asp Pro Thr Thr Thr Ser Asp Asp Asp Phe Glu
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Ser Asp Pro Asp Pro Leu Thr Asn Lys Arg Lys Arg Leu Glu Phe
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<210> SEQ ID NO 26
<211> LENGTH: 228
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 26
Met Pro Glu His Trp Glu Glu Ala Trp Leu Glu Ala Thr Lys Gly Trp
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His Asp Leu Asp Cys Arg Cys Asn Trp Glu Asp His Leu Trp Leu
20 25 30
Leu Leu Ala Asp Gly Asp Ala Leu Ala Ala Ala Val Asp Ala Ile
35 40 45
Glu Arg Asp Ala Met Gly Gly Glu Asp Val Thr Thr Ala Thr Asp Arg
50 55 60
Val Thr Ile Gly Asp Gly Cys Leu Ala Val Asn Thr Ser His Glu
65 70 75 80
Gln Val Ser Ala Ile Pro Ala Leu Ile His Glu Pro Ile Leu Cys Arg
85 90 95
Ser Gln Glu Val Leu His Thr Pro Asn Ser Pro Glu Arg Ala Glu Cys
100 105 110
Ser Arg Lys Gln Thr Val Val Glu Val Ser Leu Leu Pro Leu Pro Glu Pro
115 120 125
Ser Val Gln Ile His Pro Pro Lys Gln Arg Lys Val His Phe Ser Glu
130 135 140
Gly Thr Arg Lys Arg Lys Glu Arg Lys Pro Arg Lys Pro Arg His Arg
145 150 155 160
Pro Val Ser Arg Val Pro Lys Ala Leu Leu Arg Glu Met Asp Arg Leu
165 170 175
Met Met Lys Arg Gln Ser Asp Ala Glu Gly Gly Gly Ser Asp Ser
180 185 190
Asp Gly Trp Ser Asp Ser Leu Thr Asp Glu Trp Thr Thr Ser Asp
195 200 205
Ser Asp Phe Ile Asp Thr Pro Ile Arg Glu Arg Cys Leu Asn Lys Lys
210 215 220
Gln Lys Lys Arg
225

<210> SEQ ID NO 27
<211> LENGTH: 199
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 27
Met Glu Glu Arg Trp Leu Thr Val Ala Tyr Cys Ala His Gly Leu Phe
1 5 10 15
Cys Lys Asp Cys Lys Glu Pro Lys His Leu Glu Lys Cys Leu Thr Asp  
20 25 30
Cys Ala Ala Asp Ala Glu Gly Asp Arg His Gly Asp Gly Gly Thr Gly  
35 45
Gly Gly Asp Ala Thr Phe Asp Ile Gly Ile Asp Ala Leu Leu Ala Ala  
50 55 60
Ala Ala Gln Ser Gly Glu Asp Met Ala Pro Lys Asp Leu Lys Gln Pro  
65 70 75 80
Ser Glu Ile Pro Ala Pro Tyr His Val Pro Leu Asn Pro Gly Asn Gly  
85 90 95
Thr Thr Thr Gly Tyr Lys Thr Pro Pro Arg Tyr Lys Thr Gln Ser Ser  
100 105 110
Thr Leu Gly Thr Met Thr Val Thr Gly Leu Leu Glu Gln Ile Leu Ser  
115 120 125
Lys Asp Phe Ser Asn Ser Pro Gln Arg Arg Arg Arg Arg Arg Arg Arg  
130 135 140
Thr His Ser Leu Asp Lys Gln Arg Lys Ser His Tyr Gln Thr Pro  
145 150 155 160
Thr Lys Arg Ala Leu Ser Gln Ala Arg Ala Val Asp Pro Leu Lys Lys  
165 170 175
Lys Arg Arg Asp Glu Ser Thr Ser Gln Ala Ser Asp Asp Asp Asp  
180 185 190
Ser Ser Thr Ser Ser Gly Trp  
195

