103

SEQUENCE ANALYSIS OF THE NUCLEOCAPSID PROTEIN GENE OF THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS TAIWAN MD-001 STRAIN

L. L. Chueh,[1] K. H. Lee,[1] F. I. Wang,[1] V. F. Pang,[1] and C. N. Weng[2]

[1]Department of Veterinary Medicine
National Taiwan University
142 Chou San Road, Taipei, Taiwan
[2]Pig Research Institute, Taiwan
Chunan, Miaoli, Taiwan

1. ABSTRACT

The 3'-portion of the genome from a Taiwan isolate of porcine reproductive and respiratory syndrome (PRRS) virus, strain MD-001, was cloned and sequenced. The resultant 549 nucleotides contained an open reading frame with a coding capacity of 123 amino acids (predicted Mr 13 600). The predicted protein corresponds to the nucleocapsid protein, the gene product of ORF7. Comparative sequence analysis of several known PRRSV strains indicated that this protein showed the highest degree of amino acid similarity to the US VR2332 and the Canadian IAF-Exp91 strains (92.7%) and the least to the Dutch Lelystad strain (56.5%). The phylogenic trees constructed on the basis of the known PRRSV nucleotide sequences indicated that MD-001 strain belongs to the North American strain cluster and that it is distinct from the European virus.

2. INTRODUCTION

Porcine reproductive and respiratory syndrome has emerged in the 1990s as an important new viral disease of swine. The disease, characterized by reproductive failure in sows and respiratory symptoms in pigs of all ages, has spread rapidly and caused alarm in the swine industry worldwide (Goyal, 1993; Done *et al.*, 1996). The causative agent, a small enveloped RNA virus, was first identified in 1991 in the Netherlands (Wensvoort *et*

Coronaviruses and Arteriviruses, edited by Enjuanes *et al.*
Plenum Press, New York, 1998

al.). Based on the size of the virion, viral genome organization, and replication strategy, the PRRS virus (PRRSV) has been provisionally classified in the Arterivirus group (Meulenberg *et al.*, 1993; Plagemann and Moennig, 1992; den Boon *et al.*, 1991; Spaan *et al.*, 1988).

The nucleocapsid (N) protein is encoded by ORF 7 which is located at the 3' end of the PRRSV genome. This protein is highly immunogenic (Yoon *et al.*, 1995). PRRS was first identified from an outbreak in Taiwan in 1991. The causative virus was isolated (Chang *et al.*, 1993a) and the disease subsequently reproduced in pathogen/free piglets (Chang *et al.*, 1993b). The present study reports the cloning and nucleotide sequence analysis of the nucleocapsid gene of this Taiwan isolate and its genetic relationship to the known PRRSV strains.

3. MATERIALS AND METHODS

3.1. Virus and Cells

Virus strain, MD-001 (Chang *et al.*, 1993), was grown in a continuous cell line (MARC-145). Virus stocks were prepared following three rounds of purification by limited dilution. After complete degeneration of the monolayers, supernatant fluid was clarified and the viral particles were concentrated by ultracentrifugation through a sucrose gradient.

3.2. RNA Preparation, cDNA Synthesis, and Cloning

The viral genomic RNA was isolated according to the method described (Chomczynki and Sacchi, 1987). To clone the 3' end of the viral genome, oligo (dT) primed cDNA was produced using the Gibco BRL cDNA synthesis kit. *Eco*RI /*Not*I adaptor ligation and phosphorylation were performed. Purified cDNA was cloned into the pUC19 plasmid vector, expanded in bacteria, purified and used directly for Southern hybridization.

3.3. Southern Hybridization

DIG-labeled DNA probes (Boehringer Mannheim) were prepared using RT-PCR from the viral genomic RNA as a template. Primers (F1: 5'GGGAATGGCCAGCCAGTCAATCAACTGT3' and R1: 5'TGTAGAAGTCACGCGAATCAGGCGCACT3') targeted to nucleocapsid gene were modified from Suarez *et al.* (1994). Colony hybridization to screen the cDNA library was performed as described (Sambrook *et al.*, 1989). Positive colonies were selected and subjected to sequence analysis.

