Transmissible Gastroenteritis (TGE) of Swine: The Possible Role of Dogs in the Epizootiology of TGE

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SUMMARY

Nine of 11 six-to-eight-week-old beagle puppies from a colony started from hysterectomy-derived breeding stock developed a serological response to a transmissible gastroenteritis (TGE)-associated virus following exposure to TGE-infected pig intestinal tissue. The virus was not isolated on swine testis (ST) cells from dog rectal swabs, except in one instance; however, when composites of rectal swabs from all 11 dogs taken seven days and fourteen days postexposure were fed to piglets, they developed signs of TGE and died within seven days. This TGE-associated virus was readily isolated on ST cells from the rectal swabs of the exposed piglets.

RÉSUMÉ

Neuf chiots beagle âgés de six à huit semaines, dans un groupe de 11 sujets nés par hystérectomie, développèrent une réaction sérologique contre un virus associé à la gastro-entérite contagieuse, (TGE) après avoir été exposés à du tissu intestinal de porc atteint de gastro-entérite contagieuse (TGE). A une exception près, on n'isola pas de virus dans des cultures de cellules testiculaires (ST) de porcs inoculées à partir de frottis rectaux effectués chez des porcelets ayant absorbé une nourriture contenant des éléments qui provenaient de frottis rectaux d'un chien préalablement exposé à l'infection. Cependant, lorsqu'un mélange des frottis rectaux des onze chiots, prélevés à sept et à quatorze jours après l'exposition à la maladie, fut placé dans les aliments des porcelets, ils développèrent les symptômes cliniques de gastro-entérite contagieuse et moururent au cours des sept jours suivants.

Ce virus associé à la gastro-entérite contagieuse fut facilement isolé dans des cellules testiculaires de porcs à partir de frottis rectaux effectués sur les porcelets exposés à la maladie.

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INTRODUCTION

Norman *et al* (4) reported antibodies against a virus commonly isolated from TGE-virus-infected tissues in the sera of 73 of 76 kennel raised puppies. As the puppies had no contact with swine and had been fed only commercially prepared dog food, it was suggested that the dog might be a natural reservoir for the virus.

A second step toward defining the role that dogs may play in the epizootiology of TGE is the subject of this report.

MATERIALS AND METHODS

Source of Experimental Cytopathic TGE-Associated Virus

The TGE-associated virus was the Nebraska isolate[1] obtained in 1966. Results of experimental studies on pigs, cell cultures, and of serological tests indicated that this virus had characteristics similar to TGE-associated viruses previously reported (2, 3).

Source, Maintenance and Treatment of Dogs

Eleven six- to eight-week-old beagle puppies were purchased from a colony that had been started from hysterectomy-derived (HD) breeding stock.[2] We established that the sera of the puppies and the adult dogs in this colony contained no antibody against the TGE-associated virus (4).

Dogs were paired and maintained in isolation cages (2) for the duration of the experiment. The experimental isolation requirements limited monitoring for clinical signs to daily observations of the dogs through the transparent plexiglass cages for appetite, physical activity and examination of excreta under the wire mesh floor

[1]Kindly provided by Dr. Norman R. Underdahl, Department of Veterinary Science, University of Nebraska, Lincoln, Nebraska.

[2]Dr. William Reece, Physiology Dept., College of Veterinary Medicine, Iowa State University of Science and Technology, Ames, Iowa.

for evidence of vomiting or diarrhea.

Each dog was fed 35 ml of a 1:10 suspension of bacteria-free (filtered through a 0.45 micron Millipore filter) pig intestinal tissue infected with the Nebraska isolate of TGE. Twenty-one days later the dogs were fed 20 ml of the same virus suspension.

Sera were tested for virus neutralization by a plaque reduction method before administering the TGE virus and at 57 days post-infection.

Rectal swabs were also taken pre-exposure and on the second, fifth, seventh, and 14th day after the first exposure and on the seventh, 14th, 21st and 36th day after the second exposure. The swabs were placed in a tube containing 3 ml of diluent (2) and frozen. Before tests in cell culture and in pigs, the swabs were thawed and the fluid filtered through a 0.45 micron Millipore filter to remove the bacteria.

Experimental Inoculation of Piglets

Ten ml of a composite of filtrates from the rectal swabs of all 11 dogs taken before exposure and on the seventh and 14th day after the first exposure, and on the 36th day after the second exposure were each fed to one of four two-day-old HD piglets.