<210> SEQ ID NO 28
<211> LENGTH: 199
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 28
Met Glu Glu Arg Trp Leu Thr Val Ala Tyr Cys Ala His Gly Leu Phe  
1 5 10 15
Cys Gly Cys Lys Asp Pro Lys His Leu Glu Lys Cys Leu Thr Asp  
20 25 30
Cys Ala Ala Asp Ala Glu Gly Asp Arg His Gly Asp Gly Gly Thr Gly  
35 45
Gly Gly Asp Ala Ser Phe Asp Ile Gly Ile Asp Ala Leu Leu Ala Ala  
50 55 60
Ala Ala Gln Ser Gly Glu Ala Thr Glu Asp Ser Lys Gln Pro  
65 70 75 80
Ser Glu Ile Pro Ala Pro Tyr His Val Pro Leu Asn Pro Gly Asn Gly  
85 90 95
Thr Thr Thr Gly Tyr Lys Thr Pro Pro Arg Tyr Lys Thr Gln Ser Ser  
100 105 110
Thr Leu Gly Thr Met Thr Val Thr Gly Leu Leu Glu Gln Ile Leu Ser  
115 120 125
Lys Asp Phe Ser Asn Ser Pro Gln Arg Arg Arg Arg Arg Arg Arg Arg  
130 135 140
Thr His Ser Leu Asp Lys Gln Arg Lys Ser His Tyr Gln Thr Pro  
145 150 155 160
Thr Lys Arg Ala Leu Ser Gln Ala Arg Ala Val Asp Pro Leu Lys Lys  
165 170 175
Lys Arg Arg Glu Glu Asp Ser Thr Ser Gln Ala Ser Asp Asp Asp  
180 185 190
Ser Ser Thr Ser Ser Gly Trp

<210> SEQ ID NO 29
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 29
tccgaatggc tgagtttatg c

<210> SEQ ID NO 30
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 30
tcgcctcagc tgtctct

<210> SEQ ID NO 31
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 31
ggtgtaaag agatgaa

<210> SEQ ID NO 32
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 32
atagatgagc acacgag

<210> SEQ ID NO 33
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 33
tatcggcac gcacgat
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<210> SEQ ID NO 34
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 34
taggggccg cctcagct

<210> SEQ ID NO 35
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 35
cctacatgaa ggagaaac t

<210> SEQ ID NO 36
<211> LENGTH: 17
<212> TYPE: DNA
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<td>36</td>
<td>17</td>
<td>DNA</td>
<td>Torque teno virus</td>
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<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 44
tatgggagcag

<210> SEQ ID NO 45
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 45
Thr Ala Cys Ala Cys Thr Thr Cys Gly Gly Gly Thr Cys Ala
1  5 10 15
Gly Gly Ala Gly Gly Cys Thr
20

11

<210> SEQ ID NO 46
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 46
acctgcccatcoggaacccac

<210> SEQ ID NO 47
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 47
caatttggctggttcgtgcgc

<210> SEQ ID NO 48
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 48
tacttatagtgtgtggagcc

<210> SEQ ID NO 49
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 49
agttacacataaccacccac

<210> SEQ ID NO 50
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 50
attaccacagtacccacccccc

<210> SEQ ID NO 51
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 51
<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 52

cttgactccg ctctcaggag

<210> SEQ ID NO 53
<211> LENGTH: 2878
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus

<400> SEQUENCE: 53
tacacttcgg ggtcaggag gctcaatttg
aggcgcact tgaagcagac taatttcatg
taatatttag cagcttttct gagttggagt
gactaggttg taaccactca gatttaaggc
tagttcggat ggtctagttt atgcagcagcc
gggctcagag gacgatggag gctgataaat
tgacagag gagtagtggt gcttggggct
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ggcaacagt 2700
ggcaacagt 2760
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<210> SEQ ID NO 54
<211> LENGTH: 635
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 54
Met Arg Phe Arg Arg Arg Arg Phe Gly Arg Arg Arg Arg Tyr Tyr Arg 1 5 10 15
Lys Arg Arg Gly Gly Trp Arg Arg Phe Arg Ile Arg Arg Arg Arg Arg Arg 20 25 30
Pro Trp Arg Arg Trp Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg 35 40 45
Arg Arg Gly Arg Arg Ala Arg Pro Tyr Arg Ile Ser Ala Ala Trp Aen Pro 50 55 60
Lys Val Leu Arg Arg Cys Arg Ile Thr Gly Trp Trp Trp Val Ile Gln 65 70 75 80
Cys Met Asp Gly Met Glu Trp Ile Lys Tyr Lys Pro Met Asp Leu Arg 85 90 95
Val Ala Ala Arg Ile Phe Asp Lys Gln Gly Ser Lys Ile Glu Thr 100 105 110
Glu Glu Met Gly Tyr Leu Met Gln Tyr Gly Gly Gly Trp Ser Ser Gly 115 120 125
Val Ile Ser Leu Gly Gly Leu Phe Aen Glu Aen Arg Leu Trp Arg Aen 130 135 140
Ile Trp Ser Lys Ser Aen Gly Met Asp Leu Val Arg Tyr Phe Gly 145 150 155 160
Cys Arg Ile Arg Leu Tyr Pro Thr Glu Aen Gln Gly Tyr Leu Phe Trp 165 170 175
Tyr Asp Thr Glu Phe Asp Glu Glu Gln Arg Arg Met Leu Asp Glu Tyr

