Characterization of a variant of transmissible gastroenteritis virus (TGEV)

by

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ABSTRACT

A TGEV isolate, VMRI 5170, and a PRCV isolate, NVSL 5170, originating from a TGE outbreak on a swine farm in 1995, were characterized biologically, antigenically and genetically. Their growth characteristics were compared with the standard Miller strain of TGEV. The growth curves for the three viruses were similar. However, the average plaque size of the PRCV isolate NVSL 5170 (0.99 +/- 0.31 mm) was smaller than that for the TGEV isolate VMRI 5170 (2.33 +/- 0.56 mm) and the TGEV isolate Miller (2.47 +/- 0.50 mm). These isolates reacted in virus neutralization tests with both hyperimmune sera raised against Miller strain of TGEV and the MAbs against the conserved epitopes on the S glycoprotein of TGEV. For genetic characterization of these isolates, the S and 3/3.1 genes were sequenced and compared with known sequences of TGEV and PRCV isolates. The S gene of the TGEV isolate VMRI 5170 showed a 96 - 97 % homology with the published sequences of TGEV, with 120 - 169 nucleotide differences. The identity between the S gene sequence of the PRCV isolate NVSL 5170 and that of other PRCV isolates was also 96 - 97 %. The PRCV isolate NVSL 5170 had a truncated S gene with a 714 nucleotide deletion. This is the largest deletion detected thus far in PRCV isolates. Without accounting for the deletion, TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 showed a very high level of homology in the S gene with only 6 nucleotide differences between all 4353 nucleotides. At the amino acid level, the difference was only 4 amino acids. The protein profiles of these isolates by radioimmunoprecipitation assay also confirmed that the M and N proteins of TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 were similar in size but the S glycoprotein of PRCV isolate NVSL 5170 was smaller. The ORF 3 and 3.1 genes of PRCV isolate NVSL 5170 were intact with only 2 nucleotide differences in this region when compared to TGEV isolate VMRI 5170. However, the first different nucleotide in the 3.1 gene of NVSL 5170 created a stop codon which may have resulted in a truncated 3.1 protein. In conclusion, TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 are closely related to each other in both antigenic and genetic properties as well as biological characteristics. In addition, Phylogenetic analysis of the sequences demonstrated a very close relationship among these two isolates and presented strong evidence that PRCV isolate NVSL 5170 emerged from TGEV isolate VMRI 5170 by a single deletion. This deletion could possibly be the cause of the smaller S glycoprotein and the smaller plaque size of PRCV isolate, NVSL 5170.

1. INTRODUCTION

Transmissible gastroenteritis (TGE) disease in swine was first detected by Doyle in 1946. The causative agent was referred to as transmissible gastroenteritis virus (TGEV) which was shown to be in the family of coronaviridae (Siddell et al. 1983a). TGEV produces watery diarrhea in swine of all ages; however, the disease is most severe in pigs less than 3 weeks of age. The severity of disease depends on the immune status and the age of the piglets (Hill, 1988). By negative staining electron microscopic examination, coronavirus particles are 60 - 160 nm in diameter and are spherical to pleomorphic (Holmes, 1990; Saif and Wesley, 1992). The TGEV is enveloped with widely spaced club-shaped peplomers, 12 - 25 nm in length (Saif and Wesley, 1992). The TGEV has 3 major structural proteins, the nucleocapsid protein (N), the integral membrane glycoprotein (M) and the peplomer glycoprotein (S) (Spaan et al., 1988). The N protein is a basic phosphoprotein to which the genomic RNA binds to form a helically symmetrical nucleocapsid. The M and S proteins are glycosylated transmembrane proteins.

As a member of coronaviridae, TGEV contains a large, positive - sense, single stranded RNA genome (Siddell et al., 1983). During productive infection, TGEV synthesizes at least 8 subgenomic mRNAs (Sethna et al., 1989; Wesley et al., 1989), arranged as a nested set which have a common 3' poly-A termini, with different base sequences on the 5' end (Spaan et al., 1988). The products of 8 subgenomic mRNAs are: polymerase from mRNA 1, the peplomer or spike protein (S) from mRNA 2, a 7.9 kD protein from mRNA 3, a 27.7 kD protein from mRNA 4, a 9.3 kD protein from mRNA 5, an integral membrane from mRNA 6, nucleocapsid from mRNA 7 and a 14 kD polypeptide from mRNA 8.

The TGEV is closely related to porcine respiratory coronavirus (PRCV), because PRCV was neutralized *in vitro* by antiserum against TGEV (Callebaut et al.,1988). However, some of the monoclonal antibodies against the S protein epitopes of TGEV do not recognize PRCV. The close antigenic relatedness between these viruses is due to the similarity of their genomic RNAs. The differences that have been observed between TGEV and PRCV are deletions in the S gene and the nonstructural ORF 3 gene of PRCV (Laude et al., 1993; Russchaert et al., 1990; Vaughn et al., 1995). Thus, PRCV may be regarded as a TGEV variant. However, TGEV and PRCV isolates from the same pigs are not available to conclusively determine if PRCV originated from TGEV.

The TGEV isolate, VMRI 5170, and the PRCV isolate, NVSL 5170, provide the opportunity to study the genetic and antigenic relationship between TGEV and PRCV. The VMRI 5170 and NVSL 5170 isolates originated from the same TGE outbreak in a swine herd. However, they were later determined to be different viruses. Therefore, the hypothesis of this study is that the PRCV isolate, NVSL 5170, emerged from the TGEV isolate, VMRI 5170, caused by a deletion mutation. In addition, the purpose of the study is also to determine how the mutation influences some biological properties of the viruses. To achieve the objective, the two viruses will be characterized in comparison to the standard Miller strain of TGEV. The characteristics to be examined include:

1. growth characterization
    1.1. one step growth curve
    1.2. plaque size measurement
2. antigenic characterization using viral neutralization test
3. viral protein profiles by radioimmunoprecipitation assay
4. genetic characterization by PCR and sequence analysis

It is expected that this study should present strong evidence of the emergence of PRCV isolate, NVSL 5170, from TGEV isolate, VMRI 5170, caused by a deletion mutation.

2. LITERATURE REVIEW

Coronaviruses

Coronaviruses are large pleomorphic single - stranded positive RNA viruses (Tyrrell et al., 1978). The viruses in this genus have an unique morphology which is a pleomorphic spherical virion with club - shaped peplomers, when examined by negative stained electronmicroscopy. Their genomic nucleotides are plus - stranded RNAs which replicate by a unique mechanism. Coronaviruses infect humans and a wide range of animals causing either systemic or local diseases. However, the viruses can be divided into 3 antigenic groups (Table 1) in which there are some degrees of cross - reactivity within each group.

Table 1: *Coronaviruses*, antigenic groups and diseases. (from Holmes and Lai, 1996, *Coronaviridae* : The Virus and Their Replication)

antigenic group	Virus	Host	Respiratory infection	Enteric infection	Hepatitis	Neurologic infection
1	HCV-229E	Human	X			
	TGEV	Pig	X	X		
	PRCV	Pig	X			
	CCV	Dog		X		
	FECV	Cat		X		
	FIPV	Cat	X	X		
	RbCV	Rabbit		X	X	X
2	HCV-OC43	Human	X			
	MHV	Mouse	X	X	X	X
	SDAV	Rat				X
	HEV	Pig	X	X		X
	BCV	Cow		X		
	BRCV	Cow	X			
	RbEVC	Rabbit		X		
	TCV	Turkey	X	X		
3	IBV	Chicken	X		X	
	BDV	Turkey		X		

Note: HCV-229E, human respiratory coronavirus; TGEV, porcine transmissible gastroenteritis virus; PRCV, porcine respiratory coronavirus; CCV, canine coronavirus; FECV, Feline enteric coronavirus; FIPV, feline infectious peritonitis virus; TCV, turkey coronavirus; HCV-OC43, human respiratory coronavirus; MHV, mouse hepatitis virus; SDAV, sialodacryoadenitis virus; HEV, porcine hemagglutinating encephalomyelitis virus; BCV, bovine coronavirus; BRCV, bovine respiratory coronavirus; RbCV, rabbit coronavirus.

Coronavirus Properties

Coronaviruses are separated from other groups of viruses according to their distinct morphology. Their genomes are single plus stranded RNAs, 27 - 32 kb in size, which are 5' end capped and 3' end polyadenylated (Spaan et al., 1988; Lai, 1990). The genomic RNA of coronavirus is associated with nucleocapsid phosphoprotein to form a helical ribonucleoprotein about 9 - 11 nm in diameter. The ribonucleocapsid is surrounded by an envelope, derived from a host intracellular membrane and viral structural proteins. All coronaviruses possess 3 major structural proteins; a nucleocapsid protein (N; 50 - 60 kD), a membrane glycoprotein (M or E1; 23 - 29 kD) and a spike glycoprotein (S or E2; 170 - 220 kD). Trimers of S glycoproteins held by a noncovalent bond form long petal - shaped spikes which are embedded in and projected from the viral envelope. Therefore, the morphology of the coronaviruses is similar to a solar corona when examined by negative staining EM. The size of coronavirus particles is about 100 nm. However, they are pleomorphic and range in size from 75 - 160 nm.

Antigenic group II coronaviruses also have a fourth structural protein, hemagglutinin - esterase glycoprotein (HE, E3 or gp65; 62 - 65 kD) (Holmes and Lai, 1996). The HE dimer protein linked by a disulfide bond forms a short spike on the envelope which is homologous to that of influenza C virus. Coronaviruses that possess HE have hemagglutination, hemadsorption and acetylesterase activities.

Virions attach to receptors on the host cell membrane via the S protein. Coronaviruses are endocytosed into the cytoplasm where they replicate (Fenner et al., 1993). The genomic RNA is transcribed to a minus - stranded RNA which in turn is transcribed to a nested set of mRNA with a common 3' end. The translated proteins mature in the endoplasmic reticulum followed by assembly in and budding from Golgi cysternae. The budding viruses do not contain RNA - directed RNA polymerase (Siddell et al., 1981).

Transmissible Gastroenteritis Virus

Virion structure

TGEV is a virus in the genus coronaviruses, under the family coronaviridae (Siddell et al. 1983a) with pleomorphic spherical morphology and a diameter of about 60 - 160 nm (Okaniwa et al., 1968; Philip et al., 1971). Like other coronaviruses, TGEV also has a corona like morphology because the S glycoproteins form club - shaped surface projections, 12 - 25 nm in length which are scattered on the virus envelope. Without projections, the size of the viral particle is around 65 - 90 nm. (Thake, 1968; Pensaert et al., 1970b; Wagner et al., 1973).

Intact virions have a buoyant density of 1.18 - 1.20 g/ml in a sucrose gradient (Briton et al., 1980; Jimenez et al., 1986).

The genomic RNA of TGEV encodes 4 structural proteins which include the small integral protein (sM), nucleocapsid protein (N), membrane glycoprotein (M), and the spike glycoprotein (S) (Spann, 1988; Laude et al., 1993; Holmes and Lai, 1996). These structural proteins incorporate into the virion and have different functions, as discussed below.

Small integral protein (sM)

Godet et al. (1992) reported that ORF 4 of genomic RNA encodes a 10 kD polypeptide called the small integral membrane (sM). This sM is incorporated into the virus envelope as an integral protein, however, its function is unknown.

Nucleocapsid Protein (N)

The N protein is a 47 kD phosphoprotein bound with RNA to form the ribonucleoprotein (Laude et al., 1990). These proteins are basic as they contain clusters of basic residues, but their C termini are acidic (Kapke and Brian, 1986; Spaan, 1988). Around 8 - 10 % of the total amino acid residues are serine. In fact, most of the serine residues on the N protein are phosphorylated. The N protein has 3 structural domains; the middle domain binds to the RNA (Master, 1992) to form a helical nucleocapsid. *In vitro* studies reveal that N binds to the intracytoplasmic domain of the M protein during virus budding (Sturman et al., 1980). This leads N to facilitate encapsidation of the genomic RNA. In addition, it is now known that N protein also participates in RNA replication since antibody against N significantly inhibits genomic RNA synthesis (Compton et al., 1987; Spaan et al., 1988). The N protein is also known to elicit cell - mediated immunity (Holmes and Lai, 1996).

Membrane Glycoprotein (M)

The M is a 29 - 36 kD protein which functions as a matrix protein (Laude et al., 1993). It is composed of a 245 amino acid residue polypeptide that folds into 3 domains; hydrophilic N terminal domain, transmembrane domain, and C terminal intracytoplasmic membrane domain (Spaan et al., 1988). The N terminus domain of M, about 10 % of the M molecule, is N - linked glycosylated and is exposed on the outer surface of the envelope. Around 17 residues of the N - terminus of the M glycoprotein form a signal peptide which is recognized by the signal recognition particle for membrane insertion. This signal peptide targets M protein to the golgi complex.

The transmembrane domain is about one third of the M protein. It spans 3 times in the envelope while folding into 3 hydrophobic alpha helices (Spaan et al., 1988). This domain functions as the matrix for the viral envelope. Approximately half of the M molecule is a C

terminus intracytoplasmic domain which lies under the intracellular bilayer. This part associates with the N protein during viral budding.

The M protein not only serves as a matrix protein, but also participates in other TGEV properties. Hydrophilic N terminus which is exposed on the outer surface is responsible for mediating complement - dependent neutralization and interferon induction (Charley and Laude, 1988; Woods et al., 1988). M is also important for viral maturation, assembly and budding of the virus. The supportive evidence is that M appears to accumulate in the golgi apparatus where the virus buds in infected cells.

Spike Glycoprotein (S)

Spike or peplomer is a large membrane - anchored glycoprotein which is 220 kD of relative mass (Laude et al., 1993). S glycoprotein contains 1447 amino acid residues which form the N - to C - terminus containing a 16 amino acid residue, long N - terminal signal sequence, two large external domains (S_1 & S_2), a transmembrane domain, and a short C - terminal intracytoplasmic domain. S protein contains a large number of N - linked glycosylation sites (Rasschaert and Laude, 1987; Jacob et al., 1987). The Intracytoplasmic domain which is rich in cysteine residues may direct S glycoproteins to be incorporated into the viral envelope and interact with other structural proteins (Holmes and Lai, 1996). The S_2 segment, which connects to the cytoplasmic domain, is the carboxyl half of the S molecule. This part forms the alpha helix secondary structure with 2 heptad repeated motifs that tend to fold to an intra - chain coiled coil structure of the peplomer. Unlike antigenic group II coronaviruses, TGEV does not have a trypsin cleavage motif between S_2 and S_1. The S_1 is a N terminal polypeptide which forms a globular glycoprotein. Trimers of S_1 and S_2 hold together by non covalent bonds to form petal - shaped spikes projecting from the envelope.

S glycoprotein has many biological functions (Holmes and Lai, 1996). It binds to *aminopeptidase N*, a specific host cell surface receptor glycoprotein, during viral attachment. Inhibition of cell fusion by monoclonal antibodies against S glycoprotein suggests that S induces cell fusion of infected cells (Spaan et al., 1988). Furthermore, S glycoprotein possesses neutralizing epitopes as antibodies raised against it can neutralize the viruses at multiple steps in the viral replication cycle (Nguyen et al., 1986; Sune et al. 1990). Presentation of the S protein on infected cells also induces cellular mediated immune response (Holmes et al., 1986; Welsh et al., 1986).

Genomic Structure and Organization

The genome of coronavirus is a large single stranded RNA of positive polarity (Spaan et al., 1988; Laude et al., 1993). It is about 27 - 30 kb in length, which is the largest known genome of all RNA viruses (Spaan et al., 1988). The genomic RNA is 5' capped and 3' polyadenylated, therefore, it is infectious when introduced into host cells (Lai, 1990). TGEV genome contains 7 genes and a 60 - 80 nucleotide leader sequence at its 5' end. Each gene may have 1 or more ORFs which are separated by intergenic sequences (IS) which contain signals for transcription of a nested set of subgenomic RNAs (Spaan et al., 1988). The first gene from the 5' end is about 20 kb long consisting of 2 ORFs that encode viral RNA polymerase, protease, and other nonstructural proteins (Holmes and Lai, 1996). The rest of the genomic RNA is approximately 8.5 kb made up of 6 genomic regions; 2 (S), 3, 4 (sM), 5 (M), 6 (N) and 7. TGEV also shares the common gene order for coronaviruses, Pol - S - M - N, (Laude et al., 1993). In addition to region 1 of the genomic RNA, gene 3 of TGEV is also bicistronic. (Spaan et al, 1988; Lai 1990).

Growth Characteristics and Physicochemical Properties

TGEV can be propagated in primary and secondary pig kidney cells, pig kidney cell line (Laude et al. 1981), and McClurkin swine testicle (ST) cell line (McClurkin and Norman, 1966). The virus also replicates in organ cultures from pig esophagus, ileum and colon (Rubenstein et al., 1970). Cytopathic effect (CPE) may not be observed in the primary isolate, so a higher viral passage may be required for CPE production. The CPE includes fusion of infected cells, rounding, enlargement or elongation of infected cells, ballooning effect of the infected cells and detachment of cells (McClurkin and Norman, 1966; Kemeny, 1978; Vaughn and Paul, 1993). TGEV can be isolated from freezing and thawing of the infected cell culture, and the titer of TGEV isolates range from 1×10^5 to 5×10^7 pfu/ml (Vaughn and Paul, 1993).

TGEV is sensitive to heat and light but is resistant to the intestinal environment. TGEV is very stable when stored frozen but is labile at room temperature (Bay et al., 1952; Young et al., 1995). The virus can be kept at -20° C for 6 months without loss of infectivity. In contrast, at 37° C, the infectivity titer of the viruses will decrease 10 fold at every 24 hour interval. In addition, TGEV is inactivated by exposure to both sunlight and UV light (Haelterman, 1963; Cartwright et al., 1965). TGEV is resistant to trypsin and bile and is stable at pH 3 (Harada et al., 1968; Moscari 1980a). Resistance of TGEV to trypsin and bile allows it to pass from the stomach to the small intestine without degradation.

Replication Strategy

The replication cycle of coronaviruses has been extensively studied for mouse hepatitis virus (MHV). The events from the very beginning to the end of the cycle include; attachment and penetration, primary translation, transcription, replication, late translation and assembly, and release. The following section summarizes a TGEV replication strategy based on a MHV replication model.

Attachment and Penetration

The first step of the replication cycle is the binding of S glycoprotein to a specific receptor on the host cell membrane. For TGEV, S glycoprotein binds with aminopeptidase N (APN), a zinc binding protease (Delmas et al., 1992a) which is abundantly present on the brush border membrane of small intestinal villi (Delmas et al., 1992a). However, protease activity is not required for viral attachment. It was found that some monoclonal antibodies against porcine and human APN can inhibit binding of S to APN. The cells that are normally resistant to TGEV become susceptible to infection when cDNA coded for APN glycoprotein was inserted in the cells (Tung et al., 1992). Viruses enter into cells by fusion of the virus envelope with either a plasma membrane or an endosomal membrane (Gallagher et al., 1991; Kooi et al., 1991).

Primary Translation

After viruses penetrate into cells, they start translation of their genomic RNA. The first translated gene encodes RNA directed RNA polymerase (Holmes and Lai, 1996). It contains 2 ORFs which are translated into a polyprotein by a ribosomal frame - shifting mechanism (Brierley et al., 1989; 1991). The polyprotein is co - translationally modified to multiple proteins including RNA directed RNA polymerase by viral and host protease. The polymerase is synthesized continuously during the replication cycle.

Transcription and replication

Positive sense stranded genomic RNA is transcribed into a minus - strand RNA which in turn serves as the template for either subgenomic mRNA or genomic RNA synthesis. All minus stranded RNAs appear as double stranded RNA in replicative intermediate forms and no free minus stranded RNA is found (Perman et al., 1986). All mRNAs and genomic RNA are 5' capped and 3' polyadenylated. TGEV have 7 subgenomic mRNAs which form a nested set of mRNA with a common 3' end. They are numbered 1 to 7 according to their sizes which decrease by the increasing number (Lai, 1990). Most of the subgenomic mRNAs except the smallest one are polycistronic. However, only the ORF at the 5' end of each mRNA is translated, with the exception of mRNA 1 and 3 which are translated into 2 proteins (Spaan et

al, 1988; Lai 1990). The subgenomic mRNAs are synthesized in unequal but constant amounts during the replication cycle (Siddell et al., 1983). The mRNAs are not processed by splicing because the replication takes place in the cytoplasm, and mRNAs are transcribed independently (Siddell et al., 1983).

As a coronavirus member, TGEV mRNAs have some specific characteristics. Although the leader sequence is on the 5' end of the genomic RNA only, all subgenomic mRNAs have the leader sequence at their 5' end. However, at the 5' end of each ORF of the TGEV genome, there is a consensus intergenic sequence of 6 - 8 nucleotides, AA**CUAAAC** (Spaan, 1986; Laude et al., 1993). This sequence is complementary to that of the 3' end of the leader sequence.

Two models can explain how coronaviruses synthesize their subgenomic mRNAs (Holmes and Lai, 1996). The first model is the discontinuous, nonprocessive leader - primed transcription (Holmes and Lai, 1996). In this model, the full length minus - strand RNA is translated from the genomic plus strand RNA. Thereafter, polymerase transcribes the antileader sequence at the 3' terminus of the full length minus - strand RNA, and then terminates with dissociation of the leader from the template. The leader with or without polymerase jumps to bind with an intergenic sequence (IS) down stream of the template, which serves as the primer for mRNA synthesis. Thus, an IS acts as the core promoter for mRNA transcription (Joo et al., 1992; Kim et al., 1993). However, the upstream sequence from the leader and 5' end sequence of subgenomic mRNA are also required for transcription initiation (Liao and Lai, 1994). Within the TGEV genome, there is a conserved sequence of 10 nucleotides, around 80 bases from the 3' end of the genomic RNA that may relate to minus - stranded template synthesis (Kapke and Brian, 1986).

Another synthesis model is discontinuous transcription during minus - stranded RNA synthesis (Sawicki and Sawicki, 1990). Transcription of minus - stranded RNA terminates when the polymerase complex reaches the 3' end of an IS, which then jumps to bind to the 3' end of the leader sequence at the 5' end of the genomic RNA. Subsequently, the minus - stranded subgenomic and genomic RNAs with an antileader at their 3' end can be continuously transcribed into subgenomic or genomic mRNAs. Therefore, ISs serve as termination sites and bind with leader sequences for jumping of RNA polymerase during minus - strand transcription (Holmes and Lai, 1996). However, this model is controversial. Since loop structures have never been found on the Replicative Intermediate molecules, therefore, jumping of polymerase by looping out of the negative - stranded template and co - or post - transcriptional ligation of subgenomic minus - stranded RNAs to leaders, should not occur.

Furthermore, by coinfection of 2 strains of coronaviruses, the combination of mRNA of one strain and the leader of another strain may occur (Spaan et al., 1988).

In vitro transcription studies suggest that RNA polymerase complexes with some proteins. These proteins may be the products of the gene 1 and N protein (Brayton et al., 1982; Dennis and Brian, 1982). The polymerases for minus and plus stranded RNAs synthesis are different (Brayton et al., 1982; Brayton et al., 1984). The two RNA polymerase complexes, the early and the late polymerases, involved in the negative - stranded RNA synthesis and the mRNA synthesis, respectively.

For replication of coronaviruses to occur the Replicative Intermediate form of the full length RNA is needed (Holmes and Lai, 1996). The genomic RNA must be transcribed continuously to the full length minus - stranded RNA which in turn will serve as the template for the plus - stranded genomic RNA synthesis. The studies on defective interfering RNA of coronavirus (mouse hepatitis virus) suggest that the replication also requires a leader sequence. However, the nucleotides in the IS for genomic RNA synthesis may differ from those for the subgenomic mRNA synthesis. In addition, about 200 nucleotides at the 3' and the 5' termini of the genomic RNA may participate in the replication.

Late Translation

During late translation, coronaviruses synthesize all structural proteins and some non structural proteins from their corresponding mRNA. Most subgenomic mRNAs of TGEV are polycistronic, but only the ORF at the 5' end is translated (Holmes and Lai, 1996). However, mRNA 1 and 3 are bicistronic (Rasschaert et al., 1987). The mRNA 3 of TGEV has 2 ORFs which are translated into 2 non structural proteins. In non virulent Purdue - 15, and virulent British FS772 strain of TGEV, the genomic RNA possess the ORF 3a and 3b which are bicistronic (Spaan et al, 1988; Laude et al., 1993). Unlikely, upstream of the ORF 3b of the virulent Miller strain of TGEV exists a hexameric IS, CUAAAC. The beginning of the ORF also has a start codon to signal for mRNA production (Laude et al, 1993). Therefore, ORF 3 of Miller strain is transcribed into 2 mRNAs, so called ORF 3/3-1 instead of ORF 3a/3b. The IS of ORF 4 is also a hexamer, CUAAAC, while those of the ORF M, N and 7 are the heptameric ACUAAAC (Britton et al., 1991).

The translated proteins are processed and transported to their target sites. N is translated on free polysomes, rapidly phosphorylated in cytosol and then bound to the genomic RNA (Stohlman et al., 1983; Baric et al., 1988). M is translated and inserted into the RER and post - translationally modified by N - linked glycan (Spaan et al., 1988). The processed M glycoproteins then accumulate in the golgi apparatus where the budding virions are located. S

proteins are N - linked glycosylated, reduced and non covalently linked to form trimers. Mature S glycoproteins also accumulate in the golgi apparatus. However, some of the excess S glycoprotein is transported to the host cell membrane which may mediate cell to cell fusion (Vennema et al., 1990; Griffiths and Rottier, 1992).

Assembly and Release

Assembly and budding of viruses takes place in specific compartments followed by release of virions by exocytosis (Holmes and Lai, 1996). N phosphoproteins may bind to specific sequences, possibly leader sequences (Stohlman et al., 1988), on the genomic RNAs to initiate the helical structure. The successive binding may not require the specific binding between the RNAs and the N protein (Robbin et al., 1986; Stohlman et al., 1988). Encapsidation of RNA may be associated with a specific sequence within gene 1b, approximately 20 kb from the 5' end of the genomic RNA (Van der Most et al., 1991; Fosmire et al., 1992). The nucleoproteins of the encapsidated particles bind to M glycoproteins incorporated on to the intracellular membrane. Thereafter, they develop from a budding compartment between the RER and the golgi apparatus (Holmes and Lai, 1996). S glycoproteins which are incorporated at the time of budding, are not necessary for viral assembly but S - naked virions are non infectious (Holmes et al., 1981).

