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(54) CAUSATIVE AGENT OF THE MYSTERY SWINE DISEASE, VACCINE COMPOSITIONS AND DIAGNOSTIC KITS

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(57) **ABSTRACT**

Composition of matter comprising the causative agent of Mystery Swine Disease, Lelystad Agent, in a live, attenuated, dead, or recombinant form, or a part or component of it. Vaccine compositions and diagnostic kits based thereon. Recombinant nucleic acid comprising a Lelystad Agent-specific nucleotide sequence. Peptides comprising a Lelystad Agent-specific amino acid sequence. Lelystad Agent-specific antibodies.

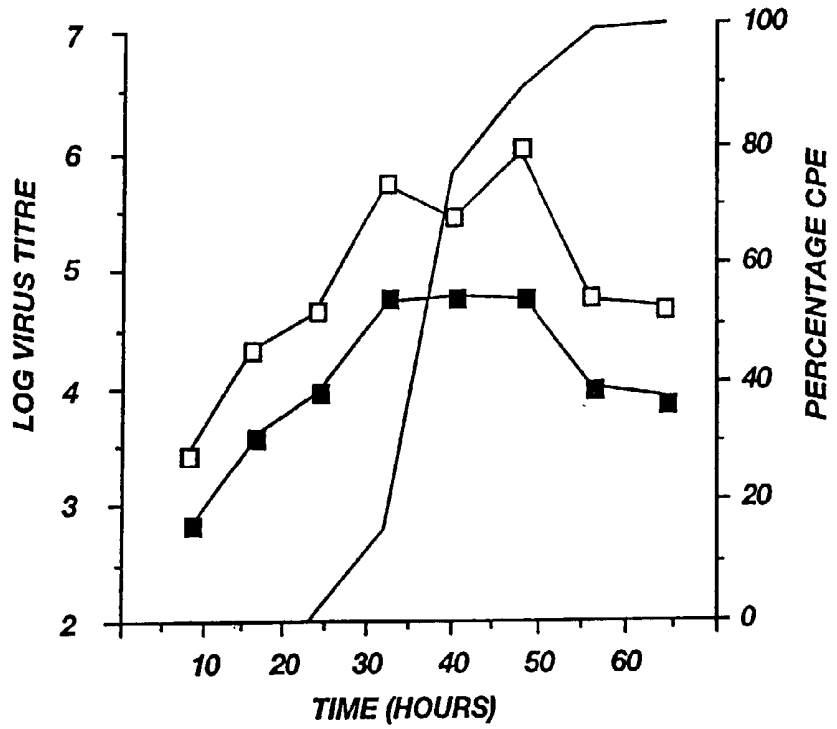

FIG. 1a

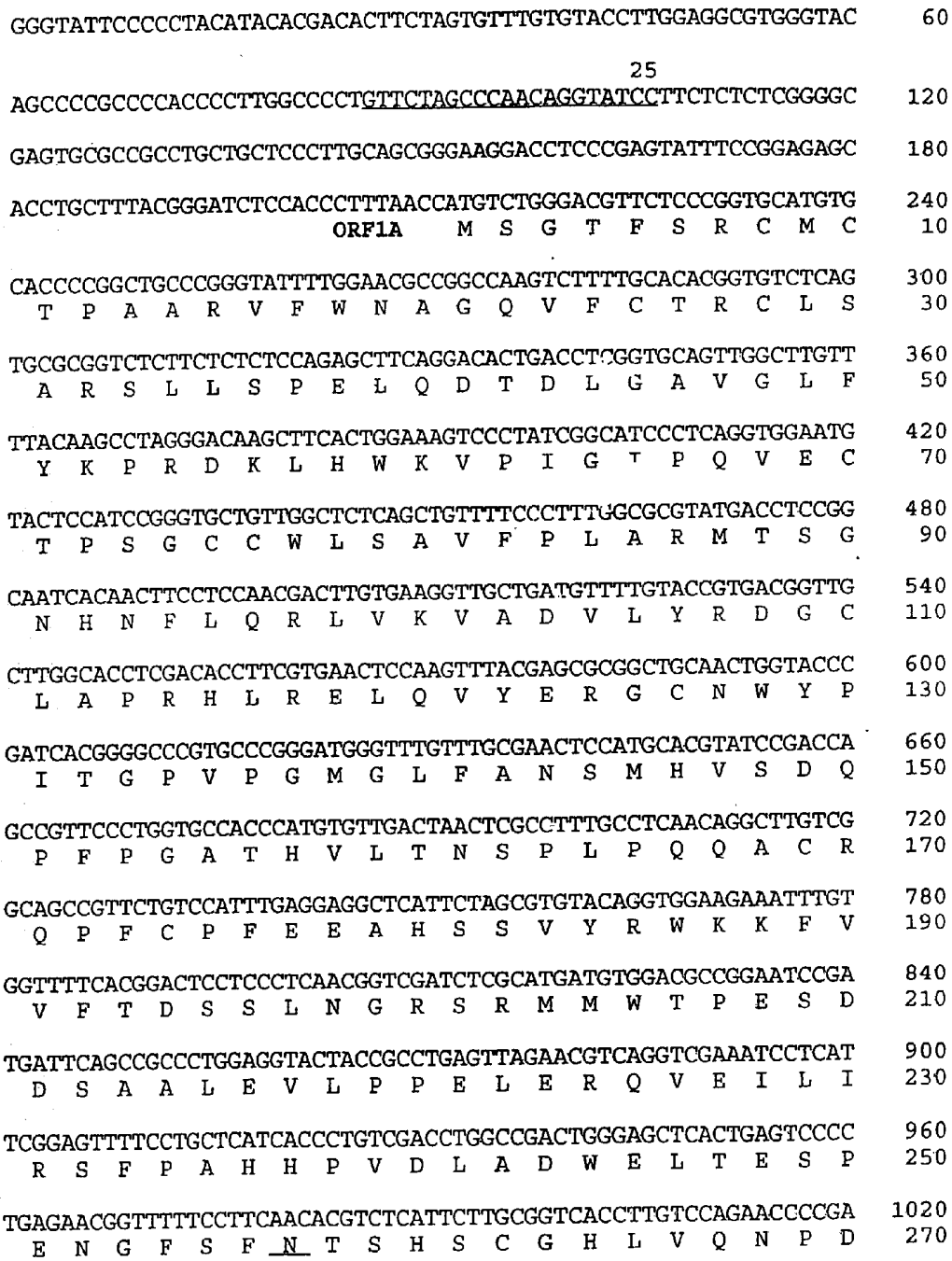
GGGTATTCCCCCTACATACACGACACTTCTAGTGTTTGTGTACCTTGGAGGCGTGGGTAC 60
25
AGCCCCGCCCCACCCCTTGGCCCCTGTTCTAGCCCAACAGGTATCCTTCTCTCTCGGGGC 120
GAGTGCGCCGCCTGCTGCTCCCTTGCAGCGGGAAGGACCTCCCGAGTATTTCCGGAGAGC 180
ACCTGCTTTACGGGATCTCCACCCTTTAACCATGTCTGGGACGTTCTCCCGGTGCATGTG 240
ORF1A M S G T F S R C M C 10
CACCCCGGCTGCCCGGGTATTTTGGAACGCCGGCCAAGTCTTTTGCACACGGTGTCTCAG 300
T P A A R V F W N A G Q V F C T R C L S 30
TGCGCGGTCTCTTCTCTCTCCAGAGCTTCAGGACACTGACCTCGGTGCAGTTGGCTTGTT 360
A R S L L S P E L Q D T D L G A V G L F 50
TTACAAGCCTAGGGACAAGCTTCACTGGAAAGTCCCTATCGGCATCCCTCAGGTGGAATG 420
Y K P R D K L H W K V P I G T P Q V E C 70
TACTCCATCCGGGTGCTGTTGGCTCTCAGCTGTTTTCCCTTTGGCGCGTATGACCTCCGG 480
T P S G C C W L S A V F P L A R M T S G 90
CAATCACAACTTCCTCCAACGACTTGTGAAGGTTGCTGATGTTTTGTACCGTGACGGTTG 540
N H N F L Q R L V K V A D V L Y R D G C 110
CTTGGCACCTCGACACCTTCGTGAACTCCAAGTTTACGAGCGCGGCTGCAACTGGTACCC 600
L A P R H L R E L Q V Y E R G C N W Y P 130
GATCACGGGGCCCGTGCCCGGGATGGGTTTGTTTGCGAACTCCATGCACGTATCCGACCA 660
I T G P V P G M G L F A N S M H V S D Q 150
GCCGTTCCCTGGTGCCACCCATGTGTTGACTAACTCGCCTTTGCCTCAACAGGCTTGTCG 720
P F P G A T H V L T N S P L P Q Q A C R 170
GCAGCCGTTCTGTCCATTTGAGGAGGCTCATTCTAGCGTGTACAGGTGGAAGAAATTTGT 780
Q P F C P F E E A H S S V Y R W K K F V 190
GGTTTTCACGGACTCCTCCCTCAACGGTCGATCTCGCATGATGTGGACGCCGGAATCCGA 840
V F T D S S L N G R S R M M W T P E S D 210
TGATTCAGCCGCCCTGGAGGTACTACCGCCTGAGTTAGAACGTCAGGTCGAAATCCTCAT 900
D S A A L E V L P P E L E R Q V E I L I 230
TCGGAGTTTTCCTGCTCATCACCCTGTCGACCTGGCCGACTGGGAGCTCACTGAGTCCCC 960
R S F P A H H P V D L A D W E L T E S P 250
TGAGAACGGTTTTTCCTTCAACACGTCTCATTCTTGCGGTCACCTTGTCCAGAACCCCGA 1020
E N G F S F N T S H S C G H L V Q N P D 270

FIG. 1b

```
CGTGTTTGATGGCAAGTGCTGGCTCTCCTGCTTTTTGGGCCAGTCGGTCGAAGTGCGCTG  1080
  V  F  D  G  K  C  W  L  S  C  F  L  G  Q  S  V  E  V  R  C   290

CCATGAGGAACATCTAGCTGACGCCTTCGGTTACCAAACCAAGTGGGGCGTGCATGGTAA  1140
  H  E  E  H  L  A  D  A  F  G  Y  Q  T  K  W  G  V  H  G  K   310

GTACCTCCAGCGCAGGCTTCAAGTTCGCGGCATTCGTGCTGTAGTCGATCCTGATGGTCC  1200
  Y  L  Q  R  R  L  Q  V  R  G  I  R  A  V  V  D  P  D  G  P   330

CATTCACGTTGAAGCGCTGTCTTGCCCCCAGTCTTGGATCAGGCACCTGACTCTGGATGA  1260
  I  H  V  E  A  L  S  C  P  Q  S  W  I  R  H  L  T  L  D  D   350

TGATGTCACCCCAGGATTCGTTCGCCTGACATCCCTTCGCATTGTGCCGAACACAGAGCC  1320
  D  V  T  P  G  F  V  R  L  T  S  L  R  I  V  P  N  T  E  P   370

TACCACTTCCCGGATCTTTCGGTTTGGAGCGCATAAGTGGTATGGCGCTGCCGGCAAACG  1380
  T  T  S  R  I  F  R  F  G  A  H  K  W  Y  G  A  A  G  K  R   390

GGCTCGTGCTAAGCGTGCCGCTAAAAGTGAGAAGGATTCGGCTCCCACCCCCAAGGTTGC  1440
  A  R  A  K  R  A  A  K  S  E  K  D  S  A  P  T  P  K  V  A   410

CCTGCCGGTCCCCACCTGTGGAATTACCACCTACTCTCCACCGACAGACGGGTCTTGTGG  1500
  L  P  V  P  T  C  G  I  T  T  Y  S  P  P  T  D  G  S  C  G   430

TTGGCATGTCCTTGCCGCCATAATGAACCGGATGATAAATGGTGACTTCACGTCCCCTCT  1560
  W  H  V  L  A  A  I  M  N  R  M  I  N  G  D  F  T  S  P  L   450

GACTCAGTACAACAGACCAGAGGATGATTGGGCTTCTGATTATGATCTTGTTCAGGCGAT  1620
  T  Q  Y  N  R  P  E  D  D  W  A  S  D  Y  D  L  V  Q  A  I   470

TCAATGTCTACGACTGCCTGCTACCGTGGTTCGGAATCGCGCCTGTCCTAACGCCAAGTA  1680
  Q  C  L  R  L  P  A  T  V  V  R  N  R  A  C  P  N  A  K  Y   490

CCTTATAAAACTTAACGGAGTTCACTGGGAGGTAGAGGTGAGGTCTGGAATGGCTCCTCG  1740
  L  I  K  L  N  G  V  H  W  E  V  E  V  R  S  G  M  A  P  R   510

CTCCCTTTCTCGTGAATGTGTGGTTGGCGTTTGCTCTGAAGGCTGTGTCGCACCGCCTTA  1800
  S  L  S  R  E  C  V  V  G  V  C  S  E  G  C  V  A  P  P  Y   530

TCCAGCAGACGGGCTACCTAAACGTGCACTCGAGGCCTTGGCGTCTGCTTACAGACTACC  1860
  P  A  D  G  L  P  K  R  A  L  E  A  L  A  S  A  Y  R  L  P   550

CTCCGATTGTGTTAGCTCTGGTATTGCTGACTTTCTTGCTAATCCACCTCCTCAGGAATT  1920
  S  D  C  V  S  S  G  I  A  D  F  L  A  N  P  P  P  Q  E  F   570

CTGGACCCTCGACAAAATGTTGACCTCCCCGTCACCAGAGCGGTCCGGCTTCTCTAGTTT  1980
  W  T  L  D  K  M  L  T  S  P  S  P  E  R  S  G  F  S  S  L   590
```

FIG. 1c

```
GTATAAATTACTATTAGAGGTTGTTCCGCAAAAATGCGGTGCCACGGAAGGGGCTTTCAT  2040
  Y  K  L  L  L  E  V  V  P  Q  K  C  G  A  T  E  G  A  F  I   610

CTATGCTGTTGAGAGGATGTTGAAGGATTGTCCGAGCTCCAAACAGGCCATGGCCCTTCT  2100
  Y  A  V  E  R  M  L  K  D  C  P  S  S  K  Q  A  M  A  L  L   630

GGCAAAAATTAAAGTTCCATCCTCAAAGGCCCCGTCTGTGTCCCTGGACGAGTGTTTCCC  2160
  A  K  I  K  V  P  S  S  K  A  P  S  V  S  L  D  E  C  F  P   650

TACGGATGTTTTAGCCGACTTCGAGCCAGCATCTCAGGAAAGGCCCCAAAGTTCCGGCGC  2220
  T  D  V  L  A  D  F  E  P  A  S  Q  E  R  P  Q  S  S  G  A   670

                                                  A
TGCTGTTGTCCTGTGTTCACCGGATGCAAAAGAGTTCGAGGAAGCAGCCCCGGAAGAAGT  2280
  A  V  V  L  C  S  P  D  A  K  E  F  E  E  A  A  P  E  E  V   690

TCAAGAGAGTGGCCACAAGGCCGTCCACTCTGCACTCCTTGCCGAGGGTCCTAACAATGA  2340
  Q  E  S  G  H  K  A  V  H  S  A  L  L  A  E  G  P  N  N  E   710

GCAGGTACAGGTGGTTGCCGGTGAGCAACTGAAGCTCGGCGGTTGTGGTTTGGCAGTCGG  2400
  Q  V  Q  V  V  A  G  E  Q  L  K  L  G  G  C  G  L  A  V  G   730

GAATGCTCATGAAGGTGCTCTGGTCTCAGCTGGTCTAATTAACCTGGTAGGCGGGAATTT  2460
  N  A  H  E  G  A  L  V  S  A  G  L  I  N  L  V  G  G  N  L   750

GTCCCCCTCAGACCCCATGAAAGAAAACATGCTCAATAGCCGGGAAGACGAACCACTGGA  2520
  S  P  S  D  P  M  K  E  N  M  L  N  S  R  E  D  E  P  L  D   770

TTTGTCCCAACCAGCACCAGCTTCCACAACGACCCTTGTGAGAGAGCAAACACCCGACAA  2580
  L  S  Q  P  A  P  A  S  T  T  T  L  V  R  E  Q  T  P  D  N   790

CCCAGGTTCTGATGCCGGTGCCCTCCCCGTCACCGTTCGAGAATTTGTCCCGACGGGGCC  2640
  P  G  S  D  A  G  A  L  P  V  T  V  R  E  F  V  P  T  G  P   810

TATACTCTGTCATGTTGAGCACTGCGGCACGGAGTCGGGCGACAGCAGTTCGCCTTTGGA  2700
  I  L  C  H  V  E  H  C  G  T  E  S  G  D  S  S  S  P  L  D   830

TCTATCTGATGCGCAAACCCTGGACCAGCCTTTAAATCTATCCCTGGCCGCTTGGCCAGT  2760
  L  S  D  A  Q  T  L  D  Q  P  L  N  L  S  L  A  A  W  P  V   850

GAGGGCCACCGCGTCTGACCCTGGCTGGGTCCACGGTAGGCGCGAGCCTGTCTTTGTAAA  2820
  R  A  T  A  S  D  P  G  W  V  H  G  R  R  E  P  V  F  V  K   870

GCCTCGAAATGCTTTCTCTGATGGCGATTCAGCCCTTCAGTTCGGGGAGCTTTCTGAATC  2880
  P  R  N  A  F  S  D  G  D  S  A  L  Q  F  G  E  L  S  E  S   890
```

FIG. 1d

```
CAGCTCTGTCATCGAGTTTGACCGGACAAAAGATGCTCCGGTGGTTGACGCCCCTGTCGA  2940
  S  S  V  I  E  F  D  R  T  K  D  A  P  V  V  D  A  P  V  D   910

CTTGACGACTTCGAACGAGGCCCTCTCTGTAGTCGATCCTTTCGAATTTGCCGAACTCAA  3000
  L  T  T  S  N  E  A  L  S  V  V  D  P  F  E  F  A  E  L  K   930

GCGCCCGCGTTTCTCCGCACAAGCCTTAATTGACCGAGGCGGTCCACTTGCCGATGTCCA  3060
  R  P  R  F  S  A  Q  A  L  I  D  R  G  G  P  L  A  D  V  H   950

TGCAAAAATAAAGAACCGGGTATATGAACAGTGCCTCCAAGCTTGTGAGCCCGGTAGTCG  3120
  A  K  I  K  N  R  V  Y  E  Q  C  L  Q  A  C  E  P  G  S  R   970

TGCAACCCCAGCCACCAGGGAGTGGCTCGACAAAATGTGGGATAGGGTGGACATGAAAAC  3180
  A  T  P  A  T  R  E  W  L  D  K  M  W  D  R  V  D  M  K  T   990

TTGGCGCTGCACCTCGCAGTTCCAAGCTGGTCGCATTCTTGCGTCCCTCAAATTCCTCCC  3240
  W  R  C  T  S  Q  F  Q  A  G  R  I  L  A  S  L  K  F  L  P  1010

TGACATGATTCAAGACACACCGCCTCCTGTTCCCAGGAAGAACCGAGCTAGTGACAATGC  3300
  D  M  I  Q  D  T  P  P  P  V  P  R  K  N  R  A  S  D  N  A  1030

CGGCCTGAAGCAACTGGTGGCACAGTGGGATAGGAAATTGAGTGTGACCCCCCCCCCAAA  3360
  G  L  K  Q  L  V  A  Q  W  D  R  K  L  S  V  T  P  P  P  K  1050

ACCGGTTGGGCCAGTGCTTGACCAGATCGTCCCTCCGCCTACGGATATCCAGCAAGAAGA  3420
  P  V  G  P  V  L  D  Q  I  V  P  P  P  T  D  I  Q  Q  E  D  1070

TGTCACCCCCTCCGATGGGCCACCCCATGCGCCGGATTTTCCTAGTCGAGTGAGCACGGG  3480
  V  T  P  S  D  G  P  P  H  A  P  D  F  P  S  R  V  S  T  G  1090

CGGGAGTTGGAAAGGCCTTATGCTTTCCGGCACCCGTCTCGCGGGGTCTATCAGCCAGCG  3540
  G  S  W  K  G  L  M  L  S  G  T  R  L  A  G  S  I  S  Q  R  1110

CCTTATGACATGGGTTTTTGAAGTTTTCTCCCACCTCCCAGCTTTTATGCTCACACTTTT  3600
  L  M  T  W  V  F  E  V  F  S  H  L  P  A  F  M  L  T  L  F  1130

CTCGCCGCGGGGCTCTATGGCTCCAGGTGATTGGTTGTTTGCAGGTGTCGTTTTACTTGC  3660
  S  P  R  G  S  M  A  P  G  D  W  L  F  A  G  V  V  L  L  A  1150

TCTCTTGCTCTGTCGTTCTTACCCGATACTCGGATGCCTTCCCTTATTGGGTGTCTTTTC  3720
  L  L  L  C  R  S  Y  P  I  L  G  C  L  P  L  L  G  V  F  S  1170

TGGTTCTTTGCGGCGTGTTCGTCTGGGTGTTTTTGGTTCTTGGATGGCTTTTGCTGTATT  3780
  G  S  L  R  R  V  R  L  G  V  F  G  S  W  M  A  F  A  V  F  1190

TTTATTCTCGACTCCATCCAACCCAGTCGGTTCTTCTTGTGACCACGATTCGCCGGAGTG  3840
  L  F  S  T  P  S  N  P  V  G  S  S  C  D  H  D  S  P  E  C  1210
```

FIG. 1e

```
TCATGCTGAGCTTTTGGCTCTTGAGCAGCGCCAACTTTGGGAACCTGTGCGCGGCCTTGT  3900
  H  A  E  L  L  A  L  E  Q  R  Q  L  W  E  P  V  R  G  L  V  1230

GGTCGGCCCCTCAGGCCTCTTATGTGTCATTCTTGGCAAGTTACTCGGTGGGTCACGTTA  3960
  V  G  P  S  G  L  L  C  V  I  L  G  K  L  L  G  G  S  R  Y  1250

TCTCTGGCATGTTCTCCTACGTTTATGCATGCTTGCAGATTTGGCCCTTTCTCTTGTTTA  4020
  L  W  H  V  L  L  R  L  C  M  L  A  D  L  A  L  S  L  V  Y  1270

TGTGGTGTCCCAGGGGCGTTGTCACAAGTGTTGGGGAAAGTGTATAAGGACAGCTCCTGC  4080
  V  V  S  Q  G  R  C  H  K  C  W  G  K  C  I  R  T  A  P  A  1290

GGAGGTGGCTCTTAATGTATTTCCTTTCTCGCGCGCCACCCGTGTCTCTCTTGTATCCTT  4140
  E  V  A  L  N  V  F  P  F  S  R  A  T  R  V  S  L  V  S  L  1310

GTGTGATCGATTCCAAACGCCAAAAGGGGTTGATCCTGTGCACTTGGCAACGGGTTGGCG  4200
  C  D  R  F  Q  T  P  K  G  V  D  P  V  H  L  A  T  G  W  R  1330

CGGGTGCTGGCGTGGTGAGAGCCCCATCCATCAACCACACCAAAAGCCCATAGCTTATGC  4260
  G  C  W  R  G  E  S  P  I  H  Q  P  H  Q  K  P  I  A  Y  A  1350

CAATTTGGATGAAAAGAAAATGTCTGCCCAAACGGTGGTTGCTGTCCCATACGATCCCAG  4320
  N  L  D  E  K  K  M  S  A  Q  T  V  V  A  V  P  Y  D  P  S  1370

TCAGGCTATCAAATGCCTGAAAGTTCTGCAGGCGGGAGGGGCCATCGTGGACCAGCCTAC  4380
  Q  A  I  K  C  L  K  V  L  Q  A  G  G  A  I  V  D  Q  P  T  1390

ACCTGAGGTCGTTCGTGTGTCCGAGATCCCCTTCTCAGCCCCATTTTTCCCAAAAGTTCC  4440
  P  E  V  V  R  V  S  E  I  P  F  S  A  P  F  F  P  K  V  P  1410

AGTCAACCCAGATTGCAGGGTTGTGGTAGATTCGGACACTTTTGTGGCTGCGGTTCGCTG  4500
  V  N  P  D  C  R  V  V  V  D  S  D  T  F  V  A  A  V  R  C  1430

                    C
CGGTTACTCGACAGCACAACTGGTTCTGGGCCGGGGCAACTTTGCCAAGTTAAATCAGAC  4560
  G  Y  S  T  A  Q  L  V  L  G  R  G  N  F  A  K  L  N  Q  T  1450

CCCCCCCAGGAACTCTATCTCCACCAAAACGACTGGTGGGGCCTCTTACACCCTTGCTGT  4620
  P  P  R  N  S  I  S  T  K  T  T  G  G  A  S  Y  T  L  A  V  1470

GGCTCAAGTGTCTGCGTGGACTCTTGTTCATTTCATCCTCGGTCTTTGGTTCACATCACC  4680
  A  Q  V  S  A  W  T  L  V  H  F  I  L  G  L  W  F  T  S  P  1490

TCAAGTGTGTGGCCGAGGAACCGCTGACCCATGGTGTTCAAATCCTTTTTCATATCCTAC  4740
  Q  V  C  G  R  G  T  A  D  P  W  C  S  N  P  F  S  Y  P  T  1510

CTATGGCCCCGGAGTTGTGTGCTCCTCTCGACTTTGTGTGTCTGCCGACGGGGTCACCCT  4800
  Y  G  P  G  V  V  C  S  S  R  L  C  V  S  A  D  G  V  T  L  1530
```

FIG. 1f

```
GCCATTGTTCTCAGCCGTGGCACAACTCTCCGGTAGAGAGGTGGGGATTTTTATTTTGGT  4860
  P  L  F  S  A  V  A  Q  L  S  G  R  E  V  G  I  F  I  L  V  1550

GCTCGTCTCCTTGACTGCTTTGGCCCACCGCATGGCTCTTAAGGCAGACATGTTAGTGGT  4920
  L  V  S  L  T  A  L  A  H  R  M  A  L  K  A  D  M  L  V  V  1570

CTTTTCGGCTTTTTGTGCTTACGCCTGGCCCATGAGCTCCTGGTTAATCTGCTTCTTTCC  4980
  F  S  A  F  C  A  Y  A  W  P  M  S  S  W  L  I  C  F  F  P  1590

TATACTCTTGAAGTGGGTTACCCTTCACCCTCTTACTATGCTTTGGGTGCACTCATTCTT  5040
  I  L  L  K  W  V  T  L  H  P  L  T  M  L  W  V  H  S  F  L  1610

GGTGTTTTGTCTGCCAGCAGCCGGCATCCTCTCACTAGGGATAACTGGCCTTCTTTGGGC  5100
  V  F  C  L  P  A  A  G  I  L  S  L  G  I  T  G  L  L  W  A  1630

AATTGGCCGCTTTACCCAGGTTGCCGGAATTATTACACCTTATGACATCCACCAGTACAC  5160
  I  G  R  F  T  Q  V  A  G  I  I  T  P  Y  D  I  H  Q  Y  T  1650

CTCTGGGCCACGTGGTGCAGCTGCTGTGGCCACAGCCCCAGAAGGCACTTATATGGCCGC  5220
  S  G  P  R  G  A  A  A  V  A  T  A  P  E  G  T  Y  M  A  A  1670

CGTCCGGAGAGCTGCTTTAACTGGGCGAACTTTAATCTTCACCCCGTCTGCAGTTGGATC  5280
  V  R  R  A  A  L  T  G  R  T  L  I  F  T  P  S  A  V  G  S  1690

CCTTCTCGAAGGTGCTTTCAGGACTCATAAACCCTGCCTTAACACCGTGAATGTTGTAGG  5340
  L  L  E  G  A  F  R  T  H  K  P  C  L  N  T  V  N  V  V  G  1710

CTCTTCCCTTGGTTCCGGAGGGGTTTTCACCATTGATGGCAGAAGAACTGTCGTCACTGC  5400
  S  S  L  G  S  G  G  V  F  T  I  D  G  R  R  T  V  V  T  A  1730

TGCCCATGTGTTGAACGGCGACACAGCTAGAGTCACCGGCGACTCCTACAACCGCATGCA  5460
  A  H  V  L  N  G  D  T  A  R  V  T  G  D  S  Y  N  R  M  H  1750

CACTTTCAAGACCAATGGTGATTATGCCTGGTCCCATGCTGATGACTGGCAGGGCGTTGC  5520
  T  F  K  T  N  G  D  Y  A  W  S  H  A  D  D  W  Q  G  V  A  1770

CCCTGTGGTCAAGGTTGCGAAGGGGTACCGCGGTCGTGCCTACTGGCAAACATCAACTGG  5580
  P  V  V  K  V  A  K  G  Y  R  G  R  A  Y  W  Q  T  S  T  G  1790

TGTCGAACCCGGTATCATTGGGGAAGGGTTCGCCTTCTGTTTTACTAACTGCGGCGATTC  5640
  V  E  P  G  I  I  G  E  G  F  A  F  C  F  T  N  C  G  D  S  1810

GGGGTCACCCGTCATCTCAGAATCTGGTGATCTTATTGGAATCCACACCGGTTCAAACAA  5700
  G  S  P  V  I  S  E  S  G  D  L  I  G  I  H  T  G  S  N  K  1830

ACTTGGTTCTGGTCTTGTGACAACCCCTGAAGGGGAGACCTGCACCATCAAAGAAACCAA  5760
  L  G  S  G  L  V  T  T  P  E  G  E  T  C  T  I  K  E  T  K  1850
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FIG. 1g

```
GCTCTCTGACCTTTCCAGACATTTTGCAGGCCCAAGCGTTCCTCTTGGGGACATTAAATT  5820
  L  S  D  L  S  R  H  F  A  G  P  S  V  P  L  G  D  I  K  L  1870

GAGTCCGGCCATCATCCCTGATGTAACATCCATTCCGAGTGACTTGGCATCGCTCCTAGC  5880
  S  P  A  I  I  P  D  V  T  S  I  P  S  D  L  A  S  L  L  A  1890

CTCCGTCCCTGTAGTGGAAGGCGGCCTCTCGACCGTTCAACTTTTGTGTGTCTTTTTCCT  5940
  S  V  P  V  V  E  G  G  L  S  T  V  Q  L  L  C  V  F  F  L  1910

TCTCTGGCGCATGATGGGCCATGCCTGGACACCCATTGTTGCCGTGGGCTTCTTTTTGCT  6000
  L  W  R  M  M  G  H  A  W  T  P  I  V  A  V  G  F  F  L  L  1930

GAATGAAATTCTTCCAGCAGTTTTGGTCCGAGCCGTGTTTTCTTTTGCACTCTTTGTGCT  6060
  N  E  I  L  P  A  V  L  V  R  A  V  F  S  F  A  L  F  V  L  1950

TGCATGGGCCACCCCCTGGTCTGCACAGGTGTTGATGATTAGACTCCTCACGGCATCTCT  6120
  A  W  A  T  P  W  S  A  Q  V  L  M  I  R  L  L  T  A  S  L  1970

CAACCGCAACAAGCTTTCTCTGGCGTTCTACGCACTCGGGGGTGTCGTCGGTTTGGCAGC  6180
  N  R  N  K  L  S  L  A  F  Y  A  L  G  G  V  V  G  L  A  A  1990

TGAAATCGGGACTTTTGCTGGCAGATTGTCTGAATTGTCTCAAGCTCTTTCGACATACTG  6240
  E  I  G  T  F  A  G  R  L  S  E  L  S  Q  A  L  S  T  Y  C  2010

CTTCTTACCTAGGGTCCTTGCTATGACCAGTTGTGTTCCCACCATCATCATTGGTGGACT  6300
  F  L  P  R  V  L  A  M  T  S  C  V  P  T  I  I  I  G  G  L  2030

                        G
CCATACCCTCGGTGTGATTCTGTGGTTATTCAAATACCGGTGCCTCCACAACATGCTGGT  6360
  H  T  L  G  V  I  L  W  L  F  K  Y  R  C  L  H  N  M  L  V  2050

TGGTGATGGGAGTTTTTCAAGCGCCTTCTTCCTACGGTATTTTGCAGAGGGTAATCTCAG  6420
  G  D  G  S  F  S  S  A  F  F  L  R  Y  F  A  E  G  N  L  R  2070

AAAAGGTGTTTCACAGTCCTGTGGCATGAATAACGAGTCCCTAACGGCTGCTTTAGCTTG  6480
  K  G  V  S  Q  S  C  G  M  N  N  E  S  L  T  A  A  L  A  C  2090

CAAGTTGTCACAGGCTGACCTTGATTTTTTGTCCAGCTTAACGAACTTCAAGTGCTTTGT  6540
  K  L  S  Q  A  D  L  D  F  L  S  S  L  T  N  F  K  C  F  V  2110

ATCTGCTTCAAACATGAAAAATGCTGCCGGCCAGTACATTGAAGCAGCGTATGCCAAGGC  6600
  S  A  S  N  M  K  N  A  A  G  Q  Y  I  E  A  A  Y  A  K  A  2130

CCTGCGCCAAGAGTTGGCCTCTCTAGTTCAGATTGACAAAATGAAAGGAGTTTTGTCCAA  6660
  L  R  Q  E  L  A  S  L  V  Q  I  D  K  M  K  G  V  L  S  K  2150
```

FIG. 1h

```
GCTCGAGGCCTTTGCTGAAACAGCCACCCCGTCCCTTGACATAGGTGACGTGATTGTTCT  6720
  L  E  A  F  A  E  T  A  T  P  S  L  D  I  G  D  V  I  V  L  2170

GCTTGGGCAACATCCTCACGGATCCATCCTCGATATTAATGTGGGGACTGAAAGGAAAAC  6780
  L  G  Q  H  P  H  G  S  I  L  D  I  N  V  G  T  E  R  K  T  2190

TGTGTCCGTGCAAGAGACCCGGAGCCTAGGCGGCTCCAAATTCAGTGTTTGTACTGTCGT  6840
  V  S  V  Q  E  T  R  S  L  G  G  S  K  F  S  V  C  T  V  V  2210

                          A
GTCCAACACACCCGTGGACGCCTTGACCGGCATCCCACTCCAGACACCAACCCCTCTTTT  6900
  S  N  T  P  V  D  A  L  T  G  I  P  L  Q  T  P  T  P  L  F  2230

TGAGAATGGTCCGCGTCATCGCAGCGAGGAAGACGATCTTAAAGTCGAGAGGATGAAGAA  6960
  E  N  G  P  R  H  R  S  E  E  D  D  L  K  V  E  R  M  K  K  2250

ACACTGTGTATCCCTCGGCTTCCACAACATCAATGGCAAAGTTTACTGCAAAATTTGGGA  7020
  H  C  V  S  L  G  F  H  N  I  N  G  K  V  Y  C  K  I  W  D  2270

CAAGTCTACCGGTGACACCTTTTACACGGATGATTCCCGGTACACCCAAGACCATGCTTT  7080
  K  S  T  G  D  T  F  Y  T  D  D  S  R  Y  T  Q  D  H  A  F  2290

TCAGGACAGGTCAGCCGACTACAGAGACAGGGACTATGAGGGTGTGCAAACCACCCCCCA  7140
  Q  D  R  S  A  D  Y  R  D  R  D  S  E  T  P  V  G  T  V  V  2310

ACAGGGATTTGATCCAAAGTCTGAAACCCCTGTTGGCACTGTTGTGATCGGCGGTATTAC  7200
  I  G  G  I  T  Y  Y  E  G  V  Q  T  T  P  Q  Q  G  F  D  P  2330

GTATAACAGGTATCTGATCAAAGGTAAGGAGGTTCTGGTCCCCAAGCCTGACAACTGCCT  7260
  K  N  R  Y  L  I  K  G  K  E  V  L  V  P  K  P  D  N  C  L  2350

TGAAGCTGCCAAGCTGTCCCTTGAGCAAGCTCTCGCTGGGATGGGCCAAACTTGCGACCT  7320
  E  A  A  K  L  S  L  E  Q  A  L  A  G  M  G  Q  T  C  D  L  2370

TACAGCTGCCGAGGTGGAAAAGCTAAAGCGCATCATTAGTCAACTCCAAGGTTTGACCAC  7380
  T  A  A  E  V  E  K  L  K  R  I  I  S  Q  L  Q  G  L  T  T  2390
                                                    ORF1B

TGAACAGGCTTTAAACTGTTAGCCGCCAGCGGCTTGACCCGCTGTGGCCGCGGCGGCCTA  7440
  E  Q  A  L  N  C  -                                          2396
  -  T  G  F  K  L  L  A  A  S  G  L  T  R  C  G  R  G  G  L    19

GTTGTGACTGAAACGGCGGTAAAAATTATAAAATACCACAGCAGAACTTTCACCTTAGGC  7500
  V  V  T  E  T  A  V  K  I  I  K  Y  H  S  R  T  F  T  L  G    39

CCTTTAGACCTAAAAGTCACTTCCGAGGTGGAGGTAAAGAAATCAACTGAGCAGGGCCAC  7560
  P  L  D  L  K  V  T  S  E  V  E  V  K  K  S  T  E  Q  G  H    59
```

FIG. 1i

```
GCTGTTGTGGCAAACTTATGTTCCGGTGTCATCTTGATGAGACCTCACCCACCGTCCCTT  7620
 A  V  V  A  N  L  C  S  G  V  I  L  M  R  P  H  P  P  S  L    79

GTCGACGTTCTTCTGAAACCCGGACTTGACACAATACCCGGCATTCAACCAGGGCATGGG  7680
 V  D  V  L  L  K  P  G  L  D  T  I  P  G  I  Q  P  G  H  G    99

GCCGGGAATATGGGCGTGGACGGTTCTATTTGGGATTTTGAAACCGCACCCACAAAGGCA  7740
 A  G  N  M  G  V  D  G  S  I  W  D  F  E  T  A  P  T  K  A   119

GAACTCGAGTTATCCAAGCAAATAATCCAAGCATGTGAAGTTAGGCGCGGGGACGCCCCG  7800
 E  L  E  L  S  K  Q  I  I  Q  A  C  E  V  R  R  G  D  A  P   139

AACCTCCAACTCCCTTACAAGCTCTATCCTGTTAGGGGGGATCCTGAGCGGCATAAAGGC  7860
 N  L  Q  L  P  Y  K  L  Y  P  V  R  G  D  P  E  R  H  K  G   159

CGCCTTATCAATACCAGGTTTGGAGATTTACCTTACAAAACTCCTCAAGACACCAAGTCC  7920
 R  L  I  N  T  R  F  G  D  L  P  Y  K  T  P  Q  D  T  K  S   179

GCAATCCACGCGGCTTGTTGCCTGCACCCCAACGGGGCCCCCGTGTCTGATGGTAAATCC  7980
 A  I  H  A  A  C  C  L  H  P  N  G  A  P  V  S  D  G  K  S   199

ACACTAGGTACCACTCTTCAACATGGTTTCGAGCTTTATGTCCCTACTGTGCCCTATAGT  8040
 T  L  G  T  T  L  Q  H  G  F  E  L  Y  V  P  T  V  P  Y  S   219

GTCATGGAGTACCTTGATTCACGCCCTGACACCCCTTTTATGTGTACTAAACATGGCACT  8100
 V  M  E  Y  L  D  S  R  P  D  T  P  F  M  C  T  K  H  G  T   239

TCCAAGGCTGCTGCAGAGGACCTCCAAAAATACGACCTATCCACCCAAGGATTTGTCCTG  8160
 S  K  F  V  L  P  G  V  L  R  L  V  R  R  F  I  F  A  A  A   259

CCTGGGGTCCTACGCCTAGTACGCAGATTCATCTTTGGCCATATTGGTAAGGCGCCGCCA  8220
 E  D  L  Q  K  Y  D  L  S  T  Q  G  G  H  I  G  K  A  P  P   279

TTGTTCCTCCCATCAACCTATCCCGCCAAGAACTCTATGGCAGGGATCAATGGCCAGAGG  8280
 L  F  L  P  S  T  Y  P  A  K  N  S  M  A  G  I  N  G  Q  R   299

TTCCCAACAAAGGACGTTCAGAGCATACCTGAAATTGATGAAATGTGTGCCCGCGCTGTC  8340
 F  P  T  K  D  V  Q  S  I  P  E  I  D  E  M  C  A  R  A  V   319

AAGGAGAATTGGCAAACTGTGACACCTTGCACCCTCAAGAAACAGTACTGTTCCAAGCCC  8400
 K  E  N  W  Q  T  V  T  P  C  T  L  K  K  Q  Y  C  S  K  P   339

AAAACCAGGACCATCCTGGGCACCAACAACTTTATTGCCTTGGCTCACAGATCGGCGCTC  8460
 K  T  R  T  I  L  G  T  N  N  F  I  A  L  A  H  R  S  A  L   359

AGTGGTGTCACCCAGGCATTCATGAAGAAGGCTTGGAAGTCCCCAATTGCCTTGGGGAAA  8520
 S  G  V  T  Q  A  F  M  K  K  A  W  K  S  P  I  A  L  G  K   379
```

FIG. 1j

```
AACAAATTCAAGGAGCTGCATTGCACTGTCGCCGGCAGGTGTCTTGAGGCCGACTTGGCC  8580
 N  K  F  K  E  L  H  C  T  V  A  G  R  C  L  E  A  D  L  A    399

TCCTGTGACCGCAGCACCCCCGCCATTGTAAGATGGTTTGTTGCCAACCTCCTGTATGAA  8640
 S  C  D  R  S  T  P  A  I  V  R  W  F  V  A  N  L  L  Y  E    419

CTTGCAGGATGTGAAGAGTACTTGCCTAGCTATGTGCTTAATTGCTGCCATGACCTCGTG  8700
 L  A  G  C  E  E  Y  L  P  S  Y  V  L  N  C  C  H  D  L  V    439

GCAACACAGGATGGTGCCTTCACAAAACGCGGTGGCCTGTCGTCCGGGGACCCCGTCACC  8760
 A  T  Q  D  G  A  F  T  K  R  G  G  L  S  S  G  D  P  V  T    459

AGTGTGTCCAACACCGTATATTCACTGGTAATTTATGCCCAGCACATGGTATTGTCGGCC  8820
 S  V  S  N  T  V  Y  S  L  V  I  Y  A  Q  H  M  V  L  S  A    479

TTGAAAATGGGTCATGAAATTGGTCTTAAGTTCCTCGAGGAACAGCTCAAGTTCGAGGAC  8880
 L  K  M  G  H  E  I  G  L  K  F  L  E  E  Q  L  K  F  E  D    499

CTCCTTGAAATTCAGCCTATGTTGGTATACTCTGATGATCTTGTCTTGTACGCTGAAAGA  8940
 L  L  E  I  Q  P  M  L  V  Y  S  D  D  L  V  L  Y  A  E  R    519

    C
CCCACATTTCCCAATTACCACTGGTGGGTCGAGCACCTTGACCTGATGCTGGGTTTCAGA  9000
 P  T  F  P  N  Y  H  W  W  V  E  H  L  D  L  M  L  G  F  R    539

ACGGACCCAAAGAAAACCGTCATAACTGATAAACCCAGCTTCCTCGGCTGCAGAATTGAG  9060
 T  D  P  K  K  T  V  I  T  D  K  P  S  F  L  G  C  R  I  E    559

GCAGGGCGACAGCTAGTCCCCAATCGCGACCGCATCCTGGCTGCTCTTGCATATCACATG  9120
 A  G  R  Q  L  V  P  N  R  D  R  I  L  A  A  L  A  Y  H  M    579

AAGGCGCAGAACGCCTCAGAGTATTATGCGTCTGCTGCCGCAATCCTGATGGATTCATGT  9180
 K  A  Q  N  A  S  E  Y  Y  A  S  A  A  A  I  L  M  D  S  C    599

GCTTGCATTGACCATGACCCTGAGTGGTATGAGGACCTCATCTGCGGTATTGCCCGGTGC  9240
 A  C  I  D  H  D  P  E  W  Y  E  D  L  I  C  G  I  A  R  C    619

GCCCGCCAGGATGGTTATAGCTTCCCAGGTCCGGCATTTTTCATGTCCATGTGGGAGAAG  9300
 A  R  Q  D  G  Y  S  F  P  G  P  A  F  F  M  S  M  W  E  K    639

CTGAGAAGTCATAATGAAGGGAAGAAATTCCGCCACTGCGGCATCTGCGACGCCAAAGCC  9360
 L  R  S  H  N  E  G  K  K  F  R  H  C  G  I  C  D  A  K  A    659

GACTATGCGTCCGCCTGTGGGCTTGATTTGTGTTTGTTCCATTCGCACTTTCATCAACAC  9420
 D  Y  A  S  A  C  G  L  D  L  C  L  F  H  S  H  F  H  Q  H    679
```

FIG. 1k

```
      C
TGCCCTGTCACTCTGAGCTGCGGTCACCATGCCGGTTCAAAGGAATGTTCGCAGTGTCAG   9480
 C  P  V  T  L  S  C  G  H  H  A  G  S  K  E  C  S  Q  C  Q     699

TCACCTGTTGGGGCTGGCAGATCCCCTCTTGATGCCGTGCTAAAACAAATTCCATACAAA   9540
 S  P  V  G  A  G  R  S  P  L  D  A  V  L  K  Q  I  P  Y  K     719

CCTCCTCGTACTGTCATCATGAAGGTGGGTAATAAAACAACGGCCCTCGATCCGGGGAGG   9600
 P  P  R  T  V  I  M  K  V  G  N  K  T  T  A  L  D  P  G  R     739

TACCAGTCCCGTCGAGGTCTCGTTGCAGTCAAGAGGGGTATTGCAGGCAATGAAGTTGAT   9660
 Y  Q  S  R  R  G  L  V  A  V  K  R  G  I  A  G  N  E  V  D     759

         A
CTTTCTGATGGGGACTACCAAGTGGTGCCTCTTTTGCCGACTTGCAAAGACATAAACATG   9720
 L  S  D  G  D  Y  Q  V  V  P  L  L  P  T  C  K  D  I  N  M     779

GTGAAGGTGGCTTGCAATGTACTACTCAGCAAGTTCATAGTAGGGCCACCAGGTTCCGGA   9780
 V  K  V  A  C  N  V  L  L  S  K  F  I  V  G  P  P  G  S  G     799

                                          T
AAGACCACCTGGCTACTGAGTCAAGTCCAGGACGATGATGTCATTTACACACCCACCCAT   9840
 K  T  T  W  L  L  S  Q  V  Q  D  D  D  V  I  Y  T  P  T  H     819
                                             I

CAGACTATGTTTGATATAGTCAGTGCTCTCAAAGTTTGCAGGTATTCCATTCCAGGAGCC   9900
 Q  T  M  F  D  I  V  S  A  L  K  V  C  R  Y  S  I  P  G  A     839

TCAGGACTCCCTTTCCCACCACCTGCCAGGTCCGGGCCGTGGGTTAGGCTTATTGCCAGC   9960
 S  G  L  P  F  P  P  P  A  R  S  G  P  W  V  R  L  I  A  S     859

GGGCACGTCCCTGGCCGAGTATCATACCTCGATGAGGCTGGATATTGTAATCATCTGGAC  10020
 G  H  V  P  G  R  V  S  Y  L  D  E  A  G  Y  C  N  H  L  D     879

ATTCTTAGACTGCTTTCCAAAACACCCCTTGTGTGTTTGGGTGACCTTCAGCAACTTCAC  10080
 I  L  R  L  L  S  K  T  P  L  V  C  L  G  D  L  Q  Q  L  H     899

CCTGTCGGCTTTGATTCCTACTGTTATGTGTTCGATCAGATGCCTCAGAAGCAGCTGACC  10140
 P  V  G  F  D  S  Y  C  Y  V  F  D  Q  M  P  Q  K  Q  L  T     919

ACTATTTACAGATTTGGCCCTAACATCTGCGCACGCATCCAGCCTTGTTACAGGGAGAAA  10200
 T  I  Y  R  F  G  P  N  I  C  A  R  I  Q  P  C  Y  R  E  K     939

CTTGAATCTAAGGCTAGGAACACTAGGGTGGTTTTTACCACCCGGCCTGTGGCCTTTGGT  10260
 L  E  S  K  A  R  N  T  R  V  V  F  T  T  R  P  V  A  F  G     959

CAGGTGCTGACACCATACCATAAAGATCGCATCGGCTCTGCGATAACCATAGATTCATCC  10320
 Q  V  L  T  P  Y  H  K  D  R  I  G  S  A  I  T  I  D  S  S     979
```

FIG. 1l

```
CAGGGGGCCACCTTTGATATTGTGACATTGCATCTACCATCGCCAAAGTCCCTAAATAAA 10380
 Q  G  A  T  F  D  I  V  T  L  H  L  P  S  P  K  S  L  N  K   999

TCCCGAGCACTTGTAGCCATCACTCGGGCAAGACACGGGTTGTTCATTTATGACCCTCAT 10440
 S  R  A  L  V  A  I  T  R  A  R  H  G  L  F  I  Y  D  P  H  1019

AACCAGCTCCAGGAGTTTTTCAACTTAACCCCTGAGCGCACTGATTGTAACCTTGTGTTC 10500
 N  Q  L  Q  E  F  F  N  L  T  P  E  R  T  D  C  N  L  V  F  1039

AGCCGTGGGGATGAGCTGGTAGTTCTGAATGCGGATAATGCAGTCACAACTGTAGCGAAG 10560
 S  R  G  D  E  L  V  V  L  N  A  D  N  A  V  T  T  V  A  K  1059

GCCCTTGAGACAGGTCCATCTCGATTTCGAGTATCAGACCCGAGGTGCAAGTCTCTCTTA 10620
 A  L  E  T  G  P  S  R  F  R  V  S  D  P  R  C  K  S  L  L  1079

GCCGCTTGTTCGGCCAGTCTGGAAGGGAGCTGTATGCCACTACCGCAAGTGGCACATAAC 10680
 A  A  C  S  A  S  L  E  G  S  C  M  P  L  P  Q  V  A  H  N  1099

CTGGGGTTTTACTTTTCCCCGGACAGTCCAACATTTGCACCTCTGCCAAAAGAGTTGGCG 10740
 L  G  F  Y  F  S  P  D  S  P  T  F  A  P  L  P  K  E  L  A  1119

CCACATTGGCCAGTGGTTACCCACCAGAATAATCGGGCGTGGCCTGATCGACTTGTCGCT 10800
 P  H  W  P  V  V  T  H  Q  N  N  R  A  W  P  D  R  L  V  A  1139

AGTATGCGCCCAATTGATGCCCGCTACAGCAAGCCAATGGTCGGTGCAGGGTATGTGGTC 10860
 S  M  R  P  I  D  A  R  Y  S  K  P  M  V  G  A  G  Y  V  V  1159

GGGCCGTCCACCTTTCTTGGTACTCCTGGTGTGGTGTCATACTATCTCACACTATACATC 10920
 G  P  S  T  F  L  G  T  P  G  V  V  S  Y  Y  L  T  L  Y  I  1179

AGGGGTGAGCCCCAGGCCTTGCCAGAAACACTCGTTTCAACAGGGCGTATAGCCACAGAT 10980
 R  G  E  P  Q  A  L  P  E  T  L  V  S  T  G  R  I  A  T  D  1199

TGTCGGGAGTATCTCGACGCGGCTGAGGAAGAGGCAGCAAAAGAACTCCCCCACGCATTC 11040
 C  R  E  Y  L  D  A  A  E  E  E  A  A  K  E  L  P  H  A  F  1219

