

# Oral transmission of transmissible gastroenteritis virus by muscle and lymph node from slaughtered pigs

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**SUMMARY:** A study was conducted in the USA to determine whether transmissible gastroenteritis (TGE) virus could be transmitted from carcasses of slaughtered pigs. Transmissible gastroenteritis virus was transmitted to 6-day-old piglets by dosing with homogenates of muscle and lymph node collected from 500 clinically normal pigs at the time of slaughter. All piglets in 2 separately housed litters showed clinical signs of TGE with 5 piglets dying within 10 d of oral dosing with homogenates. Transmissible gastroenteritis virus was isolated from 2 of these piglets and all piglets developed TGE antibody. Transmissible gastroenteritis virus was not isolated in tissue culture from muscle and lymph node homogenates, but was isolated from 4 (0.8%) of 500 tonsil samples collected from the same pigs. A survey of 250 serum samples provided an estimate of the prevalence of slaughtered pigs with TGE antibody of 34.8% in the sample population. The results indicate that carcasses of some pigs from TGE endemic areas contain viable TGE virus, and that there would be a substantial risk of introducing TGE virus into Australia by the importation of uncooked pig meat from these areas.

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## Introduction

Transmissible gastroenteritis (TGE) is a highly contagious, enteric disease of pigs caused by TGE virus, a member of the family Coronaviridae. The disease causes significant economic losses in the major pig-producing areas of North America and has been reported from countries in Central and South America, Europe, Asia and the Pacific (Saif and Bohl 1986). Australia is free from TGE, this privileged position being maintained by restrictions on the importation of pigs and pig products.

Carcasses of pigs infected with TGE can represent a source of infection for susceptible pigs. The virus is stable when frozen at  $-20^{\circ}\text{C}$  and can withstand pH changes in meat down to pH 3 (Harada *et al* 1968). Forman (1990) demonstrated experimental transmission of TGE in 7-day-old piglets dosed with homogenates of frozen meat, lymph node and bone marrow from recently infected pigs.

The present study was undertaken to provide additional data to better evaluate the risk of introducing TGE virus into Australia in uncooked pig meat. The objectives were to determine whether TGE virus could be transmitted from the carcasses of slaughtered pigs drawn from a population where TGE is endemic, and to determine the prevalence of TGE carriers and previously infected pigs in this population.

## Materials and Methods

### Animals and Housing

Two sows, each nursing 10 one-day-old piglets, were obtained from a TGE-negative herd and housed at the Veterinary Medical Research Institute, Iowa State University. Each sow and litter were isolated in separate rooms secure from infection with TGE virus.

### Collection of Samples

Five hundred pig heads were collected during March 1990 at an abattoir in Iowa and transported to Iowa State University. Samples of tonsils, brachiocephalic muscle and parotid lymph node were then collected aseptically and in a manner to avoid cross contamination between tissues. In addition, 250 serum samples were collected at the abattoir from different pigs of the same class. Sampled pigs were selected on the basis that they had passed through buying stations where overnight aggregation before going to slaughter was likely. The pigs were generally in the 100 to 120 kg bodyweight bracket.

### Processing of Samples

Muscle and lymph node (approximately 10 g and 5 g, respectively) from each of 25 pigs were pooled and prepared as a 25%

W/V homogenate in Earles medium‡, resulting in 20 homogenates. The homogenates were stored at  $-20^{\circ}\text{C}$  until assayed for the presence of TGE virus in tissue culture and by oral dosing of piglets.

Individual tonsil samples were prepared as a 10% W/V homogenate in Earles medium containing antimicrobials (500 IU penicillin, 500  $\mu\text{g}$  streptomycin, 50  $\mu\text{g}$  gentamicin and 2.5  $\mu\text{g}$  fungizone/ml). Each sample was centrifuged at 3000 RPM (2000 g) for 15 min. Supernatants were stored at  $-20^{\circ}\text{C}$  until assayed for TGE virus in tissue culture.

### Experimental Design

Each of the 20 piglets was assigned a different homogenate. From 6 days-of-age, piglets were dosed orally with 5 ml of their assigned homogenate daily for 4 d. Immediately prior to dosing, a 5 ml vial of each homogenate was thawed at  $37^{\circ}\text{C}$  and reacted for 2 h at room temperature with 1 ml of anti-pseudorabies virus (PRV) antiserum to neutralise any pseudorabies virus that may have been present in the homogenate.

The piglets were held for 4 w and observed daily for signs of clinical disease. Serum samples were collected from all piglets on the day of first dosing, and days 7, 14 and 21 post-first dosing (PD), and from the sows at the beginning and end of the study.