Genetics

RNA recombination is common among coronaviruses because of their unique replication strategy (Lai, 1992). During discontinuous transcription, RNA polymerase sometimes dissociates from a RNA template and jumps to attach to a homologous region on a different RNA template (Lai, 1992). RNA recombination leads to evolution of different strains of the same species or to different species of coronaviruses. For example, feline infectious peritonitis virus (FIPV) may have originated from the combination between TGEV and related viruses (Jacobs et al., 1987) because one domain of the S protein of FIPV and the S protein of TGEV is 93% homologous where as the other domains are somewhat different. Moreover, the homology between the amino acid sequence of the HA 1 domain of MHV - 59 and the amino acid sequence of the S protein of influenza C could be evidence of RNA recombination between 2 types of viruses (Spaan et al., 1988).

Like other RNA viruses, which have no proof - reading mechanism in their replication process, mutations frequently occur among coronaviruses (Holmes and Lai, 1996). The mutations are either point mutations or large genomic deletions. The point mutations in the S gene of MHV lead to alteration of CPE and tissue tropism (Dalziel et al., 1986; Fazakerley et

al., 1992; Gombol et al., 1993). The incidence of deletion mutations among coronaviruses is also high. The most distinctive deletion mutation is the emergence of porcine respiratory coronavirus (PRCV) from TGEV (Holmes and Lai, 1996).

Antigenicity

Antigenic Determinants

Studies on the monoclonal antibodies against TGEV structural proteins have allowed characterization of the antigenic map of TGEV. The structural S, M and N proteins are antigenic but the S glycoprotein is the primary protein that induces neutralizing antibodies (Jimenez et al., 1986; Laude et al., 1986). Antibodies against the M protein can neutralize TGEV in the presence of complement (Woods et al., 1988; Laude e al., 1988; Callebaut et al. 1988; Laude et al., 1990). There are 4 major antigenic sites on the S glycoprotein defined as site A, B, C and D (Gebauer et al., 1991). All antigenic sites are located in the N terminal half (543 amino acid residues) of the S glycoprotein (Correa et al., 1990). Only antigenic site A elicits neutralizing antibodies (Callebaut et al., 1988; Laude et al., 1988; Sanchez et al., 1990).

Antigenic site A is complex and is divided into 3 subsites, Aa, Ab and Ac (Correa et al., 1988). The amino acid residues in site A are intracellular, glycosylated and are located on the surface of TGEV. Amino acid residues involved in site A are 538, 591, and 543 for subsites Aa, Ab and Ac, respectively (Gebuaer et al., 1991). In addition, subsites Aa and Ab may overlap in residue 586 because change in residue 586 effects the conformation of both subsites. The amino acid sequence, 537 - MKSGYGQPIA - 547, which is highly conserved among TGEV may contribute partially to subsite Ac. This subsite may also contribute to protective immunity and is most likely crucial for diagnosis (Sanchez et al., 1990; Gebauer et al., 1991). Antigenic site A represents group specific epitopes which are shared by enteric TGEV and respiratory PRCV isolates (Sanchez et al., 1990).

Other antigenic sites are also characterized. Antigenic site B contains type specific epitopes which are represented by enteric TGEV isolates (Sanchez et al., 1990). It consists of at least 3 conformational epitopes two of which overlap to each other (Gebauer et al., 1991). The residues involved in antigenic site B are glycosylated residues 97 and 144. Antigenic site C contains linear epitopes which are non glycosylated (Correa et. al., 1990; Gebauer et al., 1991). The amino acid residues involved in site C are residues 50 and 51. However, the consensus sequence of site C, deduced by PEPSCAN, is possibly 48 - P - P/S - N - S - D/E - 52 (Gebauer et al., 1991). In contrast to site A, B and D, antigenic site C is not accessible in the native form. Most TGEV isolates are conserved at antigenic sites B and C but vary in site D

(Wesley et al., 1990a). The residues involved in site D are residues 381 (Gebauer et al., 1991) to 392 (Pothumus et al., 1990; Delmas et al. , 1990).

Antigenic Relationship

There is only one serotype of TGEV; however, TGEV is related to other coronaviruses (Saif and Wesley, 1992). TGEV and PRCV are closely related because hyperimmune serum against TGEV can neutralize PRCV. In contrast, TGEV shows no antigenic relationship to other porcine coronaviruses, porcine epidemic diarrhea virus or hemagglutinating encephalomyelitis virus. TGEV is related to feline infectious peritonitis virus (FIPV) and canine coronavirus (CCV) showing cross reactivity with TGEV to some degree by IFA and VN. However, they can be differentiated using a two way cross neutralization test (Reynolds et al., 1986). The monoclonal antibodies against non neutralizing epitopes of the spike protein of TGEV can recognize TGEV; however, it does not recognize FIPV, CCV or PRCV (Laude et al., 1988; Callebaut et al., 1988; Sanchez et al., 1990).

Porcine Respiratory Coronavirus (PRCV)

History

From the early 1980's, the incidence of TGE, the disease caused by TGEV, has decreased considerably in Europe. However, the serostatus of swine herd for TGEV increased without evidence of any clinical enteric disease (Pensaert et al., 1986; Jestin et al., 1987b). A coronavirus, isolated from nasal swabs, was neutralized by antiserum to TGEV (Penseart et al., 1986), and was found to infect cells of the respiratory tract (Pensaert et al., 1989). In 1990, the TGEV-like virus was also isolated from swine herds in the US (Hill, 1989; Wesley et al., 1990a). Recent studies revealed that the virus seemed to be a TGEV like - mutant since there were deletion mutations of the viral genome when compared with those of TGEV. The virus was named porcine respiratory coronavirus because of its respiratory tropism (Pensaert et al., 1986; Wesley et al., 1990a; Paul et al., 1995). It is not clear whether PRCV emerged from the recombination of TGEV and related viruses or a mutation within the TGEV genome itself. Nevertheless, the evidence from genetic sequencing suggests that PRCV originated from TGEV (Laude et al., 1993). In fact, the defective RNAs, discontinuous parts of the genomic RNAs, are normally found in infected cells. Thus, it is possible that dissociated RNA polymerase together with a nascent RNA may reassociate with the template downstream of the pause site, resulting in a deletion.

Genetic Relationship between TGEV and PRCV

The pairwise alignments of the genomic RNAs and the translated ORFs of TGEV and PRCV show only a 3 % nucleotide and amino acid difference (Laude et al., 1993). This diversity results from deletion mutations and point mutations which are limited within the 5' half of the S gene and ORF 3a (Rasschaert et al., 1990; Britton et al., 1991; Page et al., 1991). Indeed, there are some differences in the mutations between European PRCV and USA PRCV isolates. Subsequently, both USA and European PRCV may have emerged from different mutational events (Laude et al., 1993; Paul et al., 1995). However, both of them possess S genes encoding the N terminus truncating S glycoproteins, and non - translated ORF 3a psuedogenes (Laude et al., 1993). The evolutionary tree of 6 European PRCV and 5 TGEV isolates suggests that PRCV and TGEV have a common ancestor (Sanchez et al., 1992).

The mutation within the S gene of PRCV is a large deletion of 672 - 681 nucleotides at the 5' end of the S gene of TGEV (Laude et al., 1993). All European PRCV isolates have a 672 nucleotide deletion of the S gene (Sanchez et al., 1992). The deletions occur in the same position and cause a 224 amino acid truncated S glycoprotein. The number of deleted bases within the S gene of USA PRCV vary greatly. It is a 681 nucleotide deletion within the S gene of USA PRCV, ISU 1, which corresponds to 227 amino acid residues (Laude et al., 1993). Other USA PRCV isolates have 621 - 681 nucleotide deletions within the S gene (Vaughn et al., 1994; Vaughn et al., 1995). Without accounting for the deleted amino acids, the S proteins of PRCV and TGEV show a 98 % homology (Britton et al. 1991). Therefore, the S protein of PRCV and TGEV contain about 1206 - 1209 and 1431 - 1433 amino acid residues, respectively (Laude et al., 1993). Subsequently, the S glycoprotein produced by PRCV has a relative mass of 190 kD compared with that of 220 kD for TGEV (Rasschaert et al., 1990).

The mutation within the ORF 3a of European PRCV and USA PRCV are also different, but the ORF 3b are the same (Laude et al., 1993). The ORF 3a of European PRCV has 3 mutation events; a 13 nucleotide deletion including the hexameric IS, a 22 nucleotide deletion covering the AUG initiation codon, and a 36 nucleotide deletion (Laude et al., 1993). These deletions destroy the transcription start site in which, consequently the ORF 3a is a pseudogene. In the ORF 3a of USA PRCV, there is a 5 nucleotide deletion, but IS or the initiation codon is intact which does not effect transcription. However, the consensus sequence is CUAAAU instead of CUAAAC which may cause ineffective transcription. In contrast, the ORF 3b of the PRCV genome has both IS and the start codon like that of TGEV. Thus, it can be transcribed into the 3 - 1 non structural protein (Wesley et al., 1989). In fact, gene 3 of

Purdue - 115, and FS 772 TGEV do not have the CUAAAC sequence downstream of the ORF 3b but their ORF 3b encodes the same 3 - 1 products by a RNA framshifting mechanism.

The ORF 4, M, N and 7 of PRCV and TGEV are 98 % homologous (Britton, 1991). The ORF 4 of PRCV shows 96 % homology to FS 772 and Purdue 115, but 100 % identity to Miller TGEV (Rasschaert et al., 1987; Britton et al., 1989; Wesley et al., 1989). There is no deletion or insertion within the N and 7 gene of PRCV when compared with those of TGEV. The relative mass of the M and N protein produced by PRCV and TGEV infected cells are similar (Rasschaert et al., 1990).

Antigenic Relationship between TGEV and PRCV

It has been known since 1984 that TGEV and PRCV are closely related, as polyclonal antibodies were not able to distinguish between TGEV and PRCV. By one way and two way viral neutralization tests, both viruses showed complete cross reactivity (Callebaut et al., 1988). By immunoblotting using polyclonal antiserum, their antigenicities could not be differentiated using S, M and N antigens (Callebaut et al., 1988). However, monoclonal antibodies elicited to some epitopes of TGEV were unique for TGEV which would therefore differentiate PRCV from TGEV.

TGEV and PRCV have several common antigenic determinants, but recent studies show that some epitopes are not present on PRCV. Antigenic site A with neutralizing activity is fully shared between TGEV and PRCV because monoclonal antibodies against these antigenic sites neutralize both TGEV and PRCV (Callebaut, 1988; Laude et al., 1988; Sanchez et al., 1990). PRCV possesses the deleted S gene whose products are the truncated S glycoprotein (Rasschaert et al., 1990; Britton et al. 1991; Wesley et al., 1991). The deletions are 224 to 227 amino acid residues which may include antigenic sites B, C and D since the monoclonal antibodies against the epitopes within these sites do not recognize PRCV (Callebaut, 1988; Laude et al., 1988; Sanchez et al., 1990). Indeed, Laude et al. (1988) found that there is some cross reactivity at site D between TGEV and PRCV, as some residues involved in the conformational epitopes of site D come from outside the truncated domain.

In addition to the S antigen, TGEV and PRCV also exhibit M and N antigens. Monoclonal antibodies against the epitopes within the M and N protein of TGEV can recognize PRCV (Callebaut, 1988; Laude et al., 1988; Sanchez et al., 1990). However, about 30 residues of the N terminus of the M protein of TGEV which are extruded from the virion envelope do not react with 3 PRCV isolates (Laude et al., 1988). On the other hand, the epitopes within the C terminus of the M protein are conserved between TGEV and PRCV because of their cross reactivities.

Tissue Tropism

TGEV causes an enteric disease because the virus itself has a tropism for the gastrointestinal tract, but some strains of TGEV replicate in other organs. TGEV receptors on the host cell membrane are *aminopeptidase N* (APN) which are abundant on the brush border of the intestinal villi (Delmas et al., 1992a). Therefore, TGEV can infect the mucosal epithelial cells of intestinal villi. However, most of TGEV strains also replicate in the cells of the respiratory tract and alveolar macrophage (Wesley, 1990b; Britton, 1992; Laude et al., 1993). The Nebraska strains of TGEV are found to have respiratory tropism, so called respiratory TGEV (Underdhal et al., 1978; Laude et al., 1993). The antigenic sites of TGEV for APN receptors are likely to be antigenic sites A/D and B/C on the globular domain or N - terminal half of the S glycoprotein (Sanchez et al., 1992) because monoclonal antibodies against site A and D inhibit virus binding (Sanchez et al., 1992) and decrease multiplicity of TGEV in ST cells (Sune et al., 1990).

The cell receptor for PRCV seems to be APN, as it is for TGEV (Laude et al., 1993). The APN is also expressed on epithelial cells of the respiratory tract. In fact, anti APN monoclonal antibodies can inhibit the multiplication of PRCV (Delmas et al., 1992b). Additionally, cells resistant to PRCV replication when transfected with cDNA encoded for APN could support growth of PRCV (Laude et al., 1993). Interestingly, PRCV has respiratory tropism instead of enteric tropism. However, it can replicate to a limited extent in epithelial cells of the intestinal villi (Paul et al, 1995).

The mechanism of the difference in tissue tropism of TGEV and PRCV is unclear but it may be due to genetic deletions. The deletion region in the S gene of PRCV includes B and C antigenic sites (Sanchez et al., 1992) which may be the enteric receptor binding sites that TGEV uses for attachment. The four residue changes in the S protein of respiratory TGEV (residue 219 of NEB 72 and residues 92, 94 and 218 of TOY 56) are located within the deletion region of the PRCV S protein. This assumption might not be true since receptors on host cell membrane for both TGEV and PRCV are APN which are expressed in either respiratory tract or enteric tract (Laude et al., 1993). However, the deletion in the S gene of PRCV may result in an unstability of the globular part of the S glycoprotein in gastroenteric tract (Laude et al. 1993) which could effect the attachment of viruses to cells. In addition, the deletion of ORF 3a may lead to respiratory tropism of PRCV (Laude et al. 1993) since the TGEV adapted strains, which produce small plaque (SP) size, have a reduced ability to grow in intestinal cells (Wesley et al., 1990b). SP strains of TGEV also have a deletion of 462 nucleotides downstream of the S gene including ORF 3a but have a normal S gene (Wesley et

al., 1990b; Britton et al., 1992). Indeed, several cell types, which are conducted to stably express APN, could support growth of TGEV in different levels (Delmas et al., 1992b). Other factors that influence the replication cycle of the viruses may effect tissue tropism of viruses (Laude et al., 1993).

Transmissible Gastroenteritis (TGE)

Transmissible Gastroenteritis (TGE) is a disease caused by TGEV. This disease is classified in to 2 forms, epizootic and enzootic TGE (Saif and Wesley, 1992). The epizootic feature seems to be seasonal in appearance which is most prevalent in winter. This may be due to the characteristics of the virus which is easily labile at warm temperature and to sun light (Haelterman, 1962). The susceptible herds may become infected by addition of carrier pigs from infected herds. The infected pigs can shed TGEV in their feces for up to 2 weeks (Pensaert et al., 1970a) and via respiratory tract for up to 11 days (Kemeny et al., 1975).

Clinical Signs

Epizootic TGE occurs in swine herds in which most or all animals are susceptible (Saif and Wesley, 1992). The disease spreads rapidly to swine of all ages, but high mortality occurs in suckling pigs under 2 weeks of age. However, pigs over 3 weeks of age normally survive. The typical clinical signs in piglets are; transient vomiting, concomitantly or rapidly followed by profuse watery diarrhea, rapid weight loss and dehydration (Saif and Wesley, 1992). Clinical signs in growing and finishing pigs are inappetance and diarrhea for a few days. Some lactating sows may show a very sick appearance with fever, agalactia, vomiting, inappetance and diarrhea (Saif and Wesley, 1992). The incubation period of the virus is approximately 18 hours to 3 days. Therefore, most of the pigs in the herd will be affected within 2 - 3 days.

Enzootic TGE refers to a persistence of the virus and disease in a herd which periodically results in an outbreak in susceptible animals such as weaning piglets and replacement swine (Saif and Wesley, 1992). The susceptibility of animals and severity of the disease are associated with the immune status of those animals. In herd replacements, TGEV spreads slowly among adult swine. The outbreak in piglets after weaning is common because viral exposure exceeds the passive immunity of pigs (Saif and Wesley, 1992). The pigs will show signs of TGE after weaning from 6 days to 2 weeks. Clinical signs of enzootic TGE are similar to but are less severe than those of epizootic TGE. Mortality is also low. The disease will perpetuate in the herd as long as susceptible animals or immune deprived piglets are exposed to TGEV.

Pathogenesis

In the gastrointestinal tract, TGEV can survive in acidic condition and in the presence of proteolytic enzymes (Saif and Wesley, 1992). Subsequently, virus particles attach to epithelial cells of the villi of the small intestine. The infected cells are rapidly destroyed and lose their functions in digestion and absorption (Moon, 1978), resulting in diarrhea. The extensive destruction by viruses results in atrophy of villi which is most severe in jejunum and ileum, but is seldom found in the proximal part of duodenum (Hooper and Haelterman, 1966). Both virus production and villous atrophy are severe in newborn piglets rather than in piglets over 3 weeks of age (Moon et al., 1973) because the 3 - week old pigs replace villous epithelial cells 3 times more rapidly than neonatal pigs (Moon, 1978). The immune status also plays an important role in protecting cells from viral infection since the older pigs are more resistant to TGE.

Although the enteric tract is the most important replication site of TGEV, virus can multiply in other organs. TGEV was found in alveolar macrophages of infected neonatal pigs and cell culture adapted TGEV can replicate in alveolar macrophage cultures (Laude et al., 1984). Some TGEV such as a highly attenuated strain of TGEV has been found in the respiratory tract of pigs. TGEV can also replicate in the mammary glands and is shed in milk (Kemeny and Woods, 1977), which serves as a source of infection for piglets.

The most severe TGEV - induced lesions are found in the gastrointestinal tract of suckling piglets with severe dehydration (Saif and Wesley, 1992). The stomach are full of curdled milk. The small intestine is distended with yellow and foamy fluid and the intestinal wall is thin due to villous atrophy. A lack of chyle absorption is observed in lacteals of mesentery. The shortened villi appear in both the jejunum and the ileum. The ratio of the length of jejunal villi, and the depths of crypts of Lieberkuhn, decreases from 3:1 to 1:1 in severe cases of TGEV - induced villous atrophy. Transmission EM of TGEV infected epithelial cells reveals that the viral particles are in cytoplasmic vacuoles within villous enterocytes, as well as in M cells, lymphocytes and macrophages in Peyer's patches (Thake, 1968; Wagner et al., 1973; Chu et al., 1982a).

PRCV Associated Disease

Although PRCV was first isolated from normal swine and thought to be non - pathogenic, some experiments and field observations have shown that, in young piglets, it can cause a mild to moderate respiratory disease without enteric signs (O' Toole et al., 1989; Cox et al., 1990a; Laval et al., 1991; Halbur et al., 1993). Anorexia, fever and coughing are the main clinical signs. In severe cases, dyspnea, polypnea, short lasting fever and prostration may

appear (Vannier, 1990). Young piglets are much more susceptible to the disease than adults. Therefore, the older pigs may be asymptomatic following aerosal infection (Cox et al., 1990b). The virus can be isolated from nasal mucosa, tonsils, trachea, lung, stomach and small intestines (O' Toole et al., 1989; Cox et al., 1990a). However, in aerosol infected piglets, the viruses are found in mesenteric lymph nodes and in the colon. The virus particles may reach the intestine via ingestion or viremia from the respiratory tract (Laude et al., 1993).

Diagnosis

TGE shows very distinctive clinical signs and characteristic lesion of villous atrophy (Bohl 1981). Differential diagnosis should include rotavirus, porcine epidemic diarrhea virus and coccidia which may produce profuse watery diarrhea with villous atrophy. Laboratory diagnosis of TGEV may be achieved by one or more methods, such as detection of viral antigen, detection of viral nucleic acid, identification of the virus or detection of antibody response. Yet, PRCV is closely related to TGEV in both genetic and antigenic properties which requires more specific differential procedures.

The viral particles can be detected in feces and in the intestinal contents of infected animals by negative - contrast transmission EM (Saif et al., 1977). Sensitivity of diagnosis may be enhanced using immune EM (IEM) to differentiate TGEV from other enteric viruses. TGEV and PRCV may be distinguished using monoclonal antibodies.

TGEV antibodies have been detected by several different serological tests (Saif and Wesley, 1992). The most common serological method is the VN test. However, polyclonal antibodies and some monoclonal antibodies can not discriminate between TGEV and PRCV. In addition, a variety of serological techniques such as IFA, immunodiffusion, passive HA and ELISA have been applied for diagnosis. Other recently developed methods are, blocking ELISA, indirect immunoperoxidase, radioimmunoprecipitation and modified autoradiography (Saif and Wesley, 1992).

A competitive inhibition ELISA or blocking ELISA can differentiate antibodies to PRCV from those to TGEV with the same sensitivity as when detected by a viral neutralization (VN) test (Callebaut et al., 1989). The competitive inhibition ELISA has been developed using TGEV as the coating - antigen. The dilutions of test sera are reacted with the fixed antigen. Anti - TGEV serum blocks the binding of mouse monoclonal antibody raised against antigenic site B of S glycoprotein (Callebaut et al., 1988). Therefore, it gives a negative result when detected with peroxidase - mouse IgG conjugate. In contrast, anti - PRCV serum does not recognize the antigenic site B of S glycoprotein, giving a positive signal. By this method, pigs infected with

PRCV can be differentiated from those infected with TGEV (Callebaut et al., 1989; Laude et al., 1993).

Viral antigen can be detected in epithelial cells of the small intestine (Saif and Wesley, 1992). Infected pigs should be euthanized at the early stages of diarrhea for collection of mucosal scrapings or frozen sections from jejuni and ileum. These specimens are examined by FA, IFA or a immunoperoxidase method. Cross reactions may occur among TGEV, PRCV, FIPV and CCV.

TGEV could be differentiated from PRCV based on genetic differences. Both PCR and hybridization techniques have been developed to detect TGEV genomic RNA in fecal samples or infected tissues (Shockley et al., 1987; Benfield et al., 1991; Vaughn et al., 1994). Since PRCV has a 672 - 681 nucleotide deletion in the S gene, the relative mass of the PCR product of the PRCV S gene is lower than that of the TGEV S gene (Vaughn et al., 1994). Moreover, RNA probes for hybridization have also been derived from the 5' end of the S gene of TGEV which can differentiate between TGEV and PRCV. Recently, *in situ* hybridization (ISH) has been developed that can detect nucleic acid of TGEV in formalin - fixed tissue (Sirinarumitr et al., 1995). This technique applies not only to diagnostic testing for the differentiation of TGEV and PRCV, but also in studies of virus pathogenesis.

Isolation and Identification of Virus

A swine testicle cell line has been used for detecting field strains of TGEV and PRCV (McClurkin, 1966; Kemeny, 1978; Bohl, 1979; Pensaert and Cox, 1989; Vaughn et al., 1993). The presence of the virus in the cells may be observed by CPE, plaque production, VN and IFA. The CPE or plaque formation may be enhanced by using older cells (Stark et al., 1975) and adding pancreatin or trypsin to the cell culture media (Bohl, 1979; Woods, 1982). The CPE produced by PRCV resembles that of TGEV plus syncytia formation (Pensaert and Cox, 1989).

Immunity

Adult swine infected with TGEV are immune against TGEV but only local immunity is protective (Saif and Wesley, 1992). Swine infected orally develop both serum and mucosal antibodies. Serum antibodies can be detected in serum for 6 months to several years after infection (Stepanek et al., 1979), but serum antibodies provide little protection against TGEV reinfection (Haelterman, 1965; Harada, 1969). In contrast, local mucosal immunity, induced by oral but not parenteral inoculation with TGEV can protect swine from subsequent TGEV exposure (Kodama, 1980; Sprino and Ristic, 1982). The prominent class of local

immunoglobulin is secretory IgA (sIgA) which covers along the gut mucosa (Kodama, 1980). CMI also appears in infected swine but no direct evidence has been presented as to the role of CMI in the resistance of swine against TGEV (Saif and Wesley, 1992). However, it is believed that CMI may play a role in either recovery from TGEV infection or resistance to reinfection.

Sows recovered from TGE can transmit passive immunity to their suckling piglets via colostrum (Saif and Wesley, 1992). Since newborn piglets lack immunity to TGEV, passive immunity is important for immediate protection against TGEV. In the first week of parturition, the IgG class is dominant in colostrum which crosses piglets' enterocytes and provides serum antibodies (Porter and Allen, 1972; Bourne, 1973). The circulatory antibodies protect against systemic infection but not intestinal infection (Hooper and Haelterman, 1966). After a week, IgG in milk decreases while sIgA in milk is predominant (Porter and Allen, 1972). Secretory IgA will not be absorbed by the piglets but provides local immunity against TGEV in the gut tract (Roux et al., 1977), by neutralizing ingested TGEV. IgA class is produced only by oral immunization of sows but not by parenteral or systemic infection.

Vaccines have been developed to induce protective immunity for both piglets and sows. Live attenuated and inactivated TGEV vaccines are available for oral or intraperitoneal administration after birth (Saif and Wesley, 1992). Orally vaccinated newborn piglets require 5 days for active immunity development which obviously can not provide immediate protection against TGEV for the first few days of life (Pensaert, 1979). Immunization of suckling or feeder pigs could decrease mortality rate of enzootic TGEV. However, the presence of maternal antibodies in these pigs can suppress active immunity (Furuuchi et al., 1978; Hess et al., 1982). Vaccination of pregnant swine increases passive immunity for suckling piglets via colostrum and milk. There are several vaccine preparations for immunization of pregnant dams such as virulent, attenuated, inactivated and subunit vaccines which may be inoculated via oral, intranasal, intramuscular and intramammary routes (Saif and Wesley, 1992). Oral administration of virulent autogenous viruses induces the highest level of immunity, consistently producing higher titers of persisting IgA in milk (Saif and Wesley, 1992; Paul et al., 1988).

The wide prevalence of PRCV in swine herds seems to overcome the prevalence of epizootic TGE, since TGE outbreaks have declined concomitantly with the increases in the occurrence of PRCV infection (Pensaert and Cox, 1989). This suggests that PRCV infected pigs are partially immune to TGEV infection (Pensaert, 1989; Pensaert and Cox, 1989). Sows oronasally infected with PRCV after natural exposure to PRCV secrete sIgA in their milk but the level of antibody rapidly decrease approximately 24 weeks after infection (Laude et al., 1993).

However, natural infection of sows with TGEV followed with PRCV infection during pregnancy stimulates sIgA production against TGEV which can protect offspring (Duen et al., 1990). Sows first infected with PRCV develop rapid secondary immune response against TGEV with higher lactogenic IgA (Pensaert, 1989, Pensaert and Cox, 1989). Lactogenic protection in piglets from TGEV immune sows is higher than in piglets from PRCV immune sows (De Diego et al., 1992). However, Paton and Brian (1990) reported that no cross protection occurs between PRCV and TGEV via sow' s milk.