3.4. DNA Sequencing

cDNA clones were sequenced on both strands by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) using T7 DNA polymerase and an Automated Laser Fluorescent DNA sequence analyser (Pharmacia LKB). Sequence analysis was performed on an Apple Macintosh computer using the Seqman and Megalign sequence analysis programs (DNASTAR).

4. RESULTS AND DISCUSSION

The nucleotide sequence of the 3'-terminal 549 nt of the MD-001 strain of PRRSV was determined. This genomic region encompassed a large ORF encoding a polypeptide of 123 amino acids with a predicted Mr of 13 600, consistent with the estimated Mr of the nucleocapsid (N) protein (Meulenberg *et al.,* 1995; Mardassi *et al.*, 1994). Comparative sequence analysis with other known PRRSV strains indicated that the highest degree of amino acid similarity with the US VR2332 and the Canadian IAF-Exp91 strains (92.7%). The Japanese EDRD-1 together with the US VR2385 strains showed 91.9% amino acid similarity to the N protein of the MD-001 strain whereas all the European strains exhibited only 56.5 % similarity. Such relatively high divergence resulted from a number of nucleotide substitutions, insertions or deletions, making the MD-001 N protein five amino acids shorter than that of the European strains (Fig.1). Two amino acid streches, TAPM and QGAS, situated at the N-terminal and C-terminal regions respectively of the N protein of four analysed European isolates, were from the MD-001 N protein. These two missing amino acid domains were also observed in the American VR2332, Canadian IAF-exp91 and Japanese EDRD-1 strains (Fig.1).

The Clustal method was used to construct a phylogenetic tree of nine PRRSV isolates. The results indicated that these isolates can be divided into two proups. The first genotype was represent by European Lelystad, Boxmeer, No 1 and Olot/91 strains. Asian strains, MD-001 together with EDRD-1 strains, were in the same group as the North American strains and represented the second genotype (Fig. 2). The genetic relatedness of our PRRSV to the North American strain may be due to the importation of the carrier breeder pigs from those geographical areas.

ACKNOWLEDGMENTS

This work was supported by National Science Council of Republic of China project grant NSC85-2321-B-002-066-A13.

```
MD-001      MPNNNGKQQN KKK****GDG QPVNQLCQML GKIIAQQSQS RVKGPGRKNK KKNPEKPHFP LATEDDVRHH
IAF-exp91   ------R--K ---------- ---------- -------N-- -G----K--- ---------- ----------
VR2332      --------TE E--------- ---------- -------N-- -G----K--- ---------- ----------
VR2385      ----T----K R--------- ---------- -----H-N-- -G----K--- ---------- ----------
EDRD-1      ---------K R-T-----N- ---------- ---------- -G----N--- ---------- ----Y-----
No1         -AGK-QS-KK --STAPMGN- --------L- -AM-KS-*** -QQPR-GQA- --K------- --A---I---
Olot/91     -AGK-QS-KK --SAAPMGN- --------L- -AM-KS-*** -QQPR-GQA- --K------- --A---I---
Boxmeer10   -AGK-QS-KK --STAPMGN- --------L- -AM-KS-*** -QQPR-GQA- --K------- --A---I---
Lelystad    -AGK-QS-KK --STAPMGN- --------L- -AM-KS-*** -QQPR-GQA- --K------- --A---I---

MD-001      FTPSERQLCL SSIQTAFNQG AGTCILSDSG RISYTVEFSL PTHHTVRLIR VTAPPSA       123
IAF-exp91   ---------- ---------- ----T----- ----A----- ---------- ---S---       123
VR2332      ---------- ---------- ----T----- ---------- ---------- ---S---       123
VR2385      ---------- ---------- ----T----- ---------- ---------- ---S---       123
EDRD-1      ---------- ---------- ----T----- ---------- ---------- ---S---       123
No1         L-QT--S--- Q--------- ---AS--S-- KV-FQ---M- -VA------- --STSASQGA S 128
Olot/91     L-QT--S--- Q--------- ---AS--S-- KV-FQ---M- -VA------- --STSASQGA S 128
Boxmeer10   L-QT--S--- Q--------- ---AS--S-- KV-FQ---M- -VA------- --STSASQGA S 128
Lelystad    L-QT--S--- Q--------- ---AS--S-- KV-FQ---M- -VA------- --STSASQGA S 128
```