ATTGGCGATGTCAAAGGTACCACGGTTGGGGGGTGTCATCACATTACATCAAAATACCTA 11100
 I  G  D  V  K  G  T  T  V  G  G  C  H  H  I  T  S  K  Y  L  1239

CCTAGGTCCCTGCCTAAGGACTCTGTTGCCGTAGTTGGAGTAAGTTCGCCCGGCAGGGCT 11160
 P  R  S  L  P  K  D  S  V  A  V  V  G  V  S  S  P  G  R  A  1259

GCTAAAGCCGTGTGCACTCTCACCGATGTGTACCTCCCCGAACTCCGGCCATATCTGCAA 11220
 A  K  A  V  C  T  L  T  D  V  Y  L  P  E  L  R  P  Y  L  Q  1279

CCTGAGACGGCATCAAAATGCTGGAAACTCAAATTAGACTTCAGGGACGTCCGACTAATG 11280
 P  E  T  A  S  K  C  W  K  L  K  L  D  F  R  D  V  R  L  M  1299
```

FIG. 1m

```
GTCTGGAAAGGAGCCACCGCCTATTTCCAGTTGGAAGGGCTTACATGGTCGGCGCTGCCC 11340
 V  W  K  G  A  T  A  Y  F  Q  L  E  G  L  T  W  S  A  L  P   1319

              C
GACTATGCCAGGTTTATTCAGCTGCCCAAGGATGCCGTTGTATACATTGATCCGTGTATA 11400
 D  Y  A  R  F  I  Q  L  P  K  D  A  V  V  Y  I  D  P  C  I   1339

GGACCGGCAACAGCCAACCGTAAGGTCGTGCGAACCACAGACTGGCGGGCCGACCTGGCA 11460
 G  P  A  T  A  N  R  K  V  V  R  T  T  D  W  R  A  D  L  A   1359

GTGACACCGTATGATTACGGTGCCCAGAACATTTTGACAACAGCCTGGTTCGAGGACCTC 11520
 V  T  P  Y  D  Y  G  A  Q  N  I  L  T  T  A  W  F  E  D  L   1379

GGGCCGCAGTGGAAGATTTTGGGGTTGCAGCCCTTTAGGCGAGCATTTGGCTTTGAAAAC 11580
 G  P  Q  W  K  I  L  G  L  Q  P  F  R  R  A  F  G  F  E  N   1399

ACTGAGGATTGGGCAATCCTTGCACGCCGTATGAATGACGGCAAGGACTACACTGACTAT 11640
 T  E  D  W  A  I  L  A  R  R  M  N  D  G  K  D  Y  T  D  Y   1419

AACTGGAACTGTGTTCGAGAACGCCCACACGCCATCTACGGGCGTGCTCGTGACCATACG 11700
 N  W  N  C  V  R  E  R  P  H  A  I  Y  G  R  A  R  D  H  T   1439

TATCATTTTGCCCCTGGCACAGAATTGCAGGTAGAGCTAGGTAAACCCCGGCTGCCGCCT 11760
 Y  H  F  A  P  G  T  E  L  Q  V  E  L  G  K  P  R  L  P  P   1459

GGGCAAGTGCCGTGAATTCGGGGGTGATGCAATGGGGTCACTGTGGAGTAAAATCAGCCAG 11820
 G  Q  V  P  -                                                 1463
             ORF2        M  Q  W  G  H  C  G  V  K  S  A  S     12

                                                   T
CTGTTCGTGGACGCCTTCACTGAGTTCCTTGTTAGTGTGGTTGATATTGCCATTTTCCTT 11880
  C  S  W  T  P  S  L  S  S  L  L  V  W  L  I  L  P  F  S  L   32
                                                   S

GCCATACTGTTTGGGTTCACCGTCGCAGGATGGTTACTGGTCTTTCTTCTCAGAGTGGTT 11940
  P  Y  C  L  G  S  P  S  Q  D  G  Y  W  S  F  F  S  E  W  F   52

TGCTCCGCGCTTCTCCGTTCGCGCTCTGCCATTCACTCTCCCGAACTATCGAAGGTCCTA 12000
  A  P  R  F  S  V  R  A  L  P  F  T  L  P  N  Y  R  R  S  Y   72

TGAAGGCTTGTTGCCCAACTGCAGACCGGATGTCCCACAATTTGCAGTCAAGCACCCATT 12060
  E  G  L  L  P  N  C  R  P  D  V  P  Q  F  A  V  K  H  P  L   92

 C                                          G
GGGTATGTTTTGGCACATGCGAGTTTCCCACTTGATTGATGAGATGGTCTCTCGTCGCAT 12120
  G  M  F  W  H  M  R  V  S  H  L  I  D  E  M  V  S  R  R  I   112
                                             V
```

FIG. 1n

```
TTACCAGACCATGGAACATTCAGGTCAAGCGGCCTGGAAGCAGGTGGTTGGTGAGGCCAC 12180
  Y  Q  T  M  E  H  S  G  Q  A  A  W  K  Q  V  V  G  E  A  T   132

TCTCACGAAGCTGTCAGGGCTCGATATAGTTACTCATTTCCAACACCTGGCCGCAGTGGA 12240
  L  T  K  L  S  G  L  D  I  V  T  H  F  Q  H  L  A  A  V  E   152

GGCGGATTCTTGCCGCTTTCTCAGCTCACGACTCGTGATGCTAAAAAATCTTGCCGTTGG 12300
  A  D  S  C  R  F  L  S  S  R  L  V  M  L  K  N  L  A  V  G   172

CAATGTGAGCCTACAGTACAACACCACGTTGGACCGCGTTGAGCTCATCTTCCCCACGCC 12360
  N  V  S  L  Q  Y  N  T  T  L  D  R  V  E  L  I  F  P  T  P   192

AGGTACGAGGCCCAAGTTGACCGATTTCAGACAATGGCTCATCAGTGTGCACGCTTCCAT 12420
  G  T  R  P  K  L  T  D  F  R  Q  W  L  I  S  V  H  A  S  I   212
                          ORF3  M  A  H  Q  C  A  R  F  H       9

TTTTTCCTCTGTGGCTTCATCTGTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGC 12480
  F  S  S  V  A  S  S  V  T  L  F  I  V  L  W  L  R  I  P  A   232
  F  F  L  C  G  F  I  C  Y  L  V  H  S  A  L  A  S  N  S  S    29

TCTACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACACATCATTCGAGCTGACCATC 12540
  L  R  Y  V  F  G  F  H  W  P  T  A  T  H  H  S  S  -         249
  S  T  L  C  F  W  F  P  L  A  H  G  N  T  S  F  E  L  T  I    49

AACTACACCATATGCATGCCCTGTTCTACCAGTCAAGCGGCTCGCCAAAGGCTCGAGCCC 12600
  N  Y  T  I  C  M  P  C  S  T  S  Q  A  A  R  Q  R  L  E  P    69

GGTCGTAACATGTGGTGCAAAATAGGGCATGACAGGTGTGAGGAGCGTGACCATGATGAG 12660
  G  R  N  M  W  C  K  I  G  H  D  R  C  E  E  R  D  H  D  E    89

TTGTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGG 12720
  L  L  M  S  I  P  S  G  Y  D  N  L  K  L  E  G  Y  Y  A  W   109

CTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGG 12780
  L  A  F  L  S  F  S  Y  A  A  Q  F  H  P  E  L  F  G  I  G   129

AATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGATGGA 12840
  N  V  S  R  V  F  V  D  K  R  H  Q  F  I  C  A  E  H  D  G   149

CACAATTCAACCGTATCTACCGGACACAACATCTCCGCATTATATGCGGCATATTACCAC 12900
  H  N  S  T  V  S  T  G  H  N  I  S  A  L  Y  A  A  Y  Y  H   169

CACCAAATAGACGGGGGCAATTGGTTCCATTTGGAATGGCTGCGGCCACTCTTTTCTTCC 12960
  H  Q  I  D  G  G  N  W  F  H  L  E  W  L  R  P  L  F  S  S   189
                          ORF4  M  A  A  A  T  L  F  F           8
```

FIG. 1o

```
TGGCTGGTGCTCAACATATCATGGTTTCTGAGGCGTTCGCCTGTAAGCCCTGTTTCTCGA 13020
 W  L  V  L  N  I  S  W  F  L  R  R  S  P  V  S  P  V  S  R    209
L  A  G  A  Q  H  I  M  V  S  E  A  F  A  C  K  P  C  F  S     28

CGCATCTATCAGATATTGAGACCAACACGACCGCGGCTGCCGGTTTCATGGTCCTTCAGG 13080
 R  I  Y  Q  I  L  R  P  T  R  P  R  L  P  V  S  W  S  F  R    229
T  H  L  S  D  I  E  T  N  T  T  A  A  A  G  F  M  V  L  Q     48

ACATCAATTGTTTCCGACCTCACGGGGTCTCAGCAGCGCAAGAGAAAATTTCCTTCGGAA 13140
 T  S  I  V  S  D  L  T  G  S  Q  Q  R  K  R  K  F  P  S  E    249
D  I  N  C  F  R  P  H  G  V  S  A  A  Q  E  K  I  S  F  G     68

AGTCGTCCCAATGTCGTGAAGCCGTCGGTACTCCCCAGTACATCACGATAACGGCTAACG 13200
 S  R  P  N  V  V  K  P  S  V  L  P  S  T  S  R  -             265
K  S  S  Q  C  R  E  A  V  G  T  P  Q  Y  I  T  I  T  A  N     88

TGACCGACGAATCATACTTGTACAACGCGGACCTGCTGATGCTTTCTGCGTGCCTTTTCT 13260
V  T  D  E  S  Y  L  Y  N  A  D  L  L  M  L  S  A  C  L  F    108

ACGCCTCAGAAATGAGCGAGAAAGGCTTCAAAGTCATCTTTGGGAATGTCTCTGGCGTTG 13320
Y  A  S  E  M  S  E  K  G  F  K  V  I  F  G  N  V  S  G  V    128

TTTCTGCTTGTGTCAATTTCACAGATTATGTGGCCCATGTGACCCAACATACCCAGCAGC 13380
V  S  A  C  V  N  F  T  D  Y  V  A  H  V  T  Q  H  T  Q  Q    148

ATCATCTGGTAATTGATCACATTCGGTTGCTGCATTTCCTGACACCATCTGCAATGAGGT 13440
H  H  L  V  I  D  H  I  R  L  L  H  F  L  T  P  S  A  M  R    168

GGGCTACAACCATTGCTTGTTTGTTCGCCATTCTCTTGGCAATATGAGATGTTCTCACAA 13500
W  A  T  T  I  A  C  L  F  A  I  L  L  A  I  -                183
                                ORF5     M  R  C  S  H  K      6

ATTGGGGCGTTTCTTGACTCCGCACTCTTGCTTCTGGTGGCTTTTTTTGCTGTGTACCGG 13560
  L  G  R  F  L  T  P  H  S  C  F  W  W  L  F  L  L  C  T  G    26

CTTGTCCTGGTCCTTTGCCGATGGCAACGGCGACAGCTCGACATACCAATACATATATAA 13620
  L  S  W  S  F  A  D  G  N  G  D  S  S  T  Y  Q  Y  I  Y  N    46

CTTGACGATATGCGAGCTGAATGGGACCGACTGGTTGTCCAGCCATTTTGGTTGGGCAGT 13680
  L  T  I  C  E  L  N  G  T  D  W  L  S  S  H  F  G  W  A  V    66

CGAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAAC 13740
  E  T  F  V  L  Y  P  V  A  T  H  I  L  S  L  G  F  L  T  T    86

AAGCCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCGG 13800
  S  H  F  F  D  A  L  G  L  G  A  V  S  T  A  G  F  V  G  G   106
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FIG. 1p

```
GCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGT 13860
  R  Y  V  L  C  S  V  Y  G  A  C  A  F  A  A  F  V  C  F  V   126

CATCCGTGCTGCTAAAAATTGCATGGCCTGCCGCTATGCCCGTACCCGGTTTACCAACTT 13920
  I  R  A  A  K  N  C  M  A  C  R  Y  A  R  T  R  F  T  N  F   146

CATTGTGGACGACCGGGGGAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAAAATT 13980
  I  V  D  D  R  G  R  V  H  R  W  K  S  P  I  V  V  E  K  L   166

GGGCAAAGCCGAAGTCGATGGCAACCTCGTCACCATCAAACATGTCGTCCTCGAAGGGGT 14040
  G  K  A  E  V  D  G  N  L  V  T  I  K  H  V  V  L  E  G  V   186

TAAAGCTCAACCCTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAGACGATTTTTGC 14100
  K  A  Q  P  L  T  R  T  S  A  E  Q  W  E  A  -                201
                           ORF6    M  G  G  L  D  D  F  C          8

AACGATCCTATCGCCGCACAAAAGCTCGTGCTAGCCTTTAGCATCACATACACACCTATA 14160
  N  D  P  I  A  A  Q  K  L  V  L  A  F  S  I  T  Y  T  P  I    28

ATGATATACGCCCTTAAGGTGTCACGCGGCCGACTCCTGGGGCTGTTGCACATCCTAATA 14220
  M  I  Y  A  L  K  V  S  R  G  R  L  L  G  L  L  H  I  L  I    48

TTTCTGAACTGTTCCTTTACATTCGGATACATGACATATGTGCATTTTCAATCCACCAAC 14280
  F  L  N  C  S  F  T  F  G  Y  M  T  Y  V  H  F  Q  S  T  N    68

CGTGTCGCACTTACCCTGGGGGCTGTTGTCGCCCTTCTGTGGGGTGTTTACAGCTTCACA 14340
  R  V  A  L  T  L  G  A  V  V  A  L  L  W  G  V  Y  S  F  T    88

GAGTCATGGAAGTTTATCACTTCCAGATGCAGATTGTGTTGCCTTGGCCGGCGATACATT 14400
  E  S  W  K  F  I  T  S  R  C  R  L  C  C  L  G  R  R  Y  I   108

CTGGCCCCTGCCCATCACGTAGAAAGTGCTGCAGGTCTCCATTCAATCTCAGCGTCTGGT 14460
  L  A  P  A  H  H  V  E  S  A  A  G  L  H  S  I  S  A  S  G   128

AACCGAGCATACGCTGTGAGAAAGCCCGGACTAACATCAGTGAACGGCACTCTAGTACCA 14520
  N  R  A  Y  A  V  R  K  P  G  L  T  S  V  N  G  T  L  V  P   148

GGACTTCGGAGCCTCGTGCTGGGCGGCAAACGAGCTGTTAAACGAGGAGTGGTTAACCTC 14580
  G  L  R  S  L  V  L  G  G  K  R  A  V  K  R  G  V  V  N  L   168

GTCAAGTATGGCCGGTAAAAACCAGAGCCAGAAGAAAAAGAAAAGTACAGCTCCGATGGG 14640
                                                               173
  V  K  Y  G  R  -
  ORF7  M  A  G  K  N  Q  S  Q  K  K  K  K  S  T  A  P  M  G   18

GAATGGCCAGCCAGTCAATCAACTGTGCCAGTTGCTGGGTGCAATGATAAAGTCCCAGCG 14700
  N  G  Q  P  V  N  Q  L  C  Q  L  L  G  A  M  I  K  S  Q  R   38
```