3. MATERIALS AND METHODS

Cell Culture

The swine testis (ST) cell line (McClurkin and Norman, 1966) was used to propagate TGEV and PRCV. The ST cells were cultured in Eagle's minimum essential medium (MEM; Gibco BRL, Grand Island, NY) supplemented with 10 % fetal bovine serum (FBS; Gibco BRL, Grand Island, NY), sodium bicarbonate (2.0 g/l) (Fisher Scientific, Fair Lawn, NJ), 2 % L - glutamine (Gibco, Grand Island, NY) and lactalbumin enzymatic hydrolysate (5.0g/l) (Sigma, St. Louis, MO). The ST cell lines were grown in 75 cm^2 flasks (Corning, Cambridge, MA) at 37° C in a humid 5 % CO_2 atmosphere and subcultured every 3 - 4 days.

Viruses

The Miller strain (American Type Culture Collection, Rockville, MD) was used as the standard TGEV strain in this study. The VMRI 5170 and NVSL 5170 isolates were obtained from diarrheic pigs.

VMRI 5170 and NVSL 5170 isolates are the viruses isolated from suckling pigs in a herd with enteric disease in 1995 (Halbur et al., 1995). Approximately 15 - 20 % of sows and almost 100% of weaned pigs had diarrhea which suggested periodical TGE since November, 1994. However, the causative agent was still unclear. The fecal samples and tissues from neonatal pigs with diarrhea were then sent to Iowa State University - Veterinary Diagnostic Laboratory for definitive identification of enteric pathogens. Microscopic examination of intestinal section demonstrated severe atrophic enteritis. Electron microscopic examination of feces demonstrated a large number of atypical coronavirus like particles. Fluorescent antibody examination of frozen tissues demonstrated weak positive staining using anti - TGEV polyclonal antibodies. The fecal samples were also cultured on ST cells at Veterinary Medical Reseach Institute and National Veterinary Service Laboratory. Cytopathic effect typical of TGEV was observed in both laboratories. The isolates were called VMRI 5170 and NVSL 5170. By *In situ* hybridization, performed at VMRI, the tissue sample demonstrated a weak positive signal. Finally, RT - PCR was performed on RNA isolated from the viruses propagated on ST cells. Initial results revealed that the VMRI 5170 isolate was TGEV while NVSL 5170 isolate was PRCV.

Virus Plaque Purification

The 2 viral isolates and a standard virus, Miller strain of TGEV, were plaque purified a total of three times. Ten - fold serial dilutions of the viral isolates were prepared as inoculum. Four-day-old ST cell monolayers in six-well plates were inoculated with 0.5 ml of each virus and then incubated at 37° C for 1 hour. After incubation, the inoculum was removed, and the ST cell monolayers were overlaid with 2 ml of a mixture of Eagle's basal medium (BME; Gibco BRL, Grand Island, NY) and 2% agarose (FMC Bioproducts, Rockland, ME) containing 0.0016% neutral red (Fisher Scientific, Fair Lawn, NJ) and 30 mM sodium bicarbonate. The plates were placed in the dark at room temperature until the agarose became solid and then incubated at 37° C for 2 days. The virus was collected from individual plaques by aspirating infected cells and agarose with a sterile Pasteur pipette. The agarose plugs containing TGEV-infected cells were transferred into tubes containing 1 ml of MEM with 2 % FBS and 1 % antibiotics - antimycotics (GibcoBRL, Grand Island, NY). The tubes were frozen and thawed three times and clarified by centrifugation at 2,000 rpm for 10 minutes. The viral suspension was diluted ten - fold for further plaque purification. This procedure was replicated three times. The viral stocks were stored at -70° C.

One Step Growth Curves

Each strain of virus was inoculated on 4 day old ST cells cultured in 12 well plates (Corning, Cambridge, MA) at a MOI of 1 pfu/cell. At each time point from 0 to 96 hours post inoculation, the media was collected and the infected cells were scraped and transferred into a tube. The virus - cell suspension was frozen at -70° C and thawed 3 times and then clarified by centrifugation at 2,000 rpm for 10 minutes. The virus suspension was inoculated on 2 - 3 day old ST cells seeded in 96 - well plates, 8 wells each, and then incubated at 37° C in a CO_2 incubator. After 72 hours post inoculation, the cultures were observed for CPE. The reciprocal of the highest dilution that was infectious for cell cultures was the virus titer. One step growth curves were generated for each virus.

Plaque Size Measurement

Four to five day old ST cells cultured in 6 - well plates (Corning, Cambridge, MA) were inoculated at a 0.001 MOI for each strain of virus. One hour post inoculation, the inoculum was removed and replaced with 2 % Sea Plaque agarose (FME bioproducts, Rockland, NY) in an equal amount of BME (Gibco, Grand Island, NY) containing 0.0016 % neutral red (Fisher Scientific, Fair Lawn, NJ) and 30 mM sodium bicarbonate. The plates were

placed in the dark for 15 minutes and then incubated at 37° C. At 48 hours post inoculation the diameters of plaques were randomly measured in one direction. Sixty plaques of each strain of viruses were recorded and analyzed statistically using the ANOVA procedure.

Virus Neutralization Test

Hyperimmune serum or monoclonal antibodies, MH11 and MH5, directed against conserved epitopes on the S glycoprotein of TGEV were serially diluted two - fold in 96 - well plates from 1:100 to 1:102,400. Eight wells were used for each serum dilution. Diluted serum or monoclonal antibodies were mixed with 50 µl of MEM containing 100 pfu of the virus and incubated for 1 hour at 37° C. One hundred µl of ST cell suspension at a concentration of 5 x 10^5 cells/ml were dispensed into each well. The plates were incubated at 37° C for 48 hours and the cultures were observed for CPE. The experiment was replicated 6 times. The VN titer of the tested serum, resulting from the last dilution of serum neutralizing TGEV, was calculated from the average of the 6 values by the regression analysis procedure.

Radioimmunoprecipitation Assay (RIP)

Metabolic Labeling

Radioimmunoprecipitation was used to determine differences in the migration of viral structural proteins. The ST cells infected with the Miller strain, the NVSL 5170 or the VMRI 5170 isolate, and mock-infected cells were labeled with ^{35}S-methionine-cysteine. The viruses were inoculated into 3-day-old ST cells in 25 cm^2 flasks at a MOI of 0.1 pfu/cell. Inoculum was removed after 16 hours post inoculation and Met - Cys deficient DMEM (ICN, Costa - Mesa, CA) was added. After 1 hour of incubation at 37° C, the spent media was decanted and replaced with fresh Met - Cys free DMEM containing 100 µCi/ml ^{35}S-methionine-cysteine (ICN, Costa Mesa, CA). Four hours after adding ^{35}S-methionine-cysteine, the spent media was removed and the infected cell monolayers were washed 3 times with cold PBS. Subsequently, 1 ml of lysis buffer (Cellular labeling and immunoprecipitation kit, Boehringer Mannheim, Indianapolis, IN) was added into each flask. ST cells were then scraped from the surface of the flasks and transferred into 1.5 ml microfuge tubes. The cell - lysis buffer suspensions were vortex mixed vigorously for 1 minute and then incubated on ice for 30 minutes. Then, the suspensions were centrifuged at high speed at 4° C for 15 minutes. The supernatant was collected and stored at -20° C until needed.

Immunoprecipitation

Lysate (50 µl) was clarified by incubating with 20 µl of protein A coated sepharose beads (Sigma, St. Louis, MO) for 1 hour at 4° C on a rocking platform. The clarified lysate was allowed to react with 1 µl of the hyperimmune serum or monoclonal antibody, MH11, for 3 hours at 4° C on a rocking platform. Immune complexes were collected by adding protein - A - coated sepharose beads (Sigma. St. Louis, MO) and incubated overnight at 4° C on a rocking platform. The antigen-antibody complexes were washed by rinsing twice with wash buffer I, twice with wash buffer II, once with wash buffer III and twice with deionized distilled water as the method described in the cellular labeling and immunoprecipitation kit (Boehringer Mannheim, Indianapolis, IN). These immune complexes were resuspended in 30 µl Laemmli sample buffer (Bio - Rad, Hercules, CA) and heated for 3 minutes in a boiling water bath. The protein - bead mixtures were centrifuged at high speed for 30 seconds, and the supernatants were electrophoresesed through a 10 % SDS-polyacrylamide gel at 100 volts for 15 minutes, and 150 volts for 1 hour, respectively.

Autoradirography

The electrophoresed gel was fixed in acid - methanol (1 % formic acid and 31.25 % methanol) for 15 minutes and then washed 3 times with deionized water. The radioactive signals were enhanced by incubation of the fixed gel in 50 volumes of Enlightening™ (NEN, Boston, MA) for 30 minutes on rocking platform. Subsequently, the gel was vacuum dried for 90 minutes at 65° C, and was then exposed to biomax film (Kodak, Rochester, NY) overnight at -70° C.

Sequence Analysis

RNA Extraction

Viral RNAs were isolated from TGEV or PRCV infected ST cells by using a RNA isolation kit (Strategene, La Jolla, CA). Four day old ST cells grown in 75 cm^2 flasks were inoculated with NVSL 5170 or VMRI 5170 isolates and then incubated until approximately 50 % CPE was observed. The spent media was decanted and replaced with 2 ml of cold solution D (provided by the kit) in each flask. The flasks were swirled gently for 30 seconds at room temperature to lyse the cells and denature all proteins. The suspensions in 5 flasks were transferred into a chilled polypropylene tube. Then 0.5 ml of 2 M sodium acetate and 5 ml of phenol were added into each tube immediately, and thoroughly mixed. Subsequently, 1 ml of

chloroform : isoamyl alcohol was added into the mixture, vortex mixed vigorously for 10 seconds and incubated on ice for 15 minutes. The suspension was transferred into a prechilled thick - wall Nalgene 50 - ml round - bottom centrifuge tube and centrifuged at 10,000 x g for 20 minutes at 4° C. The aqueous phase was transferred to a tube and mixed with an equal volume of isopropanol. The RNA was precipitated by chilling the RNA - isopropanol mixture at -20°C for 1 hour. The mixture was centrifuged at 10,000 x g for 20 minutes at 4° C and then the supernatant was discarded. The quality of RNA was improved by dissolving the pellet in 3 ml of solution D and precipitating with 3 ml of isopropanol. The RNA - isopropanol mixture was dispensed in 100 µl volumes into 0.5 ml microfuge tubes and stored at -20° C for 1 hour or until used. The chilled RNA - isopropanol mixture was thawed and pelleted at 10,000 rpm for 10 minutes at 4° C. The supernatant was removed and the pellet was dried under vacuum for 3 - 5 minutes. The RNA pellet was resuspended in 10 µl of sterile DEPC - treated water.

cDNA Synthesis

cDNA was synthesized using the cDNA cycle kit for RT-PCR (Invitrogen, San Diego, Calif). RNA samples in the previous step were transferred using 7 µl of each into 0.5 ml microfuge tubes. Then, 1 µl of random primer and 4 µl of DEPC - treated water was added into the tubes and mixed well. The tubes were placed in a 65° C water bath for 10 minutes to denature the secondary structure of RNAs. The tubes were then left at room temperature for a few minutes to let the primer anneal. Subsequently, 4 µl of 5 x RT buffer, 1 µl of dNTP, 1 µl of 80 mM sodium pyrophosphate, 1 µl of RNase inhibitor and 1 µl of reverse transcriptase were added into each tube and mixed well. For cDNA synthesis, the mixture was then incubated in a 42° C water bath for 60 minutes.

Polymerase Chain Reaction (PCR) and Sequence Analysis

PCR-amplified fragments were obtained using cDNA-RNA heteroduplexes as templates and following the basic PCR protocol (Gibco BRL, Gaithersburg, MD). The components of the PCR mixture in each reaction were 10 µl of 5 x PCR buffer (Gibco BRL, Gaithersburg, MD), 2 mM dNTP, 6 µl of 50 mM $MgCl_2$, 10 µl of 2 mM forward primer, 10 µl of 2 mM reverse primer, 4 µl of cDNA template, 5 units of Taq DNA polymerase (Gibco BRL, Gaithersburg, MD) and sterile distilled water to 100 µl. Thirty cycles of 92° C for 30 seconds for denaturation, 48° C for 30 seconds for annealing and 72° C for 45 seconds for primer extension were performed in a thermocycler (Gene Amp PCR system 2400, Perkin Elmer). The primers used in the PCR reaction are shown in Table 2. The PCR products were

electrophoresesed through a 0.8% agarose gel and then extracted from the gel using the QIAEX II Gel Extraction kit (QIAGEN, Germany). The extracted DNA was sequenced using the primers presented in Table 3, by an automated fluorescent method using ABI 377 at the DNA sequencing facility, ISU. The positions and primers used in PCR and sequence analysis are presented in Figure 1. The base sequences were analyzed and DNA fragments were combined using the Mac Vector program. The combined fragments were compared by the Gene Works program.

Table 2: primers and their sequences used for amplification

	name	sequence(5'-->3')	direction	base range
1	185	AGG GTA AGT TGC TCA TTA G	forward	-50 - -32
2	2F	CAA ACA ACG GTT AAA CGT AG	forward	297 - 316
3	5FC	CGC TTC ATA CCA AGA CCA	reverse	1599-1616
4	4FF	GTA TCT AGG AAC ATT ACC A	forward	1224-1242
5	6RR	GTT AGA ATA GGT TAT GAC AG	reverse	2393-2412
6	6FF	TTA CAC ATC ACT ATC AGG T	forward	2130-2148
7	4RR	CCT TGT GGG TTG ACA ACA T	reverse	3308-3326
8	4RC	AGA TGT TGT CAA CAC ACA A	forward	3306-3324
9	2R	GCC TAT TAG TAG CCA CAC	reverse	4171-4188
10	5RC	CGT TGT ACA GGT GGT TAT G	forward	2941-2959
11	3RR	CTG GAC ATC TTT AAC GAC	reverse	3736-3573
12	3RC	GTC GTT AAA GAT GTC CAG	forward	3736-3753
13	662	ATT GAT GCT AAT GAC CAT TC	reverse	5495-5514

Note : The primer 2F was used for VMRI 5170 gene amplification only

Table 3: Primers used for DNA sequencing

	name	sequence(5'-->3')	direction	base range
1	185	AGG GTA AGT TGC TCA TTA G	forward	-50 - -32
2	2F	CAA ACA ACG GTT AAA CGT AG	forward	297 - 316
3	3FF	GAT CAA TGT GCT AGT TAT G	forward	657-675
4	5FC	CGC TTC ATA CCA AGA CCA	reverse	1599-1616
5	4FF	GTA TCT AGG AAC ATT ACC A	forward	1224-1242
6	5FF	CAG GAT AAC AAC ACC GAT	forward	1672-1689
7	6RR	GTT AGA ATA GGT TAT GAC AG	reverse	2393-2412
8	6FF	TTA CAC ATC ACT ATC AGG T	forward	2130-2148
9	6RC	CGT CAC ACA TTC TGA TGG	forward	2451-2468
10	GAP1	GCT CTT GGC TAG AAG GTC	forward	2807-2824
11	4RR	CCT TGT GGG TTG ACA ACA T	reverse	3308-3326
12	4RC	AGA TGT TGT CAA CAC ACA A	forward	3306-3324
13	2R	GCC TAT TAG TAG CCA CAC	reverse	4171-4188
14	5RC	CGT TGT ACA GGT GGT TAT G	forward	2941-2959
15	3RR	CTG GAC ATC TTT AAC GAC	reverse	3736-3573
16	3RC	GTC GTT AAA GAT GTC CAG	forward	3736-3753
17	583	CTA TTG AAA AAG TGC ACG TC	reverse	
18	662	ATT GAT GCT AAT GAC CAT TC	reverse	5495-5514
19	EV048	GCA TAG GTC CTA AAA GTG TCA TTG	forward	

Note : The primer 2F was used for VMRI 5170 gene sequencing only

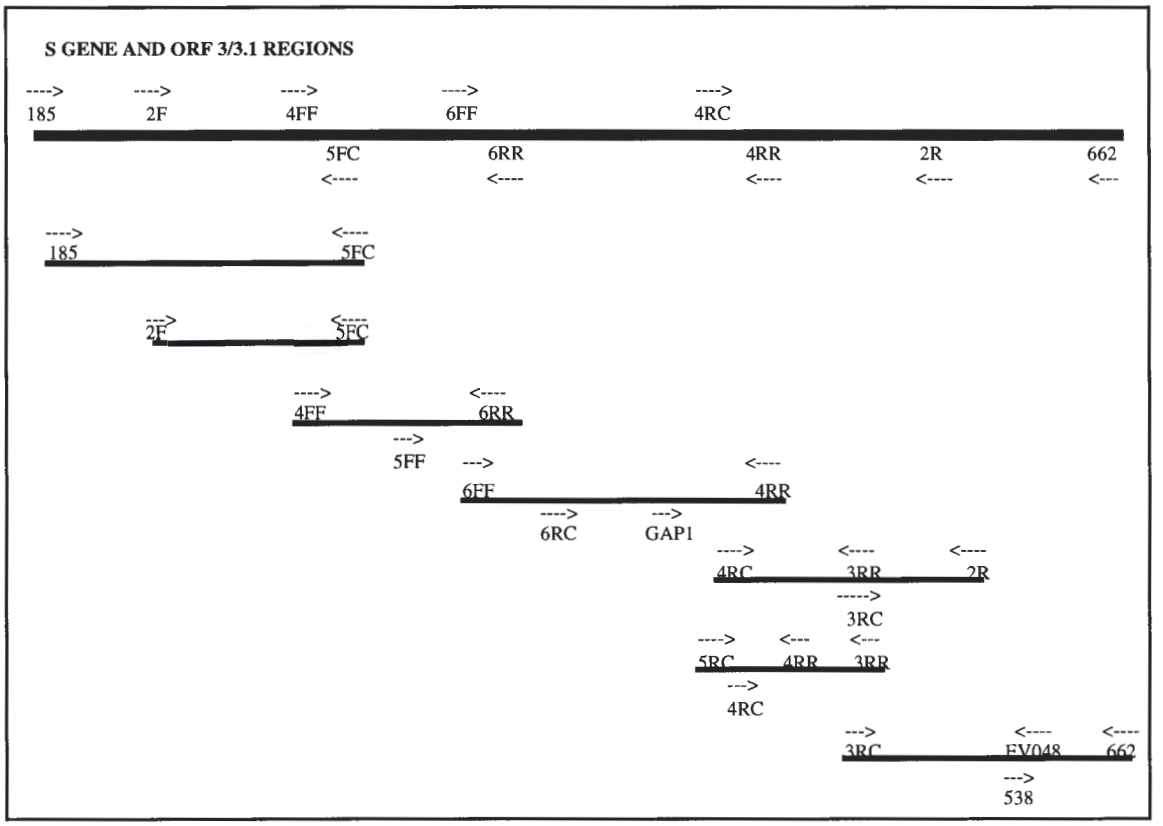

Figure 1: The positions and the primers used in DNA amplification and sequence analysis. The thick line indicates S gene and ORF 3/3.1 regions of the genome of TGEV. Each thin line shows the amplified fragment. The letters and numbers are the names of the primers while the arrows indicate the direction of the amplification leaded by the primers.

4.RESULTS

One Step Growth Curve

The titers of the three viruses exhibited the same pattern at each time point. The average titers that represented the $TCID_{50}$ of each virus and time points are shown in Table 4. However, the highest titer of each virus at a certain time point is different. The Miller strain of TGEV reached the highest titer, $10^{6.25}$, at 30 hour post inoculation while those of PRCV isolate NVSL 5170, and TGEV isolate VMRI 5170 were $10^{6.63}$ and $10^{5.63}$ at the time points of 46 and 54 hour post inoculation respectively. The $\log_{10}$ of the virus titers were plotted to create 3 growth curves demonstrated in Figure 2. There was no difference among the growth curves of the three viruses ($p = 0.63$) using one way ANOVA.

Plaque Size Measurement

The Miller strain of TGEV, TGEV isolate VMRI 5170, and PRCV isolate NVSL 5170 produced almost round plaques at 48 hour post inoculation. The diameters of the plaques of

Table 4: The average titers of the Miller strain of TGEV, the TGEV isolate, VMRI 5170, and the PRCV isolate, NVSL 5170, at each time point.

	Virus Strain		
Time(h.p.i.)	TGEV Miller	PRCV NVSL 5170	TGEV VMRI 5170
0	0	0	0
5	$10^{2.5}$	$10^{2.75}$	$10^{3.25}$
11	$10^{4.0}$	$10^{3.75}$	$10^{4.13}$
18	$10^{5.13}$	$10^{4.5}$	$10^{4.38}$
22	$10^{6.0}$	$10^{5.5}$	$10^{5.0}$
30	$10^{6.25}$	$10^{5.5}$	$10^{5.0}$
38	$10^{5.38}$	$10^{6.13}$	$10^{5.63}$
46	$10^{5.63}$	$10^{6.63}$	$10^{5.5}$
54	$10^{5.25}$	$10^{6.5}$	$10^{5.63}$
66	$10^{4.75}$	$10^{6.13}$	$10^{4.75}$
80	$10^{3.25}$	$10^{5.5}$	$10^{3.75}$
90	$10^{2.52}$	$10^{4.88}$	$10^{3.25}$

Note: Cell culture were inoculated with TGEV or PRCV at 1 MOI

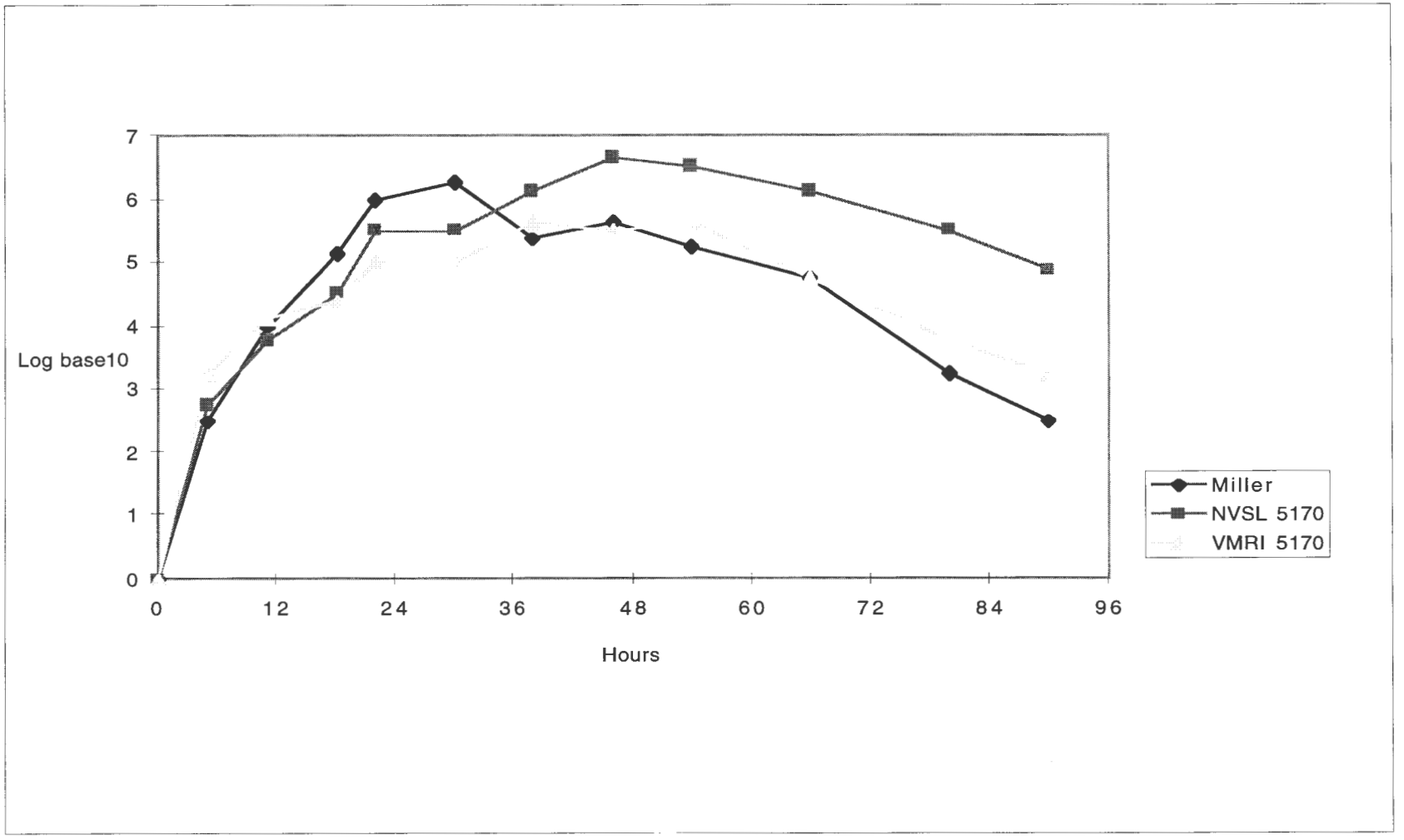

Figure 2: Growth curves of the Miller strain of TGEV, VMRI 5170 isolate of TGEV and NVSL 5170 of PRCV.

Table 5: Diameters of plaques of the Miller strain of TGEV, the TGEV isolate, VMRI 5170, and the PRCV isolate, NVSL 5170.

Plaque No.	TGEV Miller	PRCV NVSL 5170	TGEV VMRI 5170
1	1.925	0.950	2.650
2	2.500	0.950	3.200
3	2.400	0.825	2.300
4	3.375	0.500	2.900
5	2.425	1.300	3.450
6	2.400	0.400	1.600
7	2.725	1.050	2.675
8	2.575	0.850	2.700
9	2.450	0.700	2.575
10	2.975	1.200	3.000
11	3.050	1.450	2.675
12	2.000	0.975	2.000
13	2.425	1.175	3.175
14	2.450	1.175	2.200
15	3.400	1.675	2.300
16	3.175	1.000	2.450
17	2.000	1.025	2.850
18	3.350	1.000	2.800
19	3.200	1.025	1.650
20	1.925	1.500	2.000
21	2.000	1.000	2.200
22	2.500	0.900	2.900
23	2.950	1.150	1.750
24	2.225	1.500	2.200
25	2.975	1.075	2.500
26	2.600	1.175	2.600
27	2.475	1.125	2.750
28	2.400	0.900	2.700
29	2.200	1.075	3.050
30	2.700	0.575	1.975
31	2.375	0.750	2.100
32	2.875	0.575	3.425
33	2.975	0.825	2.725
34	3.125	1.000	3.900
35	2.975	0.900	2.275
36	2.125	1.250	2.350
37	2.500	1.000	2.850
38	2.000	0.800	2.300
39	2.000	1.350	2.050
40	2.325	0.650	1.975
41	2.475	0.500	1.975
42	2.125	0.700	2.600
43	1.000	0.950	2.000
44	2.250	1.000	2.525
45	2.575	1.300	1.600
46	2.725	1.300	1.500

Table 5: (continued)

Plaque No.	TGEV Miller	PRCV NVSL 5170	TGEV VMRI 5170
47	3.050	0.750	1.575
48	1.525	0.800	2.250
49	2.325	1.600	1.825
50	2.075	0.900	2.000
51	2.975	1.000	1.500
52	3.100	0.525	2.525
53	3.200	0.800	2.425
54	2.500	0.725	1.800
55	2.075	1.500	1.500
56	2.400	0.825	1.425
57	2.050	0.950	1.400
58	2.000	1.450	1.375
59	2.100	0.500	1.975
60	1.100	0.350	2.200
61	1.975	-	-
62	2.700	-	-
63	2.500	-	-
n	63	60	60

each virus is included in Table 5. Average size of plaques of Miller strain, VMRI 5170 isolate and NVSL 5170 isolate were 2.47 ± 0.50, 2.33 ± 0.56 and 0.987 ± 0.31, respectively. The raw data calculated by the ANOVA procedure revealed that the plaque sizes of these 3 viruses were different ($p < 0.0001$). However, comparison of the plaque sizes of the Miller strain and VMRI 5170 isolate showed that they were not distinguishable ($p = 0.13$). In contrast, the plaque size of the TGEV isolate, Miller strain and VMRI 5170, were significantly larger than that of PRCV isolate NVSL 5170 ($p < 0.0001$).

Virus Neutralization Test

The virus neutralization titer of the TGEV hyperimmune sera or monoclonal antibodies was calculated from an average of the replications of the highest dilution of the serum or ascites fluid that resulted in neutralization of TGEV. The VN titers are shown in Table 6. The TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 were neutralized by hyperimmune sera raised against the Miller strain of TGEV, as well as Mab against the S glycoprotein of TGEV. However, TGEV hyperimmune sera, MAb 3H11 and MAb 5A5 had lower VN titers for TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 than for the TGEV Miller strain. VN titer of MAb 5A5 for TGEV isolate VMRI 5170 was an exception.

Table 6: Neutralization of the Miller strain of TGEV, the TGEV isolate VMRI 5170, and the PRCV isolate NVSL 5170 by TGEV hyperimmune sera, and anti - TGEV MAbs, 3H11 and 5A5.

Viruses	Antibodies		
	MAb 3H11	MAb 5A5	Polyclonal anti - TGEV Ab
TGEV - Miller	1:32,948	1:34,261	1:16,574
TGEV - VMRI	1:27,199	1:64,710	1:12,761
PRCV - NVSL	1:12,295	1:12,162	1:4,575

Radioimmunoprecipitation Assay

In the radioimmunoprecipitation assay, the three viruses demonstrated the similar pattern of protein profiles (Figure 3) when reacted with hyperimmune sera, against TGEV. The molecular mass of M (28 kD) and N (46 kD) proteins were similar for the Miller strain of TGEV, the TGEV isolate VMRI 5170 and the PRCV isolate NVSL 5170. The molecular mass of the S glycoprotein of the TGEV isolate VMRI 5170 was 220 kD and was similar for the Miller strain. In contrast, the S glycoprotein of the PRCV isolate NVSL 5170 was approximately 190 kD which was less than that for the TGEV isolates Miller and VMRI 5170 (Figure 3&4).

Sequencing Analysis

The pairwise alignment of the S gene of TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 compared to other strains of TGEV are presented in Figure 5. The S gene of TGEV isolate VMRI 5170 consisted of 4353 bases while that of PRCV isolate NVSL 5170 was 3639 bases, including start and stop codons. The PRCV isolate NVSL 5170 had a 714 and 711 nucleotide deletion when aligned with the VMRI 5170 isolate and Miller strain; FS772; TF1; Purdue and NEB 72. The nucleotide and deduced amino acid homology S gene of TGEV isolate VMRI 5170 compared with those of other TGEVs are shown in Table 7. It was found that the S gene of TGEV isolate VMRI 5170 exhibited 96-97% identity to the published sequences of the S genes of TGEV with 120-169 nucleotide differences. Without accounting for the 714 nucleotide deletion, the S genes of TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 are markedly identical with only 5 nucleotide differences.

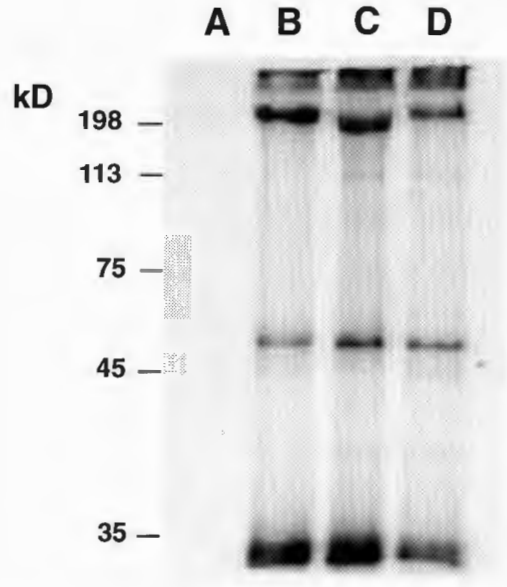

Figure 3: Immunoprecipitation of ^{35}S trans methionine - cysteine labeled structural proteins of the Miller strain of TGEV, the TGEV isolate VMRI 5170 and the PRCV isolate NVSL 5170 by hyperimmune anti - TGEV serum. The S glycoprotein of the Miller strain of TGEV and the VMRI isolate of TGEV have a molecular mass of 220 kD and that of PRCV isolate NVSL 5170 is 190 kD. The M and N proteins of the three viruses had molecular mass of 28 and 46 kD respectively.

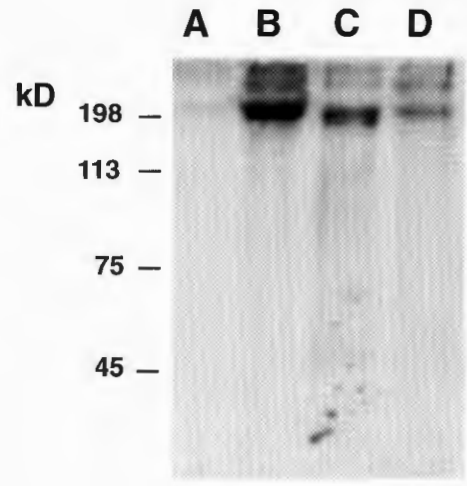

Figure 4: Immunoprecipitation of ^{35}S trans methionine - cysteine labeled S glycoprotein of the Miller strain of TGEV, TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 by MAb 3H11 against S glycoprotein of TGEV. The S glycoprotein of the Miller strain of TGEV and the TGEV isolate VMRI 5170 have M_r of 220 kD and that of PRCV isolate NVSL 5170 is 190 kD.

Note: A = mock infected cell lysate.
B = Miller strain of TGEV infected cell lysate.
C = PRCV isolate NVSL 5170 infected cell lysate.
D = TGEV isolate VMRI 5170 infected cell lysate.

Likewise, the sequence of the S gene of the TGEV isolate VMRI 5170 and the PRCV isolate NVSL 5170 were also compared with other published sequences of the S genes of PRCV. The pairwise alignments are shown in Figure 6. The S gene of NVSL 5170 isolate had a 96 - 97 % nucleic acid identity with that of the published sequences of PRCV isolates (Table 8). The position of the deletions within the S gene of PRCV isolate NVSL 5170 and that of other PRCVs are summarized in Table 9.

```
Purdue S        .......A.C ..........  ......CG..  ..G.......  ..........   50
NEB72 S         .......A.C ..........  ......CG..  ..G.......  ..........   50
TF1 S           .......A.T ..........  ......TA..  ..T.......  ..........   50
Miller S        .......A.T ..........  ......TG..  ..G.......  ..........   50
FS772 S         .......A.T ..........  ......TG..  ..G.......  ..........   50
NVSL S          .......C.C ..........  ......T---  ----------  ----------   27
VMRI S          .......C.C ..........  ......TA..  ..G.......  ..........   50
Consensus       ATGAAAAMAY TATTTGTGGT TTTGGTYRTA ATKCCATTGA TTTATGGAGA      50

Purdue S        C.........  ..........  ..........  .........C  ..C..G....   100
NEB72 S         C.........  ..........  ..........  .........C  ..C..G....   100
TF1 S           C.........  ..........  ..........  .........T  ..C..T....   100
Miller S        C.........  ..........  ..........  .........T  ..C..T....   100
FS772  S        C.........  ..........  ..........  .........T  ..C..T....   100
NVSL S          ----------  ----------  ----------  ----------  ----------   27
VMRI S          T.........  ..........  ..........  .........T  ..A..T....   100
Consensus       YAATTTTCCT TGTTCTAAAT TGACTAATAG AACTATAGGY AAMCAKTGGA      100

Purdue S        ..........  A.....C...  C....C....  ....T.....  .C........   150
NEB72 S         ..........  A.....C...  C....C....  ....T.....  .C........   150
TF1 S           ..........  A.....C...  C....T....  ....T.....  .C........   150
Miller S        ..........  A.....C...  C....T....  ....T.....  .T........   150
FS772 S         ..........  A.....C...  C....T....  ....T.....  .T........   150
NVSL S          ----------  ----------  ----------  ----------  ----------   27
VMRI S          ..........  T.....T...  A....T....  ....C.....  .C........   150
Consensus       ATCTCATTGA WACCTTYCTT MTAAAYTATA GTAGYAGGTT AYCACCTAAT      150

Purdue S        ..........  ..........  ..........  ..........  ..........   200
NEB72 S         ..........  ..........  ..........  ..........  ..........   200
TF1 S           ..........  ..........  ..........  ..........  ..........   200
Miller S        ..........  ..........  ..........  ..........  ..........   200
FS772 S         ..........  ..........  ..........  ..........  ..........   200
NVSL S          ----------  ----------  ----------  ----------  ----------   27
VMRI S          ..........  ..........  ..........  ..........  ..........   200
Consensus       TCAGATGTGG TGTTAGGTGA TTATTTTCCT ACTGTACAAC CTTGGTTTAA      200
```

Figure 5: Pairwise alignments of S genes of the TGEV isolate VMRI 5170, the PRCV isolate NVSL 5170 and other TGEV isolates.
Note: The sequences begin with the start codons and are shown as underlined bases. The position having identical nucleotides are presented as dots and the positions of deleted nucleotides are exhibited as dashes. The 5 bases that are diferent between TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 are presented as bold letters.

```
Purdue S        ...C....G. ...A...... .T........ .......C.. ..........   250
NEB72 S         ...C....G. ...A...... .T........ .......C.. ..........   250
TF1 S           ...C....G. ...A...... .T........ .......T.. ..........   250
Miller S        ...C....G. ...A...... .T........ .......T.. ..........   250
FS772   S       ...C....A. ...A...... .T........ .......T.. ..........   250
NVSL S          ---------- ---------- ---------- ---------- ----------    27
VMRI S          ...T....G. ...G...... .C........ .......T.. ..........   250
Consensus       TTGYATTCRC AATRATAGTA AYGACCTTTA TGTTACAYTG GAAAATCTTA   250

Purdue S        .....GT... ......T... ......GA.. ...T..C..G ........G-   299
NEB72 S         .....TT... ......T... ......GA.. ...T..C..G ........G-   299
TF1 S           .....TT... ......T... ......GA.. ...T..T..G ........G-   299
Miller S        .....TT... ......T... ......GA.. ...T..C..T ........A-   299
FS772 S         .....TT... ......C... ......GA.. ...G..C..G ........A-   299
NVSL S          ---------- ---------- ---------- ---------- ----------    27
VMRI S          .....TG... ......T... ......AG.. ...T..C..G ........A.   300
Consensus       AAGCAKKGTA TTGGGAYTAT GCTACARRAA ATAKCAYTTK GAATCACARG   300

Purdue S        -.-....... .......... .......... .......... ..........   347
NEB72 S         -.-....... .......... .......... .......... ..........   347
TF1 S           -.-....... .......... .......... .......... ..........   347
Miller S        -.-....... .......... .......... .......... ..........   347
FS772 S         -.-....... .......... .......... .......... ..........   347
NVSL S          ---------- ---------- ---------- ---------- ----------    27
VMRI S          .......... .......... .......... .......... ..........   350
Consensus       GACCAACGGT TAAACGTAGT CGTTAATGGA TACCCATACT CCATCACAGT   350

Purdue S        .......... .......... .....G.... .......... ..........   397
NEB72 S         .......... .......... .....G.... .......... ..........   397
TF1 S           .......... .......... .....T.... .......... ..........   397
Miller S        .......... .......... .....G.... .......... ..........   397
FS772 S         .......... .......... .....G.... .......... ..........   397
NVSL S          ---------- ---------- ---------- ---------- ----------    27
VMRI S          .......... .......... .....G.... .......... ..........   400
Consensus       TACAACAACC CGCAATTTTA ATTCTKCTGA AGGTGCTATT ATATGCATTT   400

Purdue S        .T........ .......... ........A. .A........ ..........   447
NEB72 S         .T........ .......... ........A. .A........ ..........   447
TF1 S           .C........ .......... ........C. .A........ ..........   447
Miller S        .C........ .......... ........A. .A........ ..........   447
FS772 S         .C........ .......... ........C. .A........ ..........   447
NVSL S          ---------- ---------- ---------- ---------- ----------    27
VMRI S          .C........ .......... ........C. .G........ ..........   450
Consensus       GYAAGGGCTC ACCACCTACT ACCACCACMG ARTCTAGTTT GACTTGCAAT   450

Purdue S        ........T. .......... ......T... .......... ..........   497
NEB72 S         ........T. .......... ......T... .......... ..........   497
TF1 S           ........G. .......... ......T... .......... ..........   497
Miller S        ........T. .......... ......T... .......... ..........   497
FS772 S         ........T. .......... ......T... .......... ..........   497
NVSL S          ---------- ---------- ---------- ---------- ----------    27
VMRI S          ........T. .......... ......C... .......... ..........   500
Consensus       TGGGGTAGKG AGTGCAGGTT AAACCAYAAG TTCCCTATAT GTCCTTCTAA   500
```

Figure 5: (continued)

```
Purdue S        .......... .......... .....C.... T......... ..........   547
NEB72 S         .......... .......... .....C.... T......... ..........   547
TF1 S           .......... .......... .....C.... T......... ..........   547
Miller S        .......... .......... .....C.... T......... ..........   547
FS772 S         .......... .......... .....C.... T......... ..........   547
NVSL S          ---------- ---------- ---------- ---------- ----------    27
VMRI S          .......... .......... .....T.... C......... ..........   550
Consensus       TTCAGAGGCA AATTGTGGTA ATATGYTGTA YGGCCTACAA TGGTTTGCAG   550

Purdue S        ...A...... .......... .......... .......... ..........   597
NEB72 S         ...A...... .......... .......... .......... ..........   597
TF1 S           ...C...... .......... .......... .......... ..........   597
Miller S        ...C...... .......... .......... .......... ..........   597
FS772 S         ...C...... .......... .......... .......... ..........   597
NVSL S          ---------- ---------- ---------- ---------- ----------    27
VMRI S          ...C...... .......... .......... .......... ..........   600
Consensus       ATGMGGTTGT TGCTTATTTA CATGGTGCTA GTTACCGTAT TAGTTTTGAA   600

Purdue S        ..T....... .......... C...T..... .........G ....A..A..   647
NEB72 S         ..T....... .......... C...T..... .........G ....A..A..   647
TF1 S           ..T....... .......... T...C..... .........T ....T..A..   647
Miller S        ..T....... .......... T...C..... .........G ....T..A..   647
FS772 S         ..T....... .......... T...C..... .........G ....T..A..   647
NVSL S          ---------- ---------- ---------- ---------- ----------    27
VMRI S          ..C....... .......... T...C..... .........G ....T..G..   650
Consensus       AAYCAATGGT CTGGCACTGT YACAYTTGGT GATATGCGTK CGACWACRTT   650

Purdue S        .G..GT.G.. ....C..... .......... G........T ..........   697
NEB72 S         .G..GT.T.. ....C..... .......... G........T ..........   697
TF1 S           .G..AC.G.. ....C..... .......... T........T ..........   697
Miller S        .G..AC.G.. ....C..... .......... G........T ..........   697
FS772 S         .G..AC.G.. ....C..... .......... G........T ..........   697
NVSL S          ---------- ---------- ---------- ---------- ----------    27
VMRI S          .C..AC.G.. ....T..... .......... G........C ..........   700
Consensus       ASAARYCKCT GGCAYGCTTG TAGACCTTTG KTGGTTTAAY CCTGTTTATG   700

Purdue S        .......... ......G... .......... .........C ..........   747
NEB72 S         .......... ......G... .......... .........C ..........   747
TF1 S           .......... ......A... .......... .........T ..........   747
Miller S        .......... ......A... .......... .........C ..........   747
FS772 S         .......... ......A... .......... .........C ..........   747
NVSL S          ---------- ---------- ---------- ---------- -..........    36
VMRI S          .......... ......A... .......... .........C ..........   750
Consensus       ATGTCAGTTA TTATAGRGTT AATAATAAAA ATGGTACTAY CGTAGTTTCC   750

Purdue S        .......... ....A..... .......... .......... ..AC......   797
NEB72 S         .......... ....A..... .......... .......... ..AC......   797
TF1 S           .......... ....A..... .......... .......... ..AC......   797
Miller S        .......... ....A..... .......... .......... ..AC......   797
FS772 S         .......... ....A..... .......... .......... ..AC......   797
NVSL S          .......... ....G..... .......... .......... ..GT......    86
VMRI S          .......... ....G..... .......... .......... ..GT......   800
Consensus       AATTGCACTG ATCARTGTGC TAGTTATGTG GCTAATGTTT TTRYTACACA   800
```

Figure 5: (continued)

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Purdue S        .......G.T .......... .......... .......... ..........   847
NEB72 S         .......G.T .......... .......... .......... ..........   847
TF1 S           .......G.C .......... .......... .......... ..........   847
Miller S        .......G.C .......... .......... .......... ..........   847
FS772 S         .......G.C .......... .......... .......... ..........   847
NVSL S          .......G.C .......... .......... .......... ..........   136
VMRI S          .......T.C .......... .......... .......... ..........   850
Consensus       GCCAGGAKGY TTTATACCAT CAGATTTTAG TTTTAATAAT TGGTTCCTTC   850

Purdue S        .......... .......... .......... .......... .........G   897
NEB72 S         .......... .......... .......... .......... .........G   897
TF1 S           .......... .......... .......... .......... .........A   897
Miller S        .......... .......... .......... .......... .........G   897
FS772 S         .......... .......... .......... .......... .........A   897
NVSL S          .......... .......... .......... .......... .........A   186
VMRI S          .......... .......... .......... .......... .........A   900
Consensus       TAACTAATAG CTCCACGTTG GTTAGTGGTA AATTAGTTAC CAAACAGCCR   900

Purdue S        .......... .......... .......... .......... ..........   947
NEB72 S         .......... .......... .......... .......... ..........   947
TF1 S           .......... .......... .......... .......... ..........   947
Miller S        .......... .......... .......... .......... ..........   947
FS772 S         .......... .......... .......... .......... ..........   947
NVSL S          .......... .......... .......... .......... ..........   236
VMRI S          .......... .......... .......... .......... ..........   950
Consensus       TTATTAGTTA ATTGCTTATG GCCAGTCCCT AGCTTTGAAG AAGCAGCTTC   950

Purdue S        ....T..... .....G.... ...G...... .......... ...G....T.   997
NEB72 S         ....T..... .....G.... ...G...... .......... ...G....T.   997
TF1 S           ....T..... .....A.... ...A...... .......... ...G....C.   997
Miller S        ....C..... .....A.... ...G...... .......... ...C....T.   997
FS772 S         ....T..... .....A.... ...G...... .......... ...G....T.   997
NVSL S          ....T..... .....A.... ...A...... .......... ...G....C.   286
VMRI S          ....T..... .....A.... ...A...... .......... ...G....C.   1000
Consensus       TACAYTTTGT TTTGARGGTG CTGRCTTTGA TCAATGTAAT GGTSCTGTYT   1000

Purdue S        .......T.. ......C... ..T.....C. .......... ..........   1047
NEB72 S         .......T.. ......C... ..T.....C. .......... ..........   1047
TF1 S           .......T.. ......C... ..C.....T. .......... ..........   1047
Miller S        .......C.. ......C... ..C.....T. .......... ..........   1047
FS772 S         .......T.. ......C... ..C.....T. .......... ..........   1047
NVSL S          .......T.. ......T... ..C.....T. .......... ..........   336
VMRI S          .......T.. ......T... ..C.....T. .......... ..........   1050
Consensus       TAAATAAYAC TGTAGAYGTC ATYAGGTTYA ACCTTAATTT TACTACAAAT   1050

Purdue S        .......... .......... .......... .........A ..........   1097
NEB72 S         .......... .......... .......... .........A ..........   1097
TF1 S           .......... .......... .......... .........G ..........   1097
Miller S        .......... .......... .......... .........A ..........   1097
FS772 S         .......... .......... .......... .........A ..........   1097
NVSL S          .......... .......... .......... .........A ..........   386
VMRI S          .......... .......... .......... .........A ..........   1100
Consensus       GTACAATCAG GTAAGGGTGC CACAGTGTTT TCATTGAACR CAACGGGTGG   1100
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Figure 5: (continued)

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Purdue S        .......... .....T.... ...------. ..C....... ..........   1141
NEB72 S         .......... .....T.... ...------. ..C....... ..........   1141
TF1 S           .......... .....C.... .......... ..T....... ..........   1147
Miller S        .......... .....C.... .......... ..C....... ..........   1147
FS772 S         .......... .....C.... .......... ..C....... ..........   1147
NVSL S          .......... .....C.... .......... ..C....... ..........   436
VMRI S          .......... .....C.... .......... ..C....... ..........   1150
Consensus       TGTCACTCTT GAAATYTCAT GTTATAATGA TAYAGTGAGT GACTCGAGCT   1150

Purdue S        ...T...... .........T .......... .A........ ..........   1191
NEB72 S         ...T...... .........T .......... .A........ ..........   1191
TF1 S           ...C...... .........T .......... .A........ ..........   1197
Miller S        ...C...... .........G .......... .A........ ..........   1197
FS772 S         ...C...... .........T .......... .A........ ..........   1197
NVSL S          ...C...... .........T .......... .G........ ..........   486
VMRI S          ...C...... .........T .......... .G........ ..........   1200
Consensus       TTTYCAGTTA CGGTGAAATK CCGTTCGGCG TRACTGATGG ACCACGGTAC   1200

Purdue S        .......... A......... .........T ......T... ....A.....   1241
NEB72 S         .......... A......... .........T ......T... ....A.....   1241
TF1 S           .......... T......... .........C ......C... ....G.....   1247
Miller S        .......... T......... .........T ......C... ....A.....   1247
FS772 S         .......... T......... .........T ......C... ....A.....   1247
NVSL S          .......... T......... .........T ......T... ....A.....   536
VMRI S          .......... T......... .........T ......T... ....A.....   1250
Consensus       TGTTACGTAC WCTATAATGG CACAGCTCTY AAGTATYTAG GAACRTTACC   1250

Purdue S        .......... .......... .......... ......C... ..........   1291
NEB72 S         .......... .......... .......... ......C... ..........   1291
TF1 S           .......... .......... .......... ......T... ..........   1297
Miller S        .......... .......... .......... ......C... ..........   1297
FS772 S         .......... .......... .......... ......C... ..........   1297
NVSL S          .......... .......... .......... ......C... ..........   586
VMRI S          .......... .......... .......... ......C... ..........   1300
Consensus       ACCTAGTGTC AAGGAGATTG CTATTAGTAA GTGGGGYCAT TTTTATATTA   1300

Purdue S        .......... .......... .......... .......... ......T...   1341
NEB72 S         .......... .......... .......... .......... ......T...   1341
TF1 S           .......... .......... .......... .......... ......C...   1347
Miller S        .......... .......... .......... .......... ......T...   1347
FS772 S         .......... .......... .......... .......... ......T...   1347
NVSL S          .......... .......... .......... .......... ......C...   636
VMRI S          .......... .......... .......... .......... ......C...   1350
Consensus       ATGGTTACAA TTTCTTTAGC ACATTTCCTA TTGATTGTAT ATCTTTYAAT   1350

Purdue S        .......... .T........ .......... .......... ..........   1391
NEB72 S         .......... .T........ .......... .......... ..........   1391
TF1 S           .......... .T........ .......... .......... ..........   1397
Miller S        .......... .T........ .......... .......... ..........   1397
FS772 S         .......... .T........ .......... .......... ..........   1397
NVSL S          .......... .C........ .......... .......... ..........   686
VMRI S          .......... .C........ .......... .......... ..........   1400
Consensus       TTGACCACTG GYGATAGTGA CGTTTTCTGG ACAATAGCTT ACACATCGTA   1400
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Figure 5: (continued)

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Purdue S        .........A .......... .......... .......... ..G.......   1441
NEB72 S         .........A .......... .......... .......... ..G.......   1441
TF1 S           .........G .......... .......... .......... ..T.......   1447
Miller S        .........A .......... .......... .......... ..G.......   1447
FS772 S         .........A .......... .......... .......... ..G.......   1447
NVSL S          .........G .......... .......... .......... ..G.......   736
VMRI S          .........G .......... .......... .......... ..G.......   1450
Consensus       CACTGAAGCR TTAGTACAAG TTGAAAACAC AGCTATTACA AAKGTGACGT   1450

Purdue S        .......... .C........ .......... ........A. ..........   1491
NEB72 S         .......... .C........ .......... ........A. ..........   1491
TF1 S           .......... .T........ .......... ........C. ..........   1497
Miller S        .......... .T........ .......... ........C. ..........   1497
FS772 S         .......... .T........ .......... ........C. ..........   1497
NVSL S          .......... .T........ .......... ........C. ..........   786
VMRI S          .......... .T........ .......... ........C. ..........   1500
Consensus       ATTGTAATAG TYACGTTAAT AACATTAAAT GCTCTCAAMT TACTGCTAAT   1500

