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Virus Isolation and Serum Antibody Responses After Infection of Cats With Transmissible Gastroenteritis Virus

Brief Report

By

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With 2 Figures

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Summary

Transmissible gastroenteritis virus was administered orally to cats. No clinical disease resulted but infectious virus was isolated from faeces for up to 22 days after infection and serum antibody was detected by neutralisation and immunofluorescence tests.

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The spread of transmissible gastroenteritis virus (TGEV) from infected pig herds and the recrudescence of infection after long periods have not been adequately explained. Experiments on the host range of the virus have indicated that non-porcine hosts could have a role in the epidemiology of TGE (2, 4, 7, 8, 9, 16). Indirect immunofluorescence (IIF) and virus neutralisation (VN) tests have been used recently to demonstrate antibody to TGEV in cats. In Holland and Great Britain samples from cats with clinical feline infectious peritonitis (FIP) frequently have antibody to TGEV in the IIF test (10). However, neutralising antibody has not been found in sera from Dutch cats with FIP. American and Dutch studies have recently demonstrated that sera from cats experimentally infected with FIP will cross-react, at low titre, with TGEV-infected pig cells by immunofluorescence but will not neutralise TGEV *in vitro* (10, 12). This evidence has been used to suggest that an antigenic relationship exists between TGEV and FIP virus.

The potential of the cat to excrete TGEV was studied by virus reisolation and the serological responses to TGEV and FIP were monitored; thus the possible epidemiological role of the cat in TGE could be assessed. In addition information was obtained on the possible origin and relationship of VN and IIF antibodies to TGEV which occur naturally in cats, often at high titres (10, 13).

TGEV strain KN63, used as inoculum in this experiment, was prepared as a clarified 50 per cent suspension of small intestine from an infected gnotobiotic piglet. It had a titre of 1.7×10^7 PFU/ml.

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Four twelve-week old kittens were obtained from an SPF cat colony with no evidence of neutralising antibody to TGEV and no clinical history of FIP. The kittens were housed separately in plastic isolators (14). Three animals (A, B and C) were given 1 ml of virulent TGEV orally, the fourth (D) was given 1 ml phosphate buffered saline and retained as an uninfected control. Animal A was sacrificed 3 days after infection in an attempt to detect evidence of TGEV replication in the tissues, whilst B and C were retained for 156 days. Animal D was given oral TGEV 21 days after the commencement of the experiment and 93 days later (day 114) it was hyperimmunized with 2.5 ml TGEV viral antigen produced from cell cultured virus (3), injected intraperitoneally. This dose was repeated 31 days later (day 145) and after another 10 days (day 155) the cat was killed and exsanguinated.

Control and infected animals were tested for TGEV excretion by attempted isolation of virus from faeces for up to 35 days after infection; occasionally rectal swabs were used to obtain fresh samples. Cell growth medium was used to prepare 10 per cent faecal suspensions and elute material from rectal swabs. Both types of preparation were clarified by centrifugation at $1000 \times g$ for 15 minutes and 0.2 ml of the supernate was inoculated onto monolayer cultures of secondary adult pig thyroid (APT/2) cells on glass coverslips. The medium was changed after 24 hours and 4 days later any viral cytopathic effect was recorded, the medium was removed and passaged in fresh APT/2 cultures and the coverslips were stained to demonstrate viral antigen by indirect immunofluorescence, using paired sera from a gnotobiotic piglet before and after TGEV infection, followed by FITC-conjugated rabbit anti-swine globulin (Nordic Immunological Laboratories, London). Cultures with evidence of TGEV antigen were passaged again in the presence of inhibitory levels of specific antiserum.

