Antigenic and biological variation among transmissible gastroenteritis virus isolates

by

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EXPLANATION OF THESIS FORMAT

The following thesis consists of a general introduction, a review of the literature, a separate manuscript, a general summary, literature cited, and acknowledgements. The master's candidate, Eric Martin Vaughn, is the senior author and principal investigator for the manuscript.

GENERAL INTRODUCTION

Transmissible gastroenteritis (TGE) is a common and economically important disease of neonatal swine (Bohl, 1989; Saif and Bohl, 1986). Transmissible gastroenteritis is characterized by severe diarrhea, dehydration, and high mortality in piglets under two weeks of age (Bohl, 1989; Saif and Bohl, 1986). In 1946, Doyle and Hutchings described a filterable agent that caused TGE in pigs. Later, the transmissible gastroenteritis virus (TGEV), was found to belong to the Coronaviridae family of viruses (Tajima, 1970). Being a coronavirus, TGEV is enveloped and pleomorphic with characteristic club-shaped surface projections or peplomers. The nucleic acid of TGEV is single stranded RNA of positive polarity (Brian et al., 1980). The transmissible gastroenteritis virus has three structural viral proteins, these being M, S, and N (Garwes and Pocock, 1975; Saif and Bohl, 1986). M is glycosylated with an apparent molecular mass of 25-30 kilodaltons (kDa) and is associated with the viral envelope. S, the peplomer protein, has an apparent molecular mass of 200 kDa and is also glycosylated (Garwes et al., 1976. The N protein is not glycosylated, has an apparent molecular mass of 45-50 kDa, and is also associated with the binding of viral RNA and serves as a nucleocapsid (Britton et al., 1988a). S is the viral protein that induces neutralizing antibodies (Garwes et al., 1978/79).

Two main forms of disease exist with TGE, these being the epizootic form and the enzootic form (Saif and Bohl, 1986; Paul et al., 1988). The epizootic form of TGE occurs in the winter months, usually from the middle of November to about the middle of April, with the majority of the animals in the herd susceptible to the disease. Most animals in these herds develop diarrhea with vomiting, and with pigs less than two weeks of age mortality may approach 100% (Bohl, 1989; Hill, 1989). Transmissible gastroenteritis in its enzootic form is seen among weaned pigs and is a frequent problem

in nurseries, where there are repeated additions of susceptible swine (Saif and Bohl, 1986; Paul et al., 1988; Hill, 1989). The enzootic form of TGE results in high morbidity with low mortality, diarrhea and unsatisfactory growth. In these older pigs affected with the enzootic form of TGE, achievement of market weight may be delayed by three to four weeks (Hill, 1989).

Vaccines for TGE are available and federally licensed (Saif and Bohl, 1986; Moxley and Olson, 1989). These vaccines consist of either inactivated or live attenuated virus (Saif and Bohl, 1986). The protection provided by these vaccines is variable, and it is believed that the virulent virus provides the best immunity (Moxley and Olson, 1989). In some herds where the currently available commercial vaccines have not been successful in limiting losses from TGE, autogenous vaccines have proven effective (Paul et al., 1988). These autogenous vaccines are usually composed of homogenized intestinal tissue from infected pigs and is mixed with food and fed to pregnant swine at least three weeks prior to farrowing (Saif and Bohl, 1986).

The lack of protection provided by the commercial vaccines has been in part due to the level and type of immunity in response to the vaccines (Laude et al., 1981; Bohl, 1989). Also, autogenous vaccines providing protection in some herds where commercial vaccines have failed, provides possible evidence that antigenic diversity among TGEV isolates may exist. It is believed that only one serotype of TGEV exists (Kemeny, 1976). However, antigenic variation among TGEV isolates has been demonstrated (Laude et al., 1986; Hohdatsu, 1987a). Another point to consider is the extent of biological variation among TGEV isolates. The purpose of this study was to examine a number of TGEV field isolates and to characterize the extent of antigenic and biological diversity among these isolates.

LITERATURE REVIEW

Coronaviruses

The coronaviruses are large, enveloped, plus (+) stranded RNA viruses that belong to the Coronaviridae family of viruses and have been implicated in respiratory and enteric diseases of both humans and domestic animals (Holmes, 1990; Saif and Bohl, 1986). The coronaviruses are so named because of the petal-shaped glycoproteins projecting from the envelopes of virions that reveal a "corona" or crown-like appearance when viewed by electron microscopy (McIntosh, 1990). Schalk and Hawn are believed to be the first to describe a disease that was caused by a coronavirus when avian infectious bronchitis was differentiated from other respiratory ailments of chickens in 1931 (Schalk and Hawn, 1931). Later, TGE of swine was described in 1946 by Doyle and Hutchings, and murine hepatitis virus was recognized in 1949 by Cheever et al. In 1965, Tyrrell and Bynoe described recovering an ether-labile virus recovered from a boy with a cold and its subsequent passage in human embryonic trachea organ cultures. This virus had a morphology that was very similar to that of avian infectious bronchitis virus when viewed by electron microscopy. McIntosh et al. (1967) had isolated other strains of human coronaviruses and showed that these human coronaviruses were related, both antigenically and morphologically, to murine hepatitis virus. At this point, a new genus was suggested for these viruses exhibiting a crown-like appearance (Tyrrell et al., 1968; Garwes et al., 1976). For many years, classification of coronaviruses was based solely on this characteristic morphology (Holmes 1990). However, coronaviruses can now be identified by properties of their structural proteins, genomic RNA and mRNAs, and by antigenic cross-reactivity and nucleic acid homology with known coronaviruses, in addition to their morphology (Holmes, 1990).

Coronaviruses are divided into four antigenic groups, and generally infect only one species or several closely related species (Holmes, 1990). Table 1 shows the host species infected by coronaviruses.

TABLE 1[a]. Coronaviruses: Antigenic groups, abbreviations, names, and hosts

Antigenic group	Abbreviation	Name	Host
I	HCV-229E	Human respiratory coronavirus	Human
	TGEV	Transmissible gastro-enteritis virus	Pig
	CCV	Canine coronavirus	Dog
	FECV	Feline enteric coronavirus	Cat
	FIPV	Feline infectious peritonitis virus	Cat
II	HCV-OC43	Human respiratory coronavirus	Human
	MHV	Murine hepatitis virus	Mouse
	HEV	Hemagglutinating encephalomyelitis virus	Pig
	BCV	Bovine coronavirus	Cow
	RbCV	Rabbit coronavirus	Rabbit
	SDAV	Sialodacryadentis virus	Rat
III	IBV	Infectious bronchitis virus	Chicken
IV	TCV	Turkey coronavirus	Turkey

[a] Adapted from Holmes (1990).

The Coronavirion

Examination of coronavirus particles by negative staining electron microscopy reveals that coronaviruses are generally spherical, yet pleomorphic, with a diameter

ranging from 60-160 nanometers (nm) (Holmes, 1990; Saif and Bohl, 1986). The coronavirion is enveloped with a single layer of large, widely spaced club-shaped projections called peplomers (Holmes, 1990; Saif and Bohl, 1986). These peplomers evenly cover the virion surface and are 12-25 nm in length (Saif and Bohl, 1986). Coronavirus particles have a molecular mass of 6-9 x 10^6 and a buoyant density of 1.18-1.21 g/ml in sucrose and 1.14-1.23 g/ml in CsCl (Bohl, 1989; Brian et al., 1980; Garwes and Pocock, 1975).

Three major structural proteins are found in all coronaviruses, these are 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 with an apparent molecular mass ranging from 45-60 kDa that serves to bind the genomic RNA to form a helically symmetrical nucleocapsid (Holmes, 1990). The M protein is a glycosylated transmembrane protein of an apparent molecular mass of 25-30 kDa that is deeply embedded in the host cell derived lipid bilayer (Holmes, 1990). Also the M protein serves as a base to which the nucleocapsid protein and its attached genomic RNA can anchor to the lumen of the lipid bilayer (Holmes, 1990). The peplomers are composed of the 180-200 kDa S glycoprotein. Only a small portion of the S glycoprotein is embedded in the lipid bilayer, with the majority of the glycoprotein projecting to the outside surface of the virion. The S glycoprotein is also involved in the attachment of the virion to specific cell receptors on the host cell (Holmes, 1990; Hogue et al., 1989). A small number of coronaviruses also possess an additional glycoprotein, designated HE, that is the hemagglutination-esterase glycoprotein that is involved in hemagglutination (Holmes, 1990; Hogue et al., 1989; Spaan et al., 1988). The HCV-OC43 (human respiratory coronavirus), BCV (bovine coronavirus), HEV (hemagglutinating encephalomyelitis virus), TCV (turkey coronavirus), and several strains of MHV (mouse

hepatitis virus) all contain the HE glycoprotein (Spaan et al., 1988; Holmes 1990). For members of the Coronaviridae family that do have the HE glycoprotein, this glycoprotein is also involved in virion attachment to the host cell in addition to the S glycoprotein (Holmes, 1990). The many remaining coronaviruses that lack the HE glycoprotein neither hemagglutinate nor have esterase activity (Holmes, 1990).

The genomic RNA of coronaviruses is the largest among RNA viruses, approximately 27 to 30 kilobases (kb) in size (Spaan et al., 1988). When the plus-strand genomic RNA of a coronavirus is extracted and introduced into a host cell, the genomic RNA is infectious and can serve directly as a mRNA template (Holmes 1990; Spaan et al., 1988). The genomic RNA of coronaviruses is capped at its 5' end and is polyadenylated at its 3' end (Spaan et al., 1988). Once the virus has penetrated the host cell, the genomic RNA attaches to ribosomes and a virus-specific RNA-dependent RNA polymerase is synthesized (Holmes, 1990). This RNA-dependent RNA polymerase transcribes the plus-strand genomic RNA into full-length negative-strand RNA (Spaan et al., 1988). The negative-strand RNA serves as a template for the transcription of genomic RNA and subgenomic mRNAs (Spaan et al., 1988). Five to seven subgenomic mRNAs are generated with the actual number varying among coronaviruses (Holmes, 1990; Spaan et al., 1988). The subgenomic mRNAs form a coterminal 3' nested set in which they all have common 3' ends, thus each mRNA contains all the nucleotide sequence in the next smaller mRNA plus one additional gene at the 5' end (Spaan et al., 1988; Holmes, 1990). This 5' end contains the open-reading frame that is translated into the designated protein.

Of the viral proteins generated, the N protein and several non-structural proteins are apparently made on the polysomes in the cytoplasmic matrix (Holmes, 1990). On the polysomes attached to the rough endoplasmic reticulum (RER), the synthesis and

processing of the M, S and HE (if present) glycoproteins occurs (Holmes, 1990). The S and HE glycoproteins are cotranslationally inserted through the RER membrane via a N-terminal signal peptide and glycosylated as the polypeptide grows in length (Holmes, 1990; Spaan et al., 1988). The M glycoprotein is also synthesized on membrane-bound polysomes, but its insertion and processing differ from the other viral glycoproteins (Holmes, 1990). The M glycoprotein lacks a N-terminal signal sequence, and yet is still inserted into the RER membrane (Spaan et al., 1988). This membrane insertion is dependent on internal signal recognition particles in the first or third hydrophobic domains of the M glycoprotein (Holmes, 1990; Spaan et al., 1988; Britton et al., 1988b). A putative N-terminal signal peptide of 17 residues has been identified for the transmissible gastroenteritis virus M glycoprotein (Laude et al., 1987), thus the lack of the signal sequence for insertion of the M glycoprotein may not be universal among all coronaviruses (Britton et al., 1989a). The glycosylation of the M glycoprotein occurs well after the protein is synthesized, unlike the S and HE glycoproteins that are glycosylated as they are synthesized (Holmes, 1990).

The assembly of the virions occurs with budding at the membranes between the RER and the Golgi apparatus of the infected cell (Holmes, 1990). The N protein and newly synthesized genomic RNA come together and form a helical nucleocapsid, and this nucleocapsid binds to the cytoplasmic surface of the RER and Golgi apparatus membranes. It is believed that the cytoplasmic domain of the M glycoprotein inserted in these membranes serves as an anchor for the binding of the nucleocapsid (Holmes, 1990). The S glycoprotein is transported through the Golgi apparatus and is inserted into its membrane, where it may or may not be cleaved into two 90 kDa fragments (Holmes, 1990). Whether or not this cleavage of the S glycoprotein occurs depends on the particular coronavirus and the type of host cell infected. Excess S glycoprotein is

transported to the plasma membrane of the host cell, and once at this location it will not be incorporated into any virions (Holmes, 1990). Once a complete nucleocapsid is bound to the M glycoprotein domain, a virion can then bud into the lumen of the RER and Golgi apparatus (Holmes, 1990). Virions containing a nucleocapsid, M, S, and HE (if present), are then released by cell lysis or by fusion of the Golgi apparatus derived, virion-containing, smooth-walled vesicles with the plasma membrane (Holmes, 1990). Since the M glycoprotein is restricted in its transport within the host cell (i.e., M can only be inserted in the cytoplasmic surface of the RER membrane), this accounts for the fact that coronaviruses can only bud from the RER and Golgi apparatus of the cell and not directly from the plasma membrane (Holmes, 1990). The ability of coronaviruses to bud from the RER or the Golgi apparatus, and replication of viral subgenomic and genomic RNAs via 3' co-terminal nested sets, are two very unique features of coronaviruses that enable them to be differentiated from other virus families.

The Transmissible Gastroenteritis Virion

In 1946, Doyle and Hutchings described a filterable agent that caused TGE in pigs. Later, TGEV was found to belong to the Coronaviridae family of viruses. Being a coronavirus, TGEV is enveloped and pleomorphic with characteristic club-shaped surface projections or peplomers. The nucleic acid of TGEV is single stranded RNA of positive polarity (Holmes, 1990; Spaan et al., 1988), and can be directly infectious (Norman et al., 1968). The transmissible gastroenteritis virus has three structural viral proteins, these being M, S, and N. M is glycosylated with an apparent molecular mass of 25-30 kDa and is associated with the viral envelope. S, the peplomer protein, has an apparent molecular mass of 200 kDa and is also glycosylated (Garwes et al., 1976). The N protein is not glycosylated as it is a basic phosphoprotein, has an apparent molecular

mass of 45-50 kDa, and is also associated with the binding of viral RNA and serves as a nucleocapsid (Britton et al., 1988a). S is the viral protein that induces neutralizing antibodies (Garwes et al., 1978/79). The transmissible gastroenteritis virus lacks the HE glycoprotein that is involved in the hemagglutination process of other coronaviruses (Holmes, 1990).

Being an enveloped virus, TGEV is inactivated by ether and chloroform. The transmissible gastroenteritis virus is relatively stable at pH 3 and is trypsin resistant, as are enteric viruses in general (Saif and Bohl, 1986).

The TGEV is antigenically related to the feline infectious peritonitis virus (FIPV) and canine coronavirus (CCV), as antisera produced against these viruses will react with TGEV (Horzinek et al., 1982; Sanchez et al., 1990). The recent discovery of the porcine respiratory coronavirus (PRCV) reveals that it is also very closely related antigenically to TGEV, as antisera produced against both viruses will react strongly with each of the viruses. However, the PRCV has a tropism for the respiratory tract of pigs and does not cause enteric disease symptoms (Cox et al., 1990). The TGEV shows no antigenic cross reactivity to the other porcine coronaviruses, hemagglutinating encephalomyelitis virus (HEV) and porcine epidemic diarrhea virus (PEDV or CV777) (Pensaert et al., 1981; Egerbrink et al., 1988).

The transmissible gastroenteritis virus is routinely grown in the swine testicular cell line (McClurkin and Norman, 1966), in which TGEV shows a distinctive cytopathic effect (CPE). Through the years several different cell lines were utilized in the attempt to propagate TGEV outside of the natural host. Only primary swine kidney cells (Harada et al., 1963; Witte and Easterday, 1967), primary swine thyroid cells (Witte and Easterday, 1967; Dulac et al., 1977), and swine testicular cells (McClurkin and Norman, 1966) show a distinct CPE (Bohl, 1989).

Transmissible Gastroenteritis

Transmissible gastroenteritis is a common and economically important disease of neonatal swine (Hill, 1989; Saif and Bohl, 1986). In Iowa alone it is estimated that TGE cost the pork industry about $10 million annually in 1987 and 1988 (Hill, 1989). The extent that TGE will affect a swine herd depends on the immune status and age of the swine in the herd (Hill, 1989). The virus that is the etiologic agent of TGE is the TGEV, and it is classified as a member of the Coronaviridae family of viruses.

Transmissible gastroenteritis is commonly divided into two major forms of the disease, these being the epizootic and enzootic forms (Saif and Bohl, 1986). A third minor form of TGE is classified as intermittent enzootic TGE (Saif and Bohl, 1986). The epizootic form of TGE occurs when the virus is introduced into a totally susceptible herd (Bohl, 1989), and is characterized by severe diarrhea, dehydration, vomiting, and high mortality in young piglets (Saif and Bohl, 1986). The mortality in piglets under two weeks of age may reach 100%, but in nursery age pigs, those of three to eight weeks of age, the mortality is usually less than 10 to 20% (Hill, 1989; Saif and Bohl, 1986). However, in these three to eight week old piglets infected with TGE, the damage that has occurred to their small intestinal epithelium results in a slower and less efficient growth rate (Hill, 1989). In the older pigs in an affected herd, such as the growing and finishing pigs, sows, and boars; inappetence, vomiting, and diarrhea develops that lasts from two to four days (Hill, 1989; Saif and Bohl, 1986). Agalactia commonly occurs in lactating sows infected with TGEV, which in turn contributes to the severity of the disease in suckling pigs (Bohl, 1989). In the older age groups of pigs, the morbidity may reach 100% while the mortality is usually less than 5% (Hill, 1989). The epizootic form of TGE has traditionally been thought of as usually occurring in the winter months (Saif and

Bohl, 1986), when the colder temperatures allow the labile TGEV to remain viable and be spread more readily.

The enzootic form of TGE is attributed to a continuance of the infection and the disease within a herd (Bohl, 1989). An uninterrupted source of immunologically naive swine is needed for the enzootic form of TGE to persist in a herd. A continuous farrowing schedule or the addition of feeder pigs is usually the source of susceptible pigs (Saif and Bohl, 1986). In these herds, most sows have been previously infected and can pass on a variable degree of immunity to their suckling piglets (Bohl, 1989; Saif and Bohl, 1986). Diarrhea will often occur in piglets of about 6 days of age, but the extent of disease is milder than that of the epizootic form (Hill, 1989; Bohl, 1989). Recently weaned pigs are very susceptible to enzootic TGE since they have had a sudden loss of any passive immunity to TGEV that may have been passed on to them by suckling the sow (Hill, 1989; Bohl, 1989). Mortality is usually low, around 10-20% (Hill, 1989; Bohl, 1989), but if enzootic TGE remains in a herd for over a year, the economic loses may exceed those incurred from the original epizootic outbreak (Hill, 1989). Generally, these losses are from a reduced growth rate and an increased susceptibility to other diseases (Hill, 1989).

Lastly, intermittent enzootic TGE is defined as the reentrance of TGEV into a herd that contains some immune animals, especially sows (Bohl, 1989; Saif and Bohl, 1986). Since the older sows have been previously infected, they are immune and will passively provide immunity to their suckling piglets (Bohl, 1989). However, the growing and finishing swine are susceptible since the TGEV infection from the previous winter had been terminated during the following summer and autumn months (Saif and Bohl, 1986).

There is an inverse relationship between the age of the animal infected with TGEV and the severity of the clinical signs, duration of the disease, and mortality (Bohl, 1989;

Saif and Bohl, 1986). Pigs under two weeks of age will often have a mortality rate of 100%. In most pigs under two weeks of age, death will occur in 2-7 days after the onset of symptoms (Bohl, 1989). Three-week old or older swine show much less mortality, but they will have a decreased growth rate (Saif and Bohl, 1986). Transient vomiting, watery yellowish diarrhea, rapid weight loss, and dehydration are the major clinical signs of TGE (Bohl, 1989; Saif and Bohl, 1986). The affected young pigs will have profuse, foul-smelling diarrhea which often contains small curds of undigested milk (Saif and Bohl, 1986).

Growing, finishing, or adult swine have limited clinical signs with TGE. These signs include inappetence, a mild diarrhea of short duration, and occasional vomiting (Bohl, 1989; Saif and Bohl, 1986). Agalactia can also occur in lactating sows.

The site of virus replication in the small intestine is in the jejunum, and to a lesser extent the ileum, with very little evidence of viral replication in the duodenum (Hooper and Haelterman, 1966). In the small intestine, a marked shortening or atrophy of the villi occurs and the walls of the small intestine become very thin (Hill, 1989; Garwes, 1988). The villi are shortened due to the loss and death of the villous enterocytes that are infected with TGEV (Pensaert et al., 1970). The crypt enterocytes do not appear to be infected, and as immature cells migrate up from the crypts to the tips of the villi to replace the infected villous enterocytes, they are more resistant to TGEV infection (Pensaert et al., 1970). The rate at which villous enterocytes can be replaced depends on the age of the pig. In normal one-day-old pigs, villous enterocytes can be replaced in seven to ten days, and in normal three-week-old pigs the villous enterocytes can be replaced in as little as two to four days (Moon, 1978). This difference in the time necessary to replace villous enterocytes can account for the higher mortality of newborn piglets infected with TGEV as compared to pigs of three weeks of age or older (Bohl, 1989).

There is evidence that TGEV can replicate in sites other than the gastrointestinal tract of swine. The transmissible gastroenteritis virus has been found in the milk of infected sows (Kemeny and Woods, 1977), in the respiratory tract of infected pigs (Underdahl et al., 1975) and TGEV also has been shown to replicate in alevolar macrophages (Laude et al., 1984). It is well accepted that oral ingestion of infected material is the major route for the infection and spread of TGEV. However, it is still unclear whether the extra-intestinal sites where TGEV is found are important in spreading the disease (Bohl, 1989; Saif and Bohl, 1986).

Several methods are available for the diagnosis of TGE. These include the detection of viral antigen, microscopic detection of virus, isolation and identification of virus, or serological methods (Bohl, 1989; Bernard et al., 1986; Saif and Bohl, 1986). Detection of viral antigen can be accomplished by using an immunofluorescent assay on frozen sections or mucosal scrapings of the jejunum or ileum (Saif and Bohl, 1986). Problems with detecting viral antigen can occur if the sample being tested was collected in the later stages of TGE when the infected enterocytes will have been sloughed off (Bohl, 1989; Saif and Bohl, 1986). Microscopic detection of the virus can be accomplished by negative contrast electron microscopy, and the use of immune electron microscopy can increase the sensitivity of this method (Saif and Bohl, 1986). Isolation and identification of TGEV can be accomplished in cell culture. The swine testicular cell line has been shown to be the the most sensitive cell line for the detection of TGEV (Bohl, 1989). Serological testing will determine if pigs were previously infected with TGEV. Screening for an antibody response to TGEV is most commonly accomplished by an indirect immunofluorescent assay or a virus neutralization test, the latter is considered to be the most sensitive (Bohl and Kumagai, 1965; Bohl, 1989). Also, enzyme-linked immunosorbent assay has been utilized to screen for antibodies to TGEV and correlates

closely with the virus neutralization test (Hohdatsu et al., 1987a; Van Nieuwstadt et al., 1988)

Treatment for TGE is of limited value. Prevention of dehydration and the accompanying acidosis can be attempted by supplying essential fluids, electrolytes, and nutrients to affected neonatal swine (Saif and Bohl, 1986). But under the conditions of a large farrowing operation, the intensive care necessary such as that described would be extremely difficult to implement.

The best immunity to TGE is produced by oral exposure to live, virulent TGEV (Moxley and Olson, 1989). However, oral immunization of swine with a fully virulent virus can spread the disease throughout the herd, and thus, vaccines have been developed to aid in the prevention of TGE. The commercial vaccines consist of inactivated virulent TGEV, or live attenuated TGEV (Saif and Bohl, 1986). The route of administration of these vaccines can be orally, intranasally, intramuscularly, or intramammarily (Saif and Bohl, 1986). Since TGE is an economically important disease of neonatal swine, these vaccines are meant to provide these young pigs with passively acquired immunity. This passive immunity, or lactogenic immunity as it is commonly called, is provided to the suckling piglet via the sow's colostrum or milk. If pregnant swine can be immunized at least three weeks prior to farrowing, the sows can then develop neutralizing antibodies to TGEV and then passively transfer these antibodies to their suckling piglets via the colostrum and milk. Colostral immunoglobulins (Igs) consist mostly of IgG, followed by lower concentrations of IgA, which are derived from the serum of the sow, and are transferred across the piglets intestinal epithelium and then serve as serum or humoral antibodies in the piglet (Porter and Allen, 1972). However, the passively acquired humoral antibodies of the IgG class will not provide protection against intestinal infection (Hooper and Haelterman, 1966). When IgG titers are very high in the colostrum and

milk some degree of protection can be conferred in the gut (Stone et al., 1977), but the Ig class which provides the best protection against intestinal infection is IgA. IgA has been found to very efficacious in providing protection in the gut. In order to account for the high degree of efficacy of IgA as compared to IgG, three probable explanations are presented. First, Porter and Allen (1972) found that IgA is in higher concentrations in milk. Secondly, Underdown and Dorrington (1974) found that IgA is more resistant to degradation by the naturally occurring proteolytic enzymes of the gut. Thirdly, Nagura et al. (1978) established that IgA can selectively bind to enterocytes, the same cells in the gut to which TGEV must bind to in order to cause infection. After the first week of lactation, the amount of IgG present in milk decreases markedly, while IgA concentrations remain fairly constant. The IgA present in milk is produced in the mammary tissue by cells that have migrated there from the intestinal lymphoid tissues. The process in which these IgA producing cells seed the intestinal lymphoid tissues occurs via the "gut-mammary axis" as described by Saif and Bohl (1979). Essentially, after antigenic stimulation in the gut from an intestinal infection with TGEV, immunocytes migrate to the mammary gland and then mature into plasma cells that secrete IgA into the colostrum and milk (Saif and Bohl, 1979). Unlike the IgG found in colostrum and milk, IgA is not transferred across the intestinal epithelium of the piglet, but remains in the gut where it can function in intestinal protection (Saif and Bohl, 1986). Nguyen et al. (1986) has shown that neutralizing IgA and IgG do not stop the binding of TGEV to the target cells, but rather virus neutralization is independent of viral attachment. IgA appears to be the immunoglobulin of choice in providing a high level of passively acquired protection to the neonatal pig, and the best way to achieve an IgA response to TGEV is by oral or intranasal exposure to the virus. There are commercial vaccines which are designed to be delivered orally or intranasally, with the idea that the attenuated

TGEV will replicate in the gut or the respiratory tract and induce immunocytes that will follow the "gut-mammary axis" to the intestinal lymphoid tissues where they will then mature and secrete IgA. However, the commercial vaccines are variable in the degree of protection conferred (Moxley et al., 1989), and this may be due to the fact that the level of attenuation of the virus may not allow it to adequately infect the target tissues (Laude et al., 1981) and trigger the "gut-mammary immunological link" (Welch et al., 1988; Bohl, 1989), or that the attenuated virus is more susceptible to protease digestion in the small intestine than the virulent virus (Chen, 1985). When a vaccine is administered intramammarily or intramuscularly, the immunoglobulins present in the colostrum and milk are composed of almost exclusively IgG (Bohl et al., 1972). Thus, the degree of the immune response and the class or classes of antibodies developed to TGEV that will be transferred to the suckling piglets in colostrum or milk depends on the route of administration of the vaccine.

Other types of vaccines have been developed and tested experimentally, these consist of heterologous virus vaccines, a small plaque mutant virus vaccine, stomach acid resistant TGEV mutants, and TGE viral subunit vaccines. Heterologous vaccines have been tested and consisted of FIPV (Woods, 1984; Woods and Wesley, 1986) or CCV (Woods and Pedersen, 1979). Since these two viruses are closely related to TGEV, the possibility that immunization with either one of these viruses might induce a beneficial immune response in swine was tested. Results showed that the some immunity against TGEV was evident, but that it was of poorer quality than the immunity conferred to piglets born to sows that had recovered from a natural infection (Woods and Pedersen, 1979). Woods (1978) tested a small plaque (sp) variant of TGEV for efficacy in providing protection against TGE. The sp variant "was derived from a persistently infected swine leukocyte line originally infected with a virulent TGEV", and was tested as

a live attenuated virus vaccine (Woods, 1978). Protective antibodies were elicited by either the oral/intranasal and/or the intramammary route of inoculation with the sp variant and protection was conferred to the suckling piglets as shown by challenge with a virulent TGEV. In 1981, Woods et al. reported that this sp variant was avirulent for neonatal swine, as it replicated in the laminia propria but not in the epithelial cells of the intestine. This sp variant showed appeared to be an effective vaccine for TGE, but at present its status in development for commercial use is unknown.

Aynaud et al. (1985) hypothesized that the oral vaccination failures were due to the virus being destroyed during transit through the gut by gastric and gut juices. In 1985, Aynaud et al. reported on the development of two TGEV mutants that had been selected for acid resistance by repeated growth in the presence of adult swine stomach acid. Their purpose in developing such a mutant was to ensure that a cell culture-adapted TGEV would survive gastric passage when given as an oral vaccine. Aynaud et al. (1985) found that the two acid resistant mutants had also simultaneously acquired resistance to pepsin and trypsin, two digestive enzymes known to degrade TGEV particles. The level of lactogenic immunity conferred to suckling piglets from sows inoculated by this live attenuated virus vaccine was determined as "good" (78%) by the authors. In addition, the authors felt that the passive protection provided was due to the stability of the virus particles in the digestive tract (Aynaud et al., 1985).

Gough et al. (1983), developed a 23 kDa subunit vaccine from purified virulent TGEV for intramuscular injection in pregnant swine. Immunized sows developed neutralizing antibodies in their serum and milk and were shown to confer protection in suckled piglets by challenge with virulent TGEV. The hopeful outlook of this subunit vaccine is clouded by the lack of reproducibility by other investigators in obtaining the 23 kDa fragment, and the exceedingly high costs in making large quantities of the 23 kDa

subunit. A synthetic peptide of the mouse hepatitis virus (MHV) peplomer protein has been shown to provide protection to a lethal challenge dose of MHV (Talbot et al., 1988), but as with the 23 kDa cost of producing a synthetic peptide in a large quantity would be prohibitively high.

The role that antigenic variation among TGEV isolates may play in the lack of efficacy of the commercial vaccines has been considered in the last few years (Hohdatsu et al., 1987b; Laude et al., 1986). When utilizing polyclonal antisera to TGEV, very slight differences in virus neutralization titers are detected among different TGEV strains and isolates. However, when neutralizing monoclonal antibodies (MAbs) are utilized, there are sometimes marked differences detected between virus strains and isolates. Neutralizing MAbs are directed to the S glycoprotein of TGEV (Garwes et al., 1978/1979). Monoclonal antibodies that are directed to the N protein are not neutralizing. MAbs to the M glycoprotein are not neutralizing, except for a few that require the presence of complement (Woods et al., 1988). The antigenic structure of the TGEV S glycoprotein has been defined at three levels; antigenic sites, antigenic subsites, and epitopes (Correa et al., 1988). Delmas et al. (1986) described the localization of four antigenic sites, A, B, C, and D, on the S glycoprotein of TGEV. Also in 1986, Jimenez et al. described neutralizing MAbs which recognized six distinct "critical" epitopes in two antigenic sites, A and D, on the S glycoprotein. The term "critical" is used to describe epitopes that are important in the neutralization of TGEV. Hohdatsu et al. (1987b), produced neutralizing MAbs which also recognized six distinct epitopes on the S glycoprotein. However, it is not known whether the neutralizing MAbs used in these two separate studies all recognize the same six epitopes on the S glycoprotein of TGEV. Correa et al. (1988) reported that a total of eight epitopes were found to be critical for TGEV neutralization. To date, all neutralizing MAbs directed to the S glycoprotein have

been confirmational epitopes (Correa et al., 1988). All the neutralizing MAbs described in the epitope mapping studies recognized what are generally agreed upon as the major epitopes on the S glycoprotein that need to be recognized for efficient neutralization of TGEV, yet the deficiency of some of these neutralizing MAbs to recognize and effectively neutralize some strains and isolates of TGEV was noted. This lends further evidence to the possibility that commercial vaccines may be lacking some of these "critical" epitopes and thus the lack of efficacy of the vaccines in providing protection against TGE.

ANTIGENIC AND BIOLOGICAL DIVERSITY AMONG TRANSMISSIBLE GASTROENTERITIS VIRUS ISOLATES

Summary

Twenty-four field isolates of transmissible gastroenteritis virus (TGEV) were isolated and examined for antigenic and biological characteristics. Most isolates produced a typical cytopathic effect (CPE) in swine testis (ST) cell culture, which included a ballooning or lifting away of the infected cells from the cell monolayer with heavy granulation evident. One isolate produced a novel CPE that demonstrated a more net-like appearance with less granulation present. Plaque sizes produced by the TGEV isolates varied. Protein profiles of selected TGEV isolates as determined by SDS-PAGE were essentially identical. The TGEV isolates were shown to be closely related antigenically by using hyperimmune sera in a virus neutralization (VN) test. Some antigenic diversity was detected by utilizing monoclonal antibodies (MAbs) in a VN test. Titers of the MAbs were highest with the homologous Miller TGEV, and one virus isolate was very poorly neutralized with the MAbs used in this study. Radioimmunoprecipitation (RIP) and indirect immunofluorescence assay (IFA) results were similar to those obtained by the VN test. These studies show that some biologic and antigenic diversity exists among TGEV isolates.

Introduction

Transmissible gastroenteritis (TGE) is a common and economically important disease of neonatal swine (Hill, 1989; Saif and Bohl, 1986). Transmissible gastroenteritis is characterized by severe diarrhea, dehydration, and high mortality in piglets under two weeks of age (Saif and Bohl, 1986). The transmissible gastroenteritis virus (TGEV), belongs to the Coronaviridae family of viruses. The transmissible gastroenteritis virus has three structural viral proteins, these being M, S, and N (Saif and

Bohl, 1986; Garwes et al., 1976). M is glycosylated with an apparent molecular mass of 25-30 kDa and is associated with the viral envelope. S, the peplomer protein, has an apparent molecular mass of 200 kDa and is also glycosylated (Garwes et al., 1976). The N protein is not glycosylated, has an apparent molecular mass of 45-50 kDa, and is also associated with the binding of viral RNA and serves as a nucleocapsid (Britton et al., 1988a). S is the viral protein that induces neutralizing antibodies (Garwes et al., 1978/79).

Two main forms of disease exist with TGE, these being the epizootic form and the enzootic form (Saif and Bohl, 1986). Vaccines for TGE are available and federally licensed. These vaccines consist of either inactivated or live attenuated virus. The protection provided by these vaccines is variable, and it is believed that the virulent virus provides the best immunity (Moxley and Olson, 1989). In some herds where the currently available commercial vaccines have not been successful in limiting losses to TGE, autogenous vaccines have proven effective (Paul et al., 1988).

The lack of protection provided by the commercial vaccines has been in part due to the level and type of immunity in response to the vaccines (Laude et al., 1981; Bohl, 1989). Also, autogenous vaccines providing protection in some herds where commercial vaccines have failed, provides possible evidence that antigenic diversity among TGEV isolates may exist (Paul et al., 1988). It has been thought for many years that only one serotype of TGE exists (Kemeny, 1976). However, antigenic variation among TGEV isolates has been demonstrated (Laude et al., 1986; Hohdatsu, 1987). Another point to consider is the extent of biological variation among TGEV isolates. The purpose of this study is to examine twenty-four TGEV field isolates and to characterize the extent of antigenic and biological diversity among these isolates.

Materials and Methods

Cell culture

The swine testis (ST) cell line was used to propagate and isolate TGEV (McClurkin and Norman, 1966). The ST cells were passaged and maintained in Eagle's minimum essential medium (MEM) (GIBCO, Grand Island, NY) supplemented with 10 percent fetal bovine serum (FBS) (GIBCO, Grand Island, NY) , sodium bicarbonate (2.9 g/l) (Fisher Scientific, Fair Lawn, NJ), and lactalbumin enzymatic hydrolysate (5.0 g/l) (Sigma, St. Louis, MO). The ST cell line was propagated in 75 cm^2 flasks (Costar, Cambridge, MA) at a 3-4 day interval between subculturing. The ST cell line was grown at 37 C in a humid 5% CO_2 atmosphere.

Viruses

The Miller (American Type Culture Collection, Rockville, MD), Illinois (Dr. M. Ristic, University of Illinois, Urbana, IL), and Purdue (Dr. R. Woods, National Animal Disease Center, Ames. IA) strains of TGEV were used as standard virus strains in this study.

Field samples

Small intestines of pigs with TGE were obtained from the Iowa State University Veterinary Diagnostic Laboratory. Samples were also obtained from pigs with TGE from swine herds in Kansas (Dr. Phillips, Kansas State University Veterinary Diagnostic Laboratory) and Arkansas. The samples were shown to be positive for TGEV by immunofluorescence on frozen sections of the small intestine. Approximately 10 percent suspensions of the intestinal samples were prepared in 0.05 M phosphate buffered saline (pH 7.4) and clarified by centrifugation at 200 x g for 10 minutes. The supernates were

harvested, passed through a 0.45 μm filter (Costar, Cambridge, MA) and stored at -70 C until viral isolation was attempted.

Antisera and monoclonal antibodies

The anti-TGEV hyperimmune sera were produced in eight-week-old pigs seronegative for TGE. Each TGEV isolate was grown in ST cells with MEM without FBS. Each pig was inoculated orally with 20 ml of the appropriate virus suspension adjusted to 5×10^5 p.f.u./ml. At four and eight weeks after the oral inoculation, 20 ml of the respective virus suspension was administered intravenously (IV). Two weeks after the final IV dose, blood was collected and served as the source of the anti-TGEV hyperimmune serum. The anti-TGEV (Miller strain) hyperimmune serum produced in a one-week-old gnotobiotic pig followed a similar schedule for administration of the viral dose. The gnotobiotic pig was inoculated orally with 10 ml of the Miller strain of TGEV adjusted to 5×10^6 p.f.u./ml. At four and six weeks after the oral inoculation, 10 ml of the virus suspension was administered IV. At eight weeks after the oral inoculation, 10 ml of the virus suspension was inoculated intraperitoneally (IP). Three days after the IP dose, blood was collected and served as the source of the gnotobiotic pig anti-Miller TGEV hyperimmune serum.

The anti-enterovirus hyperimmune serum was produced in eight-week-old pigs seronegative for TGE. Group 8C enterovirus was grown in ST cells with MEM without FBS. The virus suspension was adjusted to 5×10^5 p.f.u./ml and administered as described for TGEV antisera production in eight-week-old pigs.

The primary hybridomas were produced by fusion of SP2/0 myeloma cells and splenic lymphocytes of BALB/c mice (Kohler and Milstein, 1975) immunized with the Miller strain of TGEV as described by Zhu et al. (1990). Ascitic fluid containing MAb

was produced in BALB/c mice as described by Zhu et al. (1990). The MAb 1F7 was kindly provided by Dr. R. Woods, National Animal Disease Center, Ames, IA.

Virus isolation and purification

Four-day-old ST cells in 25 cm^2 flasks (Costar, Cambridge, MA) were treated with MEM containing diethylaminoethyldextran (50 µg/ml) (Sigma, St. Louis, MO) at 37 C for 30 minutes before the intestinal tissue filtrate was added. Prior to inoculation onto ST cell monolayers, 0.2 ml of each intestinal filtrate was mixed with 0.8 ml of a 1:10 dilution of porcine anti-enterovirus (Group 8C) hyperimmune serum in MEM with 2 percent FBS and antibiotics (penicillin 20,000 U/ml, streptomycin 20,000 µg/ml, and amphotericin B 50 µg/ml) (GIBCO, Grand Island, NY), and were incubated at 37 C for one hour. The ST cell monolayers were inoculated with the entire 1 ml of virus and anti-enterovirus hyperimmune serum mixture and the inoculum was adsorbed onto the ST cells for 60 minutes at 37 C, after which additional MEM with 2 percent FBS plus antibiotics was added. The cultures were incubated at 37 C for 48 hours and observed daily for cytopathic effect. After 48 hours, whether or not CPE was evident, all cultures were frozen and thawed three times, clarified by centrifugation at 200 x g for 10 minutes, and used as inoculum for the next passage.

All TGEV isolates were plaque purified a total of three times. Log dilutions of the TGEV isolates were prepared and 0.2 ml of each dilution was inoculated onto four-day-old ST cell monolayers in twelve-well plates. After one hour incubation at 37 C, the inoculum was removed, and the ST cell monolayers were overlaid with a mixture of Eagle's basal medium (BME) (GIBCO, Grand Island, NY) and agarose (FMC Bioproducts, Rockland, ME), containing 0.0016% neutral red (Fisher Scientific, Fair Lawn, NJ) and 30mM sodium bicarbonate. Two ml of the overlay was added to each well, and the overlay was allowed to solidify at room temperature, and the cultures were

incubated at 37 C for three days. Individual plaques, as visualized by the neutral red, were picked with a sterile Pasteur pipet, and the agarose plugs containing TGEV-infected cells were placed into one ml of MEM with two percent FBS and antibiotics. The agarose plugs containing TGEV-infected cells were frozen and thawed three times prior to inoculation into ST cell cultures. The inoculated ST cell cultures were observed for CPE, and the TGEV was clarified as previously described, and again diluted for further plaque purification. This was repeated until each isolate was plaque purified a total of three times. Stock virus was then prepared from the plaque purified isolates and stored at -70 C. All field isolates in this study were verified as being TGEV by an IFA with anti-TGEV (Miller strain) hyperimmune serum produced in a gnotobiotic pig.

Growth characteristics

The type of CPE induced by the TGEV isolates was observed. The Miller strain of TGEV served as the standard for the type of CPE caused by TGEV in ST cell culture to which the CPE of the isolates was compared.

Variation of plaque size among the TGEV isolates was determined as follows. Ten-fold log dilutions of plaque purified TGEV isolates were prepared and 0.2 ml of the diluted virus was allowed to adsorb on ST cell monolayers grown in six-well plates for one hour at 37 C. The inoculum was removed and the BME/agarose overlay with neutral red and sodium bicarbonate was added as previously described. After incubating for three days at 37 C, virus-induced plaques that were generally circular in shape and clearly separated from other plaques were measured for plaque diameter. Each plaque diameter was measured six times, each time at a different location than the previous measurement. A total of six plaques for each virus isolate were measured in this manner. The diameters were averaged for each isolate and then compared to the average diameters of the other isolates.

The titers of the TGEV isolates in ST cell culture were compared as the viral stocks were being produced in 75 cm^2 flasks. Each flask was infected with a multiplicity of infection (m.o.i.) of approximately 0.1 plaque-forming units (p.f.u.)/cell for each TGEV isolate. At 48 hours p.i. when CPE was essentially complete, the cultures were frozen and thawed three times, clarified by centrifugation, and titrated by plaque assay. Log dilutions of the appropriate TGEV isolate were prepared, and 0.2 ml of each dilution was inoculated onto ST cell monolayers in six-well plates (Costar, Cambridge, MA), adsorbed for one hour at 37 C, the inoculum was removed, and a BME/agarose overlay with neutral red and sodium bicarbonate was added to the cell monolayers as previously described. After three days of incubation at 37 C, the viral plaques were counted and the virus titer was expressed in p.f.u./ml. The titers of the TGEV isolates were then compared.

Electron microscopy

In order to discern any visible differences in the virion structure of selected isolates, electron microscopy was utilized. The Miller strain of TGEV, and the field isolates IA-137, IA-145, IA-156, and IA-165 were the viruses selected to be observed.

For each virus isolate, four 150 cm^2 flasks of ST cells were infected at a high m.o.i., and the flasks were frozen at 24 hours p.i. The visible cytopathic effect present when the flasks were frozen was approximately 25%. The flasks were frozen and thawed three times and clarified by centrifugation at 200 x g for 10 minutes to remove cellular debris. The remaining virus containing supernatant was then pelleted by ultra-centrifugation at 30,000 x g for 45 minutes. The viral pellet was carefully suspended in 1 ml of deionized distilled water and then stored overnight at 4 C. The pellet was resuspended, and then placed on a column containing Bio-Gel A-5m agarose beads (Biorad Laboratories, Richmond, CA) equilibrated with distilled water. The viral

suspension was then allowed to pass through the column and the first sample showing turbidity was collected until turbidity was no longer evident. This sample containing the partially purified virus was in a total volume of approximately 1 ml, and a portion of this sample was then stained with phosphotungstic acid and observed by electron microscopy.

Protein profile of TGEV isolates

Polyacrylamide gel electrophoresis was utilized to determine the extent of differences in the migration of viral structural proteins of selected isolates. The Miller and Purdue strains of TGEV, and the field isolates IA-137, IA-145, IA-156, and IA-165 were the viruses selected to be observed. Mock-infected and TGEV-infected cells were labeled with ^{35}S-methionine-cysteine (ICN Biomedicals, Irvine, CA) and partially purified as follows. Four-day-old ST cells in 75 cm^2 flasks were infected at a m.o.i. of 0.1 p.f.u./cell and incubated at 37 C. At 16 hours p.i., the medium was replaced with methionine-free MEM and the cells were incubated at 37 C for one hour. The medium was replaced with fresh methionine-free MEM with 250 µCi/ml ^{35}S-methionine-cysteine added. Seven hours after the addition of the ^{35}S-methionine-cysteine, the CPE present in the flasks was evident in approximately 25 percent of the cell monolayer, and the flasks were frozen and thawed three times. The cell lysate containing the labeled virus was clarified by centrifugation at 200 x g for 10 minutes. The virus was pelleted at 30,000 x g for 90 minutes by ultracentrifugation. The virus pellet was resuspended in one ml of TE buffer (10 mM tris-Cl, pH 7.4, 1 mM EDTA, pH 8.0) and was then overlaid on a discontinuous sucrose gradient. The concentrations of the sucrose used were 10, 25, 40, and 60 percent sucrose dissolved in TE buffer. The discontinuous sucrose gradients were then spun at 82,000 x g for 120 minutes, and the 25-40 percent interface was collected and stored at - 20 C. Twenty-five µl of the appropriately labeled

virus- or mock-infected interface were mixed with 25 µl of sample buffer (0.125M tris-HCl, ph 6.8, 4% sodium dodecyl sulfate (SDS), 20% glycerol, 10% 2-mercaptoethanol) and placed in a boiling water bath for 5 minutes. The samples were electrophoresed at 15 mA constant current through a discontinuous 10% SDS-polyacrylamide gel. The ionic strength of the acrylamide gel and running buffer were 0.375M tris-HCl (pH 8.8) and 0.025M tris-HCl (pH 8.3), respectively. High and low molecular mass proteins were used as reference standards for determination of the molecular mass of viral proteins. The acrylamide gels were placed in fixation solution for one hour, then placed in autoradiography enhancer (NEN Research Products, Boston, MA) for 30 minutes, and then rehydrated in distilled water for 30 minutes. Then the acrylamide gels were dried and film (X-OMAT, Eastman Kodak Co., Rochester, NY) exposed to the gels at -70 C for the production of autoradiographs.

The molecular mass of the TGEV proteins were determined by running ^{14}C-labeled molecular mass markers on the same gel. In calculation of molecular mass, a linear relation between log (molecular mass) and relative mobility is assumed. The relative mobility is defined as the ratio of the distance moved by the protein to the distance moved by the bromophenol blue marker.

Virus neutralization

The ability of the TGEV isolates to be neutralized by MAb or swine polyclonal anti-TGEV hyperimmune serum was determined by a plaque reduction assay. The ascitic fluid containing the MAb or the swine polyclonal anti-TGEV hyperimmune serum to be tested was heat-inactivated for 30 minutes at 56 C. The MAb or swine polyclonal anti-TGEV hyperimmune serum was diluted serially and the dilutions were mixed with an equal amount of MEM containing 1000 p.f.u. of the TGEV to be tested. The virus-antibody mixture was incubated for one hour at 37 C, and the unneutralized virus was

titrated by inoculating five-day-old ST cells in six-well plates with 0.2 ml of the virus-antibody mixture. The VN titer of the MAb or the swine polyclonal anti-TGEV hyperimmune serum was expressed as the reciprocal of the highest dilution that resulted in an 80 percent reduction of 100 p.f.u./well, as compared to the virus-medium control.

Indirect immunofluorescence assay

The titers of MAbs reacting with the TGEV isolates were determined by an IFA. Four-day-old ST cells grown in 96-well plates (Costar, Cambridge, MA) were infected with 200 p.f.u. of the appropriate virus suspended in MEM with two percent FBS and antibiotics, and incubated at 37 C. At 18-24 hours p.i., the medium was removed and the cells were washed three times with phosphate-buffered saline (PBS), pH 7.4, and then fixed with methanol for 10 minutes. After removal of the methanol, the fixed cells were allowed to dry completely. The MAbs to be tested were diluted serially, and 0.2 ml of the appropriate dilution into two wells and was incubated with the fixed cells for one hour at 37 C. The fixed cells were then washed and then stained with fluorescein-conjugated goat anti-mouse IgG (heavy and light chain) conjugate (Cappel Laboratories, Malvern, PA) for one hour at 37 C. The unbound conjugated antibodies were washed away with PBS, and glycerol:PBS (1:1) was added to each well. The plates were then examined for immunofluorescence, and the highest dilution of the MAb showing fluorescence was designated as the titer. Uninfected ST cells were processed in the same manner as above and served as negative controls.

Radioimmunoprecipitation assay

A RIP was utilized to determine the extent of differences in the migration of viral structural proteins of selected isolates. The Miller strain of TGEV, and the field isolates IA-137, IA-145, IA-156, and IA-165 were the viruses selected to be observed. These TGEV isolates and mock-infected cells were labeled with ^{35}S-methionine-cysteine and

processed as follows. Four-day-old ST cells in 25 cm^2 flasks were infected at a m.o.i. of 0.1 p.f.u./cell and incubated at 37 C. At 16 hours p.i., the medium was replaced with methionine-free MEM and the cells were incubated at 37 C for one hour. The medium was replaced with fresh methionine-free MEM with 250 µCi/ml ^{35}S-methionine-cysteine added. Four hours after the addition of the ^{35}S-methionine-cysteine, the cell monolayers scraped from the plastic flask and pelleted by centrifugation at 200 x g for 10 minutes. The cellular pellets containing labeled viral proteins and were then disrupted with 1 ml of lysis buffer (50 mM NaCl, 50 mM tris, 5mM EDTA, 1% Triton X-100, and 1mM phenylmethylsulfonyl fluoride). The lysis buffer and cellular pellet mixtures were vortexed vigorously for one minute, and placed on ice for 3 minutes. The remaining cellular residues were removed by centrifugation. The lysates were stored at -20 C until needed. One hundred and fifty µl of the appropriate lysate was mixed with 4 µl of ascitic fluid containing MAb, or 3 µl of of undiluted swine polyclonal anti-Miller TGEV hyperimmune serum and incubated overnight at 4 C. Immune complexes were then collected by the addition of sepharose beads coated with protein A (Sigma, St, Louis, MO.), and incubated for one hour at room temperature. The antigen-antibody complexes/protein A sepharose beads mixtures were then washed three times with lysis buffer and then three times with deionized distilled water, resuspended in 50 µl sample buffer, and run on a SDS-polyacrylamide gel as previously described.

Results

Virus isolation

Virus isolation was originally attempted from the small intestines of 99 pigs shown to be positive for TGEV by immunofluorescence. Transmissible gastroenteritis virus was isolated from 24 of these samples in ST cell culture for a frequency of isolation

of approximately 24%. Enterovirus contamination was a common occurrence in ST cell culture, but the use of anti-enterovirus antisera was beneficial in reducing the amount of enterovirus present so that plaque purification of the TGEV isolates could be accomplished.

Growth characteristics

All of the isolates used in this study produced a CPE in ST cell culture. The CPE was consistent among the isolates when compared to the CPE of the standard Miller strain of TGEV in ST cell culture, with the exception of the isolate IA-145. The typical CPE shown in ST cell culture consists of fusion of virally infected cells, and a ballooning effect of the cells as they detach from the cell monolayer. An uninfected cell monolayer of ST cells serves as a control and is shown in Figure 1. The typical CPE produced by the Miller strain of TGEV in ST cell culture is shown in Figure 2. The isolates IA-137, IA-156, and IA-165 also show a typical CPE in ST cell culture, as evidenced in Figures 3, 4 and 5 respectively. However, the TGEV isolate IA-145 produced a different CPE in the ST cell culture, as shown in Figure 6. The CPE induced by IA-145 is more extensive and has a more prominent net-like appearance. Also, the infected cells that comprise the CPE are less granular in appearance as compared to the CPE of the other isolates.

The average plaque sizes of the TGEV isolates and the standard Miller, Purdue, and Illinois strains is presented in Table 2. The plaque sizes ranged from 3.19 mm to 10.14 mm in diameter. The grouping of the isolates as to their plaque size is shown in Table 3. The majority of the TGEV isolates tested had average plaque sizes in Group I or II, ranging from 3.00 to 6.99 mm. One particular isolate, KS-200, had a very large plaque size 10.14 mm, that placed it in Group IV.

The titers of the TGEV isolates grown in ST cell culture are shown in Table 4. The titers of the TGEV isolates ranged from 1×10^5 to 5×10^7 p.f.u./ml.

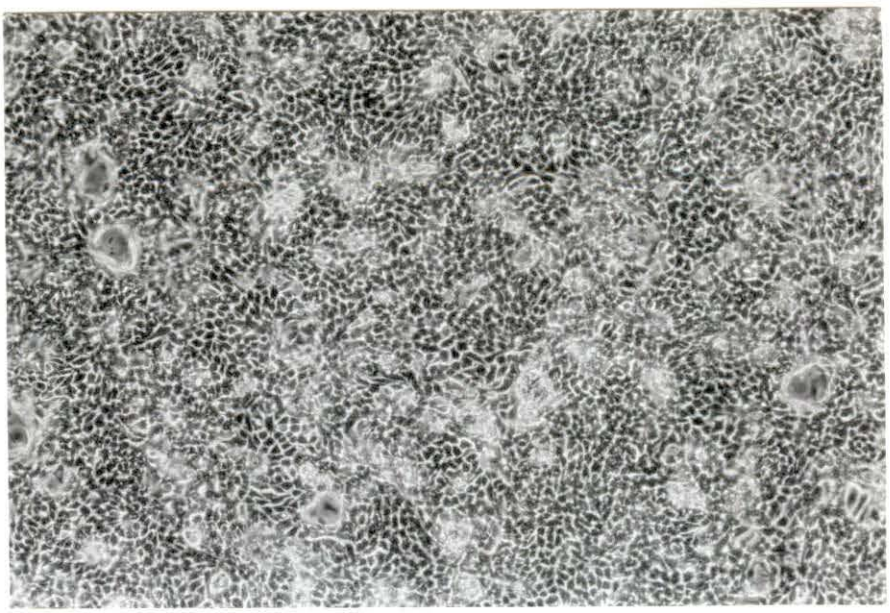

Figure 1. Uninfected four-day-old swine testis (ST) cells (Phase contrast, 100x)

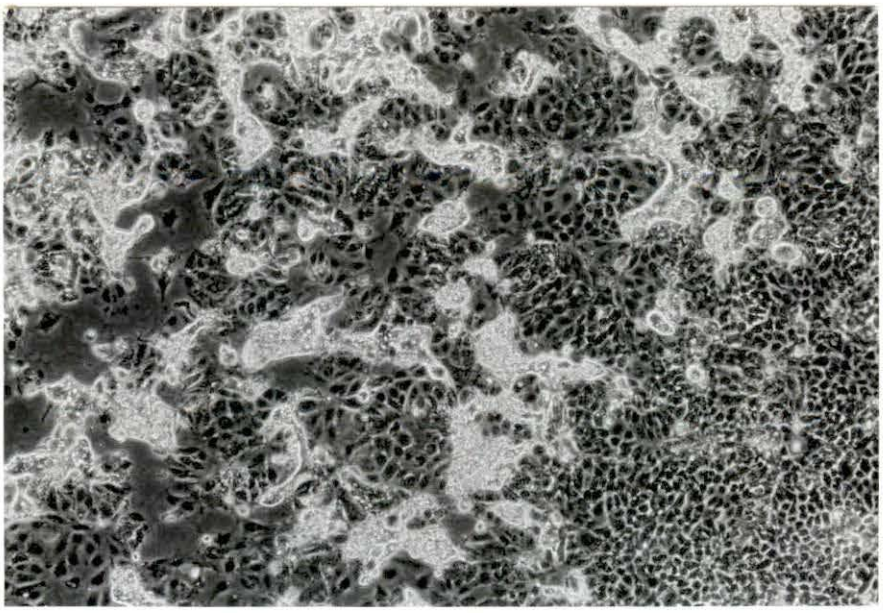

Figure 2. Cytopathic effect of the Miller strain of TGEV in four-day-old ST cells (Phase contrast, 100x)

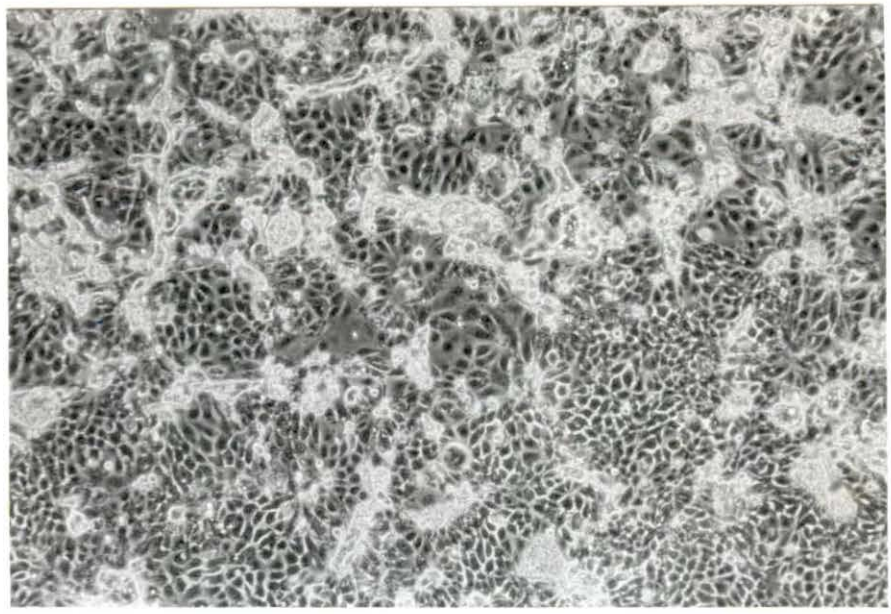

Figure 3. Cytopathic effect of IA-137 TGEV in four-day-old ST cells (Phase contrast, 100x)

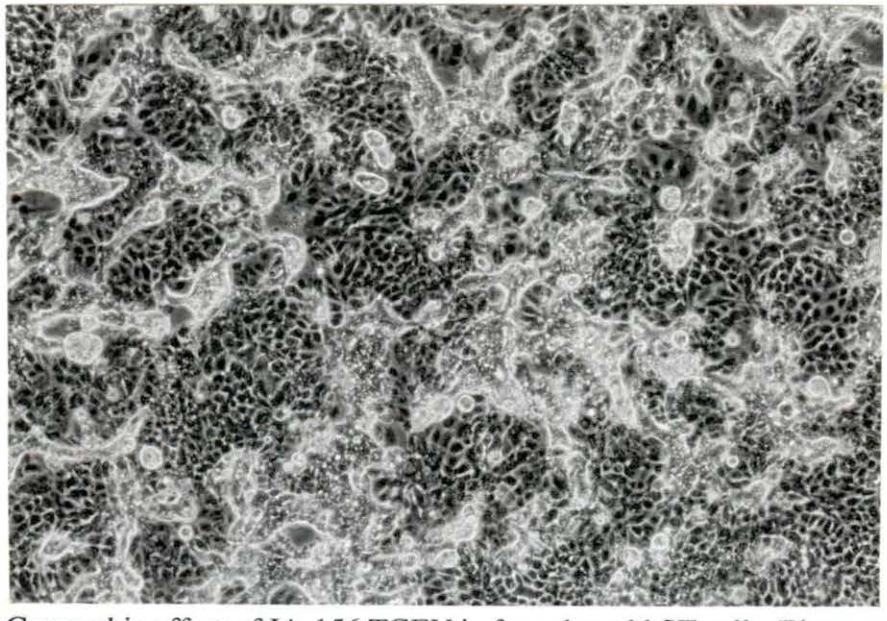

Figure 4. Cytopathic effect of IA-156 TGEV in four-day-old ST cells (Phase contrast, 100x)

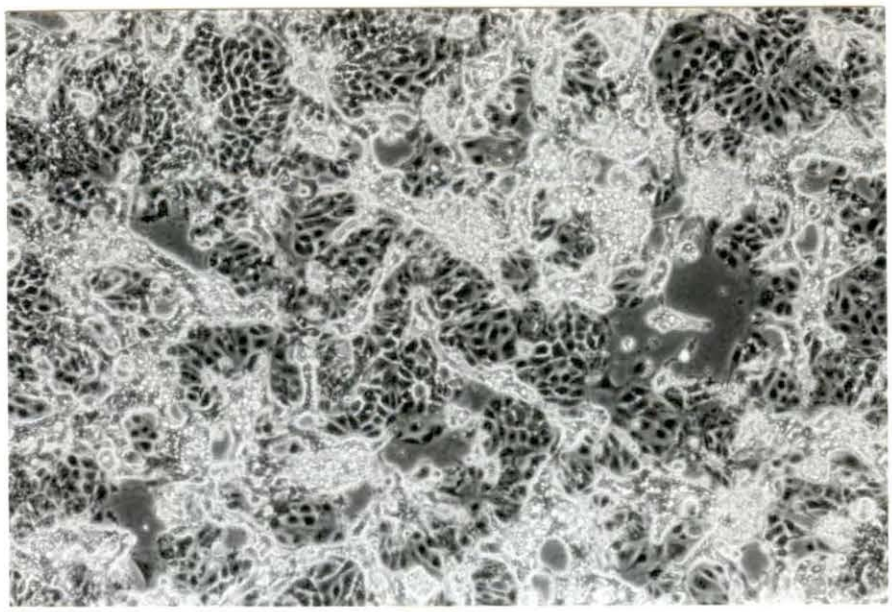

Figure 5. Cytopathic effect of IA-165 TGEV in four-day-old ST cells (Phase contrast, 100x)

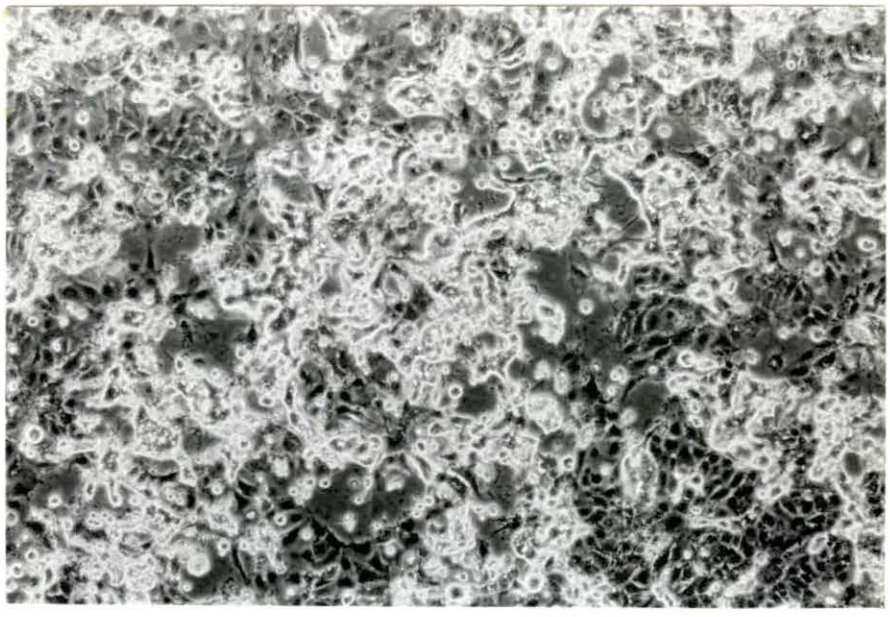

Figure 6. Cytopathic effect of IA-145 TGEV in four-day-old ST cells (Phase contrast, 100x)

Table 2. Average plaque size in millimeters of transmissible gastroenteritis virus isolates[a]

Virus	Plaque size (mm)
Miller	5.17
Illinois	3.56
Purdue	4.30
IA-100	3.97
IA-101	3.19
IA-107	4.08
IA-111	7.53
IA-114	3.44
IA-117	5.70
IA-118	7.44
IA-131	6.72
IA-136	5.50
IA-137	3.36
IA-139	8.47
IA-145	4.75
IA-148	5.08
IA-156	5.25
IA-164	3.97
IA-165	7.03
IA-166	6.94
IA-178	6.28
IA-179	4.83
IA-709	7.83
KS-200	10.14
KS-204	8.42
AR-302	6.83
AR-310	6.78

[a] Average plaque size determined in the ST cell line by measuring six different diameters of six plaques for each isolate.

Table 3. Grouping of transmissible gastroenteritis virus isolates as to the average diameter of viral plaques formed in the swine testicular cell line[a]

Group I 3.00-4.99 mm	Group II 5.00-6.99 mm	Group III 7.00-8.99 mm	Group IV 9.00-10.99 mm
Illinois	Miller	IA-111	KS-200
Purdue	IA-117	IA-118	
IA-100	IA-131	IA-139	
IA-101	IA-136	IA-165	
IA-107	IA-148	IA-709	
IA-114	IA-156	KS-204	
IA-137	IA-166	AR-302	
IA-145	IA-178	AR-310	
IA-164			
IA-179			

[a]Average plaque size determined in the ST cell line by measuring six different diameters of six plaques for each isolate.

Table 4. Titers of TGEV isolates grown in the swine testicular cell line

Virus	Titer (p.f.u./ml)
Miller	1.0×10^5
Illinois	2.0×10^6
Purdue	1.5×10^7
IA-100	2.5×10^7
IA-101	5.0×10^5
IA-107	1.5×10^7
IA-111	5.0×10^5
IA-114	2.0×10^6
IA-117	4.0×10^5
IA-118	1.5×10^6
IA-131	1.0×10^6
IA-136	3.0×10^6
IA-137	5.0×10^7
IA-139	2.5×10^6
IA-145	1.0×10^6
IA-148	1.0×10^6
IA-156	5.0×10^5
IA-164	1.5×10^7
IA-165	1.0×10^6
IA-166	5.0×10^5
IA-178	5.0×10^5
IA-179	5.0×10^7
IA-709	1.0×10^6
KS-200	1.0×10^5
KS-204	2.0×10^6
AR-302	3.0×10^6
AR-310	5.0×10^6

Virus morphology

The standard Miller strain of TGEV and the field isolates IA-137, IA-145, IA-156, and IA-165 were examined by EM. Figure 7 shows an electronmicrograph of the Miller strain of TGEV grown in ST cell culture that represents a typical coronavirus particle as seen in all the cultures observed by EM. There were no visible differences among the peplomer structures of the isolates observed by EM.

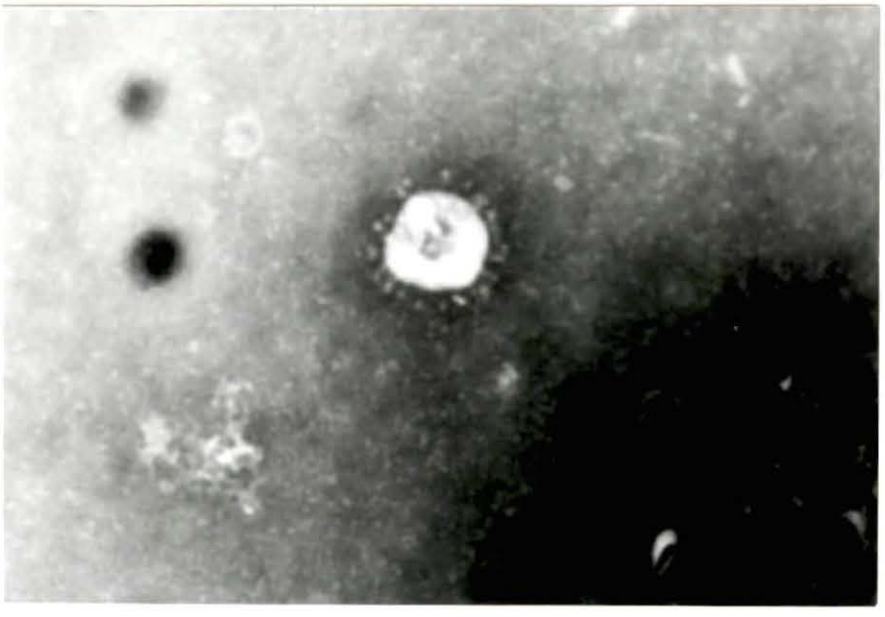

Figure 7. Electronmicrograph of a coronavirus particle of the Miller strain of TGEV grown in ST cells (Negative stain, 100,000x)

Protein profile

Viral proteins from the standard Miller strain of TGEV, and the field isolates IA-137, IA-145, IA-156, and IA-165 were radiolabeled in the presence of ^{35}S-methionine-cysteine and analyzed by SDS-PAGE, as shown in Figure 8. Three proteins were detected with calculated molecular masses of 200 kDa (S), 46 kDa (N), and 28 kDa (M). Also present was the N protein byproduct (designated here as N') with an apparent molecular mass of 42 kDa. There was no discernable difference in the migration pattern of the TGEV proteins among the isolates tested.

Antigenic relatedness

Antigenic relatedness was demonstrated by VN, IFA, and RIP with hyperimmune sera and/or MAbs.

The VN titers of the isolates with the polyclonal anti-TGEV hyperimmune sera are listed in Table 5. Variations among the homologous versus heterologous VN titers for all the hyperimmune sera ranged from 2 to 8 fold. The anti-Miller hyperimmune serum easily neutralized all isolates tested with titers of 1600 to 3200. The anti-Illinois hyperimmune serum also easily neutralized the isolates with titers of 800 to 3200. The anti-Purdue, anti-IA-111, and anti-IA-137 hyperimmune sera also neutralized the isolates tested, but the neutralization titers of these three hyperimmune sera ranged from 50 to 3200, and were generally lower than the anti-Miller and anti-Illinois hyperimmune sera. The titers of the IA-145 hyperimmune serum was the lowest of the hyperimmune sera utilized with ranges of 50 to 1600, with the majority of neutralization titers being 50 or 100 for that particular hyperimmune serum.

Figure 8. Polyacrylamide gel electrophoresis of ^{14}C-labeled molecular mass standards (kDa) (lanes 1 and 8) and ^{35}S-methionine-cysteine labeled proteins of partially purified TGEV isolates Miller (lane 3), IA-137 (lane 4), IA-145 (lane 5), IA-156 (lane 6) and IA-165 (lane 7). Mock-infected ST cells are in lane 2.

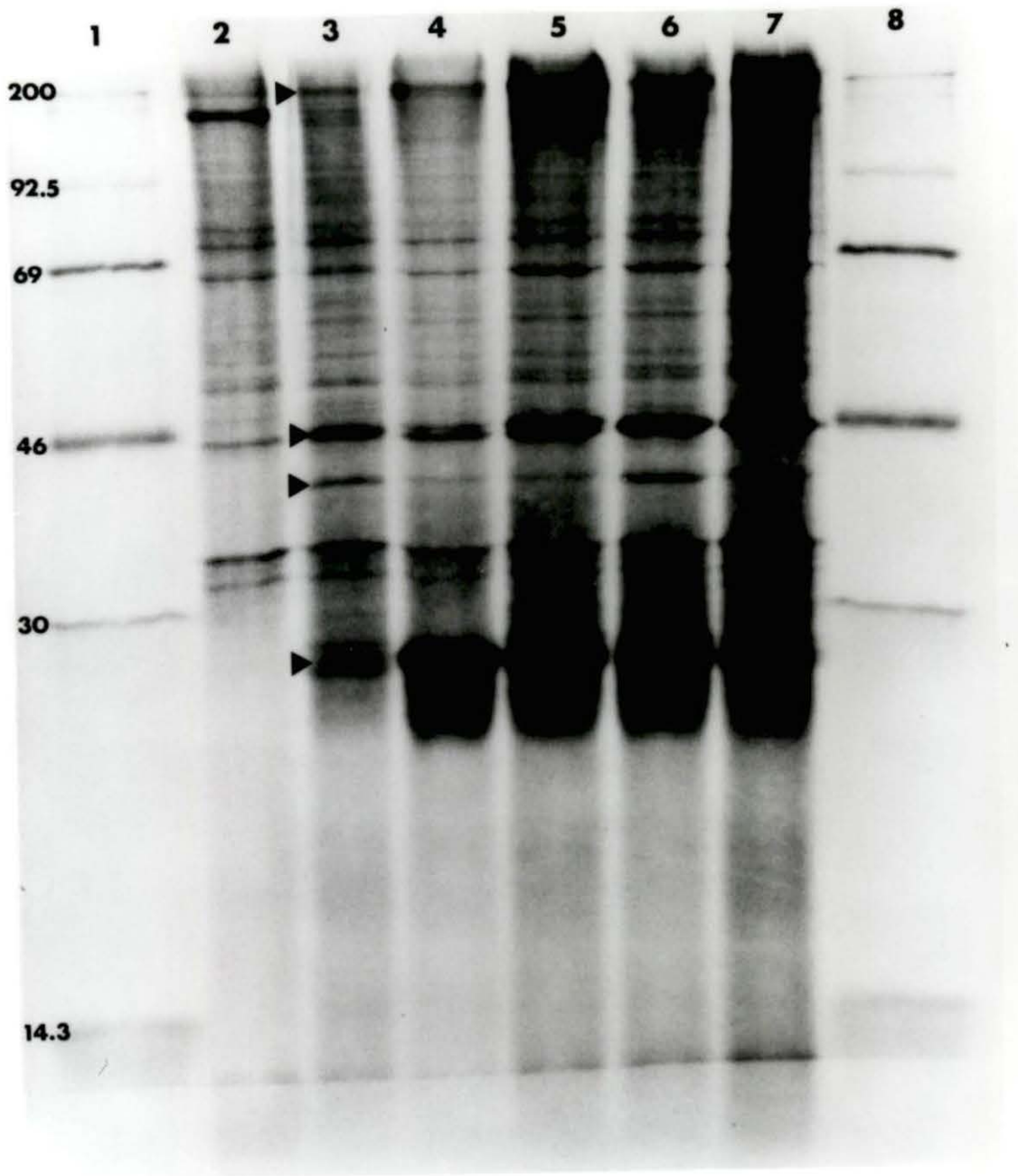
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Table 5. Neutralization of transmissible gastroenteritis isolates by polyclonal antibodies[a]

Virus	Polyclonal antibodies					
	Miller	Illinois	Purdue	IA-111	IA-137	IA-145
Miller	3200	1600	400	400	100	100
Illinois	1600	1600	200	800	50	100
Purdue	3200	3200	800	400	200	200
IA-100	3200	3200	800	400	200	200
IA-101	1600	800	200	400	50	100
IA-107	1600	1600	400	400	50	200
IA-111	3200	3200	1600	800	400	200
IA-114	3200	3200	1600	3200	400	400
IA-117	3200	3200	400	800	200	200
IA-118	1600	800	400	400	100	50
IA-131	3200	3200	3200	3200	1600	400
IA-136	3200	1600	1600	1600	400	200
IA-137	3200	1600	1600	1600	400	400
IA-139	1600	1600	800	800	100	100
IA-145	3200	1600	800	800	200	200
IA-148	3200	3200	1600	3200	400	800
IA-156	3200	3200	1600	3200	400	800
IA-164	3200	1600	400	800	100	100
IA-165	3200	1600	400	800	100	100
IA-166	1600	1600	800	800	100	100
IA-178	3200	3200	1600	400	1600	400
IA-179	1600	1600	800	800	100	100
IA-709	3200	1600	1600	200	1600	1600
KS-200	3200	1600	1600	200	1600	1600
KS-204	1600	800	800	800	100	100
AR-302	3200	3200	800	400	100	100
AR-310	1600	800	400	200	50	50

[a]Reciprocal of the last dilution of polyclonal antibodies neutralizing 80% of about 100 p.f.u. of TGEV.

The VN titers of the isolates with the MAbs MH11, MA5, and MA4 are listed in Table 6. With the exception of the field isolate IA-156, the field isolates were neutralized by the MAb MH11 with the titers ranging from 100-12800. The neutralizing titer of MH11 for the homologous Miller strain of TGEV was 51200, whereas all the field isolates and the Purdue and Illinois strains of TGEV were neutralized with 2 to 2048 fold lower titers. Again, with the exception of the field isolate IA-156, the field isolates were neutralized by the MAbs MA5 and MA4 with the titers ranging from 800-12800. The neutralizing titers of MA5 and MA4 for the homologous Miller strain of TGEV was either 25600 or 51200, whereas all the field isolates and the Purdue and Illinois strains of TGEV were neutralized with 4 to 2048 fold lower titers. When the isolates are grouped as to their VN titers with the MAbs (Tables 7 and 8) the majority of the isolates are placed into groups I and II. IA-156 was consistently placed in group IV with its low VN titer of 25.

The results of the IFA titers with the MAbs are shown in Table 9. The titers of the MAbs in the IFA are similar to those in the VN tests, with the IFA titers ranging from 2 to 16 fold higher than the VN titers. As with the VN titer for IA-156, the IFA titer with the MAbs was very low with a titer of 25.

Radioimmunoprecipitation using the swine polyclonal anti-Miller TGEV hyperimmune serum, the MAb 1F7 (directed to the N protein) and the MAb MH11 (directed to the S protein) was used to analyze the viral proteins and the antigenic composition of the standard Miller strain of TGEV and the field isolates IA-137, IA-145, IA-156, and IA-165.

The hyperimmune serum recognized the S, M, and N viral proteins of all the viruses tested, as shown in Figure 9. Also present is the N' protein with an apparent molecular mass of 42 kDa as previously described. In Figure 10, the MAb 1F7

Table 6. Neutralization of transmissible gastroenteritis virus isolates by monoclonal antibodies[a]

Virus	Monoclonal antibodies		
	MH11	MA5	MA4
Miller	51200	25600	51200
Illinois	12800	6400	6400
Purdue	3200	6400	6400
IA-100	12800	12800	12800
IA-101	12800	1600	1600
IA-107	6400	6400	6400
IA-111	100	800	800
IA-114	12800	12800	12800
IA-117	6400	12800	12800
IA-118	3200	6400	6400
IA-131	6400	12800	12800
IA-136	1600	6400	6400
IA-137	400	1600	1600
IA-139	12800	800	800
IA-145	3200	1600	3200
IA-148	12800	12800	12800
IA-156	25	25	25
IA-164	12800	12800	12800
IA-165	1600	6400	6400
IA-166	1600	12800	12800
IA-178	12800	12800	12800
IA-179	12800	6400	12800
IA-709	400	1600	3200
KS-200	1600	12800	12800
KS-204	6400	12800	12800
AR-302	12800	12800	12800
AR-310	6400	1600	1600

[a] Reciprocal of the last dilution of monoclonal antibodies neutralizing 80% of about 100 p.f.u. of TGEV.

Table 7. Grouping of transmissible gastroenteritis virus isolates as to their neutralization titers by the monoclonal antibody MH11[a]

Group I Titers of 12800-51200	Group II Titers of 1600-6400	Group III Titers of 200-800	Group IV Titers of 25-100
Miller	Purdue	IA-137	IA-111
Illinois	IA-107	IA-709	IA-156
IA-100	IA-117		
IA-101	IA-118		
IA-114	IA-131		
IA-139	IA-136		
IA-148	IA-145		
IA-164	IA-165		
IA-178	IA-166		
IA-179	KS-200		
AR-302	KS-204		
	AR-310		

[a] Reciprocal of the last dilution of monoclonal antibodies neutralizing 80% of about 100 p.f.u. of TGEV.

Table 8. Grouping of transmissible gastroenteritis virus isolates as to their neutralization titers by the monoclonal antibodies MA4 and MA5[a]

Group I Titers of 12800-51200	Group II Titers of 1600-6400	Group III Titers of 200-800	Group IV Titers of 25-100
Miller	Illinois	IA-111	IA-156
IA-100	Purdue	IA-139	
IA-114	IA-101		
IA-117	IA-107		
IA-131	IA-118		
IA-148	IA-136		
IA-164	IA-137		
IA-166	IA-145		
IA-178	IA-165		
KS-200	IA-179		
KS-204	IA-709		
AR-302	AR-310		

[a] Reciprocal of the last dilution of monoclonal antibodies neutralizing 80% of about 100 p.f.u. of TGEV.

Table 9. Immunofluorescent reactivity of transmissible gastroenteritis virus isolates with monoclonal antibodies[a]

Virus	Monoclonal antibodies		
	MH11	MA5	MA4
Miller	51200	25600	51200
Illinois	25600	25600	25600
Purdue	51200	51200	25600
IA-100	25600	25600	25600
IA-101	25600	25600	12800
IA-107	12800	25600	25600
IA-111	400	1600	1600
IA-114	25600	12800	12800
IA-117	6400	12800	12800
IA-118	3200	6400	6400
IA-131	6400	12800	12800
IA-136	6400	12800	12800
IA-137	3200	12800	12800
IA-139	51200	6400	6400
IA-145	25600	12800	12800
IA-148	51200	25600	25600
IA-156	25	25	25
IA-164	51200	25600	25600
IA-165	25600	25600	25600
IA-166	25600	51200	25600
IA-178	12800	12800	12800
IA-179	12800	12800	6400
IA-709	800	6400	3200
KS-200	6400	12800	12800
KS-204	6400	12800	12800
AR-302	25600	12800	25600
AR-310	6400	6400	6400

[a] Reciprocal of the last dilution of monoclonal antibodies showing fluorescence.

recognized the N protein of 46 kDa, the 42 kDa N' protein, and a smaller molecular mass N protein byproduct of 40 kDa (designated here as N"). In Figure 11, the MAb MH11 only recognized the S protein of the isolates, with the S protein of IA-156 being only slightly recognized. The MAb MH11 recognized what may possibly be a degradation product of the S protein of IA-156 that had an apparent molecular mass of 27 kDa. This degradation product was not evident in the other isolates tested. Also, the lack of recognition of the S protein of IA-156 with the MAb MH11 in the RIP correlates with the VN and IFA titers of MH11 with this isolate.

Figure 9. Immunoprecipitation of ^{35}S-methionine-cysteine labeled TGEV infected ST cell culture lysate with polyclonal swine anti-TGEV hyperimmune serum. ^{14}C-labeled molecular mass standards (kDa) (lanes 1 and 8), mock-infected ST cell lysate (lane 2), Miller TGEV (lane 3), IA-137 TGEV (lane 4), IA-145 TGEV (lane 5), IA-156 TGEV (lane 6), and IA-165 TGEV (lane 7).

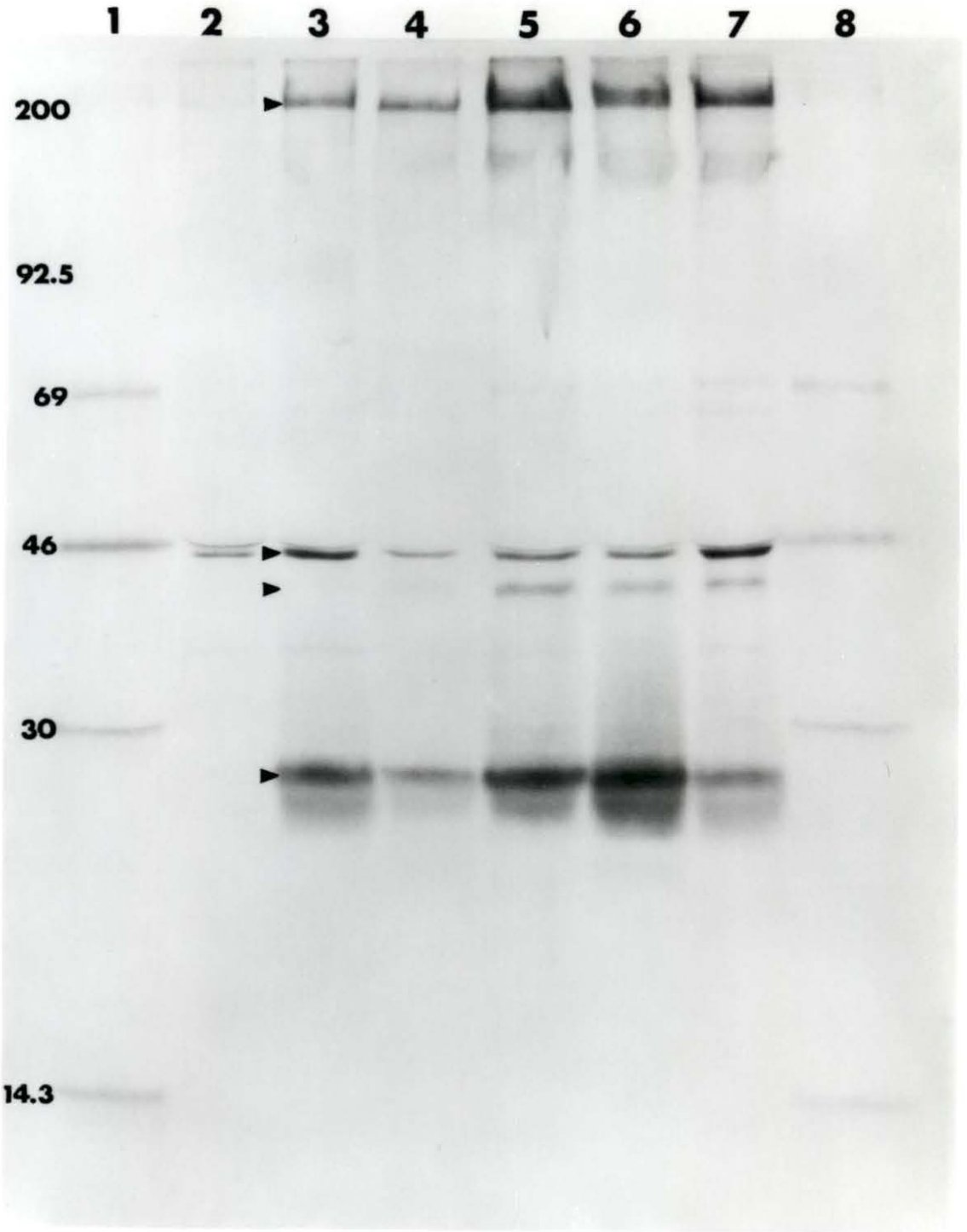
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Figure 10. Immunopreciptation of ^{35}S-methionine-cysteine labeled N protein of TGEV by the monoclonal antibody 1F7. ^{14}C-labeled molecular mass standards (kDa) (lanes 1 and 8), mock-infected ST cell lysate (lane 2), Miller TGEV (lane 3), IA-137 TGEV (lane 4), IA-145 TGEV (lane 5), IA-156 TGEV (lane 6), and IA-165 TGEV (lane 7).

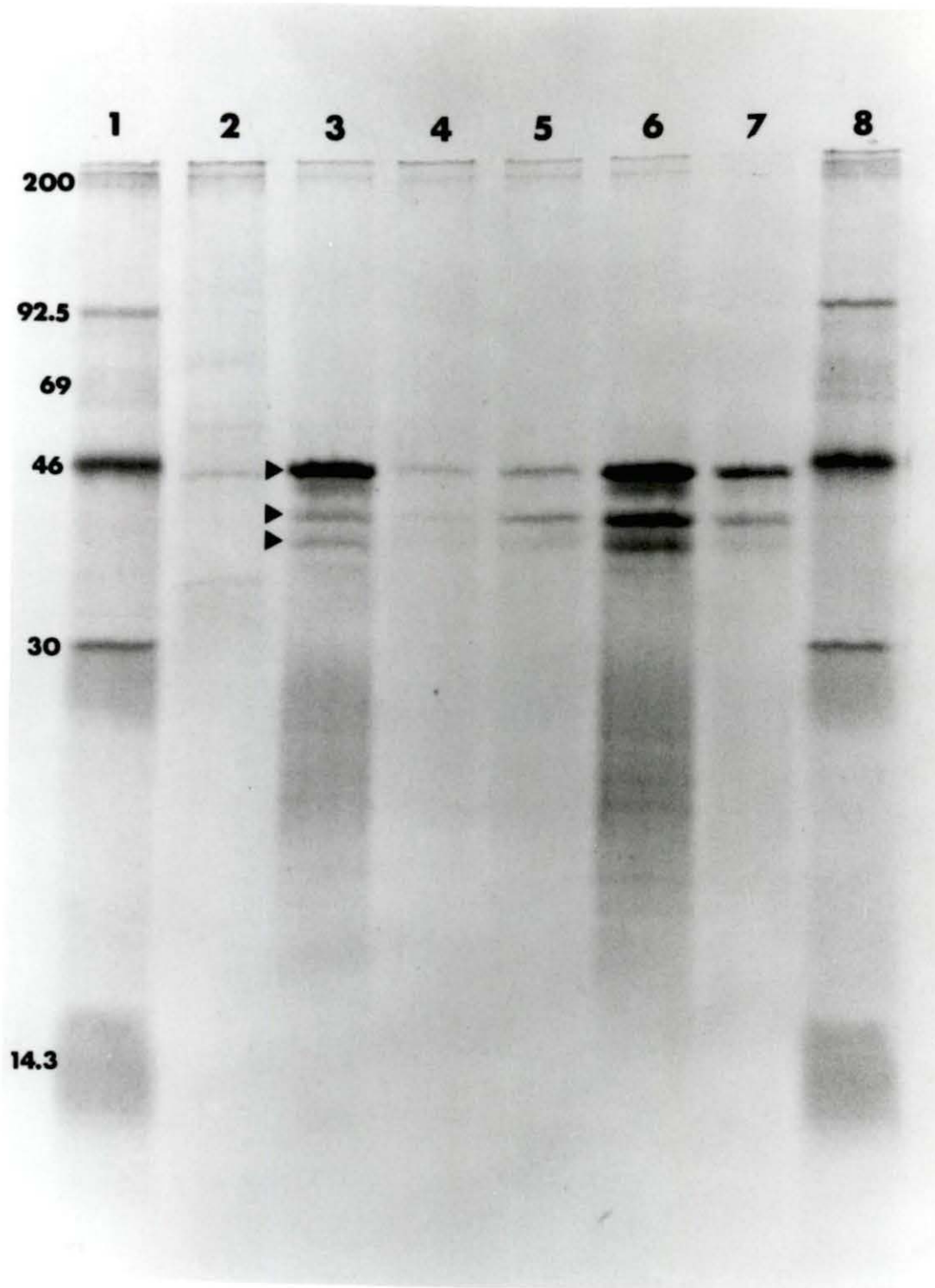
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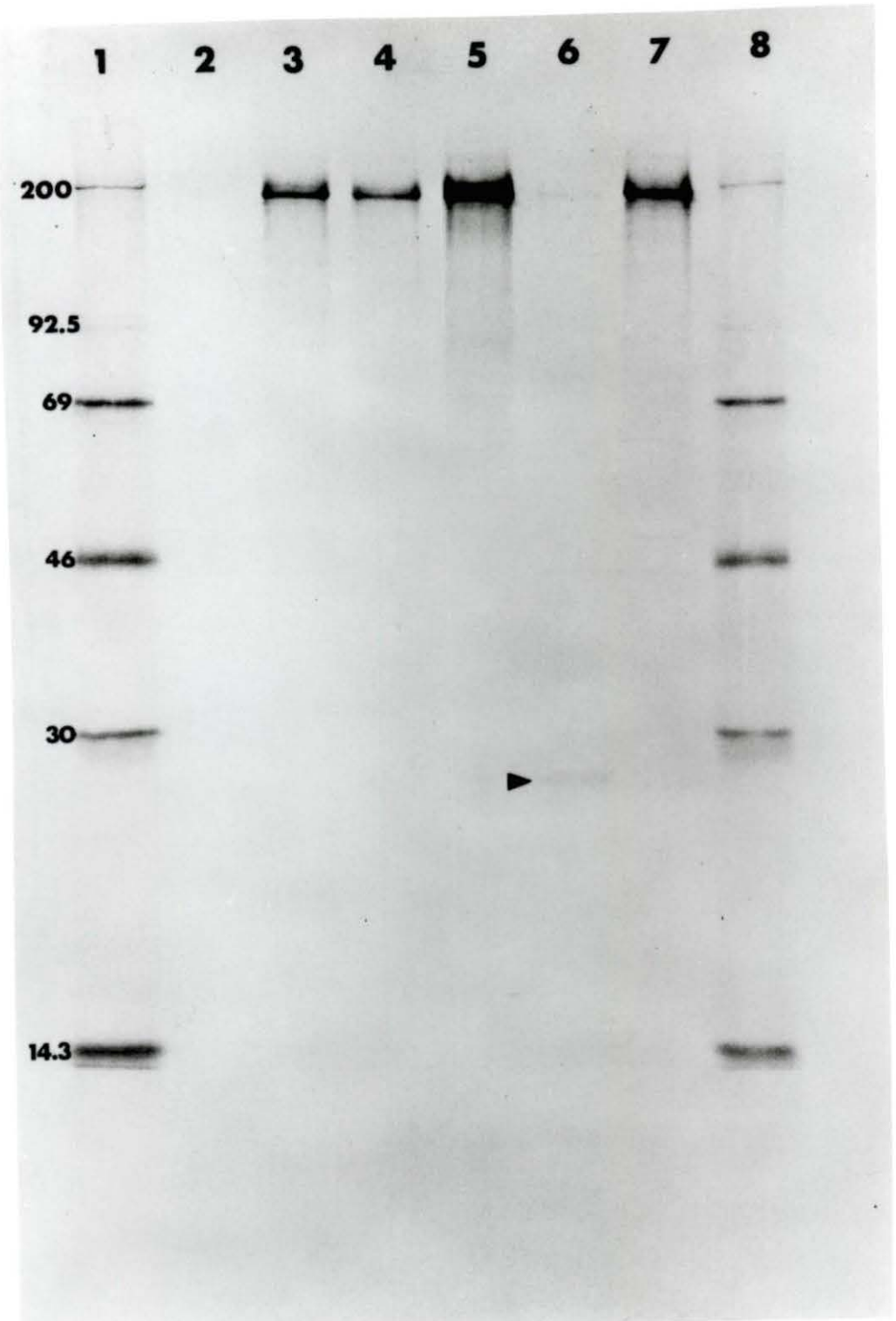
Figure 11. Immunopreciptation of ^{35}S-methionine-cysteine labeled S glycoprotein of TGEV by the monoclonal antibody MH11. ^{14}C-labeled molecular mass standards (kDa) (lanes 1 and 8), mock-infected ST cell lysate (lane 2), Miller TGEV (lane 3), IA-137 TGEV (lane 4), IA-145 TGEV (lane 5), IA-156 TGEV (lane 6), and IA-165 TGEV (lane 7).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Discussion

The results of this study show that there is antigenic and biological diversity among TGEV isolates. The biological diversity was revealed with differences in cytopathic effect and plaque size.

The cytopathic effect displayed by the isolate IA-145 was different than that of the standard Miller strain of TGEV and the remaining isolates of TGEV. The CPE of IA-145 was more extensive forming a net-like pattern and less granulation was evident in the infected cells. The significance, if any, of this type of CPE is not known. Even though IA-145 produced a different CPE in ST cell culture, it was easily neutralized by both polyclonal and monoclonal antibodies used in this study. Also, the plaque diameter formed by IA-145 in ST cell culture was similar to the majority of the isolates screened.

The TGEV isolates were grouped based on their plaque size. The majority of the isolates were placed in Group I or II, ranging from 3.00 to 6.99 mm in diameter. One particular isolate, KS-200, had a very large plaque size of 10.14 mm and was placed in Group IV. The isolate KS-200 had a normal type of CPE and was readily neutralized by both polyclonal and monoclonal antibodies used in this study. It is an accepted fact that as a particular virus adapts to growth in cell culture, the viral population changes as higher titers are achieved and the plaque size of the virus increases (Hamada et al., 1988). All of the isolates used in this study were at 9 to 12 passages in ST cell culture, so that large differences in plaque size should be due to true biological variance among isolates rather than representing a higher passaged ST cell culture adapted TGEV isolate. Carmichael et al. (1981) and Woods (1978) found that large plaque characteristics correlated with a lack of virulence. However, a large plaque size alone is not sufficient evidence to suggest that a particular isolate will be avirulent, as small plaque variants have been developed that are not virulent. Aynaud et al. (1985) developed a small plaque

variant that was resistant to digestive enzymes and Woods (1978) produced a small plaque variant from a TGEV-infected swine leukocyte line. Both of these small plaque variants were tested and found to be avirulent. However, with FIPV, avirulent isolates produce small plaques whereas virulent FIPV produces large plaques (Christianson et al., 1989). The virulence of KS-200 is not known at this time and the virulence of KS-200 would have to be proven by being tested on neonatal swine.

The plaque size of a virus in cell culture should also reflect the replication rate of the virus (Hamada et al., 1988). However, the titer of KS-200 at 48 hours p.i. was 1 x 10^5 p.f.u./ml, a relatively low titer. It is possible that the maximum titer of KS-200 was achieved prior to 48 hours p.i. and that due to the labile nature of TGEV, the titer of KS-200 was decreasing when the virus was harvested. This would have to be confirmed by performing a growth curve to measure the virus titer at various time points. Another point to consider is the fact that the Purdue strain of TGEV is a high-passaged strain (approximately 115 passages in cell culture) and produced a plaque size of 4.30 mm, while the low-passaged Miller strain had an average plaque size of 5.17 mm. The reason that the Purdue strain does not have an increased plaque size that reflects its level of cell culture passage is not known.

The titers of the isolates in ST cell culture could be construed to correlate with the level of adaptation to growth in cell culture, however as previously stated, the fact that the isolates were at similar cell passage levels should rule out this possibility. It should be noted that the listing of the titers of the isolates should in no way be construed to represent the true titer of the isolates. The titers shown are not of repeated measurements and are only meant to convey the variability in titer of the isolates grown in cell culture. In general, the more attenuated the virus, the higher the titer in cell culture. As a case in

point, the Purdue strain of TGEV had a titer of 1.5 x 10^7 p.f.u./ml after approximately 115 passages in cell culture.

Visualization of the isolates IA-137, IA-145, IA-156, and IA-165 by EM revealed that no discernible differences in virus structure was evident. It was thought that since the isolate IA-145 demonstrated a different CPE in ST cell culture that it may have a different peplomer shape. But IA-145 resembled a typical coronavirus particle just as did IA-137, IA-156, and IA-165.

Out of the 99 porcine intestinal samples processed, 24 isolates of TGEV were isolated for an efficiency of approximately 24%. The low efficiency of isolation of TGEV from clinical samples may reflect biological variability among TGEV isolates. Other factors, such as the labile nature of the TGEV, the difficulty of isolating TGEV in the presence of other naturally occurring viruses such as enteroviruses, and the lack of adaptation to ST cell culture of some of the isolates may affect TGEV isolation. When the isolates used in this study were being confirmed as being TGEV by IFA, some of the isolates showed weak positive immunofluorescence indicating the presence of TGEV, but no CPE was evident after repeated passages in ST cell culture. The isolates that failed to adapt to growth in ST cell culture potentially represent new biologic variants.

Antigenic diversity was evidenced by the results of VN tests, IFA, and RIP. It has generally been accepted that there is only one serotype of TGEV. The evidence for there being one serotype of TGEV is based on the work of Kemeny (1976) where a 32 fold difference in neutralization titers with polyclonal sera was used as an endpoint with homologous and heterologous sera for determination of serotypes. In this study, VN with polyclonal antibodies revealed 2 to 8 fold differences when tested against the TGEV isolates. The results of this study using polyclonal sera agree with the statement that there is one serotype of TGEV. However, differences were detected with MAbs as

reported by other researchers (Welch and Saif, 1988; Hohatsdu et al., 1987; Laude et al., 1986). In this study, 2 to 2048 fold differences were detected with neutralizing MAbs directed to the S glycoprotein of the TGEV. The isolate IA-156 was not neutralized by the MAbs MH11, MA4, or MA5. The IFA and RIP data confirmed these results. The RIP with MH11 showed that the isolate IA-156 lacked the epitope that MH11 recognized, as there was no detectable S glycoprotein band precipitated. If the isolate IA-156 had contained the epitope recognized by MH11 and still was not neutralized, the possibility that the epitope was inaccessible or in very small concentration on IA-156 could have been considered, but the RIP with MH11 disproved this possibility.

The lack of the MH11 epitope in the isolates IA-156 indicates that there was most likely a change in the nucleotide sequence of the S gene that resulted in a substitution or deletion of the amino acids recognized by MH11. The significance of the lack of reactivity of IA-156 with neutralizing MAbs is not clear. Almost all TGEV field isolates thus far reported are to some extent neutralized by MAbs. Additional studies are needed to determine if IA-156 is missing other epitopes that are present on other TGEV isolates.

The protein profile of partially purified TGEV isolates and the RIP of the TGEV isolates with the anti-TGEV hyperimmune serum showed that in addition to the S, N, and M proteins expected, a N' protein of 42 kDa was present. Welch and Saif (1988) demonstrated the presence of this protein and felt that the presence of the N' was related to the level of attenuation or cell adaptation of the virus. When the anti-N MAb 1F7 was utilized in a RIP, the 46 kDa N and 42 kDa N' were precipitated, but in addition, a 40 kDa N" was also present. There are two possible explanations as to why the 40 kDa N" protein was present in the 1F7 RIP and not the polyclonal RIP. First, the polyclonal serum utilized in the RIP lacked antibodies to the epitope recognized by 1F7, and thus did not precipitate the 40 kDa N" protein. This explanation, however, is unlikely. Secondly,

since 1F7 is a MAb and recognizes a distinct epitope, the kinetics of binding the N protein and its derivatives may have been shifted. The 40 kDa N" protein may have been present in such a small quantity in the infected cell lysate that the anti-N polyclonal antibodies could have been bound to the more abundant 46 kDa and 42 kDa N protein forms, and not precipitated the N" protein. Since 1F7 is a MAb and recognizes a distinct epitope, the capability of the MAb to bind and precipitate the N" protein when present in a very small amount would be increased. Whether the additional N proteins are precursors or degradation products of the 46 kDa N protein is not known.

Porcine respiratory coronavirus (PRCV) is a coronavirus that is very similar to TGEV antigenically (Pensaert, 1989). In a VN test, polyclonal swine sera from swine infected with PRCV will readily neutralize TGEV, and vice versa. Porcine respiratory coronavirus has rapidly spread through Europe and has made it necessary to develop a test to distinguish whether swine have been exposed to TGEV or PRCV, as often the sale of swine is contingent upon the herd not having antibodies to TGEV. Monoclonal antibodies developed by other researchers for detecting antigenic variation and epitope mapping of TGEV have been used in a competitive blocking ELISA to differentiate TGEV infection from a PRCV infection (Callebaut et al., 1989). The use of MAbs to detect antigenic variation among TGEV isolates will need to be further explored for future use in order to discover more epitopes that may be shared or lacking between TGEV and PRCV.

In conclusion, this study has shown antigenic and biological variation exists among TGEV isolates. Other coronaviruses have been shown to exhibit antigenic variation, i.e. turkey coronavirus (TCV) (Dea and Tijssen, 1988; Dea and Tijssen, 1989), bovine coronavirus (BCV) (El-Ghorr et al., 1989), FIPV (Fiscus and Teramoto, 1987a; 1987b), and infectious bronchitis virus (IBV) (Niesters et al., 1987). Further study will

be needed to determine what extent, if any, the presence of antigenic and biological variants have on the virulence of TGEV isolates and possibly the lack of efficacy of commercial TGEV vaccines.

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References

Aynaud, J., and T. D. Nguyen, E. Bottreau, A. Brun, and P. Vannier. 1985. Transmissible gastroenteritis (TGE) of swine: Survivor selection of TGE virus mutants in stomach juice of adult pigs. J. Gen. Virol. 66:1911-1917.

Bohl, E.H. 1989. Transmissible gastroenteritis virus (classical enteric variant). Pages 139-153 in M. B. Pensaert, ed. Virus infections of porcines. Elsevier Science Publishing Company Inc., New York, NY.

Britton, P., R. S. Carmenes, K. W. Page, D. J. Garwes, and F. Parra. 1988a. Sequence of the nucleoprotein gene from a virulent British field isolate of transmissible gastroenteritis and its expression in Saccharomyces cerevisiae. Molecular Microbiol. 2:89-99.

Callebaut, P., M. B. Pensaert, and J. Hooyberghs. 1989. A competitive ELISA for the differentiation of serum antibodies from pigs infected with transmissible gastroenteritis virus (TGEV) or with the TGEV-related porcine respiratory coronavirus. Vet. Microbiol. 20:9-19.

Carmichael, L. E., J. C. Jonbert, and R. V. H. Pollock. 1981. A modified canine parvovirus strain with novel plaque characteristics. I. Viral attenuation and dog response. Cornell Vet. 71:408-427.

Christianson, K. K., J. D. Ingersoll, R. M. Landon, N. E. Pfeiffer, and J. D. Gerber. 1989. Characterization of a temperature sensitive feline infectious peritonitis coronavirus. Arch. Virol. 109:27-33.

Dea, S., and P. Tijssen. 1988. Identification of the structural proteins of turkey enteric coronavirus. Arch. Virol. 99:173-186.

Dea, S., and P. Tijssen. 1989. Antigenic and polypeptide structure of turkey enteric coronavirus as defined by monoclonal antibodies. J. Gen. Virol. 70:1725-1741.

El-Ghorr, A. A., D. R. Snodgrass, F. M. M. Scott, and I. Campbell. 1989. A serological comparison of bovine coronavirus strains. Arch. Virol. 104:241-248.

Fiscus, S. A., and Y. A. Teramonto. 1987a. Antigenic comparison of the feline coronavirus isolates: Evidence for markedly different peplomer glycoproteins. J. Virol. 61:2607-2613.

Fiscus, S. A., and Y. A. Teramonto. 1987b. Functional differences in the peplomer glycoproteins of feline coronavirus isolates. J. Virol. 61:2655-2657.

Garwes, D. J., D. H. Pocock, and B. V. Pike. 1976. Isolation of subviral components from transmissible gastroenteritis virus. J. Gen. Virol. 32:283-294.

Garwes, D. J., M. H. Lucas, D. A. Higgins, B. V. Pike, and S. F. Cartwright. 1978/1979. Antigenicity of structural components from porcine transmissible gastroenteritis virus. Vet. Microbiol. 3:179-190.

Hamada, N., Y. Imamura, and M. Shingu. 1988. Correlation between plaque size and genetic variation of type 3 poliovirus from a vaccine. J. Med. Virol. 24:1-9.

Hill, H. T. 1989. Preventing epizootic TGE from becoming enzootic TGE. Veterinary Medicine 4:432-436.

Hohdatsu, T., Y. Eiguchi, M. Tsuchimoto, S. Ide, H. Yamagishi, and M. Matumoto. 1987. Antigenic variation of porcine transmissible gastroenteritis virus detected by monoclonal antibodies. Vet. Microbiol. 14:115-124.

Kemeny, L. J. 1976. Antibody response in pigs inoculated with transmissible gastroenteritis and cross reactions among ten isolates. Can. J. Comp. Med. 40:209-214.

Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibodies of predefined specifications. Nature (London) 256:495-498.

Laude, H., J. Gelfi, and J. M. Aynaud. 1981. In vitro properties of low- and high-passaged strains of transmissible gastroenteritis coronavirus of swine. Am. J. Vet. Res. 42:447-449.

Laude, H., J. Chapsal, J. Gelfi, S. Labian, and J. Grosclaude. 1986. Antigenic structure of transmissible gastroenteritis virus. I. Properties of monoclonal antibodies directed against virion proteins. J. Gen. Virol. 67:119-130.

McClurkin, A. W., and J. O. Norman. 1966. Studies of transmissible gastroenteritis of swine. II. Selected characteristics of a cytopathogenic virus common to five isolates from transmissible gastroenteritis. Can. J. Comp. Med. 34:347-349.

Moxley, R. A., and L. D. Olson. 1989. Clinical evaluation of transmissible gastroenteritis virus vaccines and vaccination procedures for inducing lactogenic immunity in sows. Am. J. Vet. Res. 50:111-118.

Niesters, H. G., N. M. C. Bleumink-Pluym, A. D. M. E. Osterhaus, M. C. Horzinek, and B. A. M. Zeijst. 1987. Epitopes on the peplomer protein of infectious bronchitis virus strain M41 as defined by monoclonal antibodies. Virology 161:511-519.

Paul, P. S., X. L. Zhu, and E. M. Vaughn. 1988. Current strategies for the development of efficacious vaccines for transmissible gastroenteritis in swine. Proc. U. S. Anim. Hlth. Assoc. 92:429-443.

Pensaert, M. B. 1989. Transmissible gastroenteritis virus (respiratory variant). Pages 154-165 in M. B. Pensaert, ed. Virus infections of porcines. Elsevier Science Publishing Company Inc., New York, NY.

Saif, L. J., and E. H. Bohl. 1986. Transmissible gastroenteritis. Pages 255-274 in A. D. Leman, R. D. Glock, W. L. Mengeling, R. H. C. Penny, E. Scholl, and B. Straw, eds. Diseases of swine. 6th edition. Iowa State University Press, Ames, IA.

Welch, S. W., and L. J. Saif. 1988. Monoclonal antibodies to a virulent strain of transmissible gastroenteritis: comparison of reactivity with virulent and attenuated virus. Arch. Virol. 101:221-235.

Woods, R. D. 1978. Small plaque variant transmissible gastroenteritis virus. J. Am. Vet. Med. Assoc. 173:643-647.

Zhu, X. L., P. S. Paul. E. M. Vaughn, and A. Morales. 1990. Monoclonal antibodies to the Miller strain of transmissible gastroenteritis (TGEV) of swine: Characterization and reactivity with TGEV isolates. Am. J. Vet. Res. 51:232-238.

SUMMARY AND CONCLUSIONS

Transmissible gastroenteritis remains a significant economic problem in the swine industry. Commercial TGE vaccines are available but their efficacy is variable. One explanation for this is that there is antigenic and biological diversity among TGEV isolates. In the preceding study, antigenic diversity among twenty four field isolates of TGEV was examined by virus neutralization, indirect immunofluorescence assay, and radioimmunoprecipitation. Biological diversity was examined among these TGEV isolates by observing the cytopathic effect and plaque size in cell culture, and the TGEV protein profile by SDS-PAGE.

Results from the experiment showed that antigenic and biological diversity exists among TGEV field isolates. Virus neutralization results demonstrated that antigenic variation was not as extensive with polyclonal sera as it was with monoclonal antibodies. The antigenic diversity detected by monoclonal antibodies was further substantiated by indirect immunofluorescence assay and radioimmunoprecipitation. Biological diversity existed among the TGEV isolates with different cytopathic effects and plaque sizes in cell culture. There was no apparent differences among the migration of the TGEV viral proteins by SDS-PAGE. These findings may have important implications in future vaccine development.

LITERATURE CITED

Aynaud, J., and T. D. Nguyen, E. Bottreau, A. Brun, and P. Vannier. 1985. Transmissible gastroenteritis (TGE) of swine: Survivor selection of TGE virus mutants in stomach juice of adult pigs. J. Gen. Virol. 66:1911-1917.

Bernard, S., I. Lantier, H. Laude, and J. M. Aynaud. 1986. Detection of transmissible gastroenteritis coronavirus antigens by a sandwich enzyme-linked immunosorbent assay technique. Am. J. Vet. Res. 11:2441-2444.

Bohl, E.H. 1989. Transmissible gastroenteritis virus (classical enteric variant). Pages 139-153 in M. B. Pensaert, ed. Virus infections of porcines. Elsevier Science Publishing Company Inc., New York, NY.

Bohl, E. H., and T. Kumagai. 1965. The use of cell cultures for the study of TGE virus of swine. Proc. U. S. Livestock Assoc. 69:343-350.

Bohl, E. H., P. Gupta, M. V. Fernando Olquin, and L. J. Saif. 1972. Antibody responses in serum, colostrum, and milk of swine after infection or vaccination with transmissible gastroenteritis virus. Infect. and Immun. 6:289-301.

Brian, A.B., D.E. Dennis, and J. S. Grey. 1980. Genome of porcine transmissible gastroenteritis virus. J. Virol. 34:410-415.

Britton, P., R. S. Carmenes, K. W. Page, D. J. Garwes, and F. Parra. 1988a. Sequence of the nucleoprotein gene from a virulent British field isolate of transmissible gastroenteritis and its expression in Saccharomyces cerevisiae. Molecular Microbiol. 2:89-99.

Britton, P., R. S. Carmenes, K. W. Page, and D. J. Garwes. 1988b. The integral membrane protein from a virulent isolate of transmissible gastroenteritis: molecular characterization, sequence and expression in Escherichia coli. Molecular Microbiol. 2:497-505.

Britton, P., C. Lopez Otin, J. M. Martin Alonso, and F. Parra. 1989. Sequence of the coding regions from the 3.0 kb and 3.9 kb mRNA subgenomic species from a virulent isolate of transmissible gastroenteritis virus. Arch. Virol. 105:165-178.

Callebaut, P., M. B. Pensaert, and J. Hooyberghs. 1989. A competitive ELISA for the differentiation of serum antibodies from pigs infected with transmissible gastroenteritis virus (TGEV) or with the TGEV-related porcine respiratory coronavirus. Vet. Microbiol. 20:9-19.

Carmichael, L. E., J. C. Jonbert, and R. V. H. Pollock. 1981. A modified canine parvovirus strain with novel plaque characteristics. I. Viral attenuation and dog response. Cornell Vet. 71:408-427.

Cheever, F. S., J. B. Daniels, A. M. Pappenheimer, and O. T. Bailey. 1949. A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. I. Isolation and biologic properties of the virus. J. Exp. Med. 90:181-194.

Chen, K. S. 1985. Enzymatic and acidic sensitivity profiles of selected virulent and attenuated transmissible gastroenteritis viruses of swine. Am. J. Vet. Res. 46:632-636.

Christianson, K. K., J. D. Ingersoll, R. M. Landon, N. E. Pfeiffer, and J. D. Gerber. 1989. Characterization of a temperature sensitive feline infectious peritonitis coronavirus. Arch. Virol. 109:27-33.

Correa, I., G. Jimenez, C. Sune, M. J. Bullido, and L. Enjuanes. 1988. Antigenic structure of the E2 glycoprotein from transmissible gastroenteritis coronavirus. Virus Research 10:77-94.

Cox, E., J. Hooyberghs, and M. B. Pensaert. 1990. Sites of replication of a porcine respiratory coronavirus related to transmissible gastroenteritis virus. Res. Vet. Sci. 48:165-169.

Dea, S., and P. Tijssen. 1988. Identification of the structural proteins of turkey enteric coronavirus. Arch. Virol. 99:173-186.

Dea, S., and P. Tijssen. 1989. Antigenic and polypeptide structure of turkey enteric coronavirus as defined by monoclonal antibodies. J. Gen. Virol. 70:1725-1741.

Delmas, B., J. Gelfi, and H. Laude. 1986. Antigenic structure of transmissible gastroenteritis virus. II. Domains in the peplomer glycoprotein. J. Gen. Virol. 67:1405-1418.

Doyle, L. P., and L. M. Hutchings. 1946. A transmissible gastroenteritis in pigs. J. Am. Vet. Med. Assoc. 108:257-259.

Dulac, G. C., G. M. Ruckerbauer, and P. Boulanger. 1977. Transmissible gastroenteritis: demonstration of the virus from field specimens by means of cell culture and pig inoculation. Can. J. Comp. Med. 41:357-363.

Egerbrink, H. F., J. Ederveen, P. Callebaut, and M. C. Horzinek. 1988. Characterization of the structural proteins of porcine epizootic diarrhea virus CV777. Am. J. Vet. Res. 49:1320-1324.

El-Ghorr, A. A., D. R. Snodgrass, F. M. M. Scott, and I. Campbell. 1989. A serological comparison of bovine coronavirus strains. Arch. Virol. 104:241-248.

Fiscus, S. A., and Y. A. Teramonto. 1987a. Antigenic comparison of the feline coronavirus isolates: Evidence for markedly different peplomer glycoproteins. J. Virol. 61:2607-2613.

Fiscus, S. A., and Y. A. Teramonto. 1987b. Functional differences in the peplomer glycoproteins of feline coronavirus isolates. J. Virol. 61:2655-2657.

Garwes, D. J. 1988. Transmissible gastroenteritis. Vet. Record 122:462-463.

Garwes, D. J., and D. H. Pocock. 1975. The polypeptide structure of transmissible gastroenteritis virus. J. Gen. Virol. 29:25-34.

Garwes, D. J., D. H. Pocock, and B. V. Pike. 1976. Isolation of subviral components from transmissible gastroenteritis virus. J. Gen. Virol. 32:283-294.

Garwes, D. J., M. H. Lucas, D. A. Higgins, B. V. Pike, and S. F. Cartwright. 1978/1979. Antigenicity of structural components from porcine transmissible gastroenteritis virus. Vet. Microbiol. 3:179-190.

Gough, P. M., C. J. Frank, D. G. Moore, M. A. Sagona, and C. J. Johnson. 1983. Lactogenic immunity to transmissible gastroenteritis virus induced by a subunit immunogen. Vaccine 1:37-41.

Hamada, N., Y. Imamura, and M. Shingu. 1988. Correlation between plaque size and genetic variation of type 3 poliovirus from a vaccine. J. Med. Virol. 24:1-9.

Harada, K., T. Kumagi, and J. Sasahara. 1963. Cytopathogenicity of transmissible gastroenteritis virus in pigs. Natl. Inst. Anim. Hlth. Quart. 3:166-167.

Hill, H. T. 1989. Preventing epizootic TGE from becoming enzootic TGE. Veterinary Medicine 4:432-436.

Hogue, B. G., T. E. Kienzle, and D. A. Brian. 1989. Synthesis and processing of the bovine coronavirus haemagglutinin protein. J. Gen. Virol. 70:345-352.

Hohdatsu, T., Y. Eiguchi, S. Ide, K. Baba, and H. Yamagishi. 1987a. Evaluation of an enzyme immunosorbent assay for the detection of transmissible gastroenteritis virus antibodies. Vet. Microbiol. 13:93-97.

Hohdatsu, T., Y. Eiguchi, M. Tsuchimoto, S. Ide, H. Yamagishi, and M. Matumoto. 1987b. Antigenic variation of porcine transmissible gastroenteritis virus detected by monoclonal antibodies. Vet. Microbiol. 14:115-124.

Holmes, K. V. 1990. Coronaviridae and their replication. Pages 841-856 in B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath, and B. Roizman, eds. Fields virology. 2nd edition. Raven Press, New York, NY.

Hooper, B. E., and E. O. Haelterman. 1966. Concepts of pathogenesis and passive immunity in transmissible gastroenteritis of swine. J. Am. Vet. Med. Assoc. 149:1580-1586.

Horzinek, M. C., H. Lutz, and N. C. Pedersen. 1982. Antigenic relationships among homologous structural polypeptides of porcine, feline, and canine coronaviruses. Infect. and Immun. 37:1148-1155.

Jimenez, G., I. Correa, M. P. Melgosa, M. J. Bullido, and L. Enjuanes. 1986. Critical epitopes in transmissible gastroenteritis virus neutralization. J. Virol. 60:131-139.

Kemeny, L. J. 1976. Antibody response in pigs inoculated with transmissible gastroenteritis and cross reactions among ten isolates. Can. J. Comp. Med. 40:209-214.

Kemeny, L. J., and R. D. Woods. 1977. Quantitative transmissible gastroenteritis virus shedding patterns in lactating sows. Am. J. Vet. Res. 38:307-310.

Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibodies of predefined specifications. Nature (London) 256:495-498.

Laude, H., J. Gelfi, and J. M. Aynaud. 1981. In vitro properties of low- and high-passaged strains of transmissible gastroenteritis coronavirus of swine. Am. J. Vet. Res. 42:447-449.

Laude, H., B. Charley, and J. Gelfi. 1984. Replication of transmissible gastroenteritis coronavirus (TGEV) in swine alveolar macrophages. J. Gen. Virol. 65:327-332.

Laude, H., J. Chapsal, J. Gelfi, S. Labian, and J. Grosclaude. 1986. Antigenic structure of transmissible gastroenteritis virus. I. Properties of monoclonal antibodies directed against virion proteins. J. Gen. Virol. 67:119-130.

Laude, H., D. Rasschaert, and J. C. Huet. 1987. Sequence and N-terminal processing of the transmembrane protein E1 of the coronavirus transmissible gastroenteritis virus. J. Gen. Virol. 68:1687-1693.

McClurkin, A. W., and J. O. Norman. 1966. Studies of transmissible gastroenteritis of swine. II. Selected characteristics of a cytopathogenic virus common to five isolates from transmissible gastroenteritis. Can. J. Comp. Med. 34:347-349.

McIntosh, K. 1990. Coronaviruses. Pages 857-864 in B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath, and B. Roizman, eds. Fields virology. 2nd edition. Raven Press, New York, NY.

McIntosh, K., W. B. Becker, and R. M. Chanock. 1967. Growth in suckling mouse brain of "IBV-like" viruses from patients with upper respiratory tract disease. Proc. Natl. Acad. Sci. 58:2268-2273.

Moon, H. W. 1978. Mechanisms in the pathogenesis of diarrhea: A review. J. Am. Vet. Med. Assoc. 172:443-448.

Moxley, R. A., and L. D. Olson. 1989. Clinical evaluation of transmissible gastroenteritis virus vaccines and vaccination procedures for inducing lactogenic immunity in sows. Am. J. Vet. Res. 50:111-118.

Moxley, R. A., L. D. Olson, and R. F. Solorzano. 1989. Relationship among transmissible gastroenteritis virus antibody titers in serum, colostrum, and milk from vaccinated sows, and protection in their suckling pigs. Am. J. Vet Res. 50:119-125.

Nagura, H., D. K. Nakane, and W. R. Brown. 1978. Breast milk IgA binds to jejunal epithelia in suckling rats. J. Immunol. 120:1330-1339.

Nguyen, T. D., E. Bottreau, S. Bernard, I. Lautier, and J. M. Aynaud. 1986. Neutralizing secretory IgA and IgG do not inhibit attachment of transmissible gastroenteritis virus. J. Gen. Virol. 67:939-943.

Niesters, H. G., N. M. C. Bleumink-Pluym, A. D. M. E. Osterhaus, M. C. Horzinek, and B. A. M. Zeijst. 1987. Epitopes on the peplomer protein of infectious bronchitis virus strain M41 as defined by monoclonal antibodies. Virology 161:511-519.

Norman, J. O., A. W. McClurkin, and H. L. Bachrach. 1968. Infectious nucleic acid from a transmissible agent causing gastroenteritis in pigs. J. Comp. Pathol. 78:227-235.

Paul, P. S., X. L. Zhu, and E. M. Vaughn. 1988. Current strategies for the development of efficacious vaccines for transmissible gastroenteritis in swine. Proc. U. S. Anim. Hlth. Assoc. 92:429-443.

Pensaert, M. B. 1989. Transmissible gastroenteritis virus (respiratory variant). Pages 154-165 in M. B. Pensaert, ed. Virus infections of porcines. Elsevier Science Publishing Company Inc., New York, NY.

Pensaert, M. B., E. O. Haelterman, and T. Burnstein. 1970. Transmissible gastroenteritis of swine: Virus-intestinal interactions. I. Immunofluorescence, histopathology and virus production in the small intestine through the course of infection. Arch. Gesamte Virusforsch. 31:321-334.

Pensaert, M. B., P. DeBouck, and D. J. Reynolds. 1981. An immunoelectron microscopic and immunofluorescent study on the antigenic relationship between the coronavirus-like agent, CV 777, and several coronaviruses. Arch. Virol. 68:45-52.

Porter, P., and W. D. Allen. 1972. Classes of immunoglobulins related to immunity in the pig: A review. J. Am. Vet. Med. Assoc. 160:511-518.

Saif, L. J., and E. H. Bohl. 1979. Passive immunity in transmissible gastroenteritis of swine: Immunoglobulin classes of milk antibodies after oral-intranasal inoculations of sows with a live low cell culture-passaged virus. Am. J. Vet. Res. 40:115-117.

Saif, L. J., and E. H. Bohl. 1986. Transmissible gastroenteritis. Pages 255-274 in A. D. Leman, R. D. Glock, W. L. Mengeling, R. H. C. Penny, E. Scholl, and B. Straw, eds. Diseases of swine. 6th edition. Iowa State University Press, Ames, IA.

Sanchez, C. M., G. Jimenez, M. D. Laviada, I. Correa, C. Sune, M. J. Bullido, F. Gebauer, C. Smerdou, P. Callebaut, J. Escribano, and L. Enjuanes. 1990. Antigenic homology among coronaviruses related to transmissible gastroenteritis virus. Virology 174:410-417.

Schalk, A. F., and M. C. Hawn. 1931. An apparently new respiratory disease of baby chicks. J. Am. Vet. Med. Assoc. 78:413-422.

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

Stone, S. S., L. J. Kemeny, R. D. Woods, and M. T. Jensen. 1977. Efficacy of isolated colostral IgA, IgG, and IgM(A) to protect neonatal pigs against the coronavirus of transmissible gastroenteritis. Am. J. Vet. Res. 38:1285-1288.

Tajima, M. 1970. Morphology of transmissible gastroenteritis virus of pigs. A possible member of the coronaviruses. Arch. Gestame Virusforch. 29:105-108.

Talbot, P. J., G. Dionne, and M. Lacroix. 1988. Vaccination against lethal coronavirus-induced encephalitis with a synthetic decapeptide homologous to a domain in the predicted peplomer stalk. J. Virol. 62:3032-3036.

Tyrrell, D. A. J., and M. L. Bynoe. 1965. Cultivation of a novel type of common cold virus in organ cultures. Br. Med. J. 1:1467-1470.

Tyrrell, D. A. J., J. D. Almeida, and D. M. Berry. 1968. Coronaviruses. Nature 220:650.

Underdown, B. J., and K. J. Dorrington. 1974. Studies on the structural and conformational basis for the relative resistance of serum and secretory immunoglobulin A to proteolysis. J. Immunol. 112:949-959.

Underhahl, N. R., C. A. Mebus, A. Torres-Medina. 1975. Recovery of transmissible gastroenteritis virus from chronically infected experimental pigs. Am. J. Vet. Res. 36:1473-1476.

Van Nieuwstadt, A.P., J. B. W. J. Cornelissen, and T. Zetstra. 1988. Comparison of two methods for detection of transmissible gastroenteritis virus in feces of pigs with experimentally induced infection. Am. J. Vet. Res. 11:1836-1843.

Welch, S. W., and L. J. Saif. 1988. Monoclonal antibodies to a virulent strain of transmissible gastroenteritis: comparison of reactivity with virulent and attenuated virus. Arch. Virol. 101:221-235.

Welch, S. W., L. J. Saif, and S. Ram. 1988. Cell-mediated immune responses of suckling pigs inoculated with attenuated or virulent transmissible gastroenteritis virus. Am. J. Vet. Res. 49:1228-1234.

Witte, K. H., and B. C. Easterday. 1967. Isolation and propagation of the virus of transmissible gastroenteritis of pigs in various pig cell cultures. Arch. Gestame Virusforsch. 20:327-350.

Woods, R. D. 1978. Small plaque variant transmissible gastroenteritis virus. J. Am. Vet. Med. Assoc. 173:643-647.

Woods, R. D. 1984. Efficacy of vaccination of sows with serologically related coronaviruses for control of transmissible gastroenteritis in nursing pigs. Am. J. Vet. Res. 45:1726-1729.

Woods, R. D., and N. C. Pedersen. 1979. Cross-protection studies between feline infectious peritonitis and porcine transmissible gastroenteritis viruses. Vet. Microbiol. 4:11-16.

Woods, R. D., and R. D. Wesley. 1986. Immune response in sows given transmissible gastroenteritis or canine coronavirus. Am. J. Vet. Res. 6:1239-1242.

Woods, R. D., N. F. Cheville, and J. E. Gallagher. 1981. Lesions in the small intestine of newborn pigs inoculated with porcine, feline, and canine coronaviruses. Am. J. Vet. Res. 42:1163-1169.

Woods, R. D., R. D. Wesley, and P. A. Kapke. 1988. Neutralization of porcine transmissible gastroenteritis virus by complement-dependent monoclonal antibodies. Am. J. Vet. Res. 49:300-304.

Zhu, X. L., P. S. Paul. E. M. Vaughn, and A. Morales. 1990. Monoclonal antibodies to the Miller strain of transmissible gastroenteritis (TGEV) of swine: Characterization and reactivity with TGEV isolates. Am. J. Vet. Res. 51:232-238.

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