Purdue S        .......... .......T.. .......... .......... ..C.......   1541
NEB72 S         .......... .......T.. .......... .......... ..C.......   1541
TF1 S           .......... .......T.. .......... .......... ..C.......   1547
Miller S        .......... .......T.. .......... .......... ..C.......   1547
FS772 S         .......... .......T.. .......... .......... ..T.......   1547
NVSL S          .......... .......C.. .......... .......... ..T.......   836
VMRI S          .......... .......C.. .......... .......... ..T.......   1550
Consensus       TTGAATAATG GATTTTAYCC TGTTTCTTCA AGTGAAGTTG GTYTTGTCAA   1550

Purdue S        .........T .......... ...G...... ...A...... ..........   1591
NEB72 S         .........T .......... ...G...... ...A...... ..........   1591
TF1 S           .........T .......... ...G...... ...G...... ..........   1597
Miller S        .........T .......... ...G...... ...A...... ..........   1597
FS772 S         .........T .......... ...C...... ...A...... ..........   1597
NVSL S          .........A .......... ...G...... ...G...... ..........   886
VMRI S          .........T .......... ...G...... ...G...... ..........   1600
Consensus       TAAGAGTGTW GTGTTACTAC CTASCTTTTA CACRCATACC ATTGTTAACA   1600

Purdue S        .........G .......... .......... .......... ..........   1641
NEB72 S         .........G .......... .......... .......... ..........   1641
TF1 S           .........A .......... .......... .......... ..........   1647
Miller S        .........G .......... .......... .......... ..........   1647
FS772 S         .........G .......... .......... .......... ..........   1647
NVSL S          .........G .......... .......... .......... ..........   936
VMRI S          .........A .......... .......... .......... ..........   1650
Consensus       TAACTATTGR TCTTGGTATG AAGCGTAGTG GTTATGGTCA ACCCATAGCC   1650

Purdue S        .....AT... .......C.. .......A.. ......C... ...C......   1691
NEB72 S         .....AT... .......C.. .......A.. ......C... ...C......   1691
TF1 S           .....AT... .......T.. .......G.. ......A... ...C......   1697
Miller S        .....AT... .......T.. .......A.. ......A... ...C......   1697
FS772 S         .....GT... .......T.. .......A.. ......A... ...T......   1697
NVSL S          .....TC... .......T.. .......A.. ......A... ...C......   986
VMRI S          .....TC... .......T.. .......A.. ......A... ...C......   1700
Consensus       TCAACDYTAA GTAACATYAC ACTACCARTG CAGGATMACA ACAYCGATGT   1700
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Figure 5: (continued)

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Purdue S        .......... ........C. .......... .......... ........C.    1741
NEB72 S         .......... ........C. .......... .......... ........C.    1741
TF1 S           .......... ........C. .......... .......... ........T.    1747
Miller S        .......... ........C. .......... .......... ........C.    1747
FS772 S         .......... ........C. .......... .......... ........C.    1747
NVSL S          .......... ........T. .......... .......... ........T.    1036
VMRI S          .......... ........T. .......... .......... ........T.    1750
Consensus       GTACTGTATT CGTTCTGAYC AATTTTCAGT TTATGTTCAT TCTACTTGYA    1750

Purdue S        .....G.... .......... A......... .......... ...C......    1791
NEB72 S         .....G.... .......... A......... .......... ...C......    1791
TF1 S           .....G.... .......... G......... .......... ...T......    1797
Miller S        .....T.... .......... G......... .......... ...C......    1797
FS772 S         .....G.... .......... G......... .......... ...T......    1797
NVSL S          .....G.... .......... G......... .......... ...C......    1086
VMRI S          .....G.... .......... G......... .......... ...C......    1800
Consensus       AAAGTKCTTT ATGGGACAAT RTTTTTAAGC GAAACTGCAC GGAYGTTTTA    1800

Purdue S        .......... .......... .......... .......... ..........    1841
NEB72 S         .......... .......... .......... .......... ..........    1841
TF1 S           .......... .......... .......... .......... ..........    1847
Miller S        .......... .......... .......... .......... ..........    1847
FS772 S         .......... .......... .......... .......... ..........    1847
NVSL S          .......... .......... .......... .......... ..........    1136
VMRI S          .......... .......... .......... .......... ..........    1850
Consensus       GATGCCACAG CTGTTATAAA AACTGGTACT TGTCCTTTCT CATTTGATAA    1850

Purdue S        .T........ .......... .......... ......G... ........T.    1891
NEB72 S         .T........ .......... .......... ......G... ........T.    1891
TF1 S           .T........ .......... .......... ......G... ........C.    1897
Miller S        .T........ .......... .......... ......G... ........T.    1897
FS772 S         .T........ .......... .......... ......G... ........T.    1897
NVSL S          .C........ .......... .......... ......T... ........T.    1186
VMRI S          .C........ .......... .......... ......T... ........T.    1900
Consensus       AYTGAACAAT TACTTAACTT TTAACAAGTT CTGTTTKTCG TTGAGTCCYG    1900

Purdue S        .......... .......... .......C.. .......... .A.......G    1941
NEB72 S         .......... .......... .......C.. .......... .A.......G    1941
TF1 S           .......... .......... .......C.. .......... .C.......T    1947
Miller S        .......... .......... .......C.. .......... .A.......T    1947
FS772 S         .......... .......... .......C.. .......... .G.......T    1947
NVSL S          .......... .......... .......T.. .......... .A.......T    1236
VMRI S          .......... .......... .......T.. .......... .A.......T    1950
Consensus       TTGGTGCTAA TTGTAAGTTT GATGTAGYTG CCCGTACAAG AVCCAATGAK    1950

Purdue S        .......... .......... .......... .......... ..........    1991
NEB72 S         .......... .......... .......... .......... ..........    1991
TF1 S           .......... .......... .......... .......... ..........    1997
Miller S        .......... .......... .......... .......... ..........    1997
FS772 S         .......... .......... .......... .......... ..........    1997
NVSL S          .......... .......... .......... .......... ..........    1286
VMRI S          .......... .......... .......... .......... ..........    2000
Consensus       CAGGTTGTTA GAAGTTTGTA TGTAATATAT GAAGAAGGAG ACAACATAGT    2000
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Figure 5: (continued)

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Purdue S        G......... .....T..T. .....G.G.. .......... ..........    2041
NEB72 S         G......... .....T..T. .....G.G.. .......... ..........    2041
TF1 S           G......... .....C..C. .....T.G.. .......... ..........    2047
Miller S        G......... .....T..T. .....T.A.. .......... ..........    2047
FS772 S         G......... .....C..T. .....T.G.. .......... ..........    2047
NVSL S          A......... .....C..T. .....T.G.. .......... ..........    1336
VMRI S          A......... .....C..T. .....T.G.. .......... ..........    2050
Consensus       RGGTGTACCG TCTGAYAAYA GTGGTKTRCA CGATTTGTCA GTGCTACACC    2050

Purdue S        .......C.. .........C .......... ......C... ..........    2091
NEB72 S         .......C.. .........C .......... ......C... ..........    2091
TF1 S           .......A.. .........T .......... ......C... ..........    2097
Miller S        .......C.. .........C .......... ......C... ..........    2097
FS772 S         .......A.. .........C .......... ......G... ..........    2097
NVSL S          .......A.. .........C .......... ......C... ..........    1386
VMRI S          .......A.. .........C .......... ......C... ..........    2100
Consensus       TAGATTCMTG CACAGATTAY AATATATATG GTAGAASTGG TGTTGGTATT    2100

Purdue S        ......C... .......... G......... .......... ..........    2141
NEB72 S         ......A... .......... G......... .......... ..........    2141
TF1 S           ......C... .......... A......... .......... ..........    2147
Miller S        ......C... .......... G......... .......... ..........    2147
FS772 S         ......C... .......... G......... .......... ..........    2147
NVSL S          ......C... .......... A......... .......... ..........    1436
VMRI S          ......C... .......... A......... .......... ..........    2150
Consensus       ATTAGAMAAA CTAACAGGAC RCTACTTAGT GGCTTATATT ACACATCACT    2150

Purdue S        ...A...... .......... .......... .......... G.C.......    2191
NEB72 S         ...A...... .......... .......... .......... G.C.......    2191
TF1 S           ...T...... .......... .......... .......... G.T.......    2197
Miller S        ...A...... .......... .......... .......... G.C.......    2197
FS772 S         ...T...... .......... .......... .......... G.C.......    2197
NVSL S          ...T...... .......... .......... .......... A.T.......    1486
VMRI S          ...T...... .......... .......... .......... A.T.......    2200
Consensus       ATCWGGTGAT TTGTTAGGTT TTAAAAATGT TAGTGATGGT RTYATCTACT    2200

Purdue S        .......G.. A......... ....C..... .......... ..........    2241
NEB72 S         .......G.. A......... ....C..... .......... ..........    2241
TF1 S           .......G.. A......... ....T..... .......... ..........    2247
Miller S        .......G.. A......... ....C..... .......... ..........    2247
FS772 S         .......T.. A......... ....C..... .......... ..........    2247
NVSL S          .......G.. G......... ....C..... .......... ..........    1536
VMRI S          .......G.. G......... ....C..... .......... ..........    2250
Consensus       CTGTAACKCC RTGTGATGTA AGCGYACAAG CAGCTGTTAT TGATGGTACC    2250

Purdue S        .......... ..A....... C......... ...C...... .......A..    2291
NEB72 S         .......... ..A....... C......... ...C...... .......A..    2291
TF1 S           .......... ..G....... C......... ...T...... .......A..    2297
Miller S        .......... ..A....... C......... ...C...... .......A..    2297
FS772 S         .......... ..A....... C......... ...T...... .......A..    2297
NVSL S          .......... ..A....... T......... ...T...... .......G..    1586
VMRI S          .......... ..A....... T......... ...T...... .......G..    2300
Consensus       ATAGTTGGGG CTRTCACTTC YATTAACAGT GAAYTGTTAG GTCTAACRCA    2300
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Figure 5: (continued)

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Purdue S        .......... .......... .......... .......... ..........   2341
NEB72 S         .......... .......... .......... .......... ..........   2341
TF1 S           .......... .......... .......... .......... ..........   2347
Miller S        .......... .......... .......... .......... ..........   2347
FS772 S         .......... .......... .......... .......... ..........   2347
NVSL S          .......... .......... .......... .......... ..........   1636
VMRI S          .......... .......... .......... .......... ..........   2350
Consensus       TTGGACAACA ACACCTAATT TTTATTACTA CTCTATATAT AATTACACAA   2350

Purdue S        ......G..C .......... .......... .....G.TT. ..........   2391
NEB72 S         ......G..C .......... .......... .....G.TG. ..........   2391
TF1 S           ......G..C .......... .......... .....G.CG. ..........   2397
Miller S        ......G..C .......... .......... .....G.TG. ..........   2397
FS772 S         ......T..C .......... .......... .....G.TG. ..........   2397
NVSL S          ......G..T .......... .......... .....G.CG. ..........   1686
VMRI S          ......G..T .......... .......... .....C.CG. ..........   2400
Consensus       ATGATAKGAY TCGTGGCACT GCAATTGACA GTAATSAYKT TGATTGTGAA   2400

Purdue S        .......... .......... .......... ........T. .......T..   2441
NEB72 S         .......... .......... .......... ........T. .......T..   2441
TF1 S           .......... .......... .......... ........T. .......G..   2447
Miller S        .......... .......... .......... ........T. .......G..   2447
FS772 S         .......... .......... .......... ........T. .......G..   2447
NVSL S          .......... .......... .......... ........C. .......G..   1736
VMRI S          .......... .......... .......... ........C. .......G..   2450
Consensus       CCTGTCATAA CCTATTCTAA CATAGGTGTT TGTAAAAAYG GTGCTTTKGT   2450

Purdue S        ......T..C .......... .......... .......... ..........   2491
NEB72 S         ......T..C .......... .......... .......... ..........   2491
TF1 S           ......T..T .......... .......... .......... ..........   2497
Miller S        ......T..C .......... .......... .......... ..........   2497
FS772 S         ......T..C .......... .......... .......... ..........   2497
NVSL S          ......C..C .......... .......... .......... ..........   1786
VMRI S          ......C..C .......... .......... .......... ..........   2500
Consensus       TTTTATYAAY GTCACACATT CTGATGGAGA CGTGCAACCA ATTAGCACTG   2500

Purdue S        ....T..... ......T... ..C.....C. .......... ......A..T   2541
NEB72 S         ....T..... ......T... ..C.....C. .......... ......A..T   2541
TF1 S           ....C..... ......T... ..C.....T. .......... ......G..C   2547
Miller S        ....C..... ......T... ..C.....T. .......... ......A..T   2547
FS772 S         ....C..... ......C... ..C.....T. .......... ......A..C   2547
NVSL S          ....C..... ......T... ..T.....T. .......... ......A..C   1836
VMRI S          ....C..... ......T... ..T.....T. .......... ......A..C   2550
Consensus       GTAAYGTCAC GATACCYACA AAYTTTACYA TATCCGTGCA AGTCGARTAY   2550

Purdue S        .......... .......... .......... .......... ....T.....   2591
NEB72 S         .......... .......... .......... .......... ....T.....   2591
TF1 S           .......... .......... .......... .......... ....T.....   2597
Miller S        .......... .......... .......... .......... ....T.....   2597
FS772 S         .......... .......... .......... .......... ....T.....   2597
NVSL S          .......... .......... .......... .......... ....C.....   1886
VMRI S          .......... .......... .......... .......... ....C.....   2600
Consensus       ATTCAGGTTT ACACTACACC AGTGTCAATA GACTGTTCAA GATAYGTTTG   2600
```

Figure 5: (continued)

```
Purdue S        ......T... .......... .....T.G.. .........C ..........   2641
NEB72 S         ......T... .......... .....T.G.. .........C ..........   2641
TF1 S           ......C... .......... .....C.G.. .........C ..........   2647
Miller S        ......C... .......... .....T.G.. .........C ..........   2647
FS772 S         ......C... .......... .....T.G.. .........C ..........   2647
NVSL S          ......C... .......... .....C.A.. .........T ..........   1936
VMRI S          ......C... .......... .....C.A.. .........T ..........   2650
Consensus       TAATGGYAAC CCTAGGTGTA ACAAAYTRTT AACACAATAY GTTTCTGCAT   2650

Purdue S        .......... .......... ......A... .......... ......C...   2691
NEB72 S         .......... .......... ......A... .......... ......C...   2691
TF1 S           .......... .......... ......A... .......... ......T...   2697
Miller S        .......... .......... ......A... .......... ......C...   2697
FS772 S         .......... .......... ......G... .......... ......C...   2697
NVSL S          .......... .......... ......A... .......... ......T...   1986
VMRI S          .......... .......... ......A... .......... ......T...   2700
Consensus       GTCAAACTAT TGAGCAAGCA CTTGCARTGG GTGCCAGACT TGAAAAYATG   2700

Purdue S        ..G....A.. .......G.. .......... .......... ....A.CA..   2741
NEB72 S         ..G....A.. .......G.. .......... .......... ....G.CA..   2741
TF1 S           ..A....A.. .......A.. .......... .......... ....G.GT..   2747
Miller S        ..A....G.. .......A.. .......... .......... ....G.CT..   2747
FS772 S         ..A....A.. .......A.. .......... .......... ....G.CT..   2747
NVSL S          ..A....A.. .......A.. .......... .......... ....G.CT..   2036
VMRI S          ..A....A.. .......A.. .......... .......... ....G.CT..   2750
Consensus       GARGTTGRTT CCATGTTRTT TGTTTCTGAA AATGCCCTTA AATTRGSWTC   2750

Purdue S        ...T...... .......... .....A.... ...C...... ........A.   2791
NEB72 S         ...T...... .......... .....A.... ...C...... ........A.   2791
TF1 S           ...C...... .......... .....A.... ...T...... ........A.   2797
Miller S        ...C...... .......... .....A.... ...T...... ........A.   2797
FS772 S         ...C...... .......... .....A.... ...T...... ........A.   2797
NVSL S          ...T...... .......... .....G.... ...T...... ........C.   2086
VMRI S          ...T...... .......... .....G.... ...T...... ........C.   2800
Consensus       TGTYGAAGCA TTCAATAGTT CAGAARCTTT AGAYCCTATT TACAAAGAMT   2800

Purdue S        ......A... ......T... .......... .......... C.....T...   2841
NEB72 S         ......A... ......T... .......... .......... C.....T...   2841
TF1 S           ......A... ......C... .......... .......... C.....T...   2847
Miller S        ......A... ......C... .......... .......... C.....T...   2847
FS772 S         ......A... ......C... .......... .......... C.....T...   2847
NVSL S          ......G... ......C... .......... .......... T.....G...   2136
VMRI S          ......G... ......C... .......... .......... T.....G...   2850
Consensus       GGCCTARTAT AGGTGGYTCT TGGCTAGAAG GTCTAAAATA YATACTKCCG   2850

Purdue S        ...C...... ........A. .......... .......... ..........   2891
NEB72 S         ...C...... ........A. .......... .......... ..........   2891
TF1 S           ...G...... ........A. .......... .......... ..........   2897
Miller S        ...G...... ........A. .......... .......... ..........   2897
FS772 S         ...G...... ........A. .......... .......... ..........   2897
NVSL S          ...G...... ........C. .......... .......... ..........   2186
VMRI S          ...G...... ........C. .......... .......... ..........   2900
Consensus       TCCSATAATA GCAAACGTMA GTATCGTTCA GCTATAGAGG ACTTGCTTTT   2900
```

Figure 5: (continued)

```
Purdue S        .GA..... ..... ..... .....T... .....    2941
NEB72 S         .GA..... ..... ..... .....T... .....    2941
TF1 S           .TC..... ..... ..... .....T... .....    2947
Miller S        .GC..... ..... ..... .....T... .....    2947
FS772 S         .TC..... ..... ..... .....C... .....    2947
NVSL S          .TC..... ..... ..... .....T... .....    2236
VMRI S          .TC..... ..... ..... .....T... .....    2950
Consensus       TKMTAAGGTT GTAACATCTG GTTTAGGTAC AGTTGAYGAA GATTATAAAC    2950

Purdue S        ..... .....C ........C. ....A..... ......C..T    2991
NEB72 S         ..... .....C ........C. ....A..... ......C..T    2991
TF1 S           ..... .....C ........T. ....A..... ......C..T    2997
Miller S        ..... .....C ........C. ....A..... ......C..C    2997
FS772 S         ..... .....C ........C. ....A..... ......C..C    2997
NVSL S          ..... .....T ........C. ....G..... ......T..T    2286
VMRI S          ..... .....T ........C. ....G..... ......T..T    3000
Consensus       GTTGTACAGG TGGTTATGAY ATAGCTGAYT TAGTRTGTGC TCAATAYTAY    3000

Purdue S        ..... ..... ...T...... ..... .....    3041
NEB72 S         ..... ..... ...T...... ..... .....    3041
TF1 S           ..... ..... ...C...... ..... .....    3047
Miller S        ..... ..... ...T...... ..... .....    3047
FS772 S         ..... ..... ...T...... ..... .....    3047
NVSL S          ..... ..... ...T...... ..... .....    2336
VMRI S          ..... ..... ...T...... ..... .....    3050
Consensus       AATGGCATCA TGGTGCTACC TGGYGTGGCT AATGCTGACA AAATGACTAT    3050

Purdue S        ..... ..C..T.... ..... ..... .....    3091
NEB72 S         ..... ..C..T.... ..... ..... .....    3091
TF1 S           ..... ..A..C.... ..... ..... .....    3097
Miller S        ..... ..C..C.... ..... ..... .....    3097
FS772 S         ..... ..C..C.... ..... ..... .....    3097
NVSL S          ..... ..C..C.... ..... ..... .....    2386
VMRI S          ..... ..C..C.... ..... ..... .....    3100
Consensus       GTACACAGCA TCMCTYGCAG GTGGTATAAC ATTAGGTGCA CTTGGTGGAG    3100

Purdue S        .C.....G.. ..... ..... ..... .....    3141
NEB72 S         .C.....G.. ..... ..... ..... .....    3141
TF1 S           .C.....G.. ..... ..... ..... .....    3147
Miller S        .C.....G.. ..... ..... ..... .....    3147
FS772 S         .C.....G.. ..... ..... ..... .....    3147
NVSL S          .T.....A.. ..... ..... ..... .....    2436
VMRI S          .T.....A.. ..... ..... ..... .....    3150
Consensus       GYGCCGTRGC TATACCTTTT GCAGTAGCAG TTCAGGCTAG ACTTAATTAT    3150

Purdue S        ..... ..... ...G..C... ..... .T.....    3191
NEB72 S         ..... ..... ...G..C... ..... .T.....    3191
TF1 S           ..... ..... ...A..T... ..... .C.....    3197
Miller S        ..... ..... ...G..C... ..... .C.....    3197
FS772 S         ..... ..... ...G..C... ..... .C.....    3197
NVSL S          ..... ..... ...A..C... ..... .C.....    2486
VMRI S          ..... ..... ...A..C... ..... .C.....    3200
Consensus       GTTGCTCTAC AAACTGATGT ATTRAAYAAA AACCAGCAGA TYCTGGCTAG    3200
```

Figure 5: (continued)

```
Purdue S        ......C... .......... .......... .......... ..........   3241
NEB72 S         ......C... .......... .......... .......... ..........   3241
TF1 S           ......T... .......... .......... .......... ..........   3247
Miller S        ......C... .......... .......... .......... ..........   3247
FS772 S         ......T... .......... .......... .......... ..........   3247
NVSL S          ......T... .......... .......... .......... ..........   2536
VMRI S          ......T... .......... .......... .......... ..........   3250
Consensus       TGCTTTYAAT CAAGCTATTG GTAACATTAC ACAGTCATTT GGTAAGGTTA   3250

Purdue S        .......... .....A...A ..A....... ....T..... ..........   3291
NEB72 S         .......... .......... .......... ....T..... ..........   3291
TF1 S           .......... .....A...A ..A....... ....A..... ..........   3297
Miller S        .......... .....A...A ..A....... ....A..... ..........   3297
FS772 S         .......... .....A...A ..A....... ....A..... ..........   3297
NVSL S          .......... .....A...G ..A....... ....A..... ..........   2586
VMRI S          .......... .....A...G ..A....... ....A..... ..........   3300
Consensus       ATGATGCTAT ACATCRAACR TCRCGAGGTC TTGCWACTGT TGCTAAAGCA   3300

Purdue S        .......... .......... ...CA.C.T. .......... ..........   3341
NEB72 S         .......... .......... ...C..C.T. .......... ..........   3341
TF1 S           .......... .......... ...CA.C.C. .......... ..........   3347
Miller S        .......... .......... ...CA.C.C. .......... ..........   3347
FS772 S         .......... .......... ...CA.C.C. .......... ..........   3347
NVSL S          .......... .......... ...TA.T.C. .......... ..........   2636
VMRI S          .......... .......... ...TA.T.C. .......... ..........   3350
Consensus       TTGGCAAAAG TGCAAGATGT TGTYNAYAYA CAAGGGCAAG CTTTAAGCCA   3350

Purdue S        .......... .......... .......... .......... ..T.......   3391
NEB72 S         .......... .......... .......... .......... ..T.......   3391
TF1 S           .......... .......... .......... .......... ..T.......   3397
Miller S        .......... .......... .......... .......... ..T.......   3397
FS772 S         .......... .......... .......... .......... ..T.......   3397
NVSL S          .......... .......... .......... .......... ..C.......   2686
VMRI S          .......... .......... .......... .......... ..C.......   3400
Consensus       CCTAACAGTA CAATTGCAAA ATAATTTCCA AGCCATTAGT AGYTCTATTA   3400

Purdue S        .......... C......... ..C....... .......... ...A..T...   3441
NEB72 S         .......... T......... ..C....... .......... ...A..T...   3441
TF1 S           .......... T......... ..T....... .......... ...A..C...   3447
Miller S        .......... T......... ..T....... .......... ...A..T...   3447
FS772 S         .......... T......... ..T....... .......... ...C..C...   3447
NVSL S          .......... T......... ..T....... .......... ...A..T...   2736
VMRI S          .......... T......... ..T....... .......... ...A..T...   3450
Consensus       GTGACATTTA YAATAGGCTT GAYGAATTGA GTGCTGATGC ACAMGTYGAC   3450

Purdue S        .......... .......... .......... .......... .G.....G..   3491
NEB72 S         .......... .......... .......... .......... .G.....G..   3491
TF1 S           .......... .......... .......... .......... .G.....A..   3497
Miller S        .......... .......... .......... .......... .G.....G..   3497
FS772 S         .......... .......... .......... .......... .G.....A..   3497
NVSL S          .......... .......... .......... .......... .A.....G..   2786
VMRI S          .......... .......... .......... .......... .A.....G..   3500
Consensus       AGGCTGATCA CAGGAAGACT TACAGCACTT AATGCATTTG TRTCTCARAC   3500
```

Figure 5: (continued)

```
Purdue S        .......... .....G.... .......... T......... ........C.    3541
NEB72 S         .......... .....G.... .......... T......... ........C.    3541
TF1 S           .......... .....C.... .......... T......... ........T.    3547
Miller S        .......... .....C.... .......... T......... ........C.    3547
FS772 S         .......... .....C.... .......... T......... ........C.    3547
NVSL S          .......... .....C.... .......... C......... ........C.    2836
VMRI S          .......... .....C.... .......... C......... ........C.    3550
Consensus       TCTAACCAGA CAAGCSGAGG TTAGGGCTAG YAGACAACTT GCCAAAGAYA    3550

Purdue S        .......... .......... ........T. .......... A.........    3591
NEB72 S         .......... .......... ........T. .......... A.........    3591
TF1 S           .......... .......... ........T. .......... C.........    3597
Miller S        .......... .......... ........T. .......... A.........    3597
FS772 S         .......... .......... ........T. .......... C.........    3597
NVSL S          .......... .......... ........C. .......... C.........    2886
VMRI S          .......... .......... ........C. .......... C.........    3600
Consensus       AGGTTAATGA ATGCGTTAGG TCTCAGTCYC AGAGATTCGG MTTCTGTGGT    3600

Purdue S        .......... .......... .......... .......... ..........    3641
NEB72 S         .......... .......... .......... .......... ..........    3641
TF1 S           .......... .......... .......... .......... ..........    3647
Miller S        .......... .......... .......... .......... ..........    3647
FS772 S         .......... .......... .......... .......... ..........    3647
NVSL S          .......... .......... .......... .......... ..........    2936
VMRI S          .......... .......... .......... .......... ..........    3650
Consensus       AATGGTACAC ATTTGTTTTC ACTCGCAAAT GCAGCACCAA ATGGCATGAT    3650