FIG. 1q

```
                                T
CCAGCAACCTAGGGGAGGACAGGCCAAAAAGAAAAAGCCTGAGAAGCCACATTTTCCCCT 14760
  Q  Q  P  R  G  G  Q  A  K  K  K  K  P  E  K  P  H  F  P  L    58

GGCTGCTGAAGATGACATCCGGCACCACCTCACCCAGACTGAACGCTCCCTCTGCTTGCA 14820
  A  A  E  D  D  I  R  H  H  L  T  Q  T  E  R  S  L  C  L  Q    78

                                                A
ATCGATCCAGACGGCTTTCAATCAAGGCGCAGGAACTGCGTCGCTTTCATCCAGCGGGAA 14880
  S  I  Q  T  A  F  N  Q  G  A  G  T  A  S  L  S  S  S  G  K    98

GGTCAGTTTTCAGGTTGAGTTTATGCTGCCGGTTGCTCATACAGTGCGCCTGATTCGCGT 14940
  V  S  F  Q  V  E  F  M  L  P  V  A  H  T  V  R  L  I  R  V   118

GACTTCTACATCCGCCAGTCAGGGTGCAAGTTAATTTGACAGTCAGGTGAATGGCCGCGA 15000
  T  S  T  S  A  S  Q  G  A  S  -                              128

TGGCGTGTGGCCTCTGAGTCACCTATTCAATTAGGGCGATCACATGGGGGTCATACTTAA 15060

TTCAGGCAGGAACCATGTGACCGAAATTAAAAAAAAAAAAAAAAAAAA              15088
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61
151
155
93
99
119
12
20
98
55
73
71
19
41
11
100
59
10
120
25
80
58
9
60
1
85
2
5
46
66
118
22
111
4
39
0
2
4
6
8
10
12
14
5'
3'
1a
1b
2
3
4
5
6
7
ORFs
RNA
1
2
3
4
5
6
7

Fig. 2

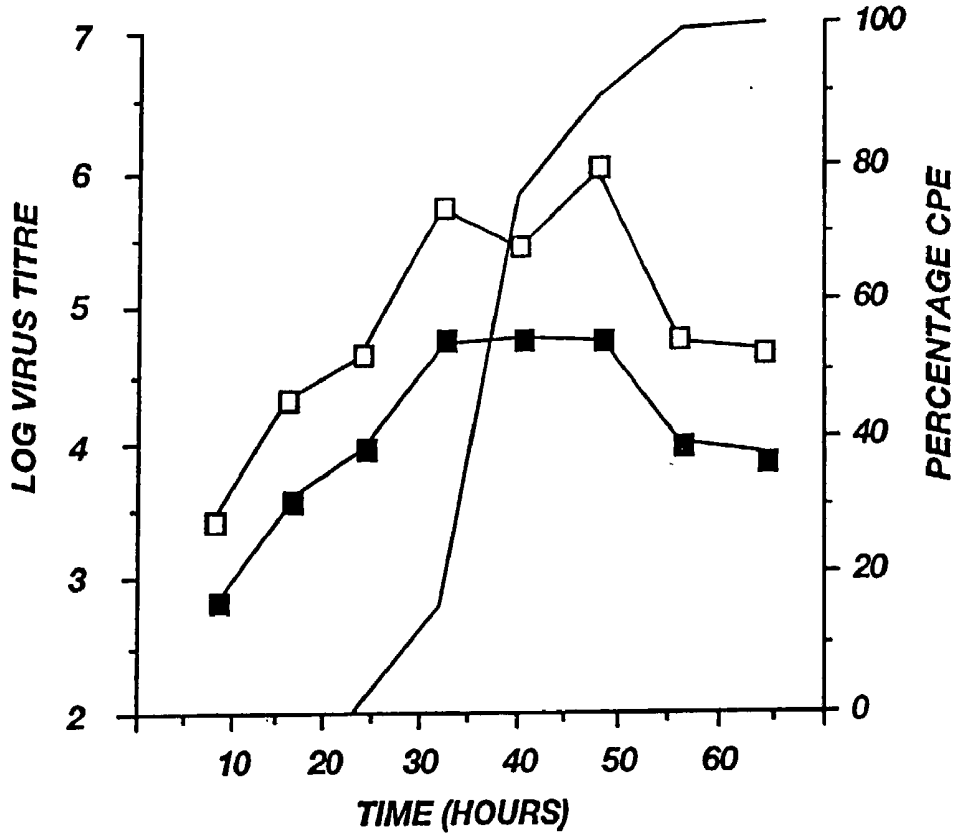
LOG VIRUS TITRE
7
6
5
4
3
2
PERCENTAGE CPE
100
80
60
40
20
0
10
20
30
40
50
60
TIME (HOURS)

Fig. 3

CAUSATIVE AGENT OF THE MYSTERY SWINE DISEASE, VACCINE COMPOSITIONS AND DIAGNOSTIC KITS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional application of co-pending U.S. application Ser. No. 09/565,864, filed May 5, 2000, now U.S. Patent _____, issued _____, which itself is a divisional application of co-pending U.S. application Ser. No. 08/747,863, filed Nov. 13, 1996, now U.S. Pat. No. 6,197,310 , issued Mar. 6, 2001, which itself is a divisional of U.S. patent application Ser. No. 08/157,005, filed Nov. 26, 1993, now U.S. Pat. No. 5,620,691, which is a U.S. National Stage under 35 U.S.C. § 371 of International Patent Application PCT/NL92/00096, filed Jun. 5, 1992, the contents of all of which are incorporated by this reference.

TECHNICAL FIELD

[0002] The invention relates to the isolation, characterization and utilization of the causative agent of the Mystery Swine Disease (MSD). The invention utilizes the discovery of the agent causing the disease and the determination of its genome organization, the genomic nucleotide sequence and the proteins encoded by the genome, for providing protection against and diagnosis of infections, in particular, protection against and diagnosis of MSD infections, and for providing vaccine compositions and diagnostic kits, either for use with MSD or with other pathogen-caused diseases.

BACKGROUND

[0003] In the winter and early spring of 1991, the Dutch pig industry was struck by a sudden outbreak of a new disease among breeding sows. Most sows showed anorexia, some aborted late in gestation (around day 110), showed stillbirths or gave birth to mummified fetuses and some had fever. Occasionally, sows with bluish ears were found, therefore, the disease was commonly named "Abortus Blauw". The disease in the sows was often accompanied by respiratory distress and death of their young piglets and often by respiratory disease and growth retardation of older piglets and fattening pigs.

[0004] The cause of this epizootic was not known, but the symptoms resembled those of a similar disease occurring in Germany since late 1990, and resembled those of the so-called "Mystery Swine Disease" as seen since 1987 in the mid-west of the United States of America and in Canada (Hill, 1990). Various other names have been used for the disease; in Germany it is known as "Seuchenhafter Spätabort der Schweine" and in North America it is also known as "Mystery Pig Disease", "Mysterious Reproductive Syndrome", and "Swine Infertility and Respiratory Syndrome". In North America, Loula (1990) described the general clinical signs as:

[0005] 1) off feed, sick animals of all ages;

[0006] 2) abortions, stillbirths, weak pigs, mummies;

[0007] 3) post-farrowing respiratory problems; and

[0008] 4) breeding problems.

[0009] No causative agent has as yet been identified, but encephalomyocarditis virus ("EMCV"), porcine parvo virus ("PPV"), pseudorabies virus ("PRV"), swine influenza virus ("SIV"), bovine viral diarrhea virus ("BVDV"), hog cholera virus ("HCV"), porcine entero viruses ("PEV"), an influenza-like virus, chlamidiae, leptospirae, have all been named as a possible cause (Loula, 1990; Mengeling and Lager, 1990; among others).

SUMMARY OF THE INVENTION

[0010] The invention provides a composition of matter comprising isolated Lelystad Agent which is the causative agent of Mystery Swine Disease, the Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, *Collection Nationale de Cultures De Microorganismes* (C.N.C.M.) 25, rue du Docteur Roux, 75724 -Paris Cedex 15, France, deposit number I-1102. The words "essentially corresponding" refer to variations that occur in nature and to artificial variations of Lelystad Agent, particularly those which still allow detection by techniques like hybridization, PCR and ELISA, using Lelystad Agent-specific materials, such as Lelystad Agent-specific DNA or antibodies.

[0011] The composition of matter may comprise live, killed, or attenuated isolated Lelystad Agent; a recombinant vector derived from Lelystad Agent; an isolated part or component of Lelystad Agent; isolated or synthetic protein (poly)peptide, or nucleic acid derived from Lelystad Agent; recombinant nucleic acid which comprises a nucleotide sequence derived from the genome of Lelystad Agent; a (poly)peptide having an amino acid sequence derived from a protein of Lelystad Agent, the (poly)peptide being produced by a cell capable of producing it due to genetic engineering with appropriate recombinant DNA; an isolated or synthetic antibody which specifically recognizes a part or component of Lelystad Agent; or a recombinant vector which contains nucleic acid comprising a nucleotide sequence coding for a protein or antigenic peptide derived from Lelystad Agent.

[0012] On the DNA level, the invention specifically provides a recombinant nucleic acid, more specifically recombinant DNA, which comprises a Lelystad Agent-specific nucleotide sequence shown in **FIG. 1** (SEQ ID NO: 1) which includes **FIGS. 1***a* ; through **1***q*. Preferably, the Lelystad Agent-specific nucleotide sequence is selected from any one of the ORFs (Open Reading Frames) shown in **FIG. 1** (SEQ ID NO: 1).

[0013] On the peptide/protein level, the invention specifically provides a peptide comprising a Lelystad Agent-specific amino acid sequence shown in **FIG. 1** (SEQ ID NO: 1).

[0014] The invention further provides a vaccine composition for vaccinating animals, in particular mammals, more in particular pigs or swine, to protect them against Mystery Swine Disease, comprising Lelystad Agent, either live, killed, or attenuated; or a recombinant vector which contains nucleic acid comprising a nucleotide sequence coding for a protein or antigenic peptide derived from Lelystad Agent; an antigenic part or component of Lelystad Agent; a protein or antigenic polypeptide derived from, or a peptide mimicking an antigenic component of, Lelystad Agent; and a suitable carrier or adjuvant.

[0015] The invention also provides a vaccine composition for vaccinating animals, in particular mammals, more in

particular pigs or swine, to protect them against a disease caused by a pathogen, comprising a recombinant vector derived from Lelystad Agent, the nucleic acid of the recombinant vector comprising a nucleotide sequence coding for a protein or antigenic peptide derived from the pathogen, and a suitable carrier or adjuvant.

[0016] The invention further provides a diagnostic kit for detecting nucleic acid from Lelystad Agent in a sample, in particular a biological sample such as blood or blood serum, sputum, saliva, or tissue, derived from an animal, in particular a mammal, more in particular a pig or swine, comprising a nucleic acid probe or primer which comprises a nucleotide sequence derived from the genome of Lelystad Agent, and suitable detection means of a nucleic acid detection assay.

[0017] The invention also provides a diagnostic kit for detecting antigen from Lelystad Agent in a sample, in particular a biological sample such as blood or blood serum, sputum, saliva, or tissue, derived from an animal, in particular a mammal, more in particular a pig or swine, comprising an antibody which specifically recognizes a part or component of Lelystad Agent, and suitable detection means of an antigen detection assay.

[0018] The invention also provides a diagnostic kit for detecting an antibody which specifically recognizes Lelystad Agent in a sample, in particular a biological sample such as blood or blood serum, sputum, saliva, or tissue, derived from an animal, in particular a mammal, more in particular a pig or swine, comprising Lelystad Agent; an antigenic part or component of Lelystad Agent; a protein or antigenic polypeptide derived from Lelystad Agent; or a peptide mimicking an antigenic component of Lelystad Agent; and suitable detection means of an antibody detection assay.

[0019] The invention also relates to a process for diagnosing whether an animal, in particular a mammal, more in particular a pig or swine, is contaminated with the causative agent of Mystery Swine Disease, comprising preparing a sample, in particular a biological sample such as blood or blood serum, sputum, saliva, or tissue, derived from the animal, and examining whether it contains Lelystad Agent nucleic acid, Lelystad Agent antigen, or antibody specifically recognizing Lelystad Agent, the Lelystad Agent being the causative agent of Mystery Swine Disease and essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

DETAILED DESCRIPTION OF THE INVENTION

[0020] The invention is a result of combined efforts of the Central Veterinary Institute (CVI) and the Regional Animal Health Services (RAHS) in the Netherlands in trying to find the cause of the new disease MSD. Farms with pigs affected by the new disease were visited by field veterinarians of the RAHS. Sick pigs, specimens of sick pigs, and sow sera taken at the time of the acute and convalescent phase of the disease were sent for virus isolation to the RAHS and the CVI. Paired sera of affected sows were tested for antibodies against ten known pig-viruses. Three different viruses, encephalomyocarditis virus, porcine entero virus type 2, porcine entero virus type 7, and an unknown agent, Lelystad Agent (LA), were isolated. Sows which had reportedly been struck with the disease mainly seroconverted to LA, and rarely to any of the other virus isolates or the known viral pathogens. In order to reproduce MSD experimentally, eight pregnant sows were inoculated intranasally with LA at day 84 of gestation. One sow gave birth to seven dead and four live but very weak piglets at day 109 of gestation; the four live piglets died one day after birth. Another sow gave birth at day 116 to three mummified fetuses, six dead piglets and three live piglets; two of the live piglets died within one day. A third sow gave birth at day 117 to two mummified fetuses, eight dead and seven live piglets. The other sows farrowed around day 115 and had less severe reproductive losses. The mean number of live piglets from all eight sows at birth was 7.3 and the mean number of dead piglets at birth was 4.6. Antibodies directed against LA were detected in 10 out of 42 serum samples collected before the pigs had sucked. LA was isolated from three piglets that died shortly after birth. These results justify the conclusion that LA is the causal agent of mystery swine disease.

[0021] LA grows with a cytopathic affect in pig lung macrophages and can be identified by staining in an immuno-peroxidase-monolayer assay (IPMA) with post-infection sera of pigs c 829 and b 822, or with any of the other post-infection sera of the SPF pigs listed in table 5. Antibodies to LA can be identified by indirect staining procedures in IPMA. LA did not grow in any other cell system tested. LA was not neutralized by homologous sera, or by sera directed against a set of known viruses (Table 3). LA did not haemagglutinate with the red blood cells tested. LA is smaller then 200 nm since it passes through a filter with pores of this size. LA is sensitive to chloroform. The above results show that Lelystad Agent is not yet identified as belonging to a certain virus group or other microbiological species. It has been deposited Jun. 5, 1991 under number I-1102 at Institute Pasteur, France.

[0022] The genome organization, nucleotide sequences, and polypeptides derived therefrom, of LA have now been found. These data together with those of others (see below) justify classification of LA (hereafter also called Lelystad Virus or LV) as a member of a new virus family, the Arteriviridae. As prototype virus of this new family we propose Equine Arteritis Virus (EAV), the first member of the new family of which data regarding the replication strategy of the genome and genome organization became available (de Vries et al., 1990, and references therein). On the basis of a comparison of our sequence data with those available for Lactate Dehydrogenase-Elevating Virus (LDV; Godeny et al., 1990), we propose that LDV is also a member of the Arteriviridae.

[0023] Given the genome organization and translation strategy of Arteriviridae, it seems appropriate to place this new virus family into the superfamily of coronaviruses (Snijder et al., 1990a).

[0024] Arteriviruses have in common that their primary target cells in respective hosts are macrophages. Replication of LDV has been shown to be restricted to macrophages in its host, the mouse; whereas this strict propensity for macrophages has not been resolved yet for EAV and LV.

[0025] Arteriviruses are spherical enveloped particles having a diameter of 45-60 nm and containing an icosahedral nucleocapsid (Brinton-Darnell and Plagemann, 1975; Horzinek et al., 1971; Hyllseth, 1973).

[0026] The genome of Arteriviridae consists of a positive stranded polyadenylated RNA molecule with a size of about 12-13 kilobases (kb) (Brinton-Darnell and Plageman, 1975; van der Zeijst et al., 1975). EAV replicates via a 3' nested set of six subgenomic mRNAs, ranging in size from 0.8 to 3.6 kb, which are composed of a leader sequence, derived from the 5' end of the genomic RNA, which is joined to the 3' terminal body sequences (de Vries et al., 1990).

[0027] Here we show that the genome organization and replication strategy of LV is similar to that of EAV, coronaviruses and toroviruses, whereas the genome sizes of the latter viruses are completely different from those of LV and EAV.

[0028] The genome of LV consists of a genomic RNA molecule of about 14.5 to 15.5 kb in length (estimated on a neutral agarose gel), which replicates via a 3' nested set of subgenomic RNAs. The subgenomic RNAs consist of a leader sequence, the length of which is yet unknown, which is derived from the 5' end of the genomic RNA and which is fused to the body sequences derived from the 3' end of the genomic RNA (**FIG. 2**).

[0029] The nucleotide sequence of the genomic RNA of LV was determined from overlapping cDNA clones. A consecutive sequence of 15,088 bp was obtained covering nearly the complete genome of LV (**FIG. 1**, SEQ ID NO: 1). In this sequence 8 open reading frames (ORFs) were identified: ORF 1A, ORF 1B, and ORFs 2 to 7.

[0030] ORF 1A and ORF 1B are predicted to encode the viral replicase or polymerase (SEQ ID NO: 2 and SEQ ID NO: 3), whereas ORFs 2 to 6 are predicted to encode structural viral membrane (envelope) associated proteins (SEQ ID NOS: 4-8). ORF 7 is predicted to encode the structural viral nucleocapsid protein (SEQ ID NO: 9).

[0031] Because the products of ORF 6 and ORF 7 of LV (SEQ ID NO: 8 and SEQ ID NO: 9) show a significant similarity with VpX and Vp1 of LDV, respectively, it is predicted that the sequences of ORFs 6 and 7 will also be highly conserved among antigenic variants of LV.

[0032] The complete nucleotide sequence of **FIG. 1** (SEQ ID NO: 1) and all the sequences and protein products encoded by ORFs 1 to 7 (SEQ ID NOS: 1-9) and possible other ORFs located in the sequence of **FIG. 1** (SEQ ID NO: 1) are especially suited for vaccine development, in whatever sense, and for the development of diagnostic tools, in whatever sense. All possible modes are well known to persons skilled in the art.

[0033] Since it is now possible to unambiguously identify LA, the causal agent of MSD, it can now be tested whether pigs are infected with LA or not. Such diagnostic tests have, until now, been unavailable.

[0034] The test can be performed by virus isolation in macrophages, or other cell culture systems in which LA might grow, and staining the infected cultures with antibodies directed against LA (such as post-infection sera c 829 or b 822), but it is also feasible to develop and employ other types of diagnostic tests.

[0035] For instance, it is possible to use direct or indirect immunohistological staining techniques, i.e., with antibodies directed to LA that are labeled with fluorescent compounds such as isothiocyanate, or labeled with enzymes such as horseradish peroxidase. These techniques can be used to detect LA antigen in tissue sections or other samples from pigs suspected to have MSD. The antibodies needed for these tests can be c 829 or b 822 or other polyclonal antibodies directed against LA, but monoclonal antibodies directed against LA can also be used.

[0036] Furthermore, since the nature and organization of the genome of LA and the nucleotide sequence of this genome have been determined, LA-specific nucleotide sequences can be identified and used to develop oligonucleotide sequences that can be used as probes or primers in diagnostic techniques such as hybridization, polymerase chain reaction, or any other techniques that are developed to specifically detect nucleotide acid sequences.

[0037] It is also possible to test for antibodies directed against LA. Table 5 shows that experimentally infected pigs rapidly develop antibodies against LA, and table 4 shows that pigs in the field also have strong antibody responses against LA. Thus, it can now also be determined whether pigs have been infected with LA in the past. Such testing is of utmost importance in determining whether pigs or pig herds or pig populations or pigs in whole regions or countries are free of LA. The test can be done by using the IPMA as described, but it is also feasible to develop and employ other types of diagnostic tests for the detection of antibodies directed against LA.

[0038] LA-specific proteins, polypeptides, and peptides, or peptide sequences mimicking antigenic components of LA, can be used in such tests. Such proteins can be derived from the LA itself, but it is also possible to make such proteins by recombinant DNA or peptide synthesis techniques. These tests can use specific polyclonal and/or monoclonal antibodies directed against LA or specific components of LA, and/or use cell systems infected with LA or cell systems expressing LA antigen. The antibodies can be used, for example, as a means for immobilizing the LA antigen (a solid surface is coated with the antibody whereafter the LA antigen is bound by the antibody) which leads to a higher specificity of the test, or can be used in a competitive assay (labeled antibody and unknown antibody in the sample compete for available LA antigen).

[0039] Furthermore, the above described diagnostic possibilities can be applied to test whether other animals, such as mammals, birds, insects or fish, or plants, or other living creatures, can be, or are, or have been infected with LA or related agents.

[0040] Since LA has now been identified as the causal agent of MSD, it is possible to make a vaccine to protect pigs against this disease. Such a vaccine can simply be made by growing LA in pig lung macrophage cultures, or in other cell systems in which LA grows. LA can then be purified or not, and killed by established techniques, such as inactivation with formaline or ultra-violet light. The inactivated LA can then be combined with adjuvantia, such as Freund's adjuvans or aluminum hydroxide or others, and this composition can then be injected in pigs.

[0041] Dead vaccines can also be made with LA protein preparations derived from LA infected cultures, or derived from cell systems expressing specifically LA protein through DNA recombinant techniques. Such subunits of LA would then be treated as above, and this would result in a subunit vaccine.

[0042] Vaccines using even smaller components of LA, such as polypeptides, peptides, or peptides mimicking antigenic components of LA, are also feasible for use as dead vaccine.

[0043] Dead vaccines against MSD can also be made by recombinant DNA techniques through which the genome of LA, or parts thereof, is incorporated in vector systems such as vaccinia virus, herpesvirus, pseudorabies virus, adeno virus, baculo virus or other suitable vector systems that can so express LA antigen in appropriate cells systems. LA antigen from these systems can then be used to develop a vaccine as above, and pigs, vaccinated with such products would develop protective immune responses against LA.

[0044] Vaccines against MSD can also be based on live preparations of LA. Since only young piglets and pregnant sows seem to be seriously affected by infection with LA, it is possible to use unattenuated LA, grown in pig lung macrophages, as vaccine for older piglets, or breeding gilts. In this way, sows can be protected against MSD before they get pregnant, which results in protection against abortions and stillbirth, and against congenital infections of piglets. Also the maternal antibody that these vaccinated sows give to their offspring would protect their offspring against the disease.

[0045] Attenuated vaccines (modified-live-vaccines) against MSD can be made by serially passaging LA in pig lung macrophages, in lung macrophages of other species, or in other cell systems, or in other animals, such as rabbits, until it has lost its pathogenicity.

[0046] Live vaccines against MSD can also be made by recombinant DNA techniques through which the genome of LA, or parts thereof, is incorporated in vector systems such as vaccinia virus, herpesvirus, pseudorabies virus, adeno virus or other suitable vector systems that can so express LA antigen. Pigs vaccinated with such live vector systems would then develop protective immune responses against LA.

[0047] Lelystad Agent itself would be specifically suited to use as a live vector system. Foreign genes could be inserted in the genome of LA and could be expressing the corresponding protein during the infection of the macrophages. This cell, which is an antigen-presenting cell, would process the foreign antigen and present it to B-lymphocytes and T-lymphocytes which will respond with the appropriate immune response.

[0048] Since LA seems to be very cell specific and possibly also very species specific, this vector system might be a very safe system, which does not harm other cells or species.

BRIEF DESCRIPTION OF THE DRAWINGS

[0049] **FIG. 1** (SEQ ID NO: 1) shows the nucleotide sequence of the LV genome. The deduced amino acid sequence of the identified ORFs (SEQ ID NOS: 2-9) are shown. The methionines encoded by the (putative) ATG start sites are indicated in bold and putative N-glycosylation sites are underlined. Differences in the nucleotide and amino acid sequence, as identified by sequencing different cDNA clones, are shown. The nucleotide sequence of primer 25, which has been used in hybridization experiments (see **FIG. 2** and section "results"), is underlined.

[0050] **FIG. 2** shows the organization of the LV genome. The cDNA clones, which have been used for the determination of the nucleotide sequence, are indicated in the upper part of the figure. The parts of the clones, which were sequenced, are indicated in black. In the lower part of the FIG. the ORFs, identified in the nucleotide sequence, and the subgenomic set of mRNAs, encoding these ORFs are shown. The dashed lines in the ORFs represent alternative initiation sites (ATGs) of these ORFs. The leader sequence of the genomic and subgenomic RNAs is indicated by a solid box.

[0051] **FIG. 3** shows the growth characteristics of LA:

[0052] empty squares—titre of cell-free virus;

[0053] solid squares—titre of cell-associated virus;

[0054] solid line—percentage cytopathic effect (CPE).

MATERIALS AND METHODS

[0055] Sample Collection

[0056] Samples and pigs were collected from farms where a herd epizootic of MSD seemed to occur. Important criteria for selecting the farm as being affected with MSD were: sows that were off feed, the occurrence of stillbirth and abortion, weak offspring, respiratory disease and death among young piglets. Samples from four groups of pigs have been investigated:

[0057] (1) tissue samples and an oral swab from affected piglets from the field (Table 1A);

[0058] (2) blood samples and oral swabs from affected sows in the field (Tables 1B and 4);

[0059] (3) tissue samples, nasal swabs and blood samples collected from specific-pathogen-free (SPF) pigs experimentally infected by contact with affected sows from the field; or

[0060] (4) tissue samples, nasal swabs and blood samples collected from specific-pathogen-free (SPF) pigs experimentally infected by inoculation with blood samples of affected sows from the field (Tables 2 and 5).

[0061] Sample Preparation

[0062] Samples for virus isolation were obtained from piglets and sows which on clinical grounds were suspected to have MSD, and from experimentally infected SPF pigs, sows and their piglets.

[0063] Tissue samples were cut on a cryostat microtome and sections were submitted for direct immunofluorescence testing (IFT) with conjugates directed against various pig pathogens.

[0064] 10% Suspensions of tissues samples were prepared in Hank's BSS supplemented with antibiotics, and oral and nasal swabs were soaked in Hank's BSS supplemented with antibiotics. After one hour at room temperature, the suspensions were clarified for 10 min at 6000 g and the supernatant was stored at −70° C. for further use. Leucocyte fractions were isolated from EDTA or heparin blood as described earlier (Wensvoort and Terpstra, 1988) and stored at −70° C. Plasma and serum for virus isolation were stored at −70° C.

[0065] Serum for serology was obtained from sows suspected to be in the acute phase of MSD, a paired serum was taken 3-9 weeks later. Furthermore, sera were taken from the experimentally infected SPF pigs at regular intervals and colostrum and serum was taken from experimentally infected sows and their piglets. Sera for serology were stored at −20° C.

[0066] Cells

[0067] Pig lung macrophages were obtained from lungs of 5-6 weeks old SPF pigs or from lungs of adult SPF sows from the Central Veterinary Institute's own herd. The lungs were washed five to eight times with phosphate buffered saline (PBS). Each aliquot of washing fluid was collected and centrifuged for 10 min at 300 g. The resulting cell pellet was washed again in PBS and resuspended in cell culture medium (160 ml medium 199, supplemented with 20 ml 2.95% tryptose phosphate, 20 ml fetal bovine serum (FBS), and 4.5 ml 1.4% sodium bicarbonate) to a concentration of 4×10^7cells/ml. The cell suspension was then slowly mixed with an equal volume of DMSO mix (6.7 ml of above medium, 1.3 ml FBS, 2 ml dimethylsulfoxide 97%), aliquoted in 2 ml ampoules and stored in liquid nitrogen.

[0068] Macrophages from one ampoule were prepared for cell culture by washing twice in Earle's MEM, and resuspended in 30 ml growth medium (Earle's MEM, supplemented with 10% FBS, 200 U/ml penicillin, 0.2 mg/ml streptomycine, 100 U/ml mycostatin, and 0.3 mg/ml glutamine). PK-15 cells (American Type Culture Collection, CCL33) and SK-6 cells (Kasza et al., 1972) were grown as described by Wensvoort et al. (1989). Secondary porcine kidney (PK2) cells were grown in Earle's MEM, supplemented with 10% FBS and the above antibiotics. All cells were grown in a cell culture cabinet at 37° C. and 5% CO^2.

[0069] Virus Isolation Procedures