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Thr Glu Pro Ser Val Met Leu Glu Asn Ser Arg Leu Ile Val
Cys Lys Glu Met Pro Ile Arg Arg Arg Val Lys Ser Ile Phe Ile
Pro Pro Pro Ala Glu Leu Thr Glu Trp Lys Phe Glu Glu Leu
Cys Glu Phe Pro Leu Phe Asn Trp Ala Cys Ile Cys Ile Asp Met Asp
Thr Pro Phe Asp Tyr Asn Ala Trp Arg Asn Ala Trp Trp Leu Met
Arg Arg Leu Glu Asn Gly Met Glu Tyr Ile Glu Arg Trp Gly Arg
Ile Pro Met Thr Gly Asp Thr Glu Leu Pro Pro Ala Asp Asp Phe Lys
Ala Gly Gly Val Asn Lys Asn Phe Lys Pro Thr Gly Ile Gin Arg Ile
Tyr Pro Ile Val Ala Val Cys Leu Val Glu Gly Asn Ile Arg Val Val
Lys Trp Ala Thr Val His Asn Gly Pro Ile Asp Arg Trp Arg Lys Lys
Gln Thr Gly Thr Leu Lys Leu Ser Ala Leu Arg Arg Leu Val Leu Arg
Val Cys Ser Glu Ser Glu Thr Tyr Tyr Lys Trp Thr Ala Ser Glu Phe
Thr Gly Ala Phe Gin Gin Asp Trp Trp Pro Val Ser Gly Thr Glu Tyr
Pro Leu Cys Thr Ile Lys Met Gin Pro Glu Phe Gin Gin Arg Pro Thr Val
Glu Val Trp Ser Trp Lys Ala Thr Ile Pro Thr Ala Gly Thr Leu Lys
Asp Tyr Phe Gly Leu Ser Ser Gly Gin Gin Trp Lys Asp Thr Asp Phe
Gly Arg Leu Gin Leu Pro Arg Ser Ser His Asn Val Asp Phe Gly His
Lys Ala Arg Phe Gly Pro Phe Cys Val Lys Pro Pro Val Glu Phe
Arg Asp Ser Ala Pro Gin Pro Leu Ann Ile Thr Val Lys Tyr Thr Phe
Tyr Phe Gin Phe Gly Gly Met Tyr Gin Pro Pro Thr Gly Ile Gin Asp
Pro Cys Thr Ser Ann Pro Thr Tyr Pro Val Arg Met Val Gly Ala Val
Thr His Pro Lys Tyr Ala Gly Gin Gly Gly Ile Ala Thr Gin Ile Gly
Asp Gin Gly Ile Thr Ala Ala Ser Leu Arg Ala Ile Ser Ala Ala Pro
Pro Ann Thr Tyr Thr Gin Ser Ala Phe Leu Lys Ala Pro Glu Thr Glu
Lys Glu Glu Arg Glu Ser Glu Thr Ser Phe Thr Ser Ala Glu Ser
Ser Ser Glu Gly Asp Gly Ser Ser Asp Asp Gin Ala Glu Arg Ala