Samples from the gastrointestinal tract were collected from piglets that died. These samples were examined histologically and attempts were made to detect TGE virus antigen and isolate TGE virus.

### Virus Isolation

An established line of swine testicle (ST) cells (McClurkin and Norman 1966) grown on glass coverslips in 24-well tissue culture plates§ was used for virus isolation. Growth medium (GM) was Eagles minimum essential medium (MEM)¶ supplemented with 10% foetal calf serum (FCS) and antimicrobials (50  $\mu\text{g}$  gentamicin and 2.5  $\mu\text{g}$  fungizone/ml). Maintenance medium (MM) differed in that it was supplemented with 2% FCS.

Before inoculation GM was removed and monolayers were overlaid with 1 ml of MEM supplemented with 50  $\mu\text{g}$  DEAE Dextran/ml# for 30 minutes. The overlay was removed and the cells were inoculated with 0.1 ml of supernatant fluid from tonsils, muscle and lymph node homogenates or tissue samples from dead piglets. These tissue samples were processed as for tonsil samples. After absorption for 1 h at  $30^{\circ}\text{C}$ , the inoculum was removed, 1 ml of MM was added and cultures were incubated at  $37^{\circ}\text{C}$  in

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TABLE 1  
Serum virus neutralising antibody to TGE virus in 250 serum samples collected from slaughtered pigs

Titre	No. Serums
Neg	163
1:4	24
1:8	22
1:16	13
> 1:32	28
TOTAL	250

an atmosphere of 95% air and 5% CO<sub>2</sub>. The ST cells were examined for cytopathic effect every 2 to 3 d, for 2-weekly passages.

#### Detection of Viral Antigen

After 4 d incubation of the final passage, all cell monolayers were fixed in acetone for 5 min at room temperature, incubated at 37°C for 30 min with an anti-TGE virus - fluorescein isothiocyanate (FITC) conjugate\*\* diluted 1:20 in PBS and further processed as for direct immunofluorescent tests (Lennette and Schmidt 1979).

Sections of small intestines collected at necropsy were frozen sectioned (Culling *et al* 1985) and processed as for monolayers.

#### Electron Microscopy

The TGE isolates from autopsied piglets were propagated on ST cells grown in 150 ml tissue culture flasks.†† These cell cultures were frozen and thawed and the clarified supernatant fluids were sprayed on Parlodion-coated grids, negatively stained with 2% phosphotungstic acid and viewed with a Hitachi HS-9 electron microscope.

#### Antibody Assays

Serum antibody to TGE virus was assayed using the serum virus neutralisation (SVN) test as described by Snyder *et al* (1981).

### Results

#### Oral Dosing of Piglets

All piglets dosed with muscle and lymph node homogenates developed clinical signs typical of TGE by 7 days PD. A profuse watery diarrhoea was accompanied by anorexia, rapid weight loss and dehydration. Vomition was observed in 7 piglets. Clinical signs were similar in severity in each litter although they were first observed on day 3 PD in litter 1 and day 6 PD in litter 2. Five piglets died, one each on days 6 and 7 PD in litter 1 and days 8, 9 and 10 PD in litter 2. Direct immunofluorescence on sections of intestinal mucosa demonstrated TGE virus antigen in piglets dying on days 7, 8 and 9 PD. Viral isolates from piglets that died on days 8 and 9 PD showed typical coronavirus morphology by electron microscopy and were confirmed as TGE virus by direct immunofluorescence of inoculated monolayers. Severe villus atrophy was evident on histological sections of small intestine from the 5 piglets that died. Sow 1 was inappetent on days 7 and 8 PD but no other clinical signs were evident in either sow. The remaining 15 piglets recovered after 6 to 9 d of illness.

No SVN antibody to TGE virus was demonstrated in the sows or piglets pre-dosing. Antibody to TGE virus was detected on day 7 PD in litter 1 and day 14 PD in litter 2. At day 21 PD, all piglets had SVN antibody titres ranging from 1:16 to 1:64. Sows 1 and 2 had titres of 1:32 and 1:8, respectively, at day 28 PD.

#### Laboratory Results

Transmissible gastroenteritis virus was isolated from 4 (0.8%) of 500 tonsil samples collected from the same pigs at the same time as the muscle and lymph node samples. Isolates were identified by demonstrating distinct cytoplasmic fluorescence in inoculated ST cells using an anti-TGE virus - FITC conjugate. No virus was isolated from muscle and lymph node homogenates.

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Of the 250 serums from slaughtered pigs sampled, 87 (34.8%) had SVN antibody to TGE virus. Results are summarised in Table 1.

### Discussion

The results of this study indicate that muscle and/or lymph nodes of some slaughtered pigs from TGE endemic areas contain TGE virus. Both litters in this study were infected with TGE virus after dosing with homogenised muscle and lymph node. Because TGE virus may have spread rapidly by contact within each litter, the result does not allow the actual prevalence of infected carcasses to be quantified. However, at least 1 homogenate fed to each litter contained virus and, therefore, the muscle or lymph node of at least 2 of 500 pigs sampled contained viable TGE virus. The slaughtered pigs were probably either incubating the disease or convalescent carriers of TGE.

All ages of pigs are susceptible to TGE virus infection, transmission being mainly by ingestion, or airborne infection (Saif and Bohl 1986). Faecal shedding under natural conditions generally lasts 2 w post-exposure (Pensaert *et al* 1970) and respiratory shedding for up to 11 d post-exposure (Kemeny *et al* 1975). Infection with TGE virus results in viraemia and localisation in a number of tissues, depending on the age of the pig (Harada *et al* 1969; Furuuchi *et al* 1978). Transmissible gastroenteritis virus has been transmitted by homogenates of kidney, spleen, liver, lungs and brain, as well as gastrointestinal tract, suggesting the virus is widely distributed in the body of infected pigs (Bay *et al* 1949). Relatively insensitive tissue culture methods (Dulac *et al* 1977) have isolated TGE virus from nasal and tracheal mucosa, oesophagus, lung, intestine, and bronchial and mesenteric lymph nodes from 4 to 5 month-old pigs 5 d after experimental exposure (Harada *et al* 1969).

Pigs infected with TGE virus may become convalescent carriers, the virus being isolated from intestinal and respiratory tissues for up to 104 d post-exposure (Underdahl *et al* 1975). However, long-term shedding of viable virus and the role of TGE virus carriers in transmitting the disease have not been fully assessed (Saif and Bohl 1986). Pre-slaughter handling stress of TGE-infected carrier pigs may lead to viraemia and virus shedding. Transmissible gastroenteritis virus has an incubation period as short as 12 to 18 h (Gillespie and Timoney 1981). Therefore, the mixing of susceptible and carrier pigs at buying stations for up to 24 h prior to slaughter could increase the likelihood of pigs incubating the disease at the time of slaughter. Forman (1990) demonstrated the presence of TGE virus in either muscle, lymph node, or bone marrow in recently infected 6-month-old pigs that had been experimentally exposed to TGE virus. The present study involved the sampling of pigs that had been exposed naturally to the virus under North American conditions. Our work shows that TGE can be transmitted to susceptible piglets by feeding carcass samples from apparently healthy pigs. The slaughtered pigs sampled in this study had no evidence of clinical disease and passed routine ante-mortem inspection.

The present survey showed a 0.8% frequency of infection from tonsil samples of 500 slaughtered pigs. This frequency is less than that recorded in an early study of slaughtered sows in Iowa (Kemeny 1978) in which TGE virus was isolated from 3% of 2058 pig tonsils. The day-to-day variation in infection rate of tonsils in that study varied widely from 0% (0/306) to 22.9% (33/144) samples being positive for TGE virus. Kemeny (1978) suggested that the prevalence he reported may have been inflated by a marked increase in the number of TGE outbreaks in the winter preceding the sampling period. As the frequency of TGE virus in samples drawn from a population in which TGE is endemic is obviously variable, it is difficult to calculate a constant risk for the presence of TGE virus in slaughtered pigs.

The prevalence of antibody to TGE virus (34.8%) found in this study is similar to the 30.9% previously reported for slaughtered pigs from this region (Egan *et al* 1982). The herd prevalence in Egan's study was 54.7%, which was similar to the reported herd prevalence of 50.6% in East Anglia, England (Pritchard 1987). These studies indicate that in areas where TGE virus has existed for some years, the herd prevalence rate approaches 50%. It is therefore reasonable to hypothesise that under the North

American marketing system, where pigs are mixed at buying stations, exposure of susceptible pigs to TGE virus may be common. Our findings strongly support this hypothesis.

Within 20 years of the first report of its occurrence in the United States in 1946 (Doyle and Hutchings 1946), TGE virus was widely dispersed over much of the Northern Hemisphere (Saif and Bohl 1986). Ferris (1973) has emphasised that the rapid intercontinental spread of TGE virus is testimony to its various mechanisms of spread. The number of infected carcasses found in this study suggests that trade in uncooked pigmeat is one possible mechanism, given that the virus survives freezing and the minimum infective dose of the virus for suckling piglets is very small. The importation into Australia of uncooked pork from any population endemically infected with TGE represents a substantial risk given the gaps in the knowledge of the pathogenesis of TGE infection, particularly with regard to the role that convalescent carriers play in transmitting the disease. The information reported in this paper should be considered in any assessment of the risk of entry of TGE virus into Australia in imported, uncooked pigmeats.

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## BOOK REVIEW

**Feeding Standards for Australian Livestock: Ruminants.** Ed. JL Corbett, CSIRO, Melbourne 266 pp. 1990, \$A50; and

**GrazFeed, a Computer Program** by M Freer and J Donnelly. Horizon Agriculture, P.O. Box 679. Manly 2095, Australia. \$375.

These two works appeared almost simultaneously as a combined study of the nutrition of grazing ruminants. The book is a detailed and critical discussion of feeding standards for grazing ruminants. The computer model is an aid to its use, with particular emphasis on energy and protein. The book is built on the reports of NRC in USA, and ARC, INRA, and others in Europe.

The whole approach is, however, oriented much more to the grazing animal than the earlier publications. This makes it more relevant to the needs of pastoral countries, but not less to countries which rely heavily on grain, and other feedstuffs, in animal production.

Seven themes are discussed. The first 5 are the requirements of energy, protein, and minerals, vitamins, and water. The prediction of intake follows, and then a brief statement on application (11 pages), including computer programs. The last section is the least adequate. The bibliography consists of about 1100 references, covering up to 1988 well, with some more recent entries. This is excellent.

Each section starts with a summary and details of the material covered is summarised in 7 pages at the beginning of the book. Unfortunately there is no index, but this deficiency is largely compensated by these summaries.

The orientation to grazing has generated some problems. The intake of grazing animals is notoriously difficult to estimate. Therefore the energy budgets of grazing animals are often based on stall feeding and calorimetry. Extrapolation from such bases must be cautious and exploratory. Moreover the effects of grazing over varying terrains, and pastures ranging in availability and composition, with selection of some parts of plants rather than others, require assumptions about animal behaviour, and the energetics of movement, prehension, and mastication, which cannot be derived wholly from "hard" data.

Nevertheless the background of calorimetry etc has enabled very useful estimates of requirements to be made, and the precision of such estimates in most circumstances has permitted confidence in planning supplementation of pastures with a wide range of feedstuffs. This is a cause for congratulations to the research workers in this field, and also to the contributors to this volume who have tackled the difficulties of synthesis and evaluation.

The section on minerals (55 pages) is thorough, as might be expected from the importance of trace elements in particular Australian grasslands. The pioneer research of Bennets, Underwood, Marston and others, developed further by Caple and contemporaries, provides an inspiring background to the section. The section on vitamins (7 pages) is probably adequate for grazing animals. The problems associated with thiaminase are of relatively recent interest.

The book, though complete in itself, receives strength from its integration with the computer program GrazFeed. This program essentially does the arithmetic necessary to combine the information from the pastures, the species of animals, the weather, and the physiological status of the animal in order to estimate what supplementation is needed for defined responses. It estimates "what happens if . . .".

The programming is elegant and user friendly. The manual is an excellent guide from first steps to the study of complex questions. It is a first class educational aid as well as a practical and useful working tool. Inevitably some defects have been found and these are being corrected by the authors. For example oat grain should be given energetic value equivalent to wheat or barley for some purposes. Some situations give rise to negative wool growth. These little problems will not disappear overnight, if past experience with computer programs is a guide.

The value of these two in on-farm decision making is necessarily limited by the difficulties in predicting events such as rainfall, and consequent effects on pasture growth and decay, and animal responses and requirements. This is especially limiting in Australia with its unreliable seasons. These have stimulated development of procedures for decision making in the face of uncertainty, which are being used routinely by farm consultants. It is a pity that they are scarcely considered in these publications.

Decisions on fodder conservation and finance, and on marketing and forward planning, depend far more on probabilities of events over which we have little or no control, than on deciding on the composition of rations or techniques of feeding. The application of the most elegant studies of nutrition must depend heavily on examination of grazing systems, including the stochastic elements.

The information in these publications is a very necessary component in rational nutrition of livestock. But it is not sufficient to advisers or their farmer clients, who must not only understand the disciplines of animal nutrition, but also evaluate future uncertainties, in order both to diagnose and deal with present problems and to take advantage of new opportunities.

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