Virus Isolation on Cell Culture

A continuous culture of swine testis (ST) cells (3) was prepared as follows: cells were suspended in F-15 medium[3] with 10% swine serum, seeded into 60 x 15 mm plastic Petri plates, and incubated at 36°C in an atmosphere of 2% CO_2 and 90% relative humidity. When the cells were confluent, the medium was removed, and they were inoculated with 0.15 to 0.2 ml of the specimen to be tested. After adsorption for 15 to 20 minutes, 8 ml of an agar overlay, consisting of 0.5% Ionagar No. 2 prepared with Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate and Eagle's amino acids and vitamins, was added and the plates returned to the incubator. In 48 to 70 hours, 5 ml of a second agar overlay containing 0.01% neutral red in 1% agar was added and after another four to six hours of incubation, the plates were examined for plaques.

[3]Grand Island Biological Company, Grand Island, New York.

Serological Techniques

Virus neutralization was performed by a plaque reduction method, using a constant virus concentration and varying serum dilutions. The Nebraska isolate of TGE virus was standardized to contain approximately 130 pfu per 0.1 ml, serum samples were inactivated at 56°C for 30 min., and serum and virus were mixed and incubated at 38°C for 30 min. Cell monolayers in plastic Petri plates were inoculated with 0.2 ml of the serum-virus mixture and allowed to adsorb for 15-20 min at room temperature. The plates were overlaid with agar and examined for plaques after incubation for approximately 60 hours. Additional plaque neutralization studies on the cytopathic virus isolated from the rectal swabs of piglets were accomplished with a porcine antiserum prepared against the Nebraska isolate.

RESULTS

Dogs fed TGE virus-infected tissue suspension filtrates showed no clinical signs of reaction.

The TGE-associated virus was not detected in ST cells inoculated with material from rectal swabs from the piglet fed a composite of material from the pre-exposure dog rectal swabs.. Virus re-isolation was accomplished from the rectal swabs of one dog only on the second day postexposure, but not from the remaining ten dogs at any time.

Composites of rectal swab material from all 11 dogs taken seven and 14 days after the first exposure each produced pathological changes typical of TGE in two-day-old HD piglets. They developed vomiting and diarrhea, and died within seven days of exposure. A cytopathic virus from the rectal swabs from these piglets was readily isolated on ST cells. This virus produced plaques which were neutralized by immune serum (Nebraska) diluted 1:1000, the highest dilution tested.

A composite of rectal swab material from the 11 dogs, taken 36 days after the last exposure, produced no reaction in a piglet. Attempts to isolate the virus from piglet rectal swabs were unsuccessful.

Pre-exposure dog serum samples were negative for neutralizing antibodies. The plaque reduction titers of the serum from each of the 11 dogs against approximately

TABLE I. Plaque Reduction Titers by Dog Sera 57 Days After the First Exposure to TGE-infected Tissues

Dog No.	Serum Dilution[a]	Dog No.	Serum Dilution
1	5	7	5
2	Neg.	8	5
3	5	9	Neg.
4	20	10	40
5	5	11	5
6	5		

[a]Reciprocal of the highest dilution of serum which would prevent the formation of virus plaques when tested against approximately 130 plaque-forming units of virus per 0.1 ml.

130 plaque-forming units of TGE-associated virus per 0.1 ml 57 days after the first exposure are shown in Table I.

DISCUSSION

The method of isolating the TGE-associated virus on ST cells described in this paper has been highly successful when applied to intestinal tissues or rectal swabs from pigs in suspected field outbreaks, or from the experimental disease. However, under the conditions of this experiment, the presence of the virus in dog rectal swabs was best demontrated by feeding the material to piglets and reisolating the virus from the fecal material of the infected piglets. We infer from this that the piglet is a more sensitive indicator for the virus than is the cell culture system.

Nine of the 11 dogs developed a serological response to the virus, but the titers were generally lower than those reported by Norman *et al* (4) in dogs for which the exact time of exposure was not known. The older dogs had the highest titers which was suggestive that recurrent infection might occur with age. Furthermore, in this experiment, the dogs were kept in isolation cages and separated from their feces, thus diminishing the intensity of possible re-exposure.

The work reported here supports the work of Haelterman (1) indicating that dogs could transmit TGE to pigs.

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