[0070] Virus isolation was performed according to established techniques using PK2, PK-15 and SK-6 cells, and pig lung macrophages. The former three cells were grown in 25 ml flasks (Greiner), and inoculated with the test sample when monolayers had reached 70-80% confluency. Macrophages were seeded in 100 μl aliquots in 96-well microtiter plates (Greiner) or in larger volumes in appropriate flasks, and inoculated with the test sample within one hour after seeding. The cultures were observed daily for cytopathic effects (CPE), and frozen at −70° C. when 50-70% CPE was reached or after five to ten days of culture. Further passages were made with freeze-thawed material of passage level 1 and 2 or higher. Some samples were also inoculated into nine to twelve day old embryonated hen eggs. Allantoic fluid was subinoculated two times using an incubation interval of three days and the harvest of the third passage was examined by haemagglutination at 4° C. using chicken red blood cells, and by an ELISA specifically detecting nucleoprotein of influenza A viruses (De Boer et al., 1990).

[0071] Serology

[0072] Sera were tested in haemagglutinating inhibition tests (HAI) to study the development of antibody against haemagglutinating encephalitis virus (HEV), and swine influenza viruses H1N1 and H3N2 according to the protocol of Masurel (1976). Starting dilutions of the sera in HAI were 1:9, after which the sera were diluted twofold.

[0073] Sera were tested in established enzyme-linked immuno-sorbent assays (ELISA) for antibodies against the glycoprotein gI of pseudorabies virus (PRV; Van Oirschot et al., 1988), porcine parvo virus (PPV; Westenbrink et al., 1989), bovine viral diarrhea virus (BVDV; Westenbrink et al., 1986), and hog cholera virus (HCV; Wensvoort et al., 1988). Starting dilutions in the ELISA's were 1:5, after which the sera were diluted twofold.

[0074] Sera were tested for neutralizing antibodies against 30-300 $TCID_{50}$ of encephalomyocarditis viruses (EMCV), porcine enteroviruses (PEV), and Lelystad Agent (LA) according to the protocol of Terpstra (1978). Starting dilutions of the sera in the serum neutralization tests (SNT) were 1:5, after which the sera were diluted twofold.

[0075] Sera were tested for binding with LA in an immuno-peroxidase-monolayer assay (IPMA). Lelystad Agent (LA; code: CDI-NL-2.91) was seeded in microtiter plates by adding 50 ml growth medium containing 100 $TCID_{50}$ LA to the wells of a microtiter plate containing freshly seeded lung macrophages. The cells were grown for two days and then fixed as described (Wensvoort, 1986). The test sera were diluted 1:10 in 0.15 M NaCl, 0.05% Tween 80, 4% horse serum, or diluted further in fourfold steps, added to the wells and then incubated for one hour at 37° C. Sheep-anti-pig immunoglobulins (Ig) conjugated to horse radish peroxidase (HRPO, DAKO) were diluted in the same buffer and used in a second incubation for one hour at 37° C., after which the plates were stained as described (Wensvoort et al., 1986). An intense red staining of the cytoplasm of infected macrophages indicated binding of the sera to LA.

[0076] Virus Identification Procedures

[0077] The identity of cytopathic isolates was studied by determining the buoyant density in CsCl, by estimating particle. size in negatively stained preparations through electron microscopy, by determining the sensitivity of the isolate to chloroform and by neutralizing the CPE of the isolate with sera with known specificity (Table 3). Whenever an isolate was specifically neutralized by a serum directed against a known virus, the isolate was considered to be a representative of this known virus.

[0078] Isolates that showed CPE on macrophage cultures were also studied by staining in IPMA with post-infection sera of pigs c 829 or b 822. The isolates were reinoculated on macrophage cultures and fixed at day 2 after inoculation before the isolate showed CPE. Whenever an isolate showed reactivity in IPMA with the post-infection sera of pigs c 829 or b 822, the isolate was considered to be a representative of the Lelystad Agent. Representatives of the other isolates grown in macrophages or uninfected macrophages were also stained with these sera to check the specificity of the sera.

[0079] Further Identification of Lelystad Agent

[0080] Lelystad Agent was further studied by haemagglutination at 4° C. and 37° C. with chicken, guinea pig, pig, sheep, or human O red blood cells. SIV, subtype H3N2, was used as positive control in the haemagglutination studies.

[0081] The binding of pig antisera specifically directed against pseudorabies virus (PRV), transmissible gastroenteritis virus (TGE), porcine epidemic diarrhea virus (PED), haemagglutinating encephalitis virus (HEV), African swine fever virus (ASFV), hog cholera virus (HCV) and swine

influenza virus (SIV) type H1N1 and H3N2, of bovine antisera specifically directed against bovine herpes viruses type 1 and 4 (BHV 1 and 4), malignant catarrhal fever (MCF), parainfluenza virus 3 (PI3), bovine respiratory syncitial virus (BRSV) and bovine leukemia virus (BLV), and of avian antisera specifically directed against avian leukemia virus (ALV) and infectious bronchitis virus (IBV) was studied with species-Ig-specific HRPO conjugates in an IPMA on LA infected and uninfected pig lung macrophages as described above.

[0082] We also tested in IPMA antisera of various species directed against mumps virus, Sendai virus, canine distemper virus, rinderpest virus, measles virus, pneumonia virus of mice, bovine respiratory syncytial virus, rabies virus, foamy virus, maedi-visna virus, bovine and murine leukemia virus, human, feline and simian immunodeficiency virus, lymphocytic choriomeningitis virus, feline infectious peritonitis virus, mouse hepatitis virus, Breda virus, Hantaan virus, Nairobi sheep disease virus, Eastern, Western and Venezuelan equine encephalomyelitis virus, rubellavirus, equine arteritis virus, lactic dehydrogenase virus, yellow fever virus, tick-born encephalitis virus and hepatitis C virus.

[0083] LA was blindly passaged in PK2, PK- 15, and SK-6 cells, and in embryonated hen eggs. After two passages, the material was inoculated again into pig lung macrophage cultures for reisolation of LA.

[0084] LA was titrated in pig lung macrophages prior to and after passing through a 0.2 micron filter (Schleicher and Schuell). The LA was detected in IPMA and by its CPE. Titres were calculated according to Reed and Muench (1938).

[0085] We further prepared pig antisera directed against LA. Two SPF pigs (21 and 23) were infected intranasally with 10^5TCID$_{50}$ of a fifth cell culture passage of LA. Two other SPF pigs (25 and 29) were infected intranasally with a fresh suspension of the lungs of an LA-infected SPF piglet containing 10^5 TCID$_{50}$ LA. Blood samples were taken at 0, 14, 28, and 42 days post-infection (dpi).

[0086] We further grew LA in porcine alveolar macrophages to determine its growth pattern over time. Porcine alveolar macrophages were seeded in F25 flasks (Greiner), infected with LA with a multiplicity of infection of 0.01 TCID$_{50}$ per cell. At 8, 16,24, 32,40,48, 56, and 64 h after infection, one flask was examined and the percentage of CPE in relation to a noninfected control culture was determined. The culture medium was then harvested and replaced with an equal volume of phosphate-buffered saline. The medium and the flask were stored at −70° C. After all cultures had been harvested, the LA titres were determined and expressed as log TCID$_{50}$ ml^{-1}.

[0087] The morphology of LA was studied by electron-microscopy. LA was cultured as above. After 48 h, the cultures were freeze-thawed and centrifuged for 10 min at 6000.times.g. An amount of 30 ml supernatant was then mixed with 0.3 ml LA-specific pig serum and incubated for 1.5 h at 37° C. After centrifugation for 30 min at 125,000× g, the resulting pellet was suspended in 1% Seakem agarose ME in phosphate-buffered saline at 40° C. After coagulation, the agarose block was immersed in 0.8% glutaraldehyde and 0.8% osmiumtetroxide (Hirsch et al., 1968) in veronal/acetate buffer, pH 7.4 (230 mOsm/kg H_2O), and fixed by microwave irradiation. This procedure was repeated once with fresh fixative. The sample was washed with water, immersed in 1% uranyl acetate, and stained by microwave irradiation. Throughout all steps, the sample was kept at 0° C. and the microwave (Samsung RE211D) was set at defrost for 5 min. Thin sections were prepared with standard techniques, stained with lead citrate (Venable et al., 1965), and examined in a Philips CM 10 electron microscope.

[0088] We further continued isolating LA from sera of pigs originating from cases of MSD. Serum samples originated from the Netherlands (field case the Netherlands 2), Germany (field cases Germany 1 and Germany 2; courtesy Drs. Berner, Müinchen and Nienhoff, Münster), and the United States [experimental case United States 1 (experiment performed with ATCC VR-2332; courtesy Drs. Collins, St. Paul and Chladek, St. Joseph), and field cases United States 2 and United States 3; courtesy Drs. van Alstine, West Lafayette and Slife, Galesburg]. All samples were sent to the "Centraal Diergeneeskundig Instituut, Lelystad" for LA diagnosis. All samples were used for virus isolation on porcine alveolar macrophages as described. Cytophatic isolates were passaged three times and identified as LA by specific immunostaining with anti-LA post infection sera b 822 and c 829.

[0089] We also studied the antigenic relationships of isolates NL1 (the first LA isolate; code CDI-NL-2.91), NL2, GE1, GE2, US1, US2, and US3. The isolates were grown in macrophages as above and were tested in IPMA with a set of field sera and two sets of experimental sera. The sera were also tested in IPMA with uninfected macrophages.

[0090] The field sera were: Two sera positive for LV (TH-187 and TO-36) were selected from a set of LA-positive Dutch field sera. Twenty-two sera were selected from field sera sent from abroad to Lelystad for serological diagnosis. The sera originated from Germany (BE-352, BE-392 and NI-f2; courtesy Dr. Bemer, München and Dr. Nienhoff, Münster), the United Kingdom (PA-141615, PA-141617 and PA-142440; courtesy Dr. Paton, Weybridge), Belgium (PE-1960; courtesy Prof. Pensaert, Gent), France (EA-2975 and EA-2985; courtesy Dr. Albina, Ploufragan), the United States (SL-441, SL-451, AL-RP9577, AL-P10814/33, AL-4994A, AL-7525, JC-MN41, JC-MN44 and JC-MN45; courtesy Dr. Slife, Galesburg, Dr. van Alstine, West Lafayette, and Dr. Collins, St. Paul), and Canada (RB-16, RB- 19, RB-22 and RB-23; courtesy Dr. Robinson, Quebec).

[0091] The experimental sera were: The above described set of sera of pigs 21, 23, 25, and 29, taken at dpi 0, 14, 28, and 42. A set of experimental sera (obtained by courtesy of Drs. Chladek, St. Joseph, and Collins, St. Paul) that originated from four six-month-old gilts that were challenged intranasally with $10^{5.1}$TCID$_{50}$ of the isolate ATCC VR-2332. Blood samples were taken from gilt 2B at 0, 20, 36, and 63 dpi; from gilt 9G at 0, 30, 44, and 68 dpi; from gilt 16W at 0, 25, 40, and 64 dpi; and from gilt 16Y at 0, 36, and 64 dpi.

[0092] To study by radio-immunoprecipitation assay (RIP; de Mazancourt et al., 1986) the proteins of LA in infected porcine alveolar macrophages, we grew LA-infected and uninfected macrophages for 16 hours in the presence of labeling medium containing ^{35}S-Cysteine. Then the labeled cells were precipitated according to standard methods with

42 dpi post-infection sera of pig b 822 and pig 23 and with serum MN 8 which was obtained 26 days after infecting a sow with the isolate ATCC VR-2332 (courtesy Dr. Collins, St. Paul). The precipitated proteins were analyzed by electrophoresis in a 12% SDS-PAGE gel and visualized by fluorography.

[0093] To characterize the genome of LA, we extracted nuclear DNA and cytoplasmatic RNA from macrophage cultures that were infected with LA and grown for 24 h or were left uninfected. The cell culture medium was discarded, and the cells were washed twice with phosphate-buffered saline. DNA was extracted as described (Strauss, 1987). The cytoplasmic RNA was extracted as described (Favaloro et al., 1980), purified by centrifugation through a 5.7 M CsCl cushion (Setzer et al., 1980), treated with RNase-free DNase (Pharmacia), and analyzed in a 0.8% neutral agarose gel (Moormann and Hulst, 1988).

[0094] Cloning and Sequencing

[0095] To clone LV RNA, intracellular RNA of LV-infected porcine lung alveolar macrophages (10 μg) was incubated with 10 mM methylmercury hydroxide for 10 minutes at room temperature. The denatured RNA was incubated at 42° C. with 50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 70 mM KCl, 0.5 mM dATP, dCTP, dGTP and dTTP, 0.6 μg calf thymus oligonucleotide primers pd(N)6 (Pharmacia) and 300 units of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) in a total volume of 100μl 20 mM EDTA was added after 1 hr; the reaction mixture was then extracted with phenol/chloroform, passed through a Sephadex G50 column and precipitated with ethanol.

[0096] For synthesis of the second cDNA strand, DNA polymerase I (Boehringer) and RNase H (Pharmacia) were used (Gübler and Hoffinan, 1983). To generate blunt ends at the termini, double-stranded cDNA was incubated with T4 DNA polymerase (Pharmacia) in a reaction mixture which contained 0.05 mM deoxynucleotide-triphosphates. Subsequently, cDNA was fractionated in a 0.8% neutral agarose gel (Moormann and Hulst, 1988). Fragments of 1 to 4 kb were electroeluted, ligated into the Smal site of pGEM-4Z (Promega), and used for transformation of *Escherichia coli* strain DH5α (Hanahan, 1985). Colony filters were hybridized with a ^{32}P-labeled single-stranded cDNA probe. The probe was reverse transcribed from LV RNA which had been fractionated in a neutral agarose gel (Moormann and Hulst, 1988). Before use, the single stranded DNA probe was incubated with cytoplasmic RNA from mock-infected lung alveolar macrophages.

[0097] The relationship between LV cDNA clones was determined by restriction enzyme analysis and by hybridization of Southern blots of the digested DNA with nick-translated cDNA probes (Sambrook et al., 1989).

[0098] To obtain the 3' end of the viral genome, we constructed a second cDNA library, using oligo $(dT)_{12-18}$ and a 3' LV-specific oligonucleotide that was complementary to the minus-strand viral genome as a primer in the first-strand reaction. The reaction conditions for first- and second-strand synthesis were identical to those described above. This library was screened with virus-specific 3' end oligonucleotide probes.

[0099] Most (>95%) of the cDNA sequences were determined with an Automated Laser Fluorescent A.L.F.™. DNA sequencer from Pharmacia LKB. Fluorescent oligonucleotide primer directed sequencing was performed on double-stranded DNA using the AutoRead™. Sequencing Kit (Pharmacia) essentially according to procedures C and D described in the Autoread™ Sequencing Kit protocol. Fluorescent primers were prepared with FluorePrime™. (Pharmacia). The remaining part of the sequence was determined via double-stranded DNA sequencing using oligonucleotide primers in conjunction with a T7 polymerase based sequencing kit (Pharmacia) and α-^{32}S-dATP (Amersham). Sequence data were analyzed using the sequence analysis programs PCGENE (Intelligenetics, Inc, Mountain View, U.S.A.) and FASTA (Pearson and Lipman, 1988).

[0100] Experimental Reproduction of MSD

[0101] Fourteen conventionally reared pregnant sows that were pregnant for 10-11 weeks were tested for antibody against LA in the IPMA. All were negative. Then two groups of four sows were formed and brought to the CVI. At week 12 of gestation, these sows were inoculated intranasally with 2 ml LA (passage level 3, titre $10^{4.8}$ $TCID_{50}$ /ml). Serum and EDTA blood samples were taken at day 10 after inoculation. Food intake, rectal temperature, and other clinical symptoms were observed daily. At farrowing, the date of birth and the number of dead and living piglets per sow were recorded, and samples were taken for virus isolation and serology.

Results

[0102] Immunofluorescence

[0103] Tissue sections of pigs with MSD were stained in an IFT with FITC-conjugates directed against African swine fever virus, hog cholera virus, pseudorabies virus, porcine parvo virus, porcine influenza virus, encephalomyocarditis virus and Chlamydia psittaci. The sections were stained, examined by fluorescent microscopy and all were found negative.

[0104] Virus Isolation from Piglets from MSD Affected Farms

[0105] Cytopathic isolates were detected in macrophage cultures inoculated with tissue samples of MSD affected, two-to-ten day old piglets. Sixteen out of 19 piglets originating from five different farms were positive (Table 1A). These isolates all reacted in IPMA with the post-infection serum of pig c 829, whereas non-inoculated control cultures did not react. The isolates, therefore, were representatives of LA. One time a cytopathic isolate was detected in an SK-6 cell culture inoculated with a suspension of an oral swab from a piglet from a sixth farm (farm VE) (Table 1A). This isolate showed characteristics of the picoma viridae and was neutralized by serum specific for PEV 2, therefore, the isolate was identified as PEV 2 (Table 3). PK2, PK-15 cells and hen eggs inoculated with samples from this group remained negative throughout.

[0106] Virus Isolation from Sows from MSD Affected Farms

[0107] Cytopathic isolates were detected in macrophage cultures inoculated with samples of MSD affected sows. 41 out of 63 sows originating from 11 farms were positive (Table 1B). These isolates all reacted in IPMA with the post-infection serum of pig b 822 and were, therefore, representatives of LA. On one occasion a cytopathic isolate

was detected in a PK2 cell culture inoculated with a suspension of a leucocyte fraction of a sow from farm HU (Table 1B). This isolate showed characteristics of the picoma viridae and was neutralized by serum specific for EMCV, therefore, the isolate was identified as EMCV (Table 3). SK-6, PK-15 cells and hen eggs inoculated with samples from this group remained negative.

[0108] Virus Isolation from SPF Pigs Kept in Contact with MSD Affected Sows

[0109] Cytopathic isolates were detected in macrophage cultures inoculated with samples of SPF pigs kept in contact with MSD affected sows. Four of the 12 pigs were positive (Table 2). These isolates all reacted in IPMA with the post-infection serum of pig c 829 and of pig b 822 and were, therefore, representatives of LA. Cytopathic isolates were also detected in PK2, PK-15 and SK-6 cell cultures inoculated with samples of these SPF pigs. Seven of the 12 pigs were positive (Table 2), these isolates were all neutralized by serum directed against PEV 7. One of these seven isolates was studied further and other characteristics also identified the isolate as PEV 7 (Table 3).

[0110] Virus Isolation from SPF Pigs Inoculated with Blood of MSD Affected Sows

[0111] Cytopathic isolates were detected in macrophage cultures inoculated with samples of SPF pigs inoculated with blood of MSD affected sows. Two out of the eight pigs were positive (Table 2). These isolates all reacted in IPMA with the post-infection serum of pig c 829 and of pig b 822 and were, therefore, representatives of LA. PK2, SK-6 and PK-15 cells inoculated with samples from this group remained negative.

[0112] Summarizing, four groups of pigs were tested for the presence of agents that could be associated with mystery swine disease (MSD).

[0113] In group one, MSD affected piglets, the Lelystad Agent (LA) was isolated from 16 out of 20 piglets; one time PEV 2 was isolated.

[0114] In group two, MSD affected sows, the Lelystad Agent was isolated from 41 out of 63 sows; one time EMCV was isolated. Furthermore, 123 out of 165 MSD affected sows seroconverted to the Lelystad Agent, as tested in the IPMA. Such massive seroconversion was not demonstrated against any of the other viral pathogens tested.

[0115] In group three, SPF pigs kept in contact with MSD affected sows, LA was isolated from four of the 12 pigs; PEV 7 was isolated from seven pigs. All 12 pigs seroconverted to LA and PEV 7.

[0116] In group four, SPF pigs inoculated with blood of MSD affected sows, the LA was isolated from two pigs. All eight pigs seroconverted to LA.

[0117] Serology of Sows from MSD Affected Farms

[0118] Paired sera from sows affected with MSD were tested against a variety of viral pathogens and against the isolates obtained during this study (Table 4). An overwhelming antibody response directed against LA was measured in the IPMA (75% of the sows seroconverted, in 23 out of the 26 farms seroconversion was found), whereas with none of the other viral pathogens a clear pattern of seroconversion was found. Neutralizing antibody directed against LA was not detected.

[0119] Serology of SPF Pigs Kept in Contact with MSD Affected Sows

[0120] All eight SPF pigs showed an antibody response in the IPMA against LA (Table 5). None of these sera were positive in the IPMA performed on uninfected macrophages. None of these sera were positive in the SNT for LA. The sera taken two weeks after contact had all high neutralizing antibody titres (>1280) against PEV 7, whereas the pre-infection sera were negative (<10), indicating that all pigs had also been infected with PEV 7.

[0121] Serology of SPF Pigs Inoculated with Blood of MSD Affected Sows

[0122] All eight SPF pigs showed an antibodyresponse in the IPMA against LA (Table 5). None of these sera were positive in the IPMA performed on uninfected macrophages. None of these sera were positive in the SNT for LA. The pre- and two weeks post-inoculation sera were negative (<10) against PEV 7.

[0123] Further Identification of Lelystad Agent

[0124] LA did not haemagglutinate with chicken, guinea pig, pig, sheep, or human O red blood cells.

[0125] LA did not react in IPMA with sera directed against PRV, TGE, PED, ASFV, etc.

[0126] After two blind passages, LA did not grow in PK2, PK-15, or SK-6 cells, or in embryonated hen eggs, inoculated through the allantoic route.

[0127] LA was still infectious after it was filtered through a 0.2 micron filter, titres before and after filitration were $10^{5.05}$ and $10^{5.3}$ $TCID_{50}$ as detected by IPMA.

[0128] Growth curve of LA (see **FIG. 3**). Maximum titres of cell-free virus were approximately $10^{5.5}TCID_{50}$ ml^{-1} from 32-48 h after inoculation. After that time the macrophages he cytopathic effect of LA.

[0129] Electronmicroscopy. Clusters of spherical LA particles were found. The particles measured 45-55 nm in diameter and contained a 30-35 nm nucleocapsid that was surrounded by a lipid bilayer membrane. LA particles were not found in infected cultures that were treated with negative serum or in negative control preparations.

[0130] Isolates from the Netherlands, Germany, and the United States. All seven isolates were isolated in porcine alveolar macrophages and passaged three to five times. All isolates caused a cytopathic effect in macrophages and could be specifically immunostained with anti-LA sera b 822 and the 42 dpi serum 23. The isolates were named NL2, GE1, GE 2, US1, US2, and US3.

[0131] Antigenic relationships ofisolates NL1, NL2, GE1, GE2, US 1, US2, and US3. None of the field sera reacted in IPMA with uninfected macrophages but all sera contained antibodies directed against one or more of the seven isolates (Table 7). None of the experimental sera reacted in IPMA with uninfected macrophages, and none of the 0 dpi experimental sera reacted with any of the seven isolates in IPMA (Table 8). All seven LA isolates reacted with all or most of the sera from the set of experimental sera of pigs 21, 23, 25,

and 29, taken after 0 dpi. Only the isolates US1, US2, and US3 reacted with all or most of the sera from the set of experimental sera of gilts 2B, 9G, 16W, and 16Y, taken after 0 dpi.

[0132] Radioimmunoprecipitation studies. Seven LA-specific proteins were detected in LA-infected macrophages but not in uninfected macrophages precipitated with the 42 dpi sera of pigs b 822 and 23. The proteins had estimated molecular weights of 65, 39, 35, 26, 19, 16, and 15 kilodalton. Only two of these LA-specific proteins, of 16 and 15 kilodalton, were also precipitated by the 26 dpi serum MN8.

[0133] Sequence and Organization of the Genome of LV

[0134] The nature of the genome of LV was determined by analyzing DNA and RNA from infected porcine lung alveolar macrophages. No LV-specific DNA was detected. However, we did detect LV-specific RNA. In a 0.8% neutral agarose gel, LV RNA migrated slightly slower than a preparation of hog cholera virus RNA of 12.3 kb (Moormann et al., 1990) did. Although no accurate size determination can be performed in neutral agarose gels, it was estimated that the LV-specific RNA is about 14.5 to 15.5 kb in length.

[0135] To determine the complexity of the LV-specific RNAs in infected cells and to establish the nucleotide sequence of the genome of LV, we prepared cDNA from RNA of LV-infected porcine lung alveolar macrophages and selected and mapped LV-specific cDNA clones as described under Materials and Methods. The specificity of the cDNA clones was reconfirmed by hybridizing specific clones, located throughout the overlapping cDNA sequence, to Northern blots carrying RNA of LV-infected and uninfected macrophages. Remarkably, some of the cDNA clones hybridized with the 14.5 to 15.5 kb RNA detected in infected macrophages only, whereas others hybridized with the 14.5 to 15.5 kb RNA as well as with a panel of 4 or 5 RNAs of lower molecular weight (estimated size, 1 to 4 kb). The latter clones were all clustered at one end of the cDNA map and covered about 4 kb of DNA. These data suggested that the genome organization of LV may be similar to that of coronaviridae (Spaan et al., 1988), Berne virus (BEV; Snijder et al., 1990b), a torovirus, and EAV (de Vries et al., 1990), i.e., besides a genomic RNA there are subgenomic mRNAs which form a nested set which is located at the 3' end of the genome. This assumption was confirmed when sequences of the cDNA clones became available and specific primers could be selected to probe the blots with. A compilation of the hybridization data obtained with cDNA clones and specific primers, which were hybridized to Northern blots carrying the RNA of LV-infected and uninfected macrophages, is shown in **FIG. 2**. Clones 12 and 20 which are located in the 5' part and the centre of the sequence, respectively, hybridize to the 14.5 to 15.5 kb genomic RNA detected in LV-infected cells only. Clones 41 and 39, however, recognize the 14.5 to 15.5 kb genomic RNA and a set of 4 and 5 RNAs of lower molecular weight, respectively. The most instructive and conclusive hybridization pattern, however, was obtained with primer 25, which is located at the ultimate 5' end in the LV sequence (compare **FIG. 1**). Primer 25 hybridized to a panel of 7 RNAs, with an estimated molecular weight ranging in size from 0.7 to 3.3 kb (subgenomic mRNAs), as well as the genomic RNA. The most likely explanation for the hybridization pattern of primer 25 is that 5' end genomic sequences, the length of which is yet unknown, fuse with the body of the mRNAs which are transcribed from the 3' end of the genome. In fact, the hybridization pattern obtained with primer 25 suggests that 5' end genomic sequences function as a so called "leader sequence" in subgenomic mRNAs. Such a transcription pattern is a hallmark of replication of coronaviridae (Spaan et al., 1988), and of EAV (de Vries et al., 1990).

[0136] The only remarkable discrepancy between LV and EAV which could be extracted from the above data is that the genome size of LV is about 2.5 kb larger than that of EAV.

[0137] The consensus nucleotide sequence of overlapping cDNA clones is shown in **FIG. 1** (SEQ ID NO: 1). The length of the sequence is 15,088 basepairs, which is in good agreement with the estimated size of the genomic LV RNA.