Recovery of TGEV was judged successful if cells showed a characteristic cytopathic effect, produced cytoplasmic fluorescence with monospecific TGEV antiserum alone and the infectivity could be neutralized by specific TGEV antiserum. TGEV was not isolated from the faeces of any animal before infection or from the uninfected control. Uninfected coverslip cultures, set up concurrently with infected samples, remained free of TGEV. TGEV was isolated from the faeces of animal B on days 7, 16, 18 and 22 after infection and from animal C on days 4, 12, 14 and 18 after infection. Virus was not isolated from faeces of animal A in the 3 days after infection nor from animal D either before or after oral administration of TGEV. There were no signs of disease in the cats after infection.

Reisolation of TGEV was attempted from animal A, killed 3 days after infection and animals B and C killed 156 days after infection. Tissue suspensions were prepared and treated as described for faecal suspensions. Tonsil, stomach, upper, middle and lower jejunum, ileum, caecum, colon, rectum, kidney, liver, spleen, lung, brain and mesenteric lymph node were the tissues used. Pooled tissue suspensions from B and C were administered orally to 3-day old SPF piglets and were also inoculated onto APT/2 monolayers for antigen detection by the IIF test. The piglets showed no signs of TGE nor was there a detectable serological response. The coverslip cultures remained negative by immunofluorescence.

The survival of infectious TGEV in the inoculum was studied at 37° and 22° C to give an indication of the probable time course of viral inactivation in the host

and in faeces. Viral antigen was demonstrated by immunofluorescence after infection of coverslip cultures by the method described for faecal virus reisolation. Infectivity titres of TGEV were determined by plaque assay on APT/2 cells in 50 mm plastic petri dishes. Infectivity was still present after 2 but not 3 days at 22° C and was lost within 24 hours at 37° C. Virus could be demonstrated by both plaque assay and immunofluorescence in infectious samples.

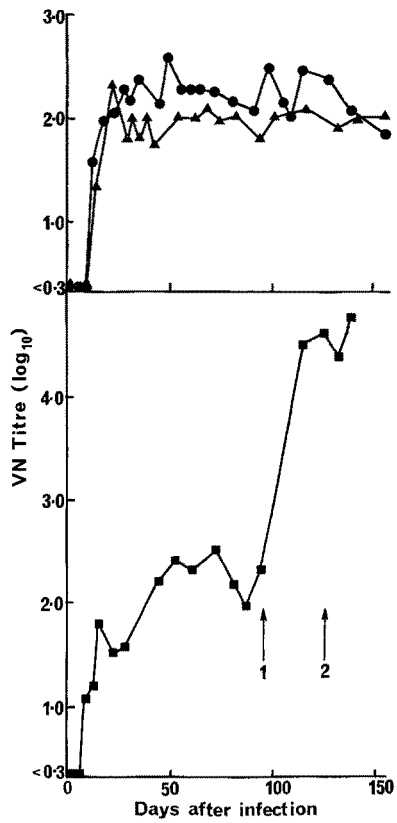

Fig. 1. TGEV virus neutralisation (VN) titres (———) of cat sera after infection with TGEV on day 0.
Symbols ▲, ●, ■ represent cats B, C and D respectively. Animal D was hyperimmunized on two occasions as indicated by arrows 1 and 2

Blood was taken from the cephalic vein of animals B, C and D before and regularly after infection to monitor the serum for VN and IIF antibodies to TGEV. A microtitre test (15) was used to assay TGEV neutralising antibody levels in serum and the responses of cats B, C and D are presented in Figure 1 as the $\log_{10}$ of the reciprocal of the end-point dilution, calculated by KÄRBER'S method (6). Antibody could not be detected prior to infection and animal A remained negative for the three days prior to sacrifice. The uninfected control also remained negative. In animals B, C and D VN antibody was first detected approximately 1 week after exposure to TGEV and the titres rose to maximum values of $10^{2.2}$—$10^{2.3}$.

Twenty-one days after first hyperimmunization cat D had developed a marked secondary response, with a VN antibody level of $10^{4.5}$ which persisted until death.