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Ala Arg Lys Arg Val Ile Lys Leu Leu Leu Lys Arg Leu Ala Asp Arg
610 615 620
Pro Val Asp Asn Lys Arg Arg Phe Ser Glu
625 630 635

<210> SEQ ID NO 55
<211> LENGTH: 73
<212> TYPE: PRT
<213> ORGANISM: Torque teno virus
<400> SEQUENCE: 55

Met Lys Glu Lys Asp Tyr Trp Glu Ala Trp Leu Thr Ser Cys Thr
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Ser Ile His Asp His His Cys Gly Ser Trp Arg Arg His Leu
20  25  30
Trp Thr Leu Cys Ala Leu Asp Ala Asp Leu Ala Ala Ala Ala Asp
35  40  45
Ile Ile Glu Arg Glu Glu Ala Asp Gly Asp Pro Gly Phe Gly Phe Val
50  55  60
Asp Gly Asp Pro Gly Asp Ala Gly Gly
65  70

<210> SEQ ID NO 56
<211> LENGTH: 2872
<212> TYPE: DNA
<213> ORGANISM: Torque teno virus
<220> FEATURE:
<221> NAME/KEY: miscifeature
<400> SEQUENCE: 56

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  120
ttgaggccag ggggctggatt ttatcaaat tattttatgc aaataaatgg gaattcgtatt
  180
ttatatttag caagtaggac ggagctcaat cggattgtgc gggcatctca aattttcatttt
  240
gcatatggtt taaccctaa gaattgaaga ggctccacag aacataaat aagtaggtga
  300
gttctccgat gctgtggatt atcgcggcag cgtagacac aacgtcttag cgacttgccg
  360
ggtgcggcgag gttctggtat cggagcctta aagggtaatat aagtagtgga
  420
agggctatag cggaccaact gggaggggaa aggattgggaa gtatcaccag ggttgggacga
  480
tttggctgac tcggcttatt gcgggcacca ccccttatgg cccactactcg cggatggaga
  540
cgggtagtgc ctgggctctg tagacgatg aaggggagtc aagttggtgt gggagcagac
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  720
gatcctgag gtagttttag ggggggtgttc ccttttttgc aagttcggcg acgggcggag
  780
gcactaagag atacaacctt cagctggtgc acacgagata agttggagtg gatcattgac
  840
tctgacagg acattgagc cctattcagca aagtttggag gttggggaag ttgctgtgtgtg
  900
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  960
gatggagttt aagttggtgtg gggctggg ccctaggtt aaggggac ggcgtttgtg
 1020
cggcacttgcacctttgg gcctagcc aaccattttc ataagggag gtcgttttga
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 1140
gaaagggcac cacatagaag aaaaatatag aaatatatttttttttccgcac ccctcgac ccctcgcagc 1200
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TYPE: PRT
ORGANISM: Torque teno virus

SEQUENCE: 60

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Phe Arg Leu Asn Gly Leu Val Phe Pro Gly Gly Gly Cys Asp Trp
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Ser Gln Trp Ser Leu Gln Asn Leu Tyr Asn Glu Leu Asn Trp Arg
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Asn Ile Trp Thr Ala Ser Asn Val Gly Met Glu Phe Ala Arg Phe
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Lys Gly Lys Phe Tyr Phe Arg His Pro Trp Arg Asn Tyr Ile Ile
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Phe Lys Pro Pro Pro Lys Leu Thr Ser Glu Leu Trp Arg Leu Ser Arg Glu
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<213> ORGANISM: Torque teno virus

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What is claimed is:

1. An immunogenic composition comprising a biologically functional plasmid or baculovirus expression vector comprising an open reading frame (ORF); wherein said ORF encodes a protein comprising amino acids 310-625 of SEQ ID NO: 16.

2. The vaccine composition according to claim 1, further comprising an adjuvant.

3. Method of eliciting an immune response in a pig against porcine torque teno virus (PTTV), comprising administering to a pig an immunologically effective amount of the composition according to claim 1.

4. The method according to claim 3, which comprises administering the composition parenterally, intranasally, intradermally, or transdermally to the pig.

5. The method according to claim 3, which comprises administering the composition intralymphoidly or intramuscularly to the pig.