[0138] Since the LV cDNA library was made by random priming of the reverse transcriptase reaction with calf thymus pd(N) 6 primers, no cDNA clones were obtained which started with a poly-A stretch at their 3' end. To clone the 3' end of the viral genome, we constructed a second cDNA library, using oligo (dT) and primer 39U183R in the reverse transcriptase reaction. Primer 39U183R is complementary to LV minus-strand RNA, which is likely present in a preparation of RNA isolated from LV-infected cells. This library was screened with virus-specific probes (nick-translated cDNA clone 119 and oligonucleotide 119R64R), resulting in the isolation of five additional cDNA clones (e.g., cDNA clone 151, **FIG. 2**). Sequencing of these cDNA clones revealed that LV contains a 3' poly(A) tail. The length of the poly(A) tail varied between the various cDNA clones, but its maximum length was twenty nucleotides. Besides clone 25 and 155 (**FIG. 2**), four additional cDNA clones were isolated at the 5' end of the genome, which were only two to three nucleotides shorter than the ultimate 5' nucleotide shown in **FIG. 1** (SEQ ID NO: 1). Given this finding and given the way cDNA was synthesized, we assume to be very close to the 5' end of the sequence of LV genomic RNA.

[0139] Nearly 75% of the genomic sequence of LV encodes ORF 1A and ORF 1B. ORF 1A probably initiates at the first AUG (nucleotide position 212, **FIG. 1**) encountered in the LV sequence. The C-terminus of ORF 1A overlaps the putative N-terminus of ORF 1 B over a small distance of 16 nucleotides. It thus seems that translation of ORF 1B proceeds via ribosomal frameshifling, a hallmark of the mode of translation of the polymerase or replicase gene of coronaviruses (Boursnell et al., 1987; Bredenbeek et al. 1990) and the torovirus BEV (Snijder et al., 1990a). The characteristic RNA pseudoknot structure which is predicted to be formed at the site of the ribosomal frameshifting is also found at this location in the sequence of LV (results not shown).

[0140] ORF 1B encodes an amino acid sequence (SEQ ID NO: 3) of nearly 1400 residues which is much smaller than ORF 1B of the coronaviruses MHV and IBV (about 3,700 amino acid residues; Bredenbeek et al., 1990; Boursnell et al., 1987) and BEV (about 2,300 amino acid residues; Snijder et al., 1990a). Characteristic features of the ORF 1B product (SEQ ID NO: 3) of members of the superfamily of coronaviridae, like the replicase motif and the Zinc finger domain, can also be found in ORF 1B of LV (results not shown).

[0141] Whereas ORF 1A and ORF 1B encode the viral polymerase (SEQ ID NO:2 and SEQ ID NO:3) and, there-

fore, are considered to encode a non-structural viral protein, ORFs 2 to 7 are believed to encode structural viral proteins (SEQ ID NOS:4-9).

[0142] The products of ORFs 2 to 6 (SEQ ID NOS:4-8) all show features reminiscent of membrane (envelope) associated proteins. ORF 2 encodes a protein (SEQ ID NO:4) of 249 amino acids containing two predicted N-linked glycosylation sites (Table 9). At the N-terminus a hydrophobic sequence, which may function as a so-called signal sequence, is identified. The C-terminus also ends with a hydrophobic sequence, which in this case may function as a transmembrane region, which anchors the ORF 2 product (SEQ ID NO:4) in the viral envelope membrane.

[0143] ORF 3 may initiate at the AUG starting at nucleotide position 12394 or at the AUG starting at nucleotide position 12556 and then encodes proteins (SEQ ID NO:5) of 265 and 211 amino acids, respectively. The protein of 265 residues contains seven putative N-linked glycosylation sites, whereas the protein of 211 residues contains four (Table 9). At the N-terminus of the protein (SEQ ID NO:5) of 265 residues a hydrophobic sequence is identified.

[0144] Judged by hydrophobicity analysis, the topology of the protein encoded by ORF 4 (SEQ ID NO:6) is similar to that encoded by ORF 2 (SEQ ID NO:4) if the product of ORF 4 (SEQ ID NO:6) initiates at the AUG starting at nucleotide position 12936. However, ORF 4 may also initiate at two other AUG codons (compare **FIGS. 1 and 2**) starting at positions 12981 and 13068 in the sequence respectively. Up to now it is unclear which start codon is used. Depending on the start codon used, ORF 4 may encode proteins (SEQ ID NO:6) of 183 amino acids containing four putative N-linked glycosylation sites, of 168 amino acids containing four putative N-linked glycosylation sites, or of 139 amino acids containing three putative N-linked glycosylation sites (Table 9).

[0145] ORF 5 is predicted to encode a protein (SEQ ID NO:7) of 201 amino acids having two putative N-linked glycosylation sites (Table 9). A characteristic feature of the ORF 5 product (SEQ ID NO:7) is the internal hydrophobic sequence between amino acid 108 to amino acid 132.

[0146] Analysis for membrane spanning segments andhydrophilicity of the product of ORF 6 (SEQ ID NO:8) shows that it contains three transmembrane spanning segments in the N-terminal 90 amino acids of its sequence. This remarkable feature is also a characteristic of the small envelope glycoprotein M or E1 of several coronaviruses, e.g., Infectious Bronchitis Virus (IBV; Boursnell et al., 1984) and Mouse Hepatitis Virus (MHV: Rottier et al., 1986). It is, therefore, predicted that the protein encoded by ORF 6 (SEQ ID NO:8) was a membrane topology analogous to that of the M or E1 protein of coronaviruses (Rottier et al., 1986). A second characteristic of the M or E1 protein is a so-called surface helix which is located immediately adjacent to the presumed third transmembrane region. This sequence of about 25 amino acids which is very well conserved among coronaviruses is also recognized, although much more degenerate, in LV. Yet we predict the product of LV ORF 6 (SEQ ID NO:8) to have an analogous membrane associated function as the coronavirus M or E1 protein. Furthermore, the protein encoded by ORF 6 (SEQ ID NO:8) showed a strong similarity (53% identical amino acids) with VpX (Godeny et al., 1990) of LDV.

[0147] The protein encoded by ORF 7 (SEQ ID NO:9) has a length of 128 amino acid residues (Table 9) which is 13 amino acids longer than Vp1 of LDV (Godeny et al., 1990). Yet a significant similarity (43% identical amino acids) was observed between the protein encoded by ORF 7 (SEQ ID NO:9) and Vp1. Another shared characteristic between the product of ORF 7 (SEQ ID NO:9) and Vp1 is the high concentration of basic residues (Arg, Lys and His) in the N-terminal half of the protein. Up to amino acid 55, the LV sequence contains 26% Arg, Lys and His. This finding is fully in line with the proposed function of the ORF 7 product (SEQ ID NO:9) or Vp1 (Godeny et al., 1990), namely encapsidation of the viral genomic RNA. On the basis of the above data, we propose the LV ORF 7 product (SEQ ID NO:9) to be the nucleocapsid protein N of the virus.

[0148] A schematic representation of the organization of the LV genome is shown in **FIG. 2**. The map of overlapping clones used to determine the sequence of LV is shown in the top panel. A linear compilation of this map indicating the 5' and 3' end of the nucleotide sequence of LV, shown in **FIG. 1** (SEQ ID NO:1), including a division in kilobases, is shown below the map of cDNA clones and allows the positioning of these clones in the sequence. The position of the ORFs identified in the LV genome is indicated below the linear map of the LV sequence. The bottom panel shows the nested set of subgenomic mRNAs, and the position of these RNAs relative to the LV sequence.

[0149] In line with the translation strategy of coronavirus, torovirus and arterivirus subgenomic mRNAs, it is predicted that ORFs 1 to 6 are translated from the unique 5' end of their genomic or mRNAs. This unique part of the mRNAs is considered to be that part of the RNA that is obtained when a lower molecular weight RNA is "subtracted" from the higher molecular weight RNA which is next in line. Although RNA 7 forms the 3' end of all the other genomic and subgenomic RNAs, and thus does not have a unique region, it is believed that ORF 7 is only translated from this smallest sized mRNA. The "leader sequence" at the 5' end of the subgenomic RNAs is indicated with a solid box. The length of this sequence is about 200 bases, but the precise site of fusion with the body of the genomic RNAs still has to be determined.

[0150] Experimental Reproduction of MSD

[0151] Eight pregnant sows were inoculated with LA and clinical signs of MSD such as inappetance and reproductive losses were reproduced in these sows. From day four to day 10-12 post-inoculation (p.i.), all sows showed a reluctance to eat. None of the sows had elevated body temperatures. Two sows had bluish ears at day 9 and 10 p.i. In Table 6 the day of birth and the number of living and dead piglets per sow is given. LA was isolated from 13 of the born piglets.

TABLE 1

Description and results of virus isolation of field samples.

A Samples of piglets suspected of infection with MSD.

farm	number of pigs	age days	material used	results*
RB	5	2	lung, tonsil, and brains	5 × LA
DV	4	3	lung, brains,	3 × LA

TABLE 1-continued

Description and results of virus isolation of field samples.

			pools of kidney, spleen	
TH	3	3–5	lung, pools of kidney, tonsil	3 × LA
DO	3	10	lung, tonsil	2 × LA
ZA	4	1	lung, tonsil	3 × LA
VE	1	?	oral swab	1 × PEV 2
TOTAL	20			16 × LA, 1 × PEV 2

B Samples of sows suspected of infection with MSD.

farm	number of sows	material used	results
TH	2	plasma and leucocytes	1 × LA
HU	5	plasma and leucocytes	2 × LA, 1 × EMCV
TS	10	plasma and leucocytes	6 × LA
HK	5	plasma and leucocytes	2 × LA
LA	6	plasma and leucocytes	2 × LA
VL	6	serum and leucocytes	5 × LA
TA	15	serum	11 × LA
LO	4	plasma and leucocytes	2 × LA
JA	8	plasma and leucocytes	8 × LA
VD	1	plasma and leucocytes	1 × LA
VW	1	serum	1 × LA
TOTAL	63		41 × LA, 1 × EMCV

*Results are given as the number of pigs from which the isolation was made. Sometimes the isolate was detected in more than one sample per pig.
LA = Lelystad Agent
PEV 2 = porcine entero virus type 2
EMCV = encephalomyocarditis virus

[0152]

TABLE 2

Description and results of virus isolation of samples of pigs with experimentally induced infections.

sow	pig@	material used	results*
A (LO) #	c 835	lung, tonsil	2 × LA
	c 836	nasal swabs	2 × PEV 7
	c 837	nasal swabs	
B (JA)	c 825	lung, tonsil	
	c 821	nasal swabs	1 × PEV 7
	c 823	nasal swabs	4 × PEV 7
C (JA)	c 833	lung, tonsil	1 × LA, 1 × PEV 7
	c 832	nasal swabs	2 × PEV 7
	c 829	nasal swabs, plasma and leucocytes	3 × LA, 2 × PEV 7
D (VD)	c 816	lung, tonsil	
	c 813	nasal swabs	1 × LA
	c 815	nasal swabs	1 × PEV 7
TOTAL isolates from contact pigs			7 × LA, 13 × PEV 7
A	b 809	nasal swabs	
	b 817	nasal swabs	
B	b 818	nasal swabs, plasma and leucocytes	1 × LA
	b 820	nasal swabs	
C	b 822	nasal swabs	
	b 826	nasal swabs	

TABLE 2-continued

Description and results of virus isolation of samples of pigs with experimentally induced infections.

sow	pig@	material used	results*
D	b 830	nasal swabs	1 × LA
	b 834	nasal swabs	
TOTAL isolates from blood inoculated pigs			2 × LA

@SPF pigs were either kept in contact (c) with a sow suspected to be infected with MSD, or were given 10 ml EDTA blood (b) of that sow intramuscularly at day 0 of the experiment. Groups of one sow and three SPF pigs (c) were kept in one pen, and all four of these groups were housed in one stable. At day 6, one SPF pig in each group was killed and tonsil and lungs were used for virus isolation. The four groups of SPF pigs inoculated with blood (b) were housed in four other pens ina separate stable. EDTA blood for virus isolation from plasma and leucocytes was taken whenever a pig had fever.
*Results are given as number of isolates per pig.
LA = Lelystad Agent
PEV 7 = procine entero virus type 7
In brackets the initials of the farm of origin of the sow are given.

[0153]

TABLE 3

Identification of viral isolates

origin and cell culture	buoyant[1] density in CsCl	particle[2] size in FM (nm)	sens[3] to chloroform	neutralized by[4] serum directed against (titre)
leucocytes sow farm HU PK-15, PK2, SK6	1.33 g/ml	28–30	not sens.	EMCV (1280)
oral swab piglet farm VE SK6	ND	28–30	not sens.	PEV 2 (>1280)
nasal swabs, tonsil SPF pigs CVI PK-15, PK2, SK6	ND	28–30	not sens.	PEV 7 (>1280)
various samples various farms pig lung macrophages	1.19 g/ml	pleomorf	sens.	none (all <5)

[1]Buoyant density in preformed linear gradients of CsCl in PBS was determined according to standard techniques (Brakke; 1967). Given is the density where the peak of infectivity was found.
[2]Infected and noninfected cell cultures of the isolate under study were freeze-thawed. Cell lysates were centrifuged for 30 min at 130,000 g, the resulting pellet was negatively stained according to standard techniques (Brenner and Horne; 1959), and studied with a Philips CM 10 electron microscope. Given is the size of particles that were present in infected and not present in non-infected cultures.
[3]Sensitivity to chloroform was determined according to standard techniques (Grist, Ross, and Bell; 1974).
[4]Hundred to 300 $TCID_{50}$ of isolates were mixed with varying dilutions of specific antisera and grown in the appropriate cell system until full CPE was observed. Sera with titres higher than 5 were retested, and sera which blocked with high titres the CPE were considered specific for the isolate. The isolates not sensitive to chloroform were tested with sera specifically directed against porcineentero viruses (PEV) 1 to 11 (courtesy Dr. Knowles, Pirbright, UK), against encephalomyocarditis virus (EMCV; courtesy Dr. Ahl, Tübingen, Germany), against porcine parvo virus, and against swine vesicular disease.
The isolate (code: CDI-NL-2.91) sensitive to chloroform was tested with antisera specifically directed against pseudorabies virus, bovine herpes virus 1, bovine herpes virus 4, malignant catarrhal virus, bovine viral diarrhea virus, hog cholera virus, swine influenza virus H1N1 and H3N2, parainfluenza 3 virus, bovine respiratory syncitial virus, transmissible gastroenteritis virus, porcine epidemic diarrhoea virus, haemagglutinating encephalitis virus, infectious bronchitis virus, bovineleukemia virus from the SPF-pigs (see Table 5).

[0154]

TABLE 4

Results of serology of paired field sera taken from sows suspected to have MSD. Sera were taken in the acute phase of the disease and 3–9 weeks later. Given is the number of sows which showed a fourfold or higher rise in titre/number of sows tested.

Farm	Interval[i] in weeks	HAI HEV	H1N1	H3N2	ELISA PPV	PPV	BVDV	HCV
TH	3	0/6	0/6	0/6	0/6	0/6	0/5	0/6
RB	5	0/13	1/13	0/13	1/9	0/7	0/6	0/9
HU	4	0/5	0/5	3/5	0/5	0/5	0/5	0/5
TS	3	1/10	0/10	0/10	0/10	0/10	0/4	0/10
VL	3	0/5	0/5	0/5	0/5	1/5	0/5	0/5
JA	3	0/11	1/11	3/11	0/11	2/11	0/11	0/11
WE	4	1/6	1/6	1/6	3/7	3/7	0/7	0/7
GI	4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
SE	5	0/8	0/8	0/8	0/8	0/6	0/3	0/8
KA	5	0/1	0/1	0/1	0/1	0/1	ND	0/1
HO	3	1/6	0/5	1/6	0/6	0/6	0/6	0/6
NY	4	0/5	1/5	1/5	0/3	0/4	0/2	0/4
JN	3	0/10	5/10	0/10	0/10	1/10	0/10	0/10
KO[f]	3	1/10	0/10	0/10	0/10	2/10	0/10	0/10
OE	9	ND	ND	ND	0/6	0/6	0/6	0/6
LO	6	ND	ND	ND	0/3	0/3	0/2	0/3
WI	4	ND	ND	ND	0/1	1/1	0/1	0/3
RR	3	ND	ND	ND	1/8	0/8	0/8	0/8
RY	4	ND	ND	ND	0/3	0/4	0/3	0/4
BE	5	ND	ND	ND	0/10	0/10	0/10	0/10
BU	3	ND	ND	ND	1/6	0/6	0/6	0/6
KR	3	ND	ND	ND	1/4	0/4	0/4	0/4
KW	5	ND	ND	ND	0/10	0/10	0/10	0/10
VR	5	ND	ND	ND	1/6	0/6	0/6	0/6
HU	4	ND	ND	ND	1/4	0/3	0/3	0/4
ME	3	ND	ND	ND	0/5	1/5	0/5	0/5
total negative[n]		19	41	29	97	16	140	165
total positive[p]		77	48	62	55	131	1	0
total sero-converted[s]		4	10	9	9	11	0	0
total tested		100	99	100	161	158	141	165

Farm	Interval in weeks	SNT EMCV	EMCVi	PEV2	PEV2i	PEV7	PEV7i	LA	IPMA LA
TH	3	0/6	0/6	0/5	0/5	0/6	0/5	0/6	6/6
RB	5	1/7	1/9	0/6	2/6	1/8	0/6	0/13	7/9
HU	4	ND	0/5	0/5	0/5	ND	0/5	0/5	5/5
TS	3	0/10	0/10	0/7	0/4	0/10	0/7	ND	10/10
VL	3	ND	ND	1/5	0/5	ND	0/5	ND	5/5
JA	3	0/11	0/11	0/11	0/11	1/11	2/11	0/5	8/11
WE	4	1/7	1/6	1/6	1/7	1/7	1/7	0/7	7/7
GI	4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	4/4
SE	5	0/8	0/8	0/6	1/8	0/8	1/5	0/8	6/8
KA	5	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
HO	3	0/6	0/6	0/6	0/6	0/6	0/6	0/6	4/6
NY	4	0/4	0/4	0/2	0/2	0/4	0/3	0/4	4/4
JN	3	0/10	0/10	1/10	0/9	0/10	0/10	0/10	5/10
KO[f]	3	0/10	0/10	2/10	2/10	1/10	3/10	ND	8/10
OE	9	0/6	0/6	1/6	1/5	ND	1/6	ND	4/6
LO	6	0/3	0/3	0/3	0/3	0/3	0/3	ND	3/3
WI	4	ND	ND	0/1	0/1	ND	0/1	ND	0/3
RR	3	0/8	1/8	0/8	0/8	0/8	0/8	ND	8/8
RY	4	0/4	ND	0/4	0/1	ND	1/4	ND	1/4
BE	5	ND	ND	0/10	0/10	ND	1/10	ND	0/10
BU	3	ND	ND	0/6	0/6	ND	0/6	ND	6/6
KR	3	ND	ND	0/4	0/4	ND	0/4	ND	1/4
KW	5	ND	ND	0/10	0/10	ND	1/10	ND	10/10
VR	5	ND	ND	0/6	1/6	ND	0/6	ND	6/6
HU	4	ND	ND	0/3	0/4	ND	0/3	ND	3/4
ME	3	ND	ND	0/5	0/5	ND	0/5	ND	2/5
total neg.[n]		15	29	0	0	2	1	69	15

TABLE 4-continued

Results of serology of paired field sera taken from sows suspected to have MSD. Sera were taken in the acute phase of the disease and 3–9 weeks later. Given is the number of sows which showed a fourfold or higher rise in titre/number of sows tested.

Farm	Interval[i] in weeks	HAI HEV	H1N1	H3N2	ELISA PPV	PPV	BVDV	HCV
total pos.[p]		88	74	144	138	90	136	0	27
total sero-converted[s]		2	3	6	8	4	10	0	123
total tested		105	107	150	146	96	147	69	165

The sera were tested in haemagglutinating inhibition (HAI) tests for the detection of antibody against haemagglutinating encephalitis virus (HEV), and swine influenza viruses H1N1 and H3N2, in enzyme-linked-immuno sorbent assays (ELISA) for the detection of antibody against the glycoprotein gI of pseudorabies virus (PRV), against porcine parvo virus (PPV), bovine viral diarrhea virus (BVDV), and hog cholera virus (HCV).
The sera were tested in serum neutralization tests (SNT) for the detection of neutralizing antibody directed against encephalomyocarditis virus (EMCV), the isolated (i) EMCV, porcine entero viruses (PEV) 2 and 7 and the PEV isolates (i), and against the Lelystad Agent (LA), and were tested in an immuno-peroxidase-monolayer-assay (IPMA) for the detection of antibody directed against the Lelystad Agent (LA).
[f]fattening pigs.
[i]time between sampling of the first and second serum.
[n]total number of pigs of which the first serum was negative in the test under study, and of which the second serum was also negative or showed a less than fourfold rise in titre.
[P]total number of pigs of which the first serum was positive and of which the second serum showed a less than fourfold rise in titre.
[s]total number of pigs of which the second serum had a fourfold or higher titre than the first serum in the test under study.
ND = not done.

[0155]

TABLE 5

Development of antibody directed against Lelystad Agent as measured by IPMA.

A contact pigs serum titres in IPMA

	Weeks post contact:				
Pig	0	2	3	4	5
c 836	0	10	640	640	640
c 837	0	10	640	640	640
c 821	0	640	640	640	640
c 823	0	160	2560	640	640
c 829	0	160	640	10240	10240
c 832	0	160	640	640	2560
c 813	0	640	2560	2560	2560
c 815	0	160	640	640	640

B blood inoculated pigs serum titres in IPMA

	Weeks post inoculation:				
Pig	0	2	3	4	6
b 809	0	640	2560	2560	2560
b 817	0	160	640	640	640
b 818	0	160	640	640	640
b 820	0	160	640	640	640
b 822	0	640	2560	2560	10240
b 826	0	640	640	640	10240
b 830	0	640	640	640	2560
b 834	0	160	640	2560	640

See Table 2 for description of the experiment. All pigs were bled at regular intervals and all sera were tested in an immuno-peroxidase-monolayer-assay (IPMA) for the detection of antibody directed against the Lelystad Agent (LA).

[0156]

TABLE 6

Experimental reproduction of MSD.

Sow	Length of gestation	No. of piglets at birth: alive (Number Ab pos)[2]	dead	No. of deaths week 1	LA[1] in piglets: born dead	died in week 1
52	113	12 (5)	3 (2)	6	2	4
965	116	3 (0)	9 (3)	2	4	
997	114	9 (0)	1 (0)	0		
1305	116	7 (0)	2 (0)	1		
134	109	4 (4)	7 (4)	4	3	
941	117	7	10			
1056	113	7 (1)	3 (0)	4		
1065	115	9	2			

[1]LA was isolated from lung, liver, spleen, kidney, or ascitic fluids.
[2]Antibodies directed against LA were detected in serum samples taken before the piglets had sucked, or were detected in ascitic fluids of piglets born dead.

[0157]

TABLE 7

Reactivity in IPMA of a collection of field sera from Europe and North America tested with LA isolates from the Netherlands (NL1 and NL2), Germany (GE1 and GE2), and the United States (US1, US2 and US3).

Isolates:	NL1	NL2	GE1	GE2	US1	US2	US3
Sera from: The Netherlands							
TH-187	3.5_t	3.5	2.5	3.5	–	–	–
TO-36	3.5	3.0	2.5	3.0	–	1.0	–

TABLE 7-continued

Reactivity in IPMA of a collection of field sera from Europe and North America tested with LA isolates from the Netherlands (NL1 and NL2), Germany (GE1 and GE2), and the United States (US1, US2 and US3).

Isolates:	NL1	NL2	GE1	GE2	US1	US2	US3
Germany							
BE-352	4.0	3.5	2.5	3.0	–	1.5	–
BE-392	3.5	3.5	2.5	2.5	1.5	1.5	0.5
NI-f2	2.5 1.5	2.0	2.5	–	–	–	
United Kingdom							
PA-141615	4.0	3.0	3.0	3.5	–	–	–
PA-141617	4.0	3.5	3.0	3.5	–	2.5	2.0
PA-142440	3.5	3.0	2.5	3.5	–	2.0	2.5
Belgium							
PE-1960	4.5	4.5	3.0	4.0	1.5	–	–
France							
EA-2975	4.0	3.5	3.0	3.0	2.0	–	–
EA-2985	3.5	3.0	3.0	2.5	–	–	–
United States							
SL-441	3.5	1.5	2.5	2.5	3.5	3.5	3.0
SL-451	3.0	2.0	2.5	2.5	3.5	4.5	4.0
AL-RP9577	1.5	–	–	1.0	3.0	4.0	2.5
AL-P10814/33	0.5	2.5	–	–	2.5	3.5	3.0
AL-4094A	–	–	–	–	1.0	2.0	0.5
AL-7525	–	–	–	–	–	1.0	–
JC-MN41	–	–	–	–	1.0	3.5	1.0
JC-MN44	–	–	–	–	2.0	3.5	2.0
JC-MN45	–	–	–	–	2.0	3.5	2.5
Canada							
RB-16	2.5	–	3.0	2.0	3.0	3.5	–
RB-19	1.0	–	1.0	–	2.5	1.5	–
RB-22	1.5	–	2.0	2.5	2.5	3.5	–
RB-23	–	–	–	–	–	3.0	–

t = titre expressed as negative log;
– = negative

[0158]

TABLE 8

Reactivity in IPMA of a collection of experimental sera raised against LA and SIRSV tested with LA isolates from the Netherlands (NL1 and NL2), Germany (GE1 and GE2), and the United States (US1, US2 and US3).

Isolates:		NL1	NL2	GE1	GE2	US1	US2	US3
Sera: anti-LA:								
21	14 dpi	2.5[t]	2.0	2.5	3.0	1.5	2.0	1.5
	28 dpi	4.0	3.5	3.5	4.0	–	2.5	1.5
	42 dpi	4.0	3.5	3.0	3.5	1.5	2.5	2.0
23	14 dpi	3.0	2.0	2.5	3.0	1.0	2.0	1.0
	28 dpi	3.5	3.5	3.5	4.0	1.5	2.0	2.0
	42 dpi	4.0	4.0	3.0	4.0	–	2.5	2.5
25	14 dpi	2.5	2.0	2.5	3.0	1.5	2.0	1.0
	28 dpi	4.0	3.5	4.0	3.5	–	1.5	2.0
	42 dpi	3.5	4.0	3.5	3.5	1.5	2.0	2.0
29	14 dpi	3.5	3.5	3.0	3.5	–	2.0	1.5
	28 dpi	3.5	3.5	3.0	3.5	–	2.5	2.0
	42 dpi	4.0	3.5	3.5	4.0	1.5	2.5	2.5

TABLE 8-continued

Reactivity in IPMA of a collection of experimental sera raised against LA and SIRSV tested with LA isolates from the Netherlands (NL1 and NL2), Germany (GE1 and GE2), and the United States (US1, US2 and US3).

Isolates:		NL1	NL2	GE1	GE2	US1	US2	US3
anti-SIRSV:								
2B	20 dpi	–	–	–	–	2.0	2.0	–
	36 dpi	–	–	–	–	1.5	2.0	–
	63 dpi	–	–	–	–	1.0	1.0	–
9G	30 dpi	–	–	–	–	2.5	3.0	–
	44 dpi	–	–	–	–	2.5	3.5	–
	68 dpi	–	–	–	–	2.0	3.5	1.5
16W	25 dpi	–	–	–	–	2.0	3.0	–
	40 dpi	–	–	–	–	2.0	3.0	–
	64 dpi	–	–	–	–	2.5	2.5	1.5
16Y	36 dpi	–	–	–	–	1.0	3.0	1.0
	64 dpi	–	–	–	–	2.5	3.0	–

t = titer expressed as negative log;
– = negative

[0159]

TABLE 9

Characteristics of the ORFs of Lelystad Virus.

ORF	Nucleotides (first-last)	No. of amino acids	Calculated size of the unmodified peptide (kDa)	number of glycosylation sites
ORF1A	212–7399	2396	260.0	3 (SEQ ID NO: 2)
ORF1B	7384–11772	1463	161.8	3 (SEQ ID NO: 3)
ORF2	11786–12532	249	28.4	2 (SEQ ID NO: 4)
ORF3	12394–13188	265	30.6	7 (SEQ ID NO: 5)
	12556–13188	211	24.5	4
ORF4	12936–13484	183	20.0	4 (SEQ ID NO: 6)
	12981–13484	168	18.4	4
	13068–13484	139	15.4	3
ORF5	13484–14086	201	22.4	2 (SEQ ID NO: 7)
ORF6	14077–14595	173	18.9	2 (SEQ ID NO: 8)
ORF7	14588–14971	128	13.8	1 (SEQ ID NO: 9)

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 9

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 212..7399
(D) OTHER INFORMATION:

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 7384..11772
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(B) LOCATION: 12394..13188
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(B) LOCATION: 12936..13484

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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GCCGGAGTGT CATGCTGAGC TTTTGGCTCT TGAGCAGCGC CAACTTTGGG AACCTGTGCG 3891

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CGAAATTAAA AAAAAAAAAA AAAAAAA 15108

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2396 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Met Ser Gly Thr Phe Ser Arg Cys Met Cys Thr Pro Ala Ala Arg Val
  1               5                  10                  15

Phe Trp Asn Ala Gly Gln Val Phe Cys Thr Arg Cys Leu Ser Ala Arg
             20                  25                  30

Ser Leu Leu Ser Pro Glu Leu Gln Asp Thr Asp Leu Gly Ala Val Gly
         35                  40                  45

Leu Phe Tyr Lys Pro Arg Asp Lys Leu His Trp Lys Val Pro Ile Gly
     50                  55                  60

Ile Pro Gln Val Glu Cys Thr Pro Ser Gly Cys Cys Trp Leu Ser Ala
 65                  70                  75                  80

Val Phe Pro Leu Ala Arg Met Thr Ser Gly Asn His Asn Phe Leu Gln
                 85                  90                  95

Arg Leu Val Lys Val Ala Asp Val Leu Tyr Arg Asp Gly Cys Leu Ala
            100                 105                 110

Pro Arg His Leu Arg Glu Leu Gln Val Tyr Glu Arg Gly Cys Asn Trp
        115                 120                 125

Tyr Pro Ile Thr Gly Pro Val Pro Gly Met Gly Leu Phe Ala Asn Ser
    130                 135                 140

Met His Val Ser Asp Gln Pro Phe Pro Gly Ala Thr His Val Leu Thr
145                 150                 155                 160

Asn Ser Pro Leu Pro Gln Gln Ala Cys Arg Gln Pro Phe Cys Pro Phe
                165                 170                 175

Glu Glu Ala His Ser Ser Val Tyr Arg Trp Lys Lys Phe Val Val Phe
            180                 185                 190

Thr Asp Ser Ser Leu Asn Gly Arg Ser Arg Met Met Trp Thr Pro Glu
        195                 200                 205

Ser Asp Asp Ser Ala Ala Leu Glu Val Leu Pro Pro Glu Leu Glu Arg
    210                 215                 220

Gln Val Glu Ile Leu Ile Arg Ser Phe Pro Ala His His Pro Val Asp
225                 230                 235                 240

Leu Ala Asp Trp Glu Leu Thr Glu Ser Pro Glu Asn Gly Phe Ser Phe
                245                 250                 255

Asn Thr Ser His Ser Cys Gly His Leu Val Gln Asn Pro Asp Val Phe
            260                 265                 270

Asp Gly Lys Cys Trp Leu Ser Cys Phe Leu Gly Gln Ser Val Glu Val
        275                 280                 285

Arg Cys His Glu Glu His Leu Ala Asp Ala Phe Gly Tyr Gln Thr Lys
    290                 295                 300

Trp Gly Val His Gly Lys Tyr Leu Gln Arg Arg Leu Gln Val Arg Gly
305                 310                 315                 320

Ile Arg Ala Val Val Asp Pro Asp Gly Pro Ile His Val Glu Ala Leu
                325                 330                 335

Ser Cys Pro Gln Ser Trp Ile Arg His Leu Thr Leu Asp Asp Asp Val
            340                 345                 350

Thr Pro Gly Phe Val Arg Leu Thr Ser Leu Arg Ile Val Pro Asn Thr
        355                 360                 365

Glu Pro Thr Thr Ser Arg Ile Phe Arg Phe Gly Ala His Lys Trp Tyr
    370                 375                 380
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Gly Ala Ala Gly Lys Arg Ala Arg Ala Lys Arg Ala Ala Lys Ser Glu
385                 390                 395                 400

Lys Asp Ser Ala Pro Thr Pro Lys Val Ala Leu Pro Val Pro Thr Cys
                405                 410                 415

Gly Ile Thr Thr Tyr Ser Pro Pro Thr Asp Gly Ser Cys Gly Trp His
            420                 425                 430

Val Leu Ala Ala Ile Met Asn Arg Met Ile Asn Gly Asp Phe Thr Ser
        435                 440                 445

Pro Leu Thr Gln Tyr Asn Arg Pro Glu Asp Asp Trp Ala Ser Asp Tyr
    450                 455                 460

Asp Leu Val Gln Ala Ile Gln Cys Leu Arg Leu Pro Ala Thr Val Val
465                 470                 475                 480

Arg Asn Arg Ala Cys Pro Asn Ala Lys Tyr Leu Ile Lys Leu Asn Gly
                485                 490                 495

Val His Trp Glu Val Glu Val Arg Ser Gly Met Ala Pro Arg Ser Leu
            500                 505                 510

Ser Arg Glu Cys Val Val Gly Val Cys Ser Glu Gly Cys Val Ala Pro
        515                 520                 525

Pro Tyr Pro Ala Asp Gly Leu Pro Lys Arg Ala Leu Glu Ala Leu Ala
    530                 535                 540

Ser Ala Tyr Arg Leu Pro Ser Asp Cys Val Ser Ser Gly Ile Ala Asp
545                 550                 555                 560

Phe Leu Ala Asn Pro Pro Pro Gln Glu Phe Trp Thr Leu Asp Lys Met
                565                 570                 575