Antibody to TGEV was not detected in the IIF test before infection. Following experimental infection with TGEV serum antibody, detectable in the IIF test, developed and persisted similarly in the three cats tested (Fig. 2). The IIF test titres were consistently lower than VN test titres. After hyperimmunization a marked increase in the IIF test titre was detected in D. This animal also developed antibody to APT/2 cell antigens in the IIF test, although the titre was four-fold lower than that of specific antiviral antibody.

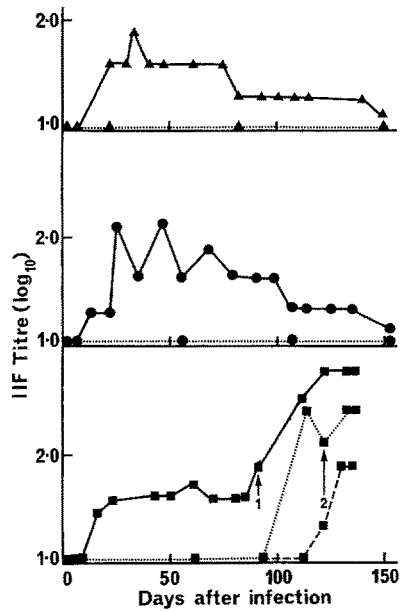

Fig. 2. IIF test antibody titres of cat sera to TGEV (———), FIP (- - - - - - - -) and uninfected pig cells (— —) after infection with TGEV on day 0.
Symbols ▲, ●, ■ represents animals B, C and D respectively. Animal D was hyperimmunized on two occasions as indicated by arrows 1 and 2

Antibody to FIP was assayed in an IIF test by Dr. N. C. PEDERSEN, University of California, Davis, U.S.A. (11) and in a suckling mouse brain-adapted FIP neutralisation test (5) by Drs. A. D. M. E. OSTERHAUS and M. C. HORZINEK, University of Utrecht, The Netherlands. No FIP-specific antibody was demonstrable in the sera before or after oral infection with TGEV. Antibody was detected by the IIF test in cat D within 21 days of the first hyperimmunization, however, reaching a titre of $10^{2.4}$ which remained constant for the following 20 days (Fig. 2) but this serum failed to neutralise FIP infectivity in the mouse brain system.

This experiment demonstrates that cats exposed to TGEV can excrete the virus for a period significantly longer than would be expected from passive transit of ingested virus. The cat could therefore constitute a practical epidemiological hazard to susceptible pigs. The ability of TGEV excreted by the cat to infect pigs or other cats was not investigated but similar work (4) demonstrated the ability

of dogs and foxes to excrete TGEV which could infect piglets. Hence, contact between these carnivores and infected pigs should be prevented. A study of the replication site of TGEV in the cat was not possible with the limited number of animals. Virus isolation from only two of the animals tested may indicate some variation in host susceptibility to infection although the serological responses were similar in all 3 animals.

FIP and TGE, two antigenically-related viruses, are now known to infect cats experimentally but result in differing immune responses. Following a single exposure to TGEV, 3 cats developed homologous antibodies, detectable by VN and IIF tests. Antibody to FIP was detected only following hyperimmunization, which resulted in extremely high anti-TGEV titres. Experimental FIP results in no TGEV neutralising antibody but antibody to both viruses is found using IIF tests with FIP titres exceeding TGEV titres. These results provide serological evidence of a distant antigenic relationship between TGEV and FIP, perhaps due to an internal group antigen not involved in virus neutralisation. This situation can be compared to the closer relationship of the canine coronavirus to TGE, agents which cross-react in VN and IIF tests (1, 12). Hyperimmunization may be important in stimulating antibody to heterologous viruses.

It is probable that infection with one or more TGEV-related viruses could result in the complex serological picture in FIP cases previously described (13). FIP virus itself may have more than one antigenic type, which would also result in the differing responses found in FIP cases in the U.K., U.S.A. and the Netherlands.

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