Purdue S        T..C...... A......... .......... T......... ..........    3691
NEB72 S         T..C...... A......... .......... T......... ..........    3691
TF1 S           C..T...... A......... .......... T......... ..........    3697
Miller S        C..C...... A......... .......... T......... ..........    3697
FS772 S         C..C...... G......... .......... T......... ..........    3697
NVSL S          T..C...... A......... .......... C......... ..........    2986
VMRI S          T..C...... A......... .......... C......... ..........    3700
Consensus       YTTYTTTCAC RCAGTGCTAT TACCAACGGC YTATGAAACT GTGACTGCTT    3700

Purdue S        ..C....... .......TC. .......... .......T.. ....G.....    3741
NEB72 S         ..C....... .......TC. .......... .......T.. ....G.....    3741
TF1 S           ..G....... .......CT. .......... .......C.. ....G.....    3747
Miller S        ..G....... .......TT. .......... .......T.. ....G.....    3747
FS772 S         ..G....... .......TT. .......... .......T.. ....G.....    3747
NVSL S          ..G....... .......TT. .......... .......T.. ....A.....    3036
VMRI S          ..G....... .......TT. .......... .......T.. ....G.....    3750
Consensus       GGSCAGGTAT TTGTGCTYYA GATGGTGATC GCACTTTYGG ACTTRTCGTT    3750

Purdue S        .......... ..T....... .......... .......... ..........    3791
NEB72 S         .......... ..T....... .......... .......... ..........    3791
TF1 S           .......... ..T....... .......... .......... ..........    3797
Miller S        .......... ..T....... .......... .......... ..........    3797
FS772 S         .......... ..T....... .......... .......... ..........    3797
NVSL S          .......... ..C....... .......... .......... ..........    3086
VMRI S          .......... ..C....... .......... .......... ..........    3800
Consensus       AAAGATGTCC AGYTGACTTT GTTTCGTAAT CTAGATGACA AGTTCTATTT    3800
```

Figure 5: (continued)

```
Purdue S        ...C..... ..........  ....T.....  T.........  .....C....   3841
NEB72 S         ...C..... ..........  ....T.....  T.........  .....C....   3841
TF1 S           ...C..... ..........  ....T.....  G.........  .....T....   3847
Miller S        ...C..... ..........  ....T.....  G.........  .....T....   3847
FS772 S         ...C..... ..........  ....C.....  G.........  .....T....   3847
NVSL S          ...T..... ..........  ....T.....  G.........  .....T....   3136
VMRI S          ...T..... ..........  ....T.....  G.........  .....T....   3850
Consensus       GACYCCCAGA ACTATGTATC AGCCYAGAGT KGCAACTAGT TCTGAYTTTG     3850

Purdue S        .......... ..........  ..........  ..........  .G........   3891
NEB72 S         .......... ..........  ..........  ..........  .G........   3891
TF1 S           .......... ..........  ..........  ..........  .G........   3897
Miller S        .......... ..........  ..........  ..........  .G........   3897
FS772 S         .......... ..........  ..........  ..........  .C........   3897
NVSL S          .......... ..........  ..........  ..........  .G........   3186
VMRI S          .......... ..........  ..........  ..........  .G........   3900
Consensus       TTCAAATTGA AGGGTGCGAT GTGCTGTTTG TTAATGCAAC TSTAAGTGAT     3900

Purdue S        .......... ..........  ..........  ........G.  ..........   3941
NEB72 S         .......... ..........  ..........  ........G.  ..........   3941
TF1 S           .......... ..........  ..........  ........G.  ..........   3947
Miller S        .......... ..........  ..........  ........G.  ..........   3947
FS772 S         .......... ..........  ..........  ........G.  ..........   3947
NVSL S          .......... ..........  ..........  ........A.  ..........   3236
VMRI S          .......... ..........  ..........  ........A.  ..........   3950
Consensus       TTGCCTAGTA TTATACCTGA TTATATTGAT ATTAATCARA CTGTTCAAGA     3950

Purdue S        ....T..... .....T....  ..........  .........G  ..........   3991
NEB72 S         ....T..... .....T....  ..........  .........G  ..........   3991
TF1 S           ....T..... .....T....  ..........  .........G  ..........   3997
Miller S        ....T..... .....T....  ..........  .........G  ..........   3997
FS772 S         ....T..... .....T....  ..........  .........G  ..........   3997
NVSL S          ....C..... .....C....  ..........  .........T  ..........   3286
VMRI S          ....C..... .....C....  ..........  .........T  ..........   4000
Consensus       CATAYTAGAA AATTTYAGAC CAAATTGGAC TGTACCTGAK TTGACATTTG     4000

Purdue S        .......... ..........  .....C....  ...G......  ..........   4041
NEB72 S         .......... ..........  .....C....  ...G......  ..........   4041
TF1 S           .......... ..........  .....C....  ...G......  ..........   4047
Miller S        .......... ..........  .....C....  ...G......  ..........   4047
FS772 S         .......... ..........  .....C....  ...G......  ..........   4047
NVSL S          .......... ..........  .....T....  ...T......  ..........   3336
VMRI S          .......... ..........  .....T....  ...T......  ..........   4050
Consensus       ACATTTTTAA CGCAACCTAT TTAAAYCTGA CTGKTGAAAT TGATGACTTA     4050

Purdue S        .......... ..........  .........C  .......A..  ..........   4091
NEB72 S         .......... ..........  .........C  .......A..  ..........   4091
TF1 S           .......... ..........  .........T  .......G..  ..........   4097
Miller S        .......... ..........  .........T  .......A..  ..........   4097
FS772 S         .......... ..........  .........T  .......A..  ..........   4097
NVSL S          .......... ..........  .........T  .......A..  ..........   3386
VMRI S          .......... ..........  .........T  .......A..  ..........   4100
Consensus       GAATTTAGGT CAGAAAAGCT ACATAACACY ACTGTAGRAC TTGCCATTCT     4100
```

Figure 5: (continued)

```
Purdue S        C.....C... .......... .......... .......... ..C.......   4141
NEB72 S         C.....C... .......... .......... .......... ..C.......   4141
TF1 S           T.....C... .......... .......... .......... ..T.......   4147
Miller S        T.....C... .......... .......... .......... ..C.......   4147
FS772 S         T.....C... .......... .......... .......... ..C.......   4147
NVSL S          T.....T... .......... .......... .......... ..T.......   3436
VMRI S          T.....T... .......... .......... .......... ..T.......   4150
Consensus       YATTGAYAAC ATTAACAATA CATTAGTCAA TCTTGAATGG CTYAATAGAA   4150

Purdue S        .......C.. .......... .......... .......... ......C...   4191
NEB72 S         .......C.. .......... .......... .......... ......C...   4191
TF1 S           .......A.. .......... .......... .......... ......T...   4197
Miller S        .......C.. .......... .......... .......... ......C...   4197
FS772 S         .......C.. .......... .......... .......... ......C...   4197
NVSL S          .......C.. .......... .......... .......... ......T...   3486
VMRI S          .......C.. .......... .......... .......... ......T...   4200
Consensus       TTGAAACMTA TGTAAAATGG CCTTGGTATG TGTGGCTACT AATAGGYTTA   4200

Purdue S        ..A....... .......... ....C..... .......... ..........   4241
NEB72 S         ..A....... .......... ....C..... .......... ..........   4241
TF1 S           ..A....... .......... ....C..... .......... ..........   4247
Miller S        ..A....... .......... ....C..... .......... ..........   4247
FS772 S         ..G....... .......... ....C..... .......... ..........   4247
NVSL S          ..A....... .......... ....T..... .......... ..........   3536
VMRI S          ..A....... .......... ....T..... .......... ..........   4250
Consensus       GTRGTAATAT TTTGCATACC ATTAYTGCTA TTTTGCTGTT GTAGTACAGG   4250

Purdue S        .......... ......G... .......... ...T..T... ........T.   4291
NEB72 S         .......... ......G... .......... ...T..T... ........T.   4291
TF1 S           .......... ......A... .......... ...C..C... ........C.   4297
Miller S        .......... ......G... .......... ...T..T... ........C.   4297
FS772 S         .......... ......G... .......... ...T..T... ........C.   4297
NVSL S          .......... ......G... .......... ...T..T... ........C.   3586
VMRI S          .......... ......G... .......... ...T..T... ........C.   4300
Consensus       TTGCTGTGGA TGCATARGTT GTTTAGGAAG TTGYTGYCAC TCTATATGYA   4300

Purdue S        .......... .......... ..C.....A. .......... ....G.....   4341
NEB72 S         .......... .......... ..C.....A. .......... ....G.....   4341
TF1 S           .......... .......... ..T.....T. .......... ....G.....   4347
Miller S        .......... .......... ..C.....T. .......... ....G.....   4347
FS772 S         .......... .......... ..C.....T. .......... ....A.....   4347
NVSL S          .......... .......... ..T.....T. .......... ....G.....   3636
VMRI S          .......... .......... ..T.....T. .......... ....G.....   4350
Consensus       GTAGAAGACA ATTTGAAAAT TAYGAACCWA TTGAAAAAGT GCACRTCCAT   4350
```

Figure 5: (continued)

Table 7: Percent nucleotide and deduced amino acid homology between S gene of TGEV isolate VMRI 5170 and that of other TGEV isolates.

Virus Strains	% homology with S gene of TGEV isolate VMRI 5170	
	Nucleic Acid Homology	Amino Acid Homology
Miller	97%	97%
FS772	97%	97%
NEB72	96%	96%
Purdue	96%	96%
TF1	97%	97%

In addition, the ORF 3/3.1 genes of VMRI 5170 and NVSL 5170 isolates were compared with those of other PRCV isolates as depicted in Figure 7. The ORF 3 of TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 were comprised of 219 bases while ORF 3.1 had 736 bases including start and stop codons. Like other coronaviruses, the ORF 3/3.1 genes of TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 had an intergenic sequence, of CUAAAC, upstream of the start codon. The base compositions within the ORF 3 of VMRI 5170 and NVSL 5170 isolates were completely identical, whereas ORF 3.1 had only 2 nucleotide differences. The first nucleotide difference within the 3.1 gene of NVSL 5170 isolate was T instead of C. Therefore, it created a stop codon which may have resulted in a truncated product of ORF 3.1 in NVSL 5170 isolate. The ORF 3/3.1 genes of TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 were shown to be similar to those of other PRCV isolates except that the ORF 3 of TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 were intact (Figure 7).

```
86/137004 S     .......A.T .......... C......G.. .......... ..........    50
HOL87 S         .......A.T .......... C......G.. .......... ..........    50
RM4 S           .......A.T .......... C......G.. .......... ..........    50
VMRI S          .......C.C .......... T......A.. .......... ..........    50
NVSL S          .......C.C .......... T......--- ---------- ----------    27
Miller S        .......A.T .......... T......G.. .......... ..........    50
Consensus       ATGAAAAMAY TATTTGTGGT YTTGGTTRTA ATGCCATTGA TTTATGGAGA    50
```

Figure 6: Pairwise alignment of S genes of TGEV isolate, Miller strain and VMRI 5170, PRCV isolate NVSL 5170 and other PRCV's S genes.
Note: The sequences begin with the start codons and are shown as underlined bases. The position having identical nucleotides are presented as dots and the positions of deleted nucleotides are exhibited as dashes. The 5 bases that are diferent between TGEV isolate VMRI 5170 and PRCV isolate NVSL 5170 are presented as bold letters.

```
86/137004 S    C..G.....- ---------- ---------- ---------- ----------    59
HOL87 S        C..G.....- ---------- ---------- ---------- ----------    59
RM4 S          C..G.....- ---------- ---------- ---------- ----------    59
VMRI S         T..T...... .......... .......... .......... ..A.......   100
NVSL S         ---------- ---------- ---------- ---------- ----------    27
Miller S       C..T...... .......... .......... .......... ..C.......   100
Consensus      YAAKTTTCCT TGTTCTAAAT TGACTAATAG AACTATAGGT AAMCATTGGA   100

86/137004 S    ---------- ---------- ---------- ---------- ----------    59
HOL87 S        ---------- ---------- ---------- ---------- ----------    59
RM4 S          ---------- ---------- ---------- ---------- ----------    59
VMRI S         .......... T.....T... A......... ....C..... .C........   150
NVSL S         ---------- ---------- ---------- ---------- ----------    27
Miller S       .......... A.....C... C......... ....T..... .T........   150
Consensus      ATCTCATTGA WACCTTYCTT MTAAATTATA GTAGYAGGTT AYCACCTAAT   150

86/137004 S    ---------- ---------- ---------- ---------- ----------    59
HOL87 S        ---------- ---------- ---------- ---------- ----------    59
RM4 S          ---------- ---------- ---------- ---------- ----------    59
VMRI S         .......... .......... .......... .......... ..........   200
NVSL S         ---------- ---------- ---------- ---------- ----------    27
Miller S       .......... .......... .......... .......... ..........   200
Consensus      TCAGATGTGG TGTTAGGTGA TTATTTTCCT ACTGTACAAC CTTGGTTTAA   200

86/137004 S    ---------- ---------- ---------- ---------- ----------    59
HOL87 S        ---------- ---------- ---------- ---------- ----------    59
RM4 S          ---------- ---------- ---------- ---------- ----------    59
VMRI S         ...T...... ...G...... .C........ .......... ..........   250
NVSL S         ---------- ---------- ---------- ---------- ----------    27
Miller S       ...C...... ...A...... .T........ .......... ..........   250
Consensus      TTGYATTCGC AATRATAGTA AYGACCTTTA TGTTACATTG GAAAATCTTA   250

86/137004 S    ---------- ---------- ---------- ---------- ----------    59
HOL87 S        ---------- ---------- ---------- ---------- ----------    59
RM4 S          ---------- ---------- ---------- ---------- ----------    59
VMRI S         ......G... .......... ......AG.. .........G ..........   300
NVSL S         ---------- ---------- ---------- ---------- ----------    27
Miller S       ......T... .......... ......GA.. .........T .........-   299
Consensus      AAGCATKGTA TTGGGATTAT GCTACARRAA ATATCACTTK GAATCACAAG   300

86/137004 S    ---------- ---------- ---------- ---------- ----------    59
HOL87 S        ---------- ---------- ---------- ---------- ----------    59
RM4 S          ---------- ---------- ---------- ---------- ----------    59
VMRI S         .......... .......... .......... .......... ..........   350
NVSL S         ---------- ---------- ---------- ---------- ----------    27
Miller S       -.-....... .......... .......... .......... ..........   347
Consensus      GACCAACGGT TAAACGTAGT CGTTAATGGA TACCCATACT CCATCACAGT   350

86/137004 S    ---------- ---------- ---------- ---------- ----------    59
HOL87 S        ---------- ---------- ---------- ---------- ----------    59
RM4 S          ---------- ---------- ---------- ---------- ----------    59
VMRI S         .......... .......... .......... .......... ..........   400
NVSL S         ---------- ---------- ---------- ---------- ----------    27
```

Figure 6: (continued)

```
Miller S        .......... .......... .......... .......... ..........   397
Consensus       TACAACAACC CGCAATTTTA ATTCTGCTGA AGGTGCTATT ATATGCATTT   400

86/137004 S     ---------- ---------- ---------- ---------- ----------    59
HOL87 S         ---------- ---------- ---------- ---------- ----------    59
RM4 S           ---------- ---------- ---------- ---------- ----------    59
VMRI S          .......... .......... ........C. .G........ ..........   450
NVSL S          ---------- ---------- ---------- ---------- ----------    27
Miller S        .......... .......... ........A. .A........ ..........   447
Consensus       GCAAGGGCTC ACCACCTACT ACCACCACMG ARTCTAGTTT GACTTGCAAT   450

86/137004 S     ---------- ---------- ---------- ---------- ----------    59
HOL87 S         ---------- ---------- ---------- ---------- ----------    59
RM4 S           ---------- ---------- ---------- ---------- ----------    59
VMRI S          .......... .......... ......C... .......... ..........   500
NVSL S          ---------- ---------- ---------- ---------- ----------    27
Miller S        .......... .......... ......T... .......... ..........   497
Consensus       TGGGGTAGTG AGTGCAGGTT AAACCAYAAG TTCCCTATAT GTCCTTCTAA   500

86/137004 S     ---------- ---------- ---------- ---------- ----------    59
HOL87 S         ---------- ---------- ---------- ---------- ----------    59
RM4 S           ---------- ---------- ---------- ---------- ----------    59
VMRI S          .......... .......... .....T.... C......... ..........   550
NVSL S          ---------- ---------- ---------- ---------- ----------    27
Miller S        .......... .......... .....C.... T......... ..........   547
Consensus       TTCAGAGGCA AATTGTGGTA ATATGYTGTA YGGCCTACAA TGGTTTGCAG   550

86/137004 S     ---------- ---------- ---------- ---------- ----------    59
HOL87 S         ---------- ---------- ---------- ---------- ----------    59
RM4 S           ---------- ---------- ---------- ---------- ----------    59
VMRI S          .......... .......... .......... .......... ..........   600
NVSL S          ---------- ---------- ---------- ---------- ----------    27
Miller S        .......... .......... .......... .......... ..........   597
Consensus       ATGCGGTTGT TGCTTATTTA CATGGTGCTA GTTACCGTAT TAGTTTTGAA   600

86/137004 S     ---------- ---------- ---------- ---------- ----------    59
HOL87 S         ---------- ---------- ---------- ---------- ----------    59
RM4 S           ---------- ---------- ---------- ---------- ----------    59
VMRI S          ..C....... .......... .......... .......... .......G..   650
NVSL S          ---------- ---------- ---------- ---------- ----------    27
Miller S        ..T....... .......... .......... .......... .......A..   647
Consensus       AAYCAATGGT CTGGCACTGT TACACTTGGT GATATGCGTG CGACTACRTT   650

86/137004 S     ---------- ---------- ---------- ---------- ----------    59
HOL87 S         ---------- ---------- ---------- ---------- ----------    59
RM4 S           ---------- ---------- ---------- ---------- ----------    59
VMRI S          .C........ ....T..... .......... .........C ..........   700
NVSL S          ---------- ---------- ---------- ---------- ----------    27
Miller S        .G........ ....C..... .......... .........T ..........   697
Consensus       ASAAACCGCT GGCAYGCTTG TAGACCTTTG GTGGTTTAAY CCTGTTTATG   700

86/137004 S     ---------- ---------- ---------- ----....T. ..........    75
HOL87 S         ---------- ---------- ---------- ----....T. ..........    75
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Figure 6: (continued)

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RM4 S          ---------- ---------- ---------- ----....T. .......... 75
VMRI S         .......... .......... .......... ........A. .......... 750
NVSL S         ---------- ---------- ---------- ---------- -......... 36
Miller S       .......... .......... .......... ........A. .......... 747
Consensus      ATGTCAGTTA TTATAGAGTT AATAATAAAA ATGGTACTWC CGTAGTTTCC 750

86/137004 S    .......... ....A..... .......... .......... ..AC..C..A 125
HOL87 S        .......... ....A..... .......... .......... ..AC..C..A 125
RM4 S          .......... ....A..... .......... .......... ..AC..T..T 125
VMRI S         .......... ....G..... .......... .......... ..GT..C..A 800
NVSL S         .......... ....G..... .......... .......... ..GT..C..A 86
Miller S       .......... ....A..... .......... .......... ..AC..C..A 797
Consensus      AATTGCACTG ATCARTGTGC TAGTTATGTG GCTAATGTTT TTRYTAYACW 800

86/137004 S    G......G.. .......... .......... .......... ......A.C. 175
HOL87 S        G......G.. .......... .......... .......... ......C.C. 175
RM4 S          A......G.. .......... .......... .......... ......C.C. 175
VMRI S         G......T.. .......... .......... .......... ......C.T. 850
NVSL S         G......G.. .......... .......... .......... ......C.T. 136
Miller S       G......G.. .......... .......... .......... ......C.T. 847
Consensus      RCCAGGAKGC TTTATACCAT CAGATTTTAG TTTTAATAAT TGGTTCMTYC 850

86/137004 S    .......... .......... ....G...C. .......... .........T 225
HOL87 S        .......... .......... ....G...T. .......... .........T 225
RM4 S          .......... .......... ....A...T. .......... .........T 225
VMRI S         .......... .......... ....G...T. .......... .........A 900
NVSL S         .......... .......... ....G...T. .......... .........A 186
Miller S       .......... .......... ....G...T. .......... .........G 897
Consensus      TAACTAATAG CTCCACGTTG GTTARTGGYA AATTAGTTAC CAAACAGCCD 900

86/137004 S    C......... .......... .......... .......... ...C...... 275
HOL87 S        C......... .......... .......... .......... ...C...... 275
RM4 S          C......... .......... .......... .......... ...T...... 275
VMRI S         T......... .......... .......... .......... ...C...... 950
NVSL S         T......... .......... .......... .......... ...C...... 236
Miller S       T......... .......... .......... .......... ...C...... 947
Consensus      YTATTAGTTA ATTGCTTATG GCCAGTCCCT AGCTTTGAAG AAGYAGCTTC 950

86/137004 S    ....T..... .......... ...A...... .......... ...G....T. 325
HOL87 S        ....T..... .......... ...A...... .......... ...G....T. 325
RM4 S          ....T..... .......... ...A...... .......... ...G....T. 325
VMRI S         ....T..... .......... ...A...... .......... ...G....C. 1000
NVSL   S       ....T..... .......... ...A...... .......... ...G....C. 286
Miller S       ....C..... .......... ...G...... .......... ...C....T. 997
Consensus      TACAYTTTGT TTTGAAGGTG CTGRCTTTGA TCAATGTAAT GGTSCTGTYT 1000

86/137004 S    .......C.. ......C... ..T.....T. .......... .......... 375
HOL87 S        .......C.. ......C... ..T.....C. .......... .......... 375
RM4 S          .......C.. ......C... ..T.....T. .......... .......... 375
VMRI S         .......T.. ......T... ..C.....T. .......... .......... 1050
NVSL S         .......T.. ......T... ..C.....T. .......... .......... 336
Miller S       .......C.. ......C... ..C.....T. .......... .......... 1047
Consensus      TAAATAAYAC TGTAGAYGTC ATYAGGTTYA ACCTTAATTT TACTACAAAT 1050
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Figure 6: (continued)

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86/137004 S    .......... .......... T......... .......... ..........   425
HOL87 S        .......... .......... T......... .......... ..........   425
RM4 S          .......... .......... T......... .......... ..........   425
VMRI S         .......... .......... C......... .......... ..........  1100
NVSL S         .......... .......... C......... .......... ..........   386
Miller S       .......... .......... C......... .......... ..........  1097
Consensus      GTACAATCAG GTAAGGGTGC YACAGTGTTT TCATTGAACA CAACGGGTGG  1100

86/137004 S    .......... .......... .......... .......... ..T.....C.   475
HOL87 S        .......... .......... .......... .......... ..T.....T.   475
RM4 S          .......... .......... .......... .......... ..T.....C.   475
VMRI S         .......... .......... .......... .......... ..C.....C.  1150
NVSL S         .......... .......... .......... .......... ..C.....C.   436
Miller S       .......... .......... .......... .......... ..C.....C.  1147
Consensus      TGTCACTCTT GAAATCTCAT GTTATAATGA TACAGTGAGT GAYTCGAGYT  1150

86/137004 S    .......... .........T ..G....... .A...A.... ..........   525
HOL87 S        .......... .........T ..T....... .A...A.... ..........   525
RM4 S          .......... .........T ..G....... .A...A.... ..........   525
VMRI S         .......... .........T ..G....... .G...G.... ..........  1200
NVSL S         .......... .........T ..G....... .G...G.... ..........   486
Miller S       .......... .........G ..G....... .A...G.... ..........  1197
Consensus      TTTCCAGTTA CGGTGAAATK CCKTTCGGCG TRACTRATGG ACCACGGTAC  1200

86/137004 S    .......... .......... .......... ......C... ..........   575
HOL87 S        .......... .......... .......... ......C... ..........   575
RM4 S          .......... .......... .......... ......C... ..........   575
VMRI S         .......... .......... .......... ......T... ..........  1250
NVSL S         .......... .......... .......... ......T... ..........   536
Miller S       .......... .......... .......... ......C... ..........  1247
Consensus      TGTTACGTAC TCTATAATGG CACAGCTCTT AAGTATYTAG GAACATTACC  1250

86/137004 S    .......... .......... .......... .......... ..........   625
HOL87 S        .......... .......... .......... .......... ..........   625
RM4 S          .......... .......... .......... .......... ..........   625
VMRI S         .......... .......... .......... .......... ..........  1300
NVSL S         .......... .......... .......... .......... ..........   586
Miller S       .......... .......... .......... .......... ..........  1297
Consensus      ACCTAGTGTC AAGGAGATTG CTATTAGTAA GTGGGGCCAT TTTTATATTA  1300

86/137004 S    .......... .......... .......... .......... ......T...   675
HOL87 S        .......... .......... .......... .......... ......T...   675
RM4 S          .......... .......... .......... .......... ......T...   675
VMRI S         .......... .......... .......... .......... ......C...  1350
NVSL S         .......... .......... .......... .......... ......C...   636
Miller S       .......... .......... .......... .......... ......T...  1347
Consensus      ATGGTTACAA TTTCTTTAGC ACATTTCCTA TTGATTGTAT ATCTTTYAAT  1350

86/137004 S    .....T.... .T........ ...C...... .......... ..........   725
HOL87 S        .....T.... .T........ ...C...... .......... ..........   725
RM4 S          .....T.... .T........ ...C...... .......... ..........   725
VMRI S         .....C.... .C........ ...T...... .......... ..........  1400
NVSL S         .....C.... .C........ ...T...... .......... ..........   686
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Figure 6: (continued)

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Miller S        .....C.... .T........ ...T...... .......... ..........   1397
Consensus       TTGACYACTG GYGATAGTGA CGTYTTCTGG ACAATAGCTT ACACATCGTA   1400

86/137004 S     .........A .......... .......... .......... ..T.......   775
HOL87 S         .........A .......... .......... .......... ..T.......   775
RM4 S           .........A .......... .......... .......... ..T.......   775
VMRI S          .........G .......... .......... .......... ..G.......   1450
NVSL S          .........G .......... .......... .......... ..G.......   736
Miller S        .........A .......... .......... .......... ..G.......   1447
Consensus       CACTGAAGCR TTAGTACAAG TTGAAAACAC AGCTATTACA AAKGTGACGT   1450

86/137004 S     .......... ...T...... .......... .......... ..........   825
HOL87 S         .......... ...T...... .......... .......... ..........   825
RM4 S           .......... ...T...... .......... .......... ..........   825
VMRI S          .......... ...C...... .......... .......... ..........   1500
NVSL S          .......... ...C...... .......... .......... ..........   786
Miller S        .......... ...C...... .......... .......... ..........   1497
Consensus       ATTGTAATAG TTAYGTTAAT AACATTAAAT GCTCTCAACT TACTGCTAAT   1500