Leu Thr Ser Pro Ser Pro Glu Arg Ser Gly Phe Ser Ser Leu Tyr Lys
            580                 585                 590

Leu Leu Leu Glu Val Val Pro Gln Lys Cys Gly Ala Thr Glu Gly Ala
        595                 600                 605

Phe Ile Tyr Ala Val Glu Arg Met Leu Lys Asp Cys Pro Ser Ser Lys
    610                 615                 620

Gln Ala Met Ala Leu Leu Ala Lys Ile Lys Val Pro Ser Ser Lys Ala
625                 630                 635                 640

Pro Ser Val Ser Leu Asp Glu Cys Phe Pro Thr Asp Val Leu Ala Asp
                645                 650                 655

Phe Glu Pro Ala Ser Gln Glu Arg Pro Gln Ser Ser Gly Ala Ala Val
            660                 665                 670

Val Leu Cys Ser Pro Asp Ala Lys Glu Phe Glu Glu Ala Ala Xaa Glu
        675                 680                 685

Glu Val Gln Glu Ser Gly His Lys Ala Val His Ser Ala Leu Leu Ala
    690                 695                 700

Glu Gly Pro Asn Asn Glu Gln Val Gln Val Val Ala Gly Glu Gln Leu
705                 710                 715                 720

Lys Leu Gly Gly Cys Gly Leu Ala Val Gly Asn Ala His Glu Gly Ala
                725                 730                 735

Leu Val Ser Ala Gly Leu Ile Asn Leu Val Gly Gly Asn Leu Ser Pro
            740                 745                 750

Ser Asp Pro Met Lys Glu Asn Met Leu Asn Ser Arg Glu Asp Glu Pro
        755                 760                 765

Leu Asp Leu Ser Gln Pro Ala Pro Ala Ser Thr Thr Thr Leu Val Arg
    770                 775                 780
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Glu Gln Thr Pro Asp Asn Pro Gly Ser Asp Ala Gly Ala Leu Pro Val
785                 790                 795                 800

Thr Val Arg Glu Phe Val Pro Thr Gly Pro Ile Leu Cys His Val Glu
                805                 810                 815

His Cys Gly Thr Glu Ser Gly Asp Ser Ser Ser Pro Leu Asp Leu Ser
            820                 825                 830

Asp Ala Gln Thr Leu Asp Gln Pro Leu Asn Leu Ser Leu Ala Ala Trp
        835                 840                 845

Pro Val Arg Ala Thr Ala Ser Asp Pro Gly Trp Val His Gly Arg Arg
    850                 855                 860

Glu Pro Val Phe Val Lys Pro Arg Asn Ala Phe Ser Asp Gly Asp Ser
865                 870                 875                 880

Ala Leu Gln Phe Gly Glu Leu Ser Glu Ser Ser Ser Val Ile Glu Phe
                885                 890                 895

Asp Arg Thr Lys Asp Ala Pro Val Val Asp Ala Pro Val Asp Leu Thr
            900                 905                 910

Thr Ser Asn Glu Ala Leu Ser Val Val Asp Pro Phe Glu Phe Ala Glu
        915                 920                 925

Leu Lys Arg Pro Arg Phe Ser Ala Gln Ala Leu Ile Asp Arg Gly Gly
    930                 935                 940

Pro Leu Ala Asp Val His Ala Lys Ile Lys Asn Arg Val Tyr Glu Gln
945                 950                 955                 960

Cys Leu Gln Ala Cys Glu Pro Gly Ser Arg Ala Thr Pro Ala Thr Arg
                965                 970                 975

Glu Trp Leu Asp Lys Met Trp Asp Arg Val Asp Met Lys Thr Trp Arg
            980                 985                 990

Cys Thr Ser Gln Phe Gln Ala Gly Arg Ile Leu Ala Ser Leu Lys Phe
        995                 1000                1005

Leu Pro Asp Met Ile Gln Asp Thr Pro Pro Pro Val Pro Arg Lys Asn
    1010                1015                1020

Arg Ala Ser Asp Asn Ala Gly Leu Lys Gln Leu Val Ala Gln Trp Asp
1025                1030                1035                1040

Arg Lys Leu Ser Val Thr Pro Pro Pro Lys Pro Val Gly Pro Val Leu
                1045                1050                1055

Asp Gln Ile Val Pro Pro Pro Thr Asp Ile Gln Gln Glu Asp Val Thr
            1060                1065                1070

Pro Ser Asp Gly Pro Pro His Ala Pro Asp Phe Pro Ser Arg Val Ser
        1075                1080                1085

Thr Gly Gly Ser Trp Lys Gly Leu Met Leu Ser Gly Thr Arg Leu Ala
    1090                1095                1100

Gly Ser Ile Ser Gln Arg Leu Met Thr Trp Val Phe Glu Val Phe Ser
1105                1110                1115                1120

His Leu Pro Ala Phe Met Leu Thr Leu Phe Ser Pro Arg Gly Ser Met
                1125                1130                1135

Ala Pro Gly Asp Trp Leu Phe Ala Gly Val Val Leu Leu Ala Leu Leu
            1140                1145                1150

Leu Cys Arg Ser Tyr Pro Ile Leu Gly Cys Leu Pro Leu Leu Gly Val
        1155                1160                1165

Phe Ser Gly Ser Leu Arg Arg Val Arg Leu Gly Val Phe Gly Ser Trp
    1170                1175                1180

Met Ala Phe Ala Val Phe Leu Phe Ser Thr Pro Ser Asn Pro Val Gly
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1185                1190                1195                1200

Ser Ser Cys Asp His Asp Ser Pro Glu Cys His Ala Glu Leu Leu Ala
                1205                1210                1215

Leu Glu Gln Arg Gln Leu Trp Glu Pro Val Arg Gly Leu Val Val Gly
            1220                1225                1230

Pro Ser Gly Leu Leu Cys Val Ile Leu Gly Lys Leu Leu Gly Gly Ser
        1235                1240                1245

Arg Tyr Leu Trp His Val Leu Leu Arg Leu Cys Met Leu Ala Asp Leu
    1250                1255                1260

Ala Leu Ser Leu Val Tyr Val Val Ser Gln Gly Arg Cys His Lys Cys
1265                1270                1275                1280

Trp Gly Lys Cys Ile Arg Thr Ala Pro Ala Glu Val Ala Leu Asn Val
                1285                1290                1295

Phe Pro Phe Ser Arg Ala Thr Arg Val Ser Leu Val Ser Leu Cys Asp
            1300                1305                1310

Arg Phe Gln Thr Pro Lys Gly Val Asp Pro Val His Leu Ala Thr Gly
        1315                1320                1325

Trp Arg Gly Cys Trp Arg Gly Glu Ser Pro Ile His Gln Pro His Gln
    1330                1335                1340

Lys Pro Ile Ala Tyr Ala Asn Leu Asp Glu Lys Lys Met Ser Ala Gln
1345                1350                1355                1360

Thr Val Val Ala Val Pro Tyr Asp Pro Ser Gln Ala Ile Lys Cys Leu
                1365                1370                1375

Lys Val Leu Gln Ala Gly Gly Ala Ile Val Asp Gln Pro Thr Pro Glu
            1380                1385                1390

Val Val Arg Val Ser Glu Ile Pro Phe Ser Ala Pro Phe Phe Pro Lys
        1395                1400                1405

Val Pro Val Asn Pro Asp Cys Arg Val Val Val Asp Ser Asp Thr Phe
    1410                1415                1420

Val Ala Ala Val Arg Cys Gly Tyr Ser Thr Ala Gln Leu Xaa Leu Gly
1425                1430                1435                1440

Arg Gly Asn Phe Ala Lys Leu Asn Gln Thr Pro Pro Arg Asn Ser Ile
                1445                1450                1455

Ser Thr Lys Thr Thr Gly Gly Ala Ser Tyr Thr Leu Ala Val Ala Gln
            1460                1465                1470

Val Ser Ala Trp Thr Leu Val His Phe Ile Leu Gly Leu Trp Phe Thr
        1475                1480                1485

Ser Pro Gln Val Cys Gly Arg Gly Thr Ala Asp Pro Trp Cys Ser Asn
    1490                1495                1500

Pro Phe Ser Tyr Pro Thr Tyr Gly Pro Gly Val Val Cys Ser Ser Arg
1505                1510                1515                1520

Leu Cys Val Ser Ala Asp Gly Val Thr Leu Pro Leu Phe Ser Ala Val
                1525                1530                1535

Ala Gln Leu Ser Gly Arg Glu Val Gly Ile Phe Ile Leu Val Leu Val
            1540                1545                1550

Ser Leu Thr Ala Leu Ala His Arg Met Ala Leu Lys Ala Asp Met Leu
        1555                1560                1565

Val Val Phe Ser Ala Phe Cys Ala Tyr Ala Trp Pro Met Ser Ser Trp
    1570                1575                1580

Leu Ile Cys Phe Phe Pro Ile Leu Leu Lys Trp Val Thr Leu His Pro
1585                1590                1595                1600
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Leu Thr Met Leu Trp Val His Ser Phe Leu Val Phe Cys Leu Pro Ala
                1605                1610                1615

Ala Gly Ile Leu Ser Leu Gly Ile Thr Gly Leu Leu Trp Ala Ile Gly
            1620                1625                1630

Arg Phe Thr Gln Val Ala Gly Ile Ile Thr Pro Tyr Asp Ile His Gln
        1635                1640                1645

Tyr Thr Ser Gly Pro Arg Gly Ala Ala Ala Val Ala Thr Ala Pro Glu
    1650                1655                1660

Gly Thr Tyr Met Ala Ala Val Arg Arg Ala Ala Leu Thr Gly Arg Thr
1665                1670                1675                1680

Leu Ile Phe Thr Pro Ser Ala Val Gly Ser Leu Leu Glu Gly Ala Phe
                1685                1690                1695

Arg Thr His Lys Pro Cys Leu Asn Thr Val Asn Val Val Gly Ser Ser
            1700                1705                1710

Leu Gly Ser Gly Gly Val Phe Thr Ile Asp Gly Arg Arg Thr Val Val
        1715                1720                1725

Thr Ala Ala His Val Leu Asn Gly Asp Thr Ala Arg Val Thr Gly Asp
    1730                1735                1740

Ser Tyr Asn Arg Met His Thr Phe Lys Thr Asn Gly Asp Tyr Ala Trp
1745                1750                1755                1760

Ser His Ala Asp Asp Trp Gln Gly Val Ala Pro Val Val Lys Val Ala
                1765                1770                1775

Lys Gly Tyr Arg Gly Arg Ala Tyr Trp Gln Thr Ser Thr Gly Val Glu
            1780                1785                1790

Pro Gly Ile Ile Gly Glu Gly Phe Ala Phe Cys Phe Thr Asn Cys Gly
        1795                1800                1805

Asp Ser Gly Ser Pro Val Ile Ser Glu Ser Gly Asp Leu Ile Gly Ile
    1810                1815                1820

His Thr Gly Ser Asn Lys Leu Gly Ser Gly Leu Val Thr Thr Pro Glu
1825                1830                1835                1840

Gly Glu Thr Cys Thr Ile Lys Glu Thr Lys Leu Ser Asp Leu Ser Arg
                1845                1850                1855

His Phe Ala Gly Pro Ser Val Pro Leu Gly Asp Ile Lys Leu Ser Pro
            1860                1865                1870

Ala Ile Ile Pro Asp Val Thr Ser Ile Pro Ser Asp Leu Ala Ser Leu
        1875                1880                1885

Leu Ala Ser Val Pro Val Val Glu Gly Gly Leu Ser Thr Val Gln Leu
    1890                1895                1900

Leu Cys Val Phe Phe Leu Leu Trp Arg Met Met Gly His Ala Trp Thr
1905                1910                1915                1920

Pro Ile Val Ala Val Gly Phe Phe Leu Leu Asn Glu Ile Leu Pro Ala
                1925                1930                1935

Val Leu Val Arg Ala Val Phe Ser Phe Ala Leu Phe Val Leu Ala Trp
            1940                1945                1950

Ala Thr Pro Trp Ser Ala Gln Val Leu Met Ile Arg Leu Leu Thr Ala
        1955                1960                1965

Ser Leu Asn Arg Asn Lys Leu Ser Leu Ala Phe Tyr Ala Leu Gly Gly
    1970                1975                1980

Val Val Gly Leu Ala Ala Glu Ile Gly Thr Phe Ala Gly Arg Leu Ser
1985                1990                1995                2000
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Glu Leu Ser Gln Ala Leu Ser Thr Tyr Cys Phe Leu Pro Arg Val Leu
                2005                2010                2015

Ala Met Thr Ser Cys Val Pro Thr Ile Ile Ile Gly Gly Leu His Thr
            2020                2025                2030

Leu Gly Val Ile Leu Trp Xaa Phe Lys Tyr Arg Cys Leu His Asn Met
        2035                2040                2045

Leu Val Gly Asp Gly Ser Phe Ser Ser Ala Phe Phe Leu Arg Tyr Phe
    2050                2055                2060

Ala Glu Gly Asn Leu Arg Lys Gly Val Ser Gln Ser Cys Gly Met Asn
2065                2070                2075                2080

Asn Glu Ser Leu Thr Ala Ala Leu Ala Cys Lys Leu Ser Gln Ala Asp
                2085                2090                2095

Leu Asp Phe Leu Ser Ser Leu Thr Asn Phe Lys Cys Phe Val Ser Ala
            2100                2105                2110

Ser Asn Met Lys Asn Ala Ala Gly Gln Tyr Ile Glu Ala Ala Tyr Ala
        2115                2120                2125

Lys Ala Leu Arg Gln Glu Leu Ala Ser Leu Val Gln Ile Asp Lys Met
    2130                2135                2140

Lys Gly Val Leu Ser Lys Leu Glu Ala Phe Ala Glu Thr Ala Thr Pro
2145                2150                2155                2160

Ser Leu Asp Ile Gly Asp Val Ile Val Leu Leu Gly Gln His Pro His
                2165                2170                2175

Gly Ser Ile Leu Asp Ile Asn Val Gly Thr Glu Arg Lys Thr Val Ser
            2180                2185                2190

Val Gln Glu Thr Arg Ser Leu Gly Gly Ser Lys Phe Ser Val Cys Thr
        2195                2200                2205

Val Val Ser Asn Thr Pro Val Asp Ala Xaa Thr Gly Ile Pro Leu Gln
    2210                2215                2220

Thr Pro Thr Pro Leu Phe Glu Asn Gly Pro Arg His Arg Ser Glu Glu
2225                2230                2235                2240

Asp Asp Leu Lys Val Glu Arg Met Lys Lys His Cys Val Ser Leu Gly
                2245                2250                2255

Phe His Asn Ile Asn Gly Lys Val Tyr Cys Lys Ile Trp Asp Lys Ser
            2260                2265                2270

Thr Gly Asp Thr Phe Tyr Thr Asp Asp Ser Arg Tyr Thr Gln Asp His
        2275                2280                2285

Ala Phe Gln Asp Arg Ser Ala Asp Tyr Arg Asp Arg Asp Tyr Glu Gly
    2290                2295                2300

Val Gln Thr Thr Pro Gln Gln Gly Phe Asp Pro Lys Ser Glu Thr Pro
2305                2310                2315                2320

Val Gly Thr Val Val Ile Gly Gly Ile Thr Tyr Asn Arg Tyr Leu Ile
                2325                2330                2335

Lys Gly Lys Glu Val Leu Val Pro Lys Pro Asp Asn Cys Leu Glu Ala
            2340                2345                2350

Ala Lys Leu Ser Leu Glu Gln Ala Leu Ala Gly Met Gly Gln Thr Cys
        2355                2360                2365

Asp Leu Thr Ala Ala Glu Val Glu Lys Leu Lys Arg Ile Ile Ser Gln
    2370                2375                2380

Leu Gln Gly Leu Thr Thr Glu Gln Ala Leu Asn Cys
2385                2390                2395
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1463 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
Thr Gly Phe Lys Leu Leu Ala Ala Ser Gly Leu Thr Arg Cys Gly Arg
  1               5                  10                  15

Gly Gly Leu Val Val Thr Glu Thr Ala Val Lys Ile Ile Lys Tyr His
             20                  25                  30

Ser Arg Thr Phe Thr Leu Gly Pro Leu Asp Leu Lys Val Thr Ser Glu
         35                  40                  45

Val Glu Val Lys Lys Ser Thr Glu Gln Gly His Ala Val Val Ala Asn
     50                  55                  60

Leu Cys Ser Gly Val Ile Leu Met Arg Pro His Pro Pro Ser Leu Val
 65                  70                  75                  80

Asp Val Leu Leu Lys Pro Gly Leu Asp Thr Ile Pro Gly Ile Gln Pro
                 85                  90                  95

Gly His Gly Ala Gly Asn Met Gly Val Asp Gly Ser Ile Trp Asp Phe
            100                 105                 110

Glu Thr Ala Pro Thr Lys Ala Glu Leu Glu Leu Ser Lys Gln Ile Ile
        115                 120                 125

Gln Ala Cys Glu Val Arg Arg Gly Asp Ala Pro Asn Leu Gln Leu Pro
    130                 135                 140

Tyr Lys Leu Tyr Pro Val Arg Gly Asp Pro Glu Arg His Lys Gly Arg
145                 150                 155                 160

Leu Ile Asn Thr Arg Phe Gly Asp Leu Pro Tyr Lys Thr Pro Gln Asp
                165                 170                 175

Thr Lys Ser Ala Ile His Ala Ala Cys Cys Leu His Pro Asn Gly Ala
            180                 185                 190

Pro Val Ser Asp Gly Lys Ser Thr Leu Gly Thr Thr Leu Gln His Gly
        195                 200                 205

Phe Glu Leu Tyr Val Pro Thr Val Pro Tyr Ser Val Met Glu Tyr Leu
    210                 215                 220

Asp Ser Arg Pro Asp Thr Pro Phe Met Cys Thr Lys His Gly Thr Ser
225                 230                 235                 240

Lys Ala Ala Ala Glu Asp Leu Gln Lys Tyr Asp Leu Ser Thr Gln Gly
                245                 250                 255

Phe Val Leu Pro Gly Val Leu Arg Leu Val Arg Arg Phe Ile Phe Gly
            260                 265                 270

His Ile Gly Lys Ala Pro Pro Leu Phe Leu Pro Ser Thr Tyr Pro Ala
        275                 280                 285

Lys Asn Ser Met Ala Gly Ile Asn Gly Gln Arg Phe Pro Thr Lys Asp
    290                 295                 300

Val Gln Ser Ile Pro Glu Ile Asp Glu Met Cys Ala Arg Ala Val Lys
305                 310                 315                 320

Glu Asn Trp Gln Thr Val Thr Pro Cys Thr Leu Lys Lys Gln Tyr Cys
                325                 330                 335

Ser Lys Pro Lys Thr Arg Thr Ile Leu Gly Thr Asn Asn Phe Ile Ala
            340                 345                 350
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Leu Ala His Arg Ser Ala Leu Ser Gly Val Thr Gln Ala Phe Met Lys
        355                 360                 365

Lys Ala Trp Lys Ser Pro Ile Ala Leu Gly Lys Asn Lys Phe Lys Glu
    370                 375                 380

Leu His Cys Thr Val Ala Gly Arg Cys Leu Glu Ala Asp Leu Ala Ser
385                 390                 395                 400

Cys Asp Arg Ser Thr Pro Ala Ile Val Arg Trp Phe Val Ala Asn Leu
                405                 410                 415

Leu Tyr Glu Leu Ala Gly Cys Glu Glu Tyr Leu Pro Ser Tyr Val Leu
            420                 425                 430

Asn Cys Cys His Asp Leu Val Ala Thr Gln Asp Gly Ala Phe Thr Lys
        435                 440                 445

Arg Gly Gly Leu Ser Ser Gly Asp Pro Val Thr Ser Val Ser Asn Thr
    450                 455                 460

Val Tyr Ser Leu Val Ile Tyr Ala Gln His Met Val Leu Ser Ala Leu
465                 470                 475                 480

Lys Met Gly His Glu Ile Gly Leu Lys Phe Leu Glu Glu Gln Leu Lys
                485                 490                 495

Phe Glu Asp Leu Leu Glu Ile Gln Pro Met Leu Val Tyr Ser Asp Asp
            500                 505                 510

Leu Val Leu Tyr Ala Glu Arg Pro Xaa Phe Pro Asn Tyr His Trp Trp
        515                 520                 525

Val Glu His Leu Asp Leu Met Leu Gly Phe Arg Thr Asp Pro Lys Lys
    530                 535                 540

Thr Val Ile Thr Asp Lys Pro Ser Phe Leu Gly Cys Arg Ile Glu Ala
545                 550                 555                 560

Gly Arg Gln Leu Val Pro Asn Arg Asp Arg Ile Leu Ala Ala Leu Ala
                565                 570                 575

Tyr His Met Lys Ala Gln Asn Ala Ser Glu Tyr Tyr Ala Ser Ala Ala
            580                 585                 590

Ala Ile Leu Met Asp Ser Cys Ala Cys Ile Asp His Asp Pro Glu Trp
        595                 600                 605

Tyr Glu Asp Leu Ile Cys Gly Ile Ala Arg Cys Ala Arg Gln Asp Gly
    610                 615                 620

Tyr Ser Phe Pro Gly Pro Ala Phe Phe Met Ser Met Trp Glu Lys Leu
625                 630                 635                 640

Arg Ser His Asn Glu Gly Lys Lys Phe Arg His Cys Gly Ile Cys Asp
                645                 650                 655

Ala Lys Ala Asp Tyr Ala Ser Ala Cys Gly Leu Asp Leu Cys Leu Phe
            660                 665                 670

His Ser His Phe His Gln His Cys Xaa Val Thr Leu Ser Cys Gly His
        675                 680                 685

His Ala Gly Ser Lys Glu Cys Ser Gln Cys Gln Ser Pro Val Gly Ala
    690                 695                 700

Gly Arg Ser Pro Leu Asp Ala Val Leu Lys Gln Ile Pro Tyr Lys Pro
705                 710                 715                 720

Pro Arg Thr Val Ile Met Lys Val Gly Asn Lys Thr Thr Ala Leu Asp
                725                 730                 735

Pro Gly Arg Tyr Gln Ser Arg Arg Gly Leu Val Ala Val Lys Arg Gly
            740                 745                 750
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Ile Ala Gly Asn Glu Val Asp Leu Ser Asp Xaa Asp Tyr Gln Val Val
        755                 760                 765

Pro Leu Leu Pro Thr Cys Lys Asp Ile Asn Met Val Lys Val Ala Cys
    770                 775                 780

Asn Val Leu Leu Ser Lys Phe Ile Val Gly Pro Pro Gly Ser Gly Lys
785                 790                 795                 800

Thr Thr Trp Leu Leu Ser Gln Val Gln Asp Asp Asp Val Ile Tyr Xaa
                805                 810                 815

Pro Thr His Gln Thr Met Phe Asp Ile Val Ser Ala Leu Lys Val Cys
            820                 825                 830

Arg Tyr Ser Ile Pro Gly Ala Ser Gly Leu Pro Phe Pro Pro Pro Ala
        835                 840                 845

Arg Ser Gly Pro Trp Val Arg Leu Ile Ala Ser Gly His Val Pro Gly
    850                 855                 860

Arg Val Ser Tyr Leu Asp Glu Ala Gly Tyr Cys Asn His Leu Asp Ile
865                 870                 875                 880

Leu Arg Leu Leu Ser Lys Thr Pro Leu Val Cys Leu Gly Asp Leu Gln
                885                 890                 895

Gln Leu His Pro Val Gly Phe Asp Ser Tyr Cys Tyr Val Phe Asp Gln
            900                 905                 910

Met Pro Gln Lys Gln Leu Thr Thr Ile Tyr Arg Phe Gly Pro Asn Ile
        915                 920                 925

Cys Ala Arg Ile Gln Pro Cys Tyr Arg Glu Lys Leu Glu Ser Lys Ala
    930                 935                 940

Arg Asn Thr Arg Val Val Phe Thr Thr Arg Pro Val Ala Phe Gly Gln
945                 950                 955                 960

Val Leu Thr Pro Tyr His Lys Asp Arg Ile Gly Ser Ala Ile Thr Ile
                965                 970                 975

Asp Ser Ser Gln Gly Ala Thr Phe Asp Ile Val Thr Leu His Leu Pro
            980                 985                 990

Ser Pro Lys Ser Leu Asn Lys Ser Arg Ala Leu Val Ala Ile Thr Arg
        995                 1000                1005

Ala Arg His Gly Leu Phe Ile Tyr Asp Pro His Asn Gln Leu Gln Glu
    1010                1015                1020

Phe Phe Asn Leu Thr Pro Glu Arg Thr Asp Cys Asn Leu Val Phe Ser
1025                1030                1035                1040

Arg Gly Asp Glu Leu Val Val Leu Asn Ala Asp Asn Ala Val Thr Thr
                1045                1050                1055

Val Ala Lys Ala Leu Glu Thr Gly Pro Ser Arg Phe Arg Val Ser Asp
            1060                1065                1070

Pro Arg Cys Lys Ser Leu Leu Ala Ala Cys Ser Ala Ser Leu Glu Gly
        1075                1080                1085

Ser Cys Met Pro Leu Pro Gln Val Ala His Asn Leu Gly Phe Tyr Phe
    1090                1095                1100

Ser Pro Asp Ser Pro Thr Phe Ala Pro Leu Pro Lys Glu Leu Ala Pro
1105                1110                1115                1120

His Trp Pro Val Val Thr His Gln Asn Asn Arg Ala Trp Pro Asp Arg
                1125                1130                1135

Leu Val Ala Ser Met Arg Pro Ile Asp Ala Arg Tyr Ser Lys Pro Met
            1140                1145                1150

Val Gly Ala Gly Tyr Val Val Gly Pro Ser Thr Phe Leu Gly Thr Pro
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        1155                1160                1165

Gly Val Val Ser Tyr Tyr Leu Thr Leu Tyr Ile Arg Gly Glu Pro Gln
    1170                1175                1180

Ala Leu Pro Glu Thr Leu Val Ser Thr Gly Arg Ile Ala Thr Asp Cys
1185                1190                1195                1200

Arg Glu Tyr Leu Asp Ala Ala Glu Glu Glu Ala Ala Lys Glu Leu Pro
                1205                1210                1215

His Ala Phe Ile Gly Asp Val Lys Gly Thr Thr Val Gly Gly Cys His
            1220                1225                1230

His Ile Thr Ser Lys Tyr Leu Pro Arg Ser Leu Pro Lys Asp Ser Val
        1235                1240                1245

Ala Val Val Gly Val Ser Ser Pro Gly Arg Ala Ala Lys Ala Val Cys
    1250                1255                1260

Thr Leu Thr Asp Val Tyr Leu Pro Glu Leu Arg Pro Tyr Leu Gln Pro
1265                1270                1275                1280

Glu Thr Ala Ser Lys Cys Trp Lys Leu Lys Leu Asp Phe Arg Asp Val
                1285                1290                1295

Arg Leu Met Val Trp Lys Gly Ala Thr Ala Tyr Phe Gln Leu Glu Gly
            1300                1305                1310

Leu Thr Trp Ser Ala Leu Pro Asp Tyr Ala Arg Xaa Ile Gln Leu Pro
        1315                1320                1325

Lys Asp Ala Val Val Tyr Ile Asp Pro Cys Ile Gly Pro Ala Thr Ala
    1330                1335                1340

Asn Arg Lys Val Val Arg Thr Thr Asp Trp Arg Ala Asp Leu Ala Val
1345                1350                1355                1360

Thr Pro Tyr Asp Tyr Gly Ala Gln Asn Ile Leu Thr Thr Ala Trp Phe
                1365                1370                1375

Glu Asp Leu Gly Pro Gln Trp Lys Ile Leu Gly Leu Gln Pro Phe Arg
            1380                1385                1390

Arg Ala Phe Gly Phe Glu Asn Thr Glu Asp Trp Ala Ile Leu Ala Arg
        1395                1400                1405

Arg Met Asn Asp Gly Lys Asp Tyr Thr Asp Tyr Asn Trp Asn Cys Val
    1410                1415                1420

Arg Glu Arg Pro His Ala Ile Tyr Gly Arg Ala Arg Asp His Thr Tyr
1425                1430                1435                1440

His Phe Ala Pro Gly Thr Glu Leu Gln Val Glu Leu Gly Lys Pro Arg
                1445                1450                1455

Leu Pro Pro Gly Gln Val Pro
            1460


(2) INFORMATION FOR SEQ ID NO: 4:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 249 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gln Trp Gly His Cys Gly Val Lys Ser Ala Ser Cys Ser Trp Thr
  1               5                  10                  15

Pro Ser Leu Ser Ser Leu Leu Val Trp Leu Ile Leu Xaa Phe Ser Leu
             20                  25                  30
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```
Pro Tyr Cys Leu Gly Ser Pro Ser Gln Asp Gly Tyr Trp Ser Phe Phe
         35                  40                  45

Ser Glu Trp Phe Ala Pro Arg Phe Ser Val Arg Ala Leu Pro Phe Thr
     50                  55                  60

Leu Pro Asn Tyr Arg Arg Ser Tyr Glu Gly Leu Leu Pro Asn Cys Arg
 65                  70                  75                  80

Pro Asp Val Pro Gln Phe Ala Val Lys His Pro Leu Xaa Met Phe Trp
                 85                  90                  95

His Met Arg Val Ser His Leu Ile Asp Glu Xaa Val Ser Arg Arg Ile
            100                 105                 110

Tyr Gln Thr Met Glu His Ser Gly Gln Ala Ala Trp Lys Gln Val Val
        115                 120                 125

Gly Glu Ala Thr Leu Thr Lys Leu Ser Gly Leu Asp Ile Val Thr His
    130                 135                 140

Phe Gln His Leu Ala Ala Val Glu Ala Asp Ser Cys Arg Phe Leu Ser
145                 150                 155                 160

Ser Arg Leu Val Met Leu Lys Asn Leu Ala Val Gly Asn Val Ser Leu
                165                 170                 175

Gln Tyr Asn Thr Thr Leu Asp Arg Val Glu Leu Ile Phe Pro Thr Pro
            180                 185                 190

Gly Thr Arg Pro Lys Leu Thr Asp Phe Arg Gln Trp Leu Ile Ser Val
        195                 200                 205

His Ala Ser Ile Phe Ser Ser Val Ala Ser Ser Val Thr Leu Phe Ile
    210                 215                 220

Val Leu Trp Leu Arg Ile Pro Ala Leu Arg Tyr Val Phe Gly Phe His
225                 230                 235                 240

Trp Pro Thr Ala Thr His His Ser Ser
                245
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 265 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Met Ala His Gln Cys Ala Arg Phe His Phe Phe Leu Cys Gly Phe Ile
  1               5                  10                  15

Cys Tyr Leu Val His Ser Ala Leu Ala Ser Asn Ser Ser Ser Thr Leu
             20                  25                  30

Cys Phe Trp Phe Pro Leu Ala His Gly Asn Thr Ser Phe Glu Leu Thr
         35                  40                  45

Ile Asn Tyr Thr Ile Cys Met Pro Cys Ser Thr Ser Gln Ala Ala Arg
     50                  55                  60

Gln Arg Leu Glu Pro Gly Arg Asn Met Trp Cys Lys Ile Gly His Asp
 65                  70                  75                  80

Arg Cys Glu Glu Arg Asp His Asp Glu Leu Leu Met Ser Ile Pro Ser
                 85                  90                  95

Gly Tyr Asp Asn Leu Lys Leu Glu Gly Tyr Tyr Ala Trp Leu Ala Phe
            100                 105                 110
```

