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Short Communication

# Studies on the survival of canine coronavirus under different environmental conditions

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## Abstract

Canine coronavirus (CCV) is a common faecal agent which is difficult to isolate. This study shows CCV to survive well at temperatures below  $-20^{\circ}\text{C}$  but not at temperatures above  $4^{\circ}\text{C}$ . The presence of faecal material markedly reduced CCV survival times at temperatures ranging from  $20^{\circ}\text{C}$  to  $-70^{\circ}\text{C}$ . Thus, it is suggested that diagnostic faecal material should be diluted 1:10 (w/v) with growth medium and examined at the earliest opportunity.

*Keywords:* Canine coronavirus; Diagnosis, Canine coronavirus; Faeces

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## 1. Introduction

Canine coronavirus (CCV) is a common enteric pathogen (Binn, et al., 1974; Carmichael and Binn 1981; Tennant et al., 1991a). The diagnosis of CCV infection relies on either demonstration of the virus by electron microscopy or by virus isolation. For the latter technique to be successful requires good survival of CCV in faeces. Although coronaviruses in general show moderate stability at temperatures below  $4^{\circ}\text{C}$  (McIntosh, 1974; Pensaert and Callebaut, 1978; Siddell et al., 1983), feline infectious peritonitis virus (FