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Antigenic and biological diversity among transmissible gastroenteritis virus isolates of swine

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ABSTRACT

Twenty-four field isolates of transmissible gastroenteritis virus (TGEV) were isolated and examined for antigenic and biological characteristics. Most TGEV 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. Minor variations in CPE were observed with one isolate, IA-145. Protein profiles of the TGEV isolates as determined by SDS-PAGE were essentially identical, with the exception of the isolate IA-101. The TGEV isolate IA-101 presented a higher molecular mass M protein and lacked an N protein doublet that was present in all other TGEV isolates. 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, IA-156, was very poorly neutralized with the mAbs used in this study. 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 (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. TGEV has three structural viral proteins, these being M, S, and N (Garwes et al., 1976; Saif and Bohl, 1986). M is glycosylated with an apparent M, of 25000–28000 and is associated with the viral envelope (Saif

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S, the spike protein, has an apparent $M_r$ of 200000 and is also glycosylated (Garwes et al., 1976). The N protein is not glycosylated, has an apparent molecular mass of 45000, and is also associated with the binding of viral RNA and serves as a nucleocapsid (Britton et al., 1988). S is the viral protein that induces neutralizing antibodies (Garwes et al., 1978/1979).

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 (Bohl, 1989; Laude et al., 1981). 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 TGEV exists (Kemeny, 1976). However, antigenic variation among TGEV isolates has been demonstrated (Hohdatsu et al., 1987; Laude et al., 1986; Zhu et al., 1990). Another point to consider is the extent of biological variation among TGEV isolates. The purpose of this study is to examine a number of 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 as described by Zhu et al. (1990).

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. The Miller strain was used at the seventh passage, and the Illinois and Purdue strains were used at the fifth and 115th respectively. The virulent gut-passaged Miller strain of TGEV (designated CHV) was obtained from the National Veterinary Services Laboratory, Ames, IA. The group 8C enterovirus was isolated from intestinal tissue of a piglet and was typed at the National Veterinary Services Laboratory, Ames, IA.
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% suspensions of the intestinal samples were prepared in 0.05 M phosphate buffered saline (pH 7.4) and clarified by centrifugation at 200 × g for 10 min. The supernatants 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 Modified Eagle’s Medium (MEM) without FBS. Each pig was inoculated orally with 20 ml of the appropriate virus suspension adjusted to 5 × 10⁵ pfu/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 × 10⁶ pfu/ml. At four and six weeks after the oral inoculation, 10 ml of the virus suspension was administered intravenously (IV). Two weeks after the final IV 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 × 10⁵ pfu/ml and administered as described for TGEV antisera production in eight-week-old pigs.

The production and specificity of the neutralizing mAbs MH11, MA5, and MA6 have been previously described (Zhu et al. 1990). The Anti-N protein 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 cm² flasks (Costar, Cambridge, MA) were treated with MEM containing diethylaminoethyl-dextran (50 μg/ml) (Sigma, St. Louis, MO) at 37°C for 30 min 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% FBS and antibiotics (penicillin 20000 U/ml, streptomycin 20000 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 min at 37°C, after which additional MEM with 2% 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×g for 10 min, and used as inoculum for the next passage.

All TGEV isolates were 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.

**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 min 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 pfu 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% reduction of 100 pfu/well, as compared with 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 pfu of the appropriate virus suspended in MEM with
2% 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 min. 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 the TGEV isolates. The Miller strain of TGEV was used as a comparative standard for the protein migration of the TGEV isolates. The TGEV isolates and mock-infected cells were labeled with 35S-methionine-cysteine and processed as follows. Four-day-old ST cells in 25 cm² flasks were infected at a m.o.i. of 0.1 pfu/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 35S-methionine-cysteine added. Four hours after the addition of the 35S-methionine-cysteine, the cell monolayers were scraped from the plastic flask and pelleted by centrifugation at 200×g for 10 min. The cellular pellets containing labeled viral proteins were then disrupted with 1 ml of lysis buffer (50 mM NaCl, 50 mM tris, 5mM EDTA, 1% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride). The lysis buffer and cellular pellet mixtures were vortexed vigorously for 1 min, and placed on ice for 3 min. 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 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 an SDS-polyacrylamide gel as described by Zhu et al. (1990).
RESULTS

Virus isolation
Virus isolation was originally attempted from the small intestines of 99 pigs shown to be positive for TGEV by immunofluorescence. TGEV 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 with the CPE of the standard Miller strain of TGEV in ST cell culture, with the exception of the isolate IA-145, which displayed minor differences in CPE. 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 Fig. 1. The typical CPE produced by the Miller strain of TGEV in ST cell culture is shown in Fig. 2. The isolate IA-156 shows a typical CPE in ST cell culture, as evidenced in Fig. 3. However, the TGEV isolate IA-145 produced a slightly different CPE in the ST cell culture, as shown in Fig. 4. The CPE induced by

Fig. 1. Uninfected four-day-old swine testis (ST) cells (Phase contrast, 100×).
Fig. 2. Cytopathic effect of the Miller strain of TGEV in four-day-old ST cells (Phase contrast, 100×).

Fig. 3. Cytopathic effect of IA-156 TGEV in four-day-old ST cells (Phase contrast, 100×).
Fig. 4. Cytopathic effect of IA-145 TGEV in four-day-old ST cells (Phase contrast, 100×).

| Virus     | 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-111    | 3200   | 3200     | 1600   | 800    | 400    | 200    |
| IA-137    | 3200   | 1600     | 1600   | 1600   | 400    | 400    |
| IA-145    | 3200   | 1600     | 800    | 800    | 200    | 200    |

*Reciprocal of the last dilution of polyclonal antibodies neutralizing 80% of about 100 pfu of TGEV. Results represent data from four determinations.

IA-145 was more extensive and had a more prominent net-like appearance. Also, the infected cells that comprise the CPE were less granular in appearance as compared with the CPE of the other isolates.

The titers of the TGEV isolates ranged from $1 \times 10^5$ to $5 \times 10^7$ pfu/ml.

**Antigenic and biological relatedness**

The VN titers of the isolates with the polyclonal anti-TGEV hyperimmune sera with homologous and heterologous isolates are shown in Table 1. Varia-
tions among the homologous versus heterologous VN titers for all the hyper-immune sera ranged from 2 to 8 fold. The anti-Miller hyperimmune serum neutralized all isolates tested with titers of 1600 to 3200. The anti-Illinois hyperimmune serum also neutralized the isolates with titers of 1600 to 3200. The anti-Purdue, anti-IA-111, anti-IA-137, and anti-IA-145 hyperimmune sera also neutralized the isolates tested, but the neutralization titers of these three hyperimmune sera ranged from 50 to 1600, and were generally lower than the anti-Miller and anti-Illinois hyperimmune sera.

The VN titers of the isolates with the mAbs MH11, MA5, and MA4 are listed in Table 2. 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

| Virus     | Monoclonal antibodies | MH11 | MA5 | MA4 |
|-----------|-----------------------|------|-----|-----|
| Miller    |                       | 51 200 | 25 600 | 51 200 |
| Illinois  |                       | 12 800 | 6400 | 6400 |
| Purdue    |                       | 3200 | 6400 | 6400 |
| IA-100    |                       | 12 800 | 12 800 | 12 800 |
| IA-101    |                       | 12 800 | 1600 | 1600 |
| IA-107    |                       | 6400 | 6400 | 6400 |
| IA-111    |                       | 100 | 800 | 800 |
| IA-114    |                       | 12 800 | 12 800 | 12 800 |
| IA-117    |                       | 6400 | 12 800 | 12 800 |
| IA-118    |                       | 3200 | 6400 | 6400 |
| IA-131    |                       | 6400 | 12 800 | 12 800 |
| IA-136    |                       | 1600 | 6400 | 6400 |
| IA-137    |                       | 400 | 1600 | 1600 |
| IA-139    |                       | 12 800 | 800 | 800 |
| IA-145    |                       | 3200 | 1600 | 3200 |
| IA-148    |                       | 12 800 | 12 800 | 12 800 |
| IA-156    |                       | 25 | 25 | 25 |
| IA-164    |                       | 12 800 | 12 800 | 12 800 |
| IA-165    |                       | 1600 | 6400 | 6400 |
| IA-166    |                       | 1600 | 12 800 | 12 800 |
| IA-178    |                       | 12 800 | 12 800 | 12 800 |
| IA-179    |                       | 12 800 | 6400 | 12 800 |
| IA-709    |                       | 400 | 1600 | 3200 |
| KS-200    |                       | 1600 | 12 800 | 12 800 |
| KS-204    |                       | 6400 | 12 800 | 12 800 |
| AR-302    |                       | 12 800 | 12 800 | 12 800 |

*Reciprocal of the last dilution of monoclonal antibodies neutralizing 80% of about 100 pfu of TGEV. Results represent data from four determinations.
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 25600 or 51200 respectively, whereas all the field isolates and the Purdue and Illinois strains of TGEV were neutralized with 4 to 2048 fold lower titers.

The results of the IFA titers with the mAbs were 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 was used to analyze the viral proteins and the antigenic composition of the standard Miller strain and all of the TGEV field isolates. The hyperimmune serum recognized the S (Mr 200000), M (Mr 28000), and N (Mr 44000) viral proteins of all the TGEV isolates, with the protein profiles of selected isolates shown in Fig. 5. One TGEV isolate, IA-101 (Fig. 5, lane 5), showed differences in its M and N proteins. The isolate IA-101 had
Fig. 6. Immunoprecipitation of 35S-methionine-cysteine labeled S glycoprotein of TGEV by the monoclonal antibody MH11. 14C-labeled molecular mass standards (kDa) (lanes 1 and 11), mock-infected ST cell lysate (lane 2), CHV-TGEV (lane 3), Miller TGEV (lane 4), Purdue TGEV (lane 5), IA-101 (lane 6), IA-137 TGEV (lane 7), IA-145 TGEV (lane 8), IA-156 TGEV (lane 9), and IA-165 TGEV (lane 10).

a slower migrating M protein with a Mr of 32000. The protein profiles of all of the TGEV isolates (data not shown), with the exception of IA-101, had a N' protein present with an apparent Mr of 42000. The lack of the N doublet for IA-101 was confirmed by the use of the anti- N protein mAb 1F7 in a RIP (data not shown). In Fig. 6, the anti-S protein mAb MH11 recognized only the S protein of the TGEV isolates selected, with the S protein of IA-156 (Fig. 6, lane 9) poorly recognized. 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.

DISCUSSION

The results of this study show that in general, TGEV isolates are highly antigenically related. However, there was some antigenic and biological diversity observed among TGEV isolates. The biological diversity was revealed with slight differences in cytopathic effect and in protein migration on SDS-PAGE.
The cytopathic effect displayed by the isolate IA-145 was slightly 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 slightly different CPE in ST cell culture, it was easily neutralized by both the polyclonal and monoclonal antibodies used in this study.

Out of the 99 porcine intestinal samples processed for this study, 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 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 could possibly represent non-cultivable biotypes of TGEV.

The protein profiles of the TGEV isolates as determined by RIP with anti-TGEV hyperimmune serum showed that in addition to the expected S, N, and M proteins, a N' protein with a Mr of 42000 was present. Welch and Saif (1988) demonstrated the presence of this protein and felt that the presence of the N' may be related to the level of attenuation or cell adaptation of the virus, since in their study the high-passaged Purdue strain exhibited more of the N' protein. To confirm this report, the virulent gut-passaged Miller CHV TGEV was included in the protein profile studies. All the isolates tested in this study, with the exception of the isolate IA-101, exhibited a N doublet with Mr of 44000 and a 42000 respectively. The levels of the N' protein detected by RIP for either the Miller or Purdue strains were essentially the same. Also, the isolate IA-156 has been inoculated into gnotobiotic piglets and was found to be a virulent TGEV isolate that produced profuse diarrhea with extensive villous atrophy in the small intestine (unpublished data). The fact that both the virulent gut-passaged Miller CHV TGEV and a virulent field isolate both produced a N doublet casts doubt on the hypothesis that the presence of such a doublet is correlated to the level of attenuation or the virulence of that particular isolate. Britton et al. (1988) published the sequence of the N protein gene of a virulent TGEV field isolate from Britain, and analysis of this sequence revealed that the open reading frame was predicted to code for a protein with a Mr of 43426. This predicted protein mass is very similar to the N proteins of the TGEV isolates in this study that had a Mr of approximately 44000. Whether the additional N protein is a precursor or degradation product of the 44000 Mr N protein is not known. In the two studies that have
presented evidence of a N protein doublet, a relatively low m.o.i. of TGEV isolates was used to infect ST cells. We have used a m.o.i. of 0.1 pfu/cell, and Welch and Saif (1988) utilized a m.o.i. of 0.02 to 0.04 pfu/cell. Other reports in which the presence of a N protein doublet was not evident, such as Laude et al. (1986) and Zhu et al. (1990), a higher m.o.i. of 50 or 100 pfu/cell was used respectively. A high m.o.i. ensures that all viable cells are infected at the same time, and the viral replication and viral protein synthesis are synchronized. However, when a low m.o.i. is used, several viral replicative cycles must occur before all the cells are infected and viral protein synthesis is occurring simultaneously. Since both studies that present evidence of an N protein doublet used a low m.o.i., it is interesting to speculate that the 42000 Mr N' protein product may be a degradation product of the 44000 Mr N protein and thus may accumulate in higher concentrations under low m.o.i. conditions. Pulse chase studies utilizing a low m.o.i. may prove beneficial in providing evidence to support this claim.

When the anti-N mAb 1F7 was utilized in a RIP (data not shown), both the 44000 Mr N and 42000 Mr N' were precipitated for all of the TGEV isolates except IA-101. The isolate IA-101 exhibited a single N protein band of 44000 Mr; a N' protein was not evident, even when the anti-N mAb 1F7 was utilized. The reason for this one isolate out of all the other TGEV isolates tested to demonstrate this phenomenon is unclear.

The M protein of the isolate IA-101 was shown to be of a higher molecular mass than those of the other TGEV isolates. The Mr of the M protein of the TGEV isolates ranged from 27000 to 28000, whereas the Mr of the IA-101 M protein was approximately 32000. The shift in the Mr of the IA-101 M protein may be due to differences in glycosylation. However, it is also possible that the M gene of IA-101 may actually have an insertion of additional nucleotides in its gene and thus have more amino acid residues present. This would most effectively be resolved by the cloning and sequencing of the M gene of IA-101 and comparing it to the published sequences of other TGEV M genes. This sequence information would also be beneficial in determining if there are more potential glycosylation sites present in the IA-101 M gene that would account for its shift in protein mobility.

Antigenic diversity was evidenced by the results of VN tests, IFA, and in the case of IA-156, by 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 homologous and heterologous TGEV isolates. The results of this study using polyclonal sera agree with the statement that there is one serotype of TGEV. However, differences among TGEV isolates detected with mAbs has been re-
ported by other researchers (Welch and Saif, 1988; Hohdatsu et al., 1987; Laude et al., 1986; Zhu et al., 1990). In this study, 2 to 2048 fold differences were detected with neutralizing mAbs directed to the S glycoprotein of the TGEV. These differences may reflect varying concentrations of the epitopes on the TGEV isolates that are recognized by the mAbs used in this study. 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 mAb MH11 showed that the isolate IA-156 lacked the epitope that mAb MH11 recognized, as there was no detectable S glycoprotein band precipitated. If the isolate IA-156 had contained the epitope recognized by mAb MH11 and still was not neutralized, the possibility that the epitope was inaccessible or was present in very low concentration on IA-156 could have been considered, but the RIP with mAb MH11 disproved this possibility.

The lack of the MH11 epitope in the isolate 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.

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