Figure 1. Alignment of amino acid sequences of the N gene of PRRSV. MD-001 sequence, strain IAF-exp91 (Mardassi *et al.,* 1995), VR2332 (Murtaugh *et al.,* 1995), VR2385 (Meng *et al.*, 1994), EDRD-1 (Saito *et al.*,1996), No1 (Drew *et al.,* 1996), Olot/91 (Plana *et al.*, 1996), Boxmeer 10 (Conzelmann *et al.*, 1993), and Lelystad (Meulenberg *et al.,* 1993). Deletions are indicated by (★) and identical residues are indicated by (–).

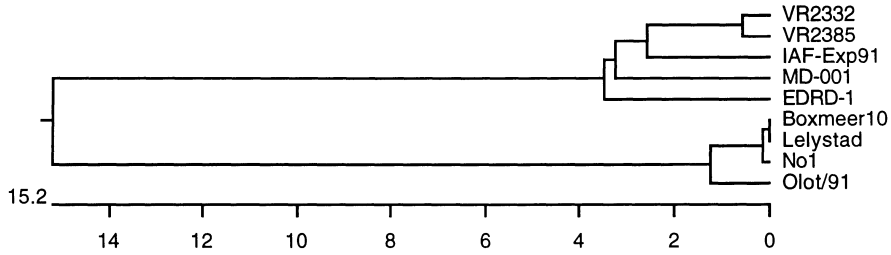

Figure 2. Phylogenetic trees based on the analysis of nucleotide sequences of the N gene of PRRSV. The tree was derived using the Clustal method and nucleotide sequence data obtained from EMBL+GeneBank for the American VR2332 (U00153) and VR2385 (U03040), Canadian IAF-Exp 91 (L40898), Taiwanese MD-001, Japanese EDRD-1 (D45852), Dutch Boxmeer 10 (L04493) and Lelystad (M96262), British No1 (L77924) and Spanish Olot/91(X92942) strains.

REFERENCES

Chang C.C., Chung W.B., Lin W.M., Weng C.N., Yang P.C., Chiu Y.T., Chang W.F. and Chu R.M., 1993a, Porcine reproductive and respiratory syndrome (PRRS) in Taiwan I. Viral isolation, *J. Chin. Soc. Vet. Sci.* **19**:268–276.

Chang C.C., Chung W.B., Lin W.M., Yang P.C., Weng C.N., Chiu Y.T., Chang W.F. and Chu R.M., 1993b, Porcine reproductive and respiratory syndrome (PRRS) in Taiwan II. Experimental reproduction of PRRS in specific-pathogen free (SPF) pigs, *J. Chin. Soc. Vet. Sci.* **19**:277–284.

Chomczynski P. and Sacchi N., 1987, Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction, *Anal. Biochem.* **162**:156–159.

Conzelmann K.K., Visser N., Woensel P.V. and Thiel H.J., 1993, Molecular characterization of porcine reproductive and respiratory syndrome virus, a member of the Arterivirus group, *Virology* **193**:329–339.

Den Boon J.A., Snijder E.J., Chirnside E.D., De Vries A.A.F., Horzinek M.C. and Spaan W.J.M., 1991, Equine arteritis virus is not a togavirus but belongs to the coronavirus-like superfamily, *J. Virol.* **65**:2910–2920.

Done S.H., Paton D.J. and White M.E.C., 1996, Porcine reproductive and respiratory syndrome (PRRS): A review, with emphasis on pathological, virological and diagnostic aspects, *Br. Vet. J.* **152**:153–174.

Drew T.W., Lowings J.P. and Yapp F., 1996, Variation in the open reading frame ORF3 and ORF7 of porcine reproductive and respiratory syndrome virus isolates in Great Britain. *Vet. Microbiol.* (in press).

Goyal S.M., 1993, Review article: Porcine reproductive and respiratory syndrome, *J. Vet. Diagn. Invest.* **5**: 656–664.

Mardassi H., Mounir S. and Dea S., 1994, Identification of major differences in the nucleocapsid protein genes of a Quebec strain and European strains of porcine reproductive and respiratory syndrome virus, *J. Gen. Virol.* **75**:681–685.

Mardassi H., Mounir S. and Dea S., 1995, Molecular analysis of the ORF 3 to 7 of porcine reproductive and respiratory syndrome virus, Quebec reference strain, *Arch. Virol.* **140**:1405–1418.

Mardassi H., Massie B. and Dea S., 1996, Intracellular synthesis, processing, and transport of proteins encoded by ORFs 5 to 7 of porcine reproductive and respiratory syndrome virus, *Virology* **221**:98–112.

Meng X.J., Paul P.S.and Halbur P.G., 1994, Molecular cloning and nucleotide sequencing of the 3'-terminal genomic RNA of the porcine reproductive and respiratory syndrome virus, *J. Gen. Virol.* **75**:1795–1801.

Meulenberg J.J.M., Hulst M.M., De Meijer E.J., Moonen P.L.J.M., Den Besten A., De Kluyver E.P., Wensvoort G. and Moormann R.J.M., 1993, Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV, *Virology* **192**:62–72.

Meulenberg J.J.M., Petersen-Den Besten A., De Kluyver E.P., Moonen R.J.M., Schaaper W.M.M. and Wensvoort G., 1995, Characterization of proteins encoded by ORF 2 to 7 of Lelystad virus, *Virology* **206**:155–163.

Murtaugh M.P., Elam M.R. and Kakach L.T., 1995, Comparison of the structural protein coding sequences of the VR-2332 and Lelystad virus strains of the PRRS virus, *Arch. Virol.* **140**:1451–1460.

Plagemann P.G.W. and Moennig V., 1992, Lactate dehydrogenase-elevating virus, equine arteritis virus and simian hemorrhagic fever virus, a new group of positive strand RNA virus, *Adv. Virus Res.* **41**:99–192.

Saito A., Kanno T., Murakami Y., Muramatsu M. and Yamaguchi S., 1996, Characteristics of major structural protein coding gene and leader-body sequence in subgenomic mRNA of porcine reproductive and respiratory syndrome virus isolated in Japan, *J. Vet. Med. Sci.* **58**:377–380.

Sambrook J., Fritsch E.F. and Maniatis T., 1989, *Molecular cloning: a laboratory manual*. 2nd ed., Cold Spring Harbor Laboratory Press, New York.

Sanger F., Nicklen S. and Coulson A.R., 1977, DNA sequencing with chain-terminating inhibitors, *Pro. Natl. Acad. Sci. USA* **74**:5463–5467.

Spaan W., Cavanagh D. and Horzinek M.C., 1988, Coronaviruses: structure and genome expression, *J. Gen. Virol.* **69**:2939–2952.

Suarez P., Zardoya R., Prieto C., Solana A., Tabares E., Bautista J.M. and Castro J.M., 1994, Direct detection of the porcine reproductive and respiratory syndrome (PRRS) virus by reverse polymerase chain reaction (RT-PCR), *Arch. Virol.* **135**: 89–99.

Wensvoort G., Terpstra C., Pol J.M., Ter Laak E.A., Bloemraad M., De Kluyver E.P., Kragten C., Van Butten L., Den Besten A., Wagenaar F., Broekhuijsen J.M., Moonen P.L.J.M., Zetstra T., De Boer E.A., Tibben H.J., De Jong M.F., Van't Veld P., Groenland G.J.R., Van Gennep J.A., Voets M.T., Verheijden J.H.M. and Braamskamp J., 1991, Mystery swine disease in the Netherlands: The isolation of Lelystad virus, *Vet. Quart.* **13**:121–130.

Yoon K.J., Zimmerman J.J., Swenson S.L., McGinley M.J., Eernisse K.A., Brevik A., Rhinehart L.L. Frey M.L., Hill H.T. and Platt K.B., 1995, Characterization of the humoral immune response to porcine reproductive and respiratory syndrome (PRRS) virus infection, *J. Vet. Diagn. Invest.* **7**:305–312.