86/137004 S     .......... .......T.. .......... .......... ..TC......   875
HOL87 S         .......... .......T.. .......... .......... ..TC......   875
RM4 S           .......... .......T.. .......... .......... ..TC......   875
VMRI S          .......... .......C.. .......... .......... ..TT......   1550
NVSL S          .......... .......C.. .......... .......... ..TT......   836
Miller S        .......... .......T.. .......... .......... ..CT......   1547
Consensus       TTGAATAATG GATTTTAYCC TGTTTCTTCA AGTGAAGTTG GTYYTGTCAA   1550

86/137004 S     .........T .......... ........CT G..A...... ..........   925
HOL87 S         .........T .......... ........CT G..A...... ..........   925
RM4 S           .........T .......... ........CT G..A...... ..........   925
VMRI S          .........T .......... ........TA C..G...... ..........   1600
NVSL S          .........A .......... ........TA C..G...... ..........   886
Miller S        .........T .......... ........TA C..A...... ..........   1597
Consensus       TAAGAGTGTW GTGTTACTAC CTAGCTTTYW SACRCATACC ATTGTTAACA   1600

86/137004 S     .........G .......... .......... .......... ..........   975
HOL87 S         .........G .......... .......... .......... ..........   975
RM4 S           .........G .......... .......... .......... ..........   975
VMRI S          .........A .......... .......... .......... ..........   1650
NVSL S          .........G .......... .......... .......... ..........   936
Miller S        .........G .......... .......... .......... ..........   1647
Consensus       TAACTATTGR TCTTGGTATG AAGCGTAGTG GTTATGGTCA ACCCATAGCC   1650

86/137004 S     .....GC... .......... .......... .......... ...C......   1025
HOL87 S         .....GC... .......... .......... .......... ...C......   1025
RM4 S           .....GC... .......... .......... .......... ...A......   1025
VMRI S          .....TC... .......... .......... .......... ...C......   1700
NVSL S          .....TC... .......... .......... .......... ...C......   986
Miller S        .....AT... .......... .......... .......... ...C......   1697
Consensus       TCAACDYTAA GTAACATTAC ACTACCAATG CAGGATAACA ACAMCGATGT   1700

86/137004 S     .......G.. ........C. .......... .......... ........C.   1075
HOL87 S         .......G.. ........C. .......... .......... ........C.   1075
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Figure 6: (continued)

```
RM4 S          .......G.. ........C. .......... .......... ........C.    1075
VMRI S         .......A.. ........T. .......... .......... ........T.    1750
NVSL S         .......A.. ........T. .......... .......... ........T.    1036
Miller S       .......A.. ........C. .......... .......... ........C.    1747
Consensus      GTACTGTRTT CGTTCTGAYC AATTTTCAGT TTATGTTCAT TCTACTTGYA    1750

86/137004 S    .....GC... .......... .......... .......... ..........    1125
HOL87 S        .....GC... .......... .......... .......... ..........    1125
RM4 S          .....GT... .......... .......... .......... ..........    1125
VMRI S         .....GC... .......... .......... .......... ..........    1800
NVSL S         .....GC... .......... .......... .......... ..........    1086
Miller S       .....TC... .......... .......... .......... ..........    1797
Consensus      AAAGTKYTTT ATGGGACAAT GTTTTTAAGC GAAACTGCAC GGACGTTTTA    1800

86/137004 S    .......... .......... .......... .......... ..........    1175
HOL87 S        .......... .......... .......... .......... ..........    1175
RM4 S          .......... .......... .......... .......... ..........    1175
VMRI S         .......... .......... .......... .......... ..........    1850
NVSL S         .......... .......... .......... .......... ..........    1136
Miller S       .......... .......... .......... .......... ..........    1847
Consensus      GATGCCACAG CTGTTATAAA AACTGGTACT TGTCCTTTCT CATTTGATAA    1850

86/137004 S    .T........ .......... .......... ......G... ........T.    1225
HOL87 S        .T........ .......... .......... ......G... ........T.    1225
RM4 S          .T........ .......... .......... ......G... ........C.    1225
VMRI S         .C........ .......... .......... ......T... ........T.    1900
NVSL S         .C........ .......... .......... ......T... ........T.    1186
Miller S       .T........ .......... .......... ......G... ........T.    1897
Consensus      AYTGAACAAT TACTTAACTT TTAACAAGTT CTGTTTKTCG TTGAGTCCYG    1900

86/137004 S    .......... .......... .......C.. .......A.. .........T    1275
HOL87 S        .......... .......... .......C.. .......C.. .........G    1275
RM4 S          .......... .......... .......C.. .......A.. .........T    1275
VMRI S         .......... .......... .......T.. .......A.. .........T    1950
NVSL S         .......... .......... .......T.. .......A.. .........T    1236
Miller S       .......... .......... .......C.. .......A.. .........T    1947
Consensus      TTGGTGCTAA TTGTAAGTTT GATGTAGYTG CCCGTACMAG AACCAATGAK    1950

86/137004 S    ...T...... .......... .......... .......... ...G......    1325
HOL87 S        ...G...... .......... .......... .......... ...G......    1325
RM4 S          ...G...... .......... .......... .......... ...G......    1325
VMRI S         ...G...... .......... .......... .......... ...A......    2000
NVSL S         ...G...... .......... .......... .......... ...A......    1286
Miller S       ...G...... .......... .......... .......... ...A......    1997
Consensus      CAGKTTGTTA GAAGTTTGTA TGTAATATAT GAAGAAGGAG ACARCATAGT    2000

86/137004 S    T......... .....C.... .......G.. .......... ..........    1375
HOL87 S        T......... .....C.... .......G.. .......... ..........    1375
RM4 S          T......... .....C.... .......G.. .......... ..........    1375
VMRI S         A......... .....C.... .......G.. .......... ..........    2050
NVSL S         A......... .....C.... .......G.. .......... ..........    1336
Miller S       G......... .....T.... .......A.. .......... ..........    2047
Consensus      DGGTGTACCG TCTGAYAATA GTGGTTTRCA CGATTTGTCA GTGCTACACC    2050
```

Figure 6: (continued)

```
86/137004 S     .......G.. .......... .......... .......... ..........   1425
HOL87 S         .......G.. .......... .......... .......... ..........   1425
RM4 S           .......G.. .......... .......... .......... ..........   1425
VMRI S          .......A.. .......... .......... .......... ..........   2100
NVSL S          .......A.. .......... .......... .......... ..........   1386
Miller S        .......C.. .......... .......... .......... ..........   2097
Consensus       TAGATTCVTG CACAGATTAC AATATATATG GTAGAACTGG TGTTGGTATT   2100

86/137004 S     .......... .......... GC........ .......... ..........   1475
HOL87 S         .......... .......... GC........ .......... ..........   1475
RM4 S           .......... .......... GA........ .......... ..........   1475
VMRI S          .......... .......... AC........ .......... ..........   2150
NVSL S          .......... .......... AC........ .......... ..........   1436
Miller S        .......... .......... GC........ .......... ..........   2147
Consensus       ATTAGACAAA CTAACAGGAC RMTACTTAGT GGCTTATATT ACACATCACT   2150

86/137004 S     ...T...... .......... ...A...... .......... G.T.......   1525
HOL87 S         ...T...... .......... ...A...... .......... G.T.......   1525
RM4 S           ...T...... .......... ...C...... .......... G.T.......   1525
VMRI S          ...T...... .......... ...A...... .......... A.T.......   2200
NVSL S          ...T...... .......... ...A...... .......... A.T.......   1486
Miller S        ...A...... .......... ...A...... .......... G.C.......   2197
Consensus       ATCWGGTGAT TTGTTAGGTT TTAMAAATGT TAGTGATGGT RTYATCTACT   2200

86/137004 S     .......... A........T .......... .....A.... .......G..   1575
HOL87 S         .......... A........T .......... .....G.... .......A..   1575
RM4 S           .......... A........T .......... .....A.... .......A..   1575
VMRI S          .......... G........A .......... .....G.... .......A..   2250
NVSL S          .......... G........A .......... .....G.... .......A..   1536
Miller S        .......... A........A .......... .....G.... .......A..   2247
Consensus       CTGTAACGCC RTGTGATGTW AGCGCACAAG CAGCTRTTAT TGATGGTRCC   2250

86/137004 S     .......... .......... C......... ...T...... C......A..   1625
HOL87 S         .......... .......... C......... ...T...... G......A..   1625
RM4 S           .......... .......... C......... ...T...... G......A..   1625
VMRI S          .......... .......... T......... ...T...... G......G..   2300
NVSL S          .......... .......... T......... ...T...... G......G..   1586
Miller S        .......... .......... C......... ...C...... G......A..   2297
Consensus       ATAGTTGGGG CTATCACTTC YATTAACAGT GAAYTGTTAG STCTAACRCA   2300

86/137004 S     ........T. .......... .......... .......... ..........   1675
HOL87 S         ........T. .......... .......... .......... ..........   1675
RM4 S           ........C. .......... .......... .......... ..........   1675
VMRI S          ........C. .......... .......... .......... ..........   2350
NVSL S          ........C. .......... .......... .......... ..........   1636
Miller S        ........C. .......... .......... .......... ..........   2347
Consensus       TTGGACAAYA ACACCTAATT TTTATTACTA CTCTATATAT AATTACACAA   2350

86/137004 S     ......A..C .......... C......G.. .....G.C.. ..A.......   1725
HOL87 S         ......A..C .......... C......A.. .....G.C.. ..G.......   1725
RM4 S           ......A..C .......... C......G.. .....G.C.. ..A.......   1725
VMRI S          ......G..T .......... G......A.. .....C.C.. ..A.......   2400
NVSL S          ......G..T .......... G......A.. .....G.C.. ..A.......   1686
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Figure 6: (continued)

```
Miller S        ......G..C .......... G......A.. .....G.T.. ..A.......    2397
Consensus       ATGATARGAY TCGTGGCACT SCAATTGRCA GTAATSAYGT TGRTTGTGAA    2400

86/137004 S     .......... .......... .......... ........T. ..........    1775
HOL87 S         .......... .......... .......... ........T. ..........    1775
RM4 S           .......... .......... .......... ........T. ..........    1775
VMRI S          .......... .......... .......... ........C. ..........    2450
NVSL S          .......... .......... .......... ........C. ..........    1736
Miller S        .......... .......... .......... ........T. ..........    2447
Consensus       CCTGTCATAA CCTATTCTAA CATAGGTGTT TGTAAAAAYG GTGCTTTGGT    2450

86/137004 S     ......T... .......... .......... .......... ..T.......    1825
HOL87 S         ......T... .......... .......... .......... ..A.......    1825
RM4 S           ......T... .......... .......... .......... ..T.......    1825
VMRI S          ......C... .......... .......... .......... ..T.......    2500
NVSL S          ......C... .......... .......... .......... ..T.......    1786
Miller S        ......T... .......... .......... .......... ..T.......    2497
Consensus       TTTTATYAAC GTCACACATT CTGATGGAGA CGTGCAACCA ATWAGCACTG    2500

86/137004 S     ....C..... .........T ..C....... .......... .........C    1875
HOL87 S         ....T..... .........T ..C....... .......... .........C    1875
RM4 S           ....C..... .........T ..C....... .......... .........C    1875
VMRI S          ....C..... .........A ..T....... .......... .........C    2550
NVSL S          ....C..... .........A ..T....... .......... .........C    1836
Miller S        ....C..... .........A ..C....... .......... .........T    2547
Consensus       GTAAYGTCAC GATACCTACW AAYTTTACTA TATCCGTGCA AGTCGAATAY    2550

86/137004 S     .......... .......... .......... .......... ....T.....    1925
HOL87 S         .......... .......... .......... .......... ....T.....    1925
RM4 S           .......... .......... .......... .......... ....T.....    1925
VMRI S          .......... .......... .......... .......... ....C.....    2600
NVSL S          .......... .......... .......... .......... ....C.....    1886
Miller S        .......... .......... .......... .......... ....T.....    2597
Consensus       ATTCAGGTTT ACACTACACC AGTGTCAATA GACTGTTCAA GATAYGTTTG    2600

86/137004 S     .......... .......... .....C.G.. .........C ..........    1975
HOL87 S         .......... .......... .....C.G.. .........C ..........    1975
RM4 S           .......... .......... .....C.G.. .........C ..........    1975
VMRI S          .......... .......... .....C.A.. .........T ..........    2650
NVSL S          .......... .......... .....C.A.. .........T ..........    1936
Miller S        .......... .......... .....T.G.. .........C ..........    2647
Consensus       TAATGGCAAC CCTAGGTGTA ACAAAYTRTT AACACAATAY GTTTCTGCAT    2650

86/137004 S     .......... .......... .......... .......... ......C...    2025
HOL87 S         .......... .......... .......... .......... ......C...    2025
RM4 S           .......... .......... .......... .......... ......C...    2025
VMRI S          .......... .......... .......... .......... ......T...    2700
NVSL S          .......... .......... .......... .......... ......T...    1986
Miller S        .......... .......... .......... .......... ......C...    2697
Consensus       GTCAAACTAT TGAGCAAGCA CTTGCAATGG GTGCCAGACT TGAAAAYATG    2700

86/137004 S     .......A.. .......... .......... .......... ..........    2075
HOL87 S         .......A.. .......... .......... .......... ..........    2075
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Figure 6: (continued)

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RM4 S          .......A.. .......... .......... .......... ..........   2075
VMRI S         .......A.. .......... .......... .......... ..........   2750
NVSL S         .......A.. .......... .......... .......... ..........   2036
Miller S       .......G.. .......... .......... .......... ..........   2747
Consensus      GAAGTTGRTT CCATGTTATT TGTTTCTGAA AATGCCCTTA AATTGGCTTC   2750

86/137004 S    ...C...... .......... .....A.... .......... ....A.G.A.   2125
HOL87 S        ...T...... .......... .....A.... .......... ....C.C.A.   2125
RM4 S          ...C...... .......... .....A.... .......... ....A.G.A.   2125
VMRI S         ...T...... .......... .....G.... .......... ....A.G.C.   2800
NVSL S         ...T...... .......... .....G.... .......... ....A.G.C.   2086
Miller S       ...C...... .......... .....A.... .......... ....A.G.A.   2797
Consensus      TGTYGAAGCA TTCAATAGTT CAGAARCTTT AGATCCTATT TACAMASAMT   2800

86/137004 S    ......A... ........T. .......... .......... C.....T...   2175
HOL87 S        ......A... ........T. .......... .......... C.....T...   2175
RM4 S          ......A... ........T. .......... .......... C.....T...   2175
VMRI S         ......G... ........C. .......... .......... T.....G...   2850
NVSL S         ......G... ........C. .......... .......... T.....G...   2136
Miller S       ......A... ........C. .......... .......... C.....T...   2847
Consensus      GGCCTARTAT AGGTGGCTYT TGGCTAGAAG GTCTAAAATA YATACTKCCG   2850

86/137004 S    .......... ........A. .......... .......... ..........   2225
HOL87 S        .......... ........A. .......... .......... ..........   2225
RM4 S          .......... ........A. .......... .......... ..........   2225
VMRI S         .......... ........C. .......... .......... ..........   2900
NVSL S         .......... ........C. .......... .......... ..........   2186
Miller S       .......... ........A. .......... .......... ..........   2897
Consensus      TCCGATAATA GCAAACGTMA GTATCGTTCA GCTATAGAGG ACTTGCTTTT   2900

86/137004 S    .T........ .......... .......... .......... .....C....   2275
HOL87 S        .T........ .......... .......... .......... .....C....   2275
RM4 S          .T........ .......... .......... .......... .....C....   2275
VMRI S         .T........ .......... .......... .......... .....T....   2950
NVSL S         .T........ .......... .......... .......... .....T....   2236
Miller S       .G........ .......... .......... .......... .....T....   2947
Consensus      TKCTAAGGTT GTAACATCTG GTTTAGGTAC AGTTGATGAA GATTAYAAAC   2950

86/137004 S    .......... .........C .......... ....A..... ......C..T   2325
HOL87 S        .......... .........C .......... ....A..... ......C..T   2325
RM4 S          .......... .........C .......... ....A..... ......C..T   2325
VMRI S         .......... .........T .......... ....G..... ......T..T   3000
NVSL S         .......... .........T .......... ....G..... ......T..T   2286
Miller S       .......... .........C .......... ....A..... ......C..C   2997
Consensus      GTTGTACAGG TGGTTATGAY ATAGCTGACT TAGTRTGTGC TCAATAYTAY   3000

86/137004 S    ........C. .......... .......... .......... ..........   2375
HOL87 S        ........C. .......... .......... .......... ..........   2375
RM4 S          ........T. .......... .......... .......... ..........   2375
VMRI S         ........C. .......... .......... .......... ..........   3050
NVSL S         ........C. .......... .......... .......... ..........   2336
Miller S       ........C. .......... .......... .......... ..........   3047
Consensus      AATGGCATYA TGGTGCTACC TGGTGTGGCT AATGCTGACA AAATGACTAT   3050
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Figure 6: (continued)

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86/137004 S    ...C..... ..........  .......... .......... C.........  2425
HOL87 S        ...T..... ..........  .......... .......... T.........  2425
RM4 S          ...C..... ..........  .......... .......... C.........  2425
VMRI S         ...C..... ..........  .......... .......... C.........  3100
NVSL S         ...C..... ..........  .......... .......... C.........  2386
Miller S       ...C..... ..........  .......... .......... C.........  3097
Consensus      GTAYACAGCA TCCCTCGCAG  GTGGTATAAC ATTAGGTGCA YTTGGTGGAG  3100

86/137004 S    .C.....GG. ..........  .......... ........A. ..........  2475
HOL87 S        .C.....GT. ..........  .......... ........C. ..........  2475
RM4 S          .C.....GG. ..........  .......... ........A. ..........  2475
VMRI S         .T.....AG. ..........  .......... ........A. ..........  3150
NVSL S         .T.....AG. ..........  .......... ........A. ..........  2436
Miller S       .C.....GG. ..........  .......... ........A. ..........  3147
Consensus      GYGCCGTRKC TATACCTTTT  GCAGTAGCAG TTCAGGCTMG ACTTAATTAT  3150

86/137004 S    .......... ..........  ...G...... .......... ..........  2525
HOL87 S        .......... ..........  ...G...... .......... ..........  2525
RM4 S          .......... ..........  ...G...... .......... ..........  2525
VMRI S         .......... ..........  ...A...... .......... ..........  3200
NVSL S         .......... ..........  ...A...... .......... ..........  2486
Miller S       .......... ..........  ...G...... .......... ..........  3197
Consensus      GTTGCTCTAC AAACTGATGT  ATTRAACAAA AACCAGCAGA TCCTGGCTAG  3200

86/137004 S    ......T... ..........  .......... .......... ..........  2575
HOL87 S        ......T... ..........  .......... .......... ..........  2575
RM4 S          ......T... ..........  .......... .......... ..........  2575
VMRI S         ......T... ..........  .......... .......... ..........  3250
NVSL S         ......T... ..........  .......... .......... ..........  2536
Miller S       ......C... ..........  .......... .......... ..........  3247
Consensus      TGCTTTYAAT CAAGCTATTG  GTAACATTAC ACAGTCATTT GGTAAGGTTA  3250

86/137004 S    .......... .........A  .......... ..A....... ..........  2625
HOL87 S        .......... .........A  .......... ..A....... ..........  2625
RM4 S          .......... .........A  .......... ..A....... ..........  2625
VMRI S         .......... .........G  .......... ..G....... ..........  3300
NVSL S         .......... .........G  .......... ..G....... ..........  2586
Miller S       .......... .........A  .......... ..G....... ..........  3297
Consensus      ATGATGCTAT ACATCAAACR  TCACGAGGTC TTRCAACTGT TGCTAAAGCA  3300

86/137004 S    .......... ..........  ...C..C... .....T.... .......A..  2675
HOL87 S        .......... ..........  ...C..C... .....T.... .......A..  2675
RM4 S          .......... ..........  ...C..C... .....T.... .......A..  2675
VMRI S         .......... ..........  ...T..T... .....G.... .......C..  3350
NVSL S         .......... ..........  ...T..T... .....G.... .......C..  2636
Miller S       .......... ..........  ...C..C... .....G.... .......C..  3347
Consensus      TTGGCAAAAG TGCAAGATGT  TGTYAAYACA CAAGGKCAAG CTTTAAGMCA  3350

86/137004 S    .......... ..........  .......... .......... ..T.......  2725
HOL87 S        .......... ..........  .......... .......... ..T.......  2725
RM4 S          .......... ..........  .......... .......... ..T.......  2725
VMRI S         .......... ..........  .......... .......... ..C.......  3400
NVSL S         .......... ..........  .......... .......... ..C.......  2686
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Figure 6: (continued)

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Miller S        .......... .......... .......... .......... ..T.......    3397
Consensus       CCTAACAGTA CAATTGCAAA ATAATTTCCA AGCCATTAGT AGYTCTATTA    3400

86/137004 S     .......... .......... .......... .......... ......C...    2775
HOL87 S         .......... .......... .......... .......... ......C...    2775
RM4 S           .......... .......... .......... .......... ......C...    2775
VMRI S          .......... .......... .......... .......... ......T...    3450
NVSL S          .......... .......... .......... .......... ......T...    2736
Miller S        .......... .......... .......... .......... ......T...    3447
Consensus       GTGACATTTA TAATAGGCTT GATGAATTGA GTGCTGATGC ACAAGTYGAC    3450

86/137004 S     .......... .......... .......... .......... .G........    2825
HOL87 S         .......... .......... .......... .......... .G........    2825
RM4 S           .......... .......... .......... .......... .G........    2825
VMRI S          .......... .......... .......... .......... .A........    3500
NVSL S          .......... .......... .......... .......... .A........    2786
Miller S        .......... .......... .......... .......... .G........    3497
Consensus       AGGCTGATCA CAGGAAGACT TACAGCACTT AATGCATTTG TRTCTCAGAC    3500

86/137004 S     .......... .......... .......... T......... ..T.......    2875
HOL87 S         .......... .......... .......... T......... ..T.......    2875
RM4 S           .......... .......... .......... T......... ..T.......    2875
VMRI S          .......... .......... .......... C......... ..C.......    3550
NVSL S          .......... .......... .......... C......... ..C.......    2836
Miller S        .......... .......... .......... T......... ..C.......    3547
Consensus       TCTAACCAGA CAAGCCGAGG TTAGGGCTAG YAGACAACTT GCYAAAGACA    3550

86/137004 S     .......... ........A. ........T. .T........ C.........    2925
HOL87 S         .......... ........G. ........T. .G........ C.........    2925
RM4 S           .......... ........G. ........T. .G........ C.........    2925
VMRI S          .......... ........G. ........C. .G........ C.........    3600
NVSL S          .......... ........G. ........C. .G........ C.........    2886
Miller S        .......... ........G. ........T. .G........ A.........    3597
Consensus       AGGTTAATGA ATGCGTTARG TCTCAGTCYC AKAGATTCGG MTTCTGTGGT    3600

86/137004 S     .......... .......... .......... .......... ..........    2975
HOL87 S         .......... .......... .......... .......... ..........    2975
RM4 S           .......... .......... .......... .......... ..........    2975
VMRI S          .......... .......... .......... .......... ..........    3650
NVSL S          .......... .......... .......... .......... ..........    2936
Miller S        .......... .......... .......... .......... ..........    3647
Consensus       AATGGTACAC ATTTGTTTTC ACTCGCAAAT GCAGCACCAA ATGGCATGAT    3650

86/137004 S     C......... .......... .......... T......... ..........    3025
HOL87 S         C......... .......... .......... T......... ..........    3025
RM4 S           C......... .......... .......... G......... ..........    3025
VMRI S          T......... .......... .......... C......... ..........    3700
NVSL S          T......... .......... .......... C......... ..........    2986
Miller S        C......... .......... .......... T......... ..........    3697
Consensus       YTTCTTTCAC ACAGTGCTAT TACCAACGGC BTATGAAACT GTGACTGCTT    3700

86/137004 S     ..T....... .......... ....G..... .......... ....G.....    3075
HOL87 S         ..T....... .......... ....G..... .......... ....G.....    3075
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Figure 6: (continued)

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RM4 S          ..T..... .......... ....T..... .......... ....G.....   3075
VMRI S         ..G..... .......... ....G..... .......... ....G.....   3750
NVSL S         ..G..... .......... ....G..... .......... ....A.....   3036
Miller S       ..G..... .......... ....G..... .......... ....G.....   3747
Consensus      GGKCAGGTAT TTGTGCTTTA GATGKTGATC GCACTTTTGG ACTTRTCGTT   3750

86/137004 S    .......... ..T..... A......... .......... .T........   3125
HOL87 S        .......... ..T..... A......... .......... .G........   3125
RM4 S          .......... ..T..... A......... .......... .G........   3125
VMRI S         .......... ..C..... G......... .......... .G........   3800
NVSL S         .......... ..C..... G......... .......... .G........   3086
Miller S       .......... ..T..... G......... .......... .G........   3797
Consensus      AAAGATGTCC AGYTGACTTT RTTTCGTAAT CTAGATGACA AKTTCTATTT   3800

86/137004 S    ...A...... .......... .......... .......... ..........   3175
HOL87 S        ...A...... .......... .......... .......... ..........   3175
RM4 S          ...A...... .......... .......... .......... ..........   3175
VMRI S         ...T...... .......... .......... .......... ..........   3850
NVSL S         ...T...... .......... .......... .......... ..........   3136
Miller S       ...C...... .......... .......... .......... ..........   3847
Consensus      GACHCCCAGA ACTATGTATC AGCCTAGAGT GGCAACTAGT TCTGATTTTG   3850

86/137004 S    .......... .......... .......... .....A.... ..........   3225
HOL87 S        .......... .......... .......... .....A.... ..........   3225
RM4 S          .......... .......... .......... .....A.... ..........   3225
VMRI S         .......... .......... .......... .....G.... ..........   3900
NVSL S         .......... .......... .......... .....G.... ..........   3186
Miller S       .......... .......... .......... .....G.... ..........   3897
Consensus      TTCAAATTGA AGGGTGCGAT GTGCTGTTTG TTAATRCAAC TGTAAGTGAT   3900

86/137004 S    .......... .......... .......... ........G. ..........   3275
HOL87 S        .......... .......... .......... ........G. ..........   3275
RM4 S          .......... .......... .......... ........G. ..........   3275
VMRI S         .......... .......... .......... ........A. ..........   3950
NVSL S         .......... .......... .......... ........A. ..........   3236
Miller S       .......... .......... .......... ........G. ..........   3947
Consensus      TTGCCTAGTA TTATACCTGA TTATATTGAT ATTAATCARA CTGTTCAAGA   3950

86/137004 S    ....T..... .....T.... .......... .........G C....TA.G.   3325
HOL87 S        ....T..... .....T.... .......... .........G C....AT.G.   3325
RM4 S          ....T..... .....T.... .......... .........G C....AT.G.   3325
VMRI S         ....C..... .....C.... .......... .........T T....AT.T.   4000
NVSL S         ....C..... .....C.... .......... .........T T....AT.T.   3286
Miller S       ....T..... .....T.... .......... .........G T....AT.T.   3997
Consensus      CATAYTAGAA AATTTYAGAC CAAATTGGAC TGTACCTGAK YTGACWWTKG   4000

86/137004 S    ..G....... .......... .....C.... ...G...... ..........   3375
HOL87 S        ..G....... .......... .....C.... ...G...... ..........   3375
RM4 S          ..G....... .......... .....C.... ...G...... ..........   3375
VMRI S         ..A....... .......... .....T.... ...T...... ..........   4050
NVSL S         ..A....... .......... .....T.... ...T...... ..........   3336
Miller S       ..A....... .......... .....C.... ...G...... ..........   4047
Consensus      ACRTTTTTAA CGCAACCTAT TTAAAYCTGA CTGKTGAAAT TGATGACTTA   4050
```

Figure 6: (continued)

```
86/137004 S    ..G..... .......... .......... .......... ..........   3425
HOL87 S        ..G..... .......... .......... .......... ..........   3425
RM4 S          ..G..... .......... .......... .......... ..........   3425
VMRI S         ..A..... .......... .......... .......... ..........   4100
NVSL S         ..A..... .......... .......... .......... ..........   3386
Miller S       ..A..... .......... .......... .......... ..........   4097
Consensus      GARTTTAGGT CAGAAAAGCT ACATAACACT ACTGTAGAAC TTGCCATTCT   4100

86/137004 S    C.....C... .......... ..T....... .......... ..T.......   3475
HOL87 S        C.....C... .......... ..T....... .......... ..T.......   3475
RM4 S          C.....C... .......... ..G....... .......... ..T.......   3475
VMRI S         T.....T... .......... ..T....... .......... ..T.......   4150
NVSL S         T.....T... .......... ..T....... .......... ..T.......   3436
Miller S       T.....C... .......... ..T....... .......... ..C.......   4147
Consensus      YATTGAYAAC ATTAACAATA CAKTAGTCAA TCTTGAATGG CTYAATAGAA   4150

86/137004 S    .......T.. .......... .......... .......... ......C...   3525
HOL87 S        .......C.. .......... .......... .......... ......C...   3525
RM4 S          .......T.. .......... .......... .......... ......C...   3525
VMRI S         .......C.. .......... .......... .......... ......T...   4200
NVSL S         .......C.. .......... .......... .......... ......T...   3486
Miller S       .......C.. .......... .......... .......... ......C...   4197
Consensus      TTGAAACYTA TGTAAAATGG CCTTGGTATG TGTGGCTACT AATAGGYTTA   4200

86/137004 S    .......... .......... ....C..... .......... ..........   3575
HOL87 S        .......... .......... ....C..... .......... ..........   3575
RM4 S          .......... .......... ....C..... .......... ..........   3575
VMRI S         .......... .......... ....T..... .......... ..........   4250
NVSL S         .......... .......... ....T..... .......... ..........   3536
Miller S       .......... .......... ....C..... .......... ..........   4247
Consensus      GTAGTAATAT TTTGCATACC ATTAYTGCTA TTTTGCTGTT GTAGTACAGG   4250

86/137004 S    .......... ..C....... .......... .......... .......T..   3625
HOL87 S        .......... ..T....... .......... .......... .......T..   3625
RM4 S          .......... ..C....... .......... .......... .......T..   3625
VMRI S         .......... ..C....... .......... .......... .......G..   4300
NVSL S         .......... ..C....... .......... .......... .......G..   3586
Miller S       .......... ..C....... .......... .......... .......G..   4297
Consensus      TTGCTGTGGA TGYATAGGTT GTTTAGGAAG TTGTTGTCAC TCTATATKCA   4300

86/137004 S    .......... A......... ..T....... .......... ..........   3675
HOL87 S        .......... G......... ..T....... .......... ..........   3675
RM4 S          .......... A......... ..T....... .......... ..........   3675
VMRI S         .......... A......... ..T....... .......... ..........   4350
NVSL S         .......... A......... ..T....... .......... ..........   3636
Miller S       .......... A......... ..C....... .......... ..........   4347
Consensus      GTAGAAGACA RTTTGAAAAT TAYGAACCTA TTGAAAAAGT GCACGTCCAT   4350
```

Figure 6: (continued)

Table 8: Percent homology of nucleotide and deduced amino acid of S gene of PRCV isolate NVSL 5170 compared to that of other PRCV isolates.

Virus Strains	% homology with S gene of PRCV isolate NVSL 5170	
	Nucleic Acid Homology	Amino Acid Homology
87/137004	96%	96%
Hol87	96%	96%
RM4	96%	96%

Table 9: The deletion positions and number of deleted nucleotides within S genes of PRCV isolates when compared to S gene of TGEV isolate VMRI 5170.

PRCV Strains	Number of deleted nucleotides with in S genes of PRCV	Base Range of Deletion
NVSL 5170	714	28 - 741
87/137004	675	60 - 734
Hol87	675	60 - 734
RM4	675	60 - 734

```
NVSL5170 (3681-4824)  .......... ......A... ....T..... .......... ..........   3730
VMRI5170 (4418-5561)  .......... ......A... ....T..... .......... ..........   4467
PRCV-IA1894 (24-1138) .......... ......A... ....T..... .......... ..........   73
PRCV-LEPP (24-1165)   .......... ......A... ....T..... .......... ..........   73
PRCV-AR310 (24-1165)  .......... ......A... ....T..... .......... ..........   73
PRCV-ISU1 (24-876)    .......... ......G... ....C..... ---------- ----------   53
Consensus             TAAATTTAAA ATGTTARTTT TATCYGCTAT AATAGCATTT GTTATTAAGG   50

NVSL5170 (3681-4824)  .......... .......... .......... ...A...... ..........   3780
VMRI5170 (4418-5561)  .......... .......... .......... ...A...... ..........   4517
PRCV-IA1894 (24-1138) .......... .......... .......... ...A...... ..........   123
PRCV-LEPP (24-1165)   .......... .......... .......... ...T...... ..........   123
PRCV-AR310 (24-1165)  .......... .......... .......... ...A...... ..........   123
PRCV-ISU1 (24-876)    ---------- ---------- ---------- ---------- ----------   53
                                              ♠♠♠♠♠
Consensus             ATGATGAATA AAGTCCTTAA GAACTAAACT TTCWGGTCAT TACAGGTCCT   100
```

Figure 7: Comparison of the nucleotide sequences of the ORF 3/3.1 region of the TGEV isolate, VMRI 5170, the PRCV isolate, NVSL 5170, and other PRCV isolates.

Note : The positions of intergenic sequences are underlined and marked with the symbol ♠. The start codons and stop codons of each ORF are underlined and labeled with |--> and <--|, respectively. The positions having identical nucleotides are presented as dots and the positions of deleted nucleotides are marked by dashes. The 2 different nucleotides among VMRI 5170 and NVSL 5170 isolates are bold letters.

```
NVSL5170 (3681-4824)  .......... ..T......C ....A..... .......... ..........   3830
VMRI5170 (4418-5561)  .......... ..T......C ....A..... .......... ..........   4567
PRCV-IA1894 (24-1138) .......... ..G......C ....T..... .......... ..........   173
PRCV-LEPP (24-1165)   .......... ..T......T ....A..... .......... ..........   173
PRCV-AR310 (24-1165)  .......... ..T......T ....A..... .......... ..........   173
PRCV-ISU1 (24-876)    ---------- ---------- ---------- ---------- ----------   53
                        |-->start ORF3
Consensus             GTATGGACAT TGKCAAATCY ATTAWTACAT CCGTGGATGC TGTACTTGAC   150

NVSL5170 (3681-4824)  .......... .......... ......T... .......... ..........   3880
VMRI5170 (4418-5561)  .......... .......... ......T... .......... ..........   4617
PRCV-IA1894 (24-1138) .......... .......... ......A... .......... ..........   223
PRCV-LEPP (24-1165)   .......... .......... ......T... .......... ..........   223
PRCV-AR310 (24-1165)  .......... .......... ......T... .......... ..........   223
PRCV-ISU1 (24-876)    ---------- ---------- ---------- ---------- --------..   55
Consensus             GAACTTGATT GTGCATACTT CGCTGTWACT CTTAAAGTAG AATTTAAGAC   200

NVSL5170 (3681-4824)  .....G.... .......... .......... .......... ..........   3930
VMRI5170 (4418-5561)  .....G.... .......... .......... .......... ..........   4667
PRCV-IA1894 (24-1138) .....A.... .......... .......... .......... ..........   273
PRCV-LEPP (24-1165)   .....A.... .......... .......... .......... ..........   273
PRCV-AR310 (24-1165)  .....A.... .......... .......... .......... ..........   273
PRCV-ISU1 (24-876)    .....A.... .......... .......... .......... ..........   105
Consensus             TGGTARATTA CTTGTGTGTA TAGGTTTTGG TGACACACTT CTTGCGGCTA   250

NVSL5170 (3681-4824)  ...A...... .......... .......... .......... ..........   3980
VMRI5170 (4418-5561)  ...A...... .......... .......... .......... ..........   4717
PRCV-IA1894 (24-1138) ...G...... ....------ ---------- -------... ..........   300
PRCV-LEPP (24-1165)   ...A...... .......... .......... .......... ..........   323
PRCV-AR310 (24-1165)  ...A...... .......... .......... .......... ..........   323
PRCV-ISU1 (24-876)    ...A...... .-----.... .......... .......... ..........   150
Consensus             GGGRTAAAGC ATATGCTAAG CTTGGTCTCG CCACTATTGA AGAAGTAAAC   300

NVSL5170 (3681-4824)  .......... .......... .......... .......... ........A.   4030
VMRI5170 (4418-5561)  .......... .......... .......... .......... ........A.   4767
PRCV-IA1894 (24-1138) .......... .......... .......... .......... ........G.   350
PRCV-LEPP (24-1165)   .......... .......... .......... .......... ........A.   373
PRCV-AR310 (24-1165)  .......... .......... .......... .......... ........A.   373
PRCV-ISU1 (24-876)    .......... .......... .......... .......... ........A.   200
                            stop ORF3 <--|
Consensus             ACACAAAATC CAAAGCATTA AGTGTTACAA AACAATTAAA GAGAGATTRT   350

NVSL5170 (3681-4824)  .......... .......... ........G. .......... ..........   4080
VMRI5170 (4418-5561)  .......... .......... ........G. .......... ..........   4817
PRCV-IA1894 (24-1138) .......... .........- ----....T. .......... ..........   395
PRCV-LEPP (24-1165)   .......... .......... ........G. .......... ..........   423
PRCV-AR310 (24-1165)  .......... .......... ........G. .......... ..........   423
PRCV-ISU1 (24-876)    .......... .......... ........G. .......... ..........   250
                                  ♠♠♠♠ ♠           |--> start ORF3.1
Consensus             AGAAAAACTG TCATTCTAAA CTTTGTGTKA AAATGATTGG TGGACTTTTT   400

NVSL5170 (3681-4824)  .......... .......T.. .......... .......... ..........   4130
VMRI5170 (4418-5561)  .......... .......T.. .......... .......... ..........   4867
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Figure 7: (continued)

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PRCV-IA1894 (24-1138) .......... .......T.. .......... .......... ..........   445
PRCV-LEPP (24-1165)   .......... .......G.. .......... .......... ..........   473
PRCV-AR310 (24-1165)  .......... .......G.. .......... .......... ..........   473
PRCV-ISU1 (24-876)    .......... .......T.. .......... .......... ..........   300
Consensus             CTTAATACTC TGAGTTTKGT AATTGTTAGT AACCATTCTA TTGTTAATAA   450

NVSL5170 (3681-4824)  C......... .....C..C. .......... .......... ...T....G.   4180
VMRI5170 (4418-5561)  C......... .....C..C. .......... .......... ...C....G.   4917
PRCV-IA1894 (24-1138) T......... .....T..T. .......... .......... ...C....T.   495
PRCV-LEPP (24-1165)   C......... .....T..T. .......... .......... ...C....G.   523
PRCV-AR310 (24-1165)  C......... .....T..T. .......... .......... ...C....G.   523
PRCV-ISU1 (24-876)    C......... .....T..T. .........- ---------- ----------   329
Consensus             YACAGCAAAT GTGCAYCAYA CACAACAAGA CCGTGTTATA GTAYAACAKC   500

NVSL5170 (3681-4824)  ........G. .......... A......... ....C...G. ..........   4230
VMRI5170 (4418-5561)  ........G. .......... A......... ....C...T. ..........   4967
PRCV-IA1894 (24-1138) ........A. .......... G......... ....T...G. ..........   545
PRCV-LEPP (24-1165)   ........G. .......... A......... ....C...G. ..........   573
PRCV-AR310 (24-1165)  ........G. .......... A......... ....C...G. ..........   573
PRCV-ISU1 (24-876)    ---------- ---------- ---------- ---------- ----------   329
Consensus             ATCAGGTTRT TAGTGCTAGA RCACAAAATT ATTAYCCAKA GTTCAGCATC   550

NVSL5170 (3681-4824)  .......... .......... CT.......T .....T...A ..........   4280
VMRI5170 (4418-5561)  .......... .......... CT.......T .....T...A ..........   5017
PRCV-IA1894 (24-1138) .......... .......... TC.......G .....C...C ..........   595
PRCV-LEPP (24-1165)   .......... .-........ CC.......G .....T...A ..........   622
PRCV-AR310 (24-1165)  .......... .-........ CC.......G .....T...A ..........   622
PRCV-ISU1 (24-876)    ---------- ---------- ---------- ---------- ------....   333
Consensus             GCTGTACTTT TTGTATCTTT YYTAGCTTTK TACCGYAGTM CAAACTTTAA   600

NVSL5170 (3681-4824)  .......... .......... .......... .......... ..........   4330
VMRI5170 (4418-5561)  .......... .......... .......... .......... ..........   5067
PRCV-IA1894 (24-1138) .......... .......... .......... .......... ..........   645
PRCV-LEPP (24-1165)   .......... .......... .......... .......... ..........   672
PRCV-AR310 (24-1165)  .......... .......... .......... .......... ..........   672
PRCV-ISU1 (24-876)    .......... .......... .......... .......... ..........   383
Consensus             GACGTGTGTC GGTATCTTAA TGTTTAAGAT TTTATCAATG ACACTTTTAG   650

NVSL5170 (3681-4824)  .......... .......... .......... .......... ..........   4380
VMRI5170 (4418-5561)  .......... .......... .......... .......... ..........   5117
PRCV-IA1894 (24-1138) .......... .......... .......... .......... ..........   695
PRCV-LEPP (24-1165)   .......... .......... .......... .......... ..........   722
PRCV-AR310 (24-1165)  .......... .......... .......... .......... ..........   722
PRCV-ISU1 (24-876)    .......... .......... .......... .......... ..........   433
Consensus             GACCTATGCT TATAGTATAT GGTTACTACA TTGATGGCAT TGTTACAACA   700

NVSL5170 (3681-4824)  ...G...... .......... .......... .......... ..........   4430
VMRI5170 (4418-5561)  ...G...... .......... .......... .......... ..........   5167
PRCV-IA1894 (24-1138) ...G...... .......... .......... .......... ..........   745
PRCV-LEPP (24-1165)   ...G...... .......... .......... .......... ..........   772
PRCV-AR310 (24-1165)  ...G...... .......... .......... .......... ..........   772
PRCV-ISU1 (24-876)    ...T...... .......... .......... .......... ..........   483
Consensus             ACTKTCTTAT CTTTAAGATT CGCCTACTTA GCATACTTTT GGTATGTTAA   750
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Figure 7: (continued)

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NVSL5170 (3681-4824)  C..........  ..........  ..........  ..........  ..........  4480
VMRI5170 (4418-5561)  C..........  ..........  ..........  ..........  ..........  5217
PRCV-IA1894 (24-1138) T..........  ..........  ..........  ..........  ..........  795
PRCV-LEPP (24-1165)   T..........  ..........  ..........  ..........  ..........  822
PRCV-AR310 (24-1165)  T..........  ..........  ..........  ..........  ..........  822
PRCV-ISU1 (24-876)    T..........  ..........  ..........  ..........  ..........  533
Consensus             YAGTAGGTTT GAATTTATTT TATACAACAC AACGACACTC ATGTTTGTAC  800

NVSL5170 (3681-4824)  ..........  ..........  ..........  ..........  ..........  4530
VMRI5170 (4418-5561)  ..........  ..........  ..........  ..........  ..........  5267
PRCV-IA1894 (24-1138) ..........  ..........  ..........  ..........  ..........  845
PRCV-LEPP (24-1165)   ..........  ..........  ..........  ..........  ..........  872
PRCV-AR310 (24-1165)  ..........  ..........  ..........  ..........  ..........  872

PRCV-ISU1 (24-876)    ..........  ..........  ..........  ..........  ..........  583
Consensus             ATGGCAGAGC TGCACCGTTT AAGAGAAGTT CTCACAGCTC TATTTATGTC  850

NVSL5170 (3681-4824)  .....A....  ..........  ..........  .........C  ...C......  4580
VMRI5170 (4418-5561)  .....A....  ..........  ..........  .........C  ...C......  5317
PRCV-IA1894 (24-1138) .....G....  ..........  ..........  .........C  ...C......  895
PRCV-LEPP (24-1165)   .....A....  ..........  ..........  .........C  ...C......  922
PRCV-AR310 (24-1165)  .....A....  ..........  ..........  .........C  ...C......  922
PRCV-ISU1 (24-876)    .....A....  ..........  ..........  .........T  ...T......  633
Consensus             ACATTRTATG GTGGCATAAA TTATATGTTT GTGAATGACY TCAYGTTGCA  900

NVSL5170 (3681-4824)  ..........  ..........  ..........  ..........  ...AA.....  4630
VMRI5170 (4418-5561)  ..........  ..........  ..........  ..........  ...AA.....  5367
PRCV-IA1894 (24-1138) ..........  ..........  ..........  ..........  ...GC.....  945
PRCV-LEPP (24-1165)   ..........  ..........  ..........  ..........  ...AC.....  972
PRCV-AR310 (24-1165)  ..........  ..........  ..........  ..........  ...AC.....  972
PRCV-ISU1 (24-876)    ..........  ..........  ..........  ..........  ...AC.....  683
Consensus             TTTTGTAGAC CCTATGCTTG TAAGCATAGC AATACGTGGC TTARMTCATG  950

NVSL5170 (3681-4824)  ..........  ..........  ..........  ..........  ..........  4680
VMRI5170 (4418-5561)  ..........  ..........  ..........  ..........  ..........  5417
PRCV-IA1894 (24-1138) ..........  ..........  ..........  ..........  ..........  995
PRCV-LEPP (24-1165)   ..........  ..........  ..........  ..........  ..........  1022
PRCV-AR310 (24-1165)  ..........  ..........  ..........  ..........  ..........  1022
PRCV-ISU1 (24-876)    ..........  ..........  ..........  ..........  ..........  733
Consensus             CTGATCTAAC TGTAGTTAGA GCAGTTGAAC TTCTCAATGG TGATTTTATT  1000

NVSL5170 (3681-4824)  ..........  .......GC. C.....C...  ..........  ..........  4730
VMRI5170 (4418-5561)  ..........  .......GC. C.....C...  ..........  ..........  5467
PRCV-IA1894 (24-1138) ..........  .......TT. T.....T...  ..........  ..........  1045
PRCV-LEPP (24-1165)   ..........  .......GC. C.....C...  ..........  ..........  1072
PRCV-AR310 (24-1165)  ..........  .......GC. C.....C...  ..........  ..........  1072
PRCV-ISU1 (24-876)    ..........  .......GC. C.....C...  ..........  ..........  783
Consensus             TATATATTTT CACAGGAKYC YGTAGTYGGT GTTTACAATG CAGCCTTTTC  1050

NVSL5170 (3681-4824)  ......G...  ..........  ..........  .......A..  ..........  4780
VMRI5170 (4418-5561)  ......G...  ..........  ..........  .......A..  ..........  5517
PRCV-IA1894 (24-1138) ......A...  ..........  ..........  .......G..  ..........  1095
PRCV-LEPP (24-1165)   ......G...  ..........  ..........  .......G..  ..........  1122
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Figure 7: (continued)

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PRCV-AR310 (24-1165)  ......G... .......... .......... .......G.. ..........   1122
PRCV-ISU1 (24-876)    ......G... .......... .......... .......G.. ..........   833
Consensus             TCAGGCRGTT CTAAACGAAA TTGACTTAAA AGAAGAARAG GGAGACCGTA   1100

NVSL5170 (3681-4824)  .......... .......... .......... ......C..C ....   4824
VMRI5170 (4418-5561)  .......... .......... .......... ......C..C ....   5561
PRCV-IA1894 (24-1138) .......... ....-..... .......... ......T..C ....   1138
PRCV-LEPP (24-1165)   .......... ....-..... .......... ......T..C ....   1165
PRCV-AR310 (24-1165)  .......... ....-..... .......... ......T..C ....   1165
PRCV-ISU1 (24-876)    .......... ....-..... .......... ......T..T ....   876
                                  stop ORF3.1 <--|
Consensus             CCTATGACGT TTCCCTAGGG CATTGACTGT CATAGAYGAY AATG   1144
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Figure 7: (continued)

5. DISCUSSION AND CONCLUSIONS

In this study we have shown that PRCV isolate NVSL 5170 differed from TGEV VMRI 5170 isolate and the standard Miller strain of TGEV. The VMRI 5170 strain was similar to the Miller strain of TGEV in growth characteristics and protein profiles. However, there was some degree of genetic and antigenic diversity when compared to other TGEV strains.

It has been indirectly proven that the TGEV variant, PRCV, has evolved from TGEV by a deletion mutation of the S gene (Rasschaert et al., 1990; Wesley et al., 1990; Wesley et al., 1991). This study presents strong evidence that the PRCV isolate NVSL 5170 is a truncated version of the TGEV isolate VMRI 5170 by a single deletion of the S gene. It should be noted that both viruses were isolated from the same TGE outbreak in a swine herd (Halbur et al., 1995). Furthermore, the pairwise alignment of the S gene and ORF 3/3.1 regions of both isolates when compared with other TGEV and PRCV isolates showed that VMRI 5170 and NVSL 5170 isolates are highly identical. With the exception of the large deletion, the homology of these regions is more than 99 %. Interestingly, the 714 nucleotide deletion of the NVSL 5170 isolate was the largest single deletion of all published sequences among PRCV isolates. Deletions in all other PRCV isolates to date range from 672 - 681 nucleotides (Laude et al., 1993; Vaughn et al., 1995).

The ORF 3/3.1 region of TGEV and PRCV isolates is normally diverse. The number of deleted bases and the positions of deletions vary among PRCV isolates (Rasschaert et al., 1990; Britton et al., 1991; Wesley et al., 1991; Vaughn et al., 1995). Some of the PRCV isolates, AR310 and LEPP, have complete ORF 3/3.1 region (Vaughn et al., 1995). Likewise, the PRCV isolate NVSL 5170 had intact ORF 3/3.1 region including the perfect IS elements and start codons. However, the first substituted nucleotide within the ORF 3.1 of the NVSL 5170 isolate created a stop codon which may have resulted in a truncated 3.1 gene product. These diversities could be a consequence of that as each PRCV isolate originates from a different TGEV ancestor. For instance, the European PRCV and the USA PRCV arose independently from different strains of TGEV (Laude et al., 1993). In this case, the TGEV isolate, VMRI 5170, seemed to be the ancestor of the PRCV isolate NVSL 5170, because their genomic sequences within the S gene and ORF 3/3.1 regions were much more alike than those of other TGEV or PRCV isolates.

The one step growth curves depict the multiplication of the three viruses in cell culture. The growth curve of VMRI 5170 and NVSL 5170 isolates were similar to that of the Miller strain of TGEV. There were differences in the plaque sizes of the TGEV and PRCV isolates.

The PRCV isolate NVSL 5170 had an average plaque size that was significantly smaller than that of VMRI and the Miller strain of TGEV. The small plaque size is possibly associated with the mutation within the S gene or the ORF 3/3.1 gene. It is believed that the small plaque size variants are due to the mutation within the S gene (Holmes and Lai, 1996) or the ORF 3/3.1 regions (Wesley et al., 1990; Vaughn et al., 1995). Thus, it is possible that the deletion within the S gene of NVSL 5170 isolate or the truncated 3.1 gene products may contribute to the small plaque size of the NVSL 5170 isolate.

Antigenic diversity among TGEV and PRCV has been demonstrated using a viral neutralization (VN) test (Kemedy, 1967; Vaughn and Paul, 1993). However, only one serotype of TGEV is recognized. In this study, the three viruses were neutralized by hyperimmune sera and monoclonal antibodies raised against the Miller strain of TGEV with different VN titers. Callebaut et al. (1988), also reported the antigenic differences between TGEV and PRCV. In addition, there are alterations of amino acid residues within the antigenic sites which arose from changes of nucleotides within the S gene of TGEV and PRCV, and the residues within antigenic sites A and D show a high number of amino acid changes (Gebauer et al., 1991; Sanchez et al., 1992). The Miller strain reacted with hyperimmune sera and MAb 3H11 and 5A5 with high VN titers, with the exception of the reaction between VMRI 5170 and MAb 5A5. This is possibly due to the substitution or deletion of nucleotides within the S genes of the VMRI 5170 and NVSL 5170 isolates. However, the alterations of the residues in the S glycoprotein of VMRI 5170 isolate that react with MAb 5A5 may increase the affinity of antigenic sites on the S gene of VMRI 5170 and MAb 5A5. Thus, the reaction between VMRI 5170 and MAb 5A5 gives very high VN titers.

Radioimmunoprecipitation assay (RIP) provides information on the major structural proteins, S, M and N, of TGEV and PRCV. Our data confirms that the S glycoprotein of PRCV is smaller than that of TGEV and is caused by the large deletion within the S gene (Rasschaert et al., 1990).

This study presented strong evidence that the PRCV isolate NVSL 5170 originated from the TGEV isolate VMRI 5170 caused by a single deletion within the 5' half of the S gene, resulting in a truncated S glycoprotein. The deletion mutation within the S gene of PRCV isolate NVSL 5170 may be the result of genetic recombination as reported for mouse hepatitis virus and other coronaviruses because the repeated IS elements along the genomic RNA could facilitate genetic recombination during RNA synthesis using a copy - choice mechanism (Lai, 1992). Therefore, deletion mutation and genetic recombination tend to play an important role in the evolution of coronaviruses and other plus - stranded RNA viruses.

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