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```
Leu Ser Phe Ser Tyr Ala Ala Gln Phe His Pro Glu Leu Phe Gly Ile
        115                 120                 125

Gly Asn Val Ser Arg Val Phe Val Asp Lys Arg His Gln Phe Ile Cys
    130                 135                 140

Ala Glu His Asp Gly His Asn Ser Thr Val Ser Thr Gly His Asn Ile
145                 150                 155                 160

Ser Ala Leu Tyr Ala Ala Tyr Tyr His His Gln Ile Asp Gly Gly Asn
                165                 170                 175

Trp Phe His Leu Glu Trp Leu Arg Pro Leu Phe Ser Ser Trp Leu Val
            180                 185                 190

Leu Asn Ile Ser Trp Phe Leu Arg Arg Ser Pro Val Ser Pro Val Ser
        195                 200                 205

Arg Arg Ile Tyr Gln Ile Leu Arg Pro Thr Arg Pro Arg Leu Pro Val
    210                 215                 220

Ser Trp Ser Phe Arg Thr Ser Ile Val Ser Asp Leu Thr Gly Ser Gln
225                 230                 235                 240

Gln Arg Lys Arg Lys Phe Pro Ser Glu Ser Arg Pro Asn Val Val Lys
                245                 250                 255

Pro Ser Val Leu Pro Ser Thr Ser Arg
            260                 265


(2) INFORMATION FOR SEQ ID NO: 6:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 183 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met Ala Ala Ala Thr Leu Phe Phe Leu Ala Gly Ala Gln His Ile Met
  1               5                  10                  15

Val Ser Glu Ala Phe Ala Cys Lys Pro Cys Phe Ser Thr His Leu Ser
             20                  25                  30

Asp Ile Glu Thr Asn Thr Thr Ala Ala Ala Gly Phe Met Val Leu Gln
         35                  40                  45

Asp Ile Asn Cys Phe Arg Pro His Gly Val Ser Ala Ala Gln Glu Lys
     50                  55                  60

Ile Ser Phe Gly Lys Ser Ser Gln Cys Arg Glu Ala Val Gly Thr Pro
 65                  70                  75                  80

Gln Tyr Ile Thr Ile Thr Ala Asn Val Thr Asp Glu Ser Tyr Leu Tyr
                 85                  90                  95

Asn Ala Asp Leu Leu Met Leu Ser Ala Cys Leu Phe Tyr Ala Ser Glu
            100                 105                 110

Met Ser Glu Lys Gly Phe Lys Val Ile Phe Gly Asn Val Ser Gly Val
        115                 120                 125

Val Ser Ala Cys Val Asn Phe Thr Asp Tyr Val Ala His Val Thr Gln
    130                 135                 140

His Thr Gln Gln His His Leu Val Ile Asp His Ile Arg Leu Leu His
145                 150                 155                 160

Phe Leu Thr Pro Ser Ala Met Arg Trp Ala Thr Thr Ile Ala Cys Leu
                165                 170                 175

Phe Ala Ile Leu Leu Ala Ile
```

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180

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 201 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met Arg Cys Ser His Lys Leu Gly Arg Phe Leu Thr Pro His Ser Cys
1 5 10 15

Phe Trp Trp Leu Phe Leu Leu Cys Thr Gly Leu Ser Trp Ser Phe Ala
20 25 30

Asp Gly Asn Gly Asp Ser Ser Thr Tyr Gln Tyr Ile Tyr Asn Leu Thr
35 40 45

Ile Cys Glu Leu Asn Gly Thr Asp Trp Leu Ser Ser His Phe Gly Trp
50 55 60

Ala Val Glu Thr Phe Val Leu Tyr Pro Val Ala Thr His Ile Leu Ser
65 70 75 80

Leu Gly Phe Leu Thr Thr Ser His Phe Phe Asp Ala Leu Gly Leu Gly
85 90 95

Ala Val Ser Thr Ala Gly Phe Val Gly Gly Arg Tyr Val Leu Cys Ser
100 105 110

Val Tyr Gly Ala Cys Ala Phe Ala Ala Phe Val Cys Phe Val Ile Arg
115 120 125

Ala Ala Lys Asn Cys Met Ala Cys Arg Tyr Ala Arg Thr Arg Phe Thr
130 135 140

Asn Phe Ile Val Asp Asp Arg Gly Arg Val His Arg Trp Lys Ser Pro
145 150 155 160

Ile Val Val Glu Lys Leu Gly Lys Ala Glu Val Asp Gly Asn Leu Val
165 170 175

Thr Ile Lys His Val Val Leu Glu Gly Val Lys Ala Gln Pro Leu Thr
180 185 190

Arg Thr Ser Ala Glu Gln Trp Glu Ala
195 200

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 173 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Met Gly Gly Leu Asp Asp Phe Cys Asn Asp Pro Ile Ala Ala Gln Lys
1 5 10 15

Leu Val Leu Ala Phe Ser Ile Thr Tyr Thr Pro Ile Met Ile Tyr Ala
20 25 30

Leu Lys Val Ser Arg Gly Arg Leu Leu Gly Leu Leu His Ile Leu Ile
35 40 45

Phe Leu Asn Cys Ser Phe Thr Phe Gly Tyr Met Thr Tyr Val His Phe
50 55 60

-continued

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Gln Ser Thr Asn Arg Val Ala Leu Thr Leu Gly Ala Val Val Ala Leu
 65                  70                  75                  80

Leu Trp Gly Val Tyr Ser Phe Thr Glu Ser Trp Lys Phe Ile Thr Ser
                 85                  90                  95

Arg Cys Arg Leu Cys Cys Leu Gly Arg Arg Tyr Ile Leu Ala Pro Ala
            100                 105                 110

His His Val Glu Ser Ala Ala Gly Leu His Ser Ile Ser Ala Ser Gly
        115                 120                 125

Asn Arg Ala Tyr Ala Val Arg Lys Pro Gly Leu Thr Ser Val Asn Gly
    130                 135                 140

Thr Leu Val Pro Gly Leu Arg Ser Leu Val Leu Gly Gly Lys Arg Ala
145                 150                 155                 160

Val Lys Arg Gly Val Val Asn Leu Val Lys Tyr Gly Arg
                165                 170


(2) INFORMATION FOR SEQ ID NO: 9:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 128 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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  1               5                  10                  15

Met Gly Asn Gly Gln Pro Val Asn Gln Leu Cys Gln Leu Leu Gly Ala
             20                  25                  30

Met Ile Lys Ser Gln Arg Gln Gln Pro Arg Gly Gly Gln Xaa Lys Lys
         35                  40                  45

Lys Lys Pro Glu Lys Pro His Phe Pro Leu Ala Ala Glu Asp Asp Ile
     50                  55                  60

Arg His His Leu Thr Gln Thr Glu Arg Ser Leu Cys Leu Gln Ser Ile
 65                  70                  75                  80

Gln Thr Ala Phe Asn Gln Gly Ala Gly Thr Ala Xaa Leu Ser Ser Ser
                 85                  90                  95

Gly Lys Val Ser Phe Gln Val Glu Phe Met Leu Pro Val Ala His Thr
            100                 105                 110

Val Arg Leu Ile Arg Val Thr Ser Thr Ser Ala Ser Gln Gly Ala Ser
        115                 120                 125
```

What is claimed is:

1. Composition of matter comprising isolated Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

2. Composition of matter comprising killed isolated Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

3. Composition of matter comprising attenuated isolated Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

4. Composition of matter comprising a recombinant vector derived from Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

5. Composition of matter comprising an isolated part or component of Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

6. Composition of matter comprising isolated or synthetic protein, (poly)peptide, or nucleic acid derived from Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

7. Composition of matter comprising recombinant nucleic acid which comprises a nucleotide sequence derived from the genome of Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

8. Composition of matter comprising recombinant nucleic acid which comprises a Lelystad Agent-specific nucleotide sequence shown in **FIG. 1**.

9. Composition of matter comprising recombinant nucleic acid which comprises a Lelystad Agent-specific nucleotide sequence selected from anyone of the Open Reading Frames shown in **FIG. 1**.

10. Composition of matter comprising a (poly)peptide having an amino acid sequence derived from a protein of Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, the (poly)peptide being produced by a cell capable of producing it due to genetic engineering with appropriate recombinant DNA.

11. Composition of matter comprising a (poly)peptide comprising a Lelystad Agent-specific amino acid sequence shown in **FIG. 1**.

12. Composition of matter comprising an isolated or synthetic antibody which specifically recognizes a part or component of Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

13. Composition of matter comprising a recombinant vector which contains nucleic acid comprising a nucleotide sequence coding for a protein or antigenic peptide derived from Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

14. Vaccine composition for vaccinating animals, in particular mammals, more in particular pigs or swine, to protect them against Mystery Swine Disease, comprising Lelystad Agent which is the causative agent of Mystery swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, and a suitable carrier or adjuvant.

15. Vaccine composition for vaccinating animals, in particular mammals, more in particular pigs or swine, to protect them against Mystery Swine Disease, comprising killed Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, and a suitable carrier or adjuvant.

16. Vaccine composition for vaccinating animals, in particular mammals, more in particular pigs or swine, to protect them against Mystery Swine Disease, comprising attenuated Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, and a suitable carrier or adjuvant.

17. Vaccine composition for vaccinating animals, in particular mammals, more in particular pigs or swine, to protect them against Mystery Swine Disease, comprising a recombinant vector which contains nucleic acid comprising a nucleotide sequence coding for a protein or antigenic peptide derived from Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.9 1) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, and a suitable carrier or adjuvant.

18. Vaccine composition for vaccinating animals, in particular mammals, more in particular pigs or swine, to protect them against Mystery Swine Disease, comprising an antigenic part or component of Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, and a suitable carrier or adjuvant.

19. Vaccine composition for vaccinating animals, in particular mammals, more in particular pigs or swine, to protect them against Mystery Swine Disease, comprising a protein or antigenic polypeptide derived from, or a peptide mimicking an antigenic component of, Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, and a suitable carrier or adjuvant.

20. Vaccine composition for vaccinating animals, in particular mammals, more in particular pigs or swine, to protect them against a disease caused by a pathogen, comprising a recombinant vector derived from Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, the nucleic acid of the recombinant vector comprising a nucleotide sequence coding for a protein or antigenic peptide derived from the pathogen, and a suitable carrier or adjuvant.

21. Diagnostic kit for detecting nucleic acid from Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, in a sample, in particular a biological samples such as blood or blood serum, sputum, saliva, or tissue, derived from an animal, in particular a mammal, more in particular a pig or swine, comprising a nucleic acid probe or primer which comprises a nucleotide sequence derived from the genome of Lelystad Agent, and suitable detection means of a nucleic acid detection assay.

22. Diagnostic kit for detecting antigen from Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to

the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, in a sample, in particular a biological sample such as blood or blood serum, sputum, saliva, or tissue, derived from an animal, in particular a mammal, more in particular a pig or swine, comprising an antibody which specifically recognizes a part or component of Lelystad Agent, and suitable detection means of an antigen detection assay.

23. Diagnostic kit for detecting an antibody which specifically recognizes Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, in a sample, in particular a biological sample such as blood or blood serum, sputum, saliva, or tissue, derived from an animal, in particular a mammal, more in particular a pig or swine, comprising an antigenic part or component of Lelystad Agent, and suitable detection means of an antibody detection assay.

24. Diagnostic kit for detecting an antibody which specifically recognizes Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, in a sample, in particular a biological sample such as blood or blood serum, sputum, saliva, or tissue, derived from an animal, in particular a mammal, more in particular a pig or swine, comprising a protein or antigenic polypeptide derived from Lelystad Agent, or a peptide mimicking an antigenic component of Lelystad Agent, and suitable detection means of an antibody detection assay.

25. Diagnostic kit for detecting an antibody which specifically recognizes Lelystad Agent which is the causative agent of Mystery Swine Disease, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, in a sample, in particular a biological sample such as blond or blond serum, sputum, saliva, or tissue, derived from an animal, in particular a mammal, more in particular a pig or swine, comprising killed, live or attenuated Lelystad Agent, and suitable detection means of an antibody detection assay.

26. A process for diagnosing whether an animal, in particular a mammal, more in particular a pig or swine, is contaminated with the causative agent of Mystery Swine Disease, comprising preparing a sample, in particular a biological sample such as blond or blond serum, sputum, saliva, or tissue, derived from the animal, and examining whether it contains Lelystad Agent nucleic acid, Lelystad Agent antigen, or antibody specifically recognizing Lelystad Agent, said Lelystad Agent being the causative agent of Mystery Swine Disease and essentially corresponding to the isolate Lelystad Agent (CDI-NL-2.91) deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

27. A diagnostic kit for detecting nucleic acid from Lelystad Agent, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, in a biological sample, derived from an animal, said diagnostic kit comprising:

a nucleic acid probe or primer which comprises a nucleotide sequence essentially corresponding to the genome of Lelystad Agent, and means for detecting nucleic acid.

28. The diagnostic kit of claim 27 wherein the means for detecting a nucleic acid comprises a hybridization of the nucleic acid probe or primer.

29. The diagnostic kit of claim 28 wherein the means for detecting a nucleic acid comprises polymerase chain reaction ("PCR").

30. A diagnostic kit for detecting an antibody which specifically recognizes Lelystad Agent in a biological sample derived from an animal, said kit comprising

an antigen selected from the group consisting of virus, protein, polypeptide, and peptide, said antigen immunoreactive with serum antibodies of a sow, said serum antibodies obtained by:

a) intranasally inoculating a specific pathogen free sow with two milliliters of a virus essentially corresponding to the virus identified as deposit number I- 1102, deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France (at passage level 3, titer $10^{4.8}$TCID$_{50}$ / milliliter); and further comprising

b) collecting serum antibodies from the thus inoculated sow after 25 to 33 days, and

means for detecting said antibody.

31. A diagnostic kit for detecting an antibody which specifically recognizes Lelystad Agent, said Lelystad Agent essentially corresponding to the isolated Lelystad Agent deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102, in a sample derived from an animal said diagnostic kit comprising:

a protein or antigenic polypeptide derived from Lelystad Agent, or a peptide mimicking an antigenic component of Lelystad Agent, and

means for detecting an antibody reacting with said protein or antigenic polypeptide derived from Lelystad Agent, or a peptide mimicking an antigenic component of Lelystad Agent.

32. A diagnostic kit for detecting an antibody which specifically recognizes a causative agent of Mystery Swine Disease, said causative agent being at least partially antigenically cross-reactive with Lelystad Agent essentially corresponding to the isolated Lelystad Agent deposited Jun. 5, 1991 with the Institut Pasteur; Paris, France, deposit number I-1102, in a sample derived from an animal, said diagnostic kit comprising

said causative agent, and

means for detecting said antibody.

33. The diagnostic kit of claim 32 wherein the causative agent is live.

34. A process for detecting a causative agent of Mystery Swine Disease in an animal, comprising preparing a sample derived from the animal, and examining whether the sample contains Lelystad Agent nucleic acid, Lelystad Agent antigen, or antibody specifically recognizing Lelystad Agent, said Lelystad Agent essentially corresponding to the isolate Lelystad Agent deposited Jun. 5, 1991 with the Institut Pasteur, Paris, France, deposit number I-1102.

35. The process according to claim 34 wherein said sample is serum taken from the animal.

* * * * *

专利名称(译)	神秘猪病的致病因子，疫苗成分和诊断试剂盒		
公开(公告)号	US20030118608A1	公开(公告)日	2003-06-26
申请号	US10/226065	申请日	2002-08-21
[标]申请(专利权)人(译)	WENSVOORT GERT 特普斯特拉CATHARINUS POL JOANNES MARIA ANTHONIS MOORMANN ROBERTUS雅克布斯MARIA MEULENBERG约翰娜雅各巴MARIA		
申请(专利权)人(译)	WENSVOORT GERT 特普斯特拉CATHARINUS POL JOANNES MARIA ANTHONIS MOORMANN ROBERTUS雅克布斯MARIA MEULENBERG约翰娜雅各巴MARIA		
当前申请(专利权)人(译)	WENSVOORT GERT 特普斯特拉CATHARINUS POL JOANNES MARIA ANTHONIS MOORMANN ROBERTUS雅克布斯MARIA MEULENBERG约翰娜雅各巴MARIA		
[标]发明人	WENSVOORT GERT TERPSTRA CATHARINUS POL JOANNES MARIA ANTHONIS MOORMANN ROBERTUS JACOBUS MARIA MEULENBERG JOHANNA JACOBA MARIA		
发明人	WENSVOORT, GERT TERPSTRA, CATHARINUS POL, JOANNES MARIA ANTHONIS MOORMANN, ROBERTUS JACOBUS MARIA MEULENBERG, JOHANNA JACOBA MARIA		
IPC分类号	A61K39/00 A61K39/12 A61K39/215 A61K39/395 A61K39/42 A61P31/12 A61P31/14 C07H21/00 C07K14/08 C07K14/435 C07K16/00 C07K16/10 C12N7/00 C12N7/01 C12N15/09 C12N15/50 C12N15/66 C12P21/00 C12P21/02 C12Q1/68 C12Q1/70 G01N33/53 G01N33/569 C07K2/00 C07K4/00 C07K5/00 C07K7/00 C07K14/00 C07K17/00 A61K38/00		
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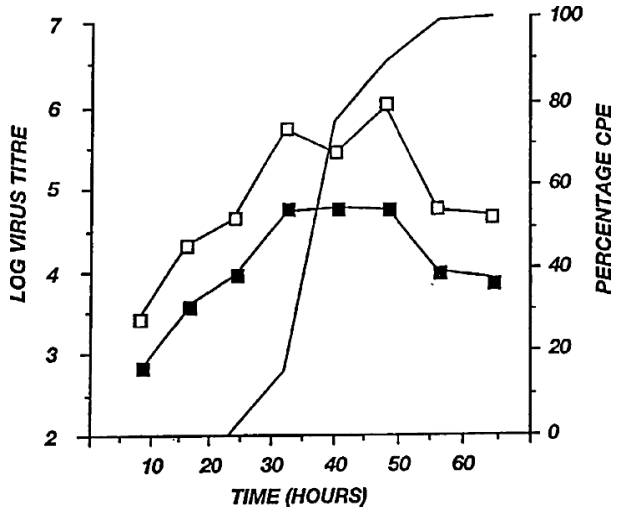
优先权	08/157005 1993-11-26 US		
其他公开文献	US6806086		
外部链接	Espacenet USPTO		

摘要(译)

包含神秘猪病，莱利斯塔德试剂的致病因子的物质组合物，其为活的，减毒的，死的或重组的形式，或其部分或组分。基于其的疫苗组合物和诊断试剂盒。包含Lelystad试剂特异性核苷酸序列的重组核酸。包含Lelystad特异性氨基酸序列的肽。 Lelystad特异性抗体。


LOG VIRUS TITRE
PERCENTAGE CPE
TIME (HOURS)
7
6
5
4
3
2
100
80
60
40
20
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10
20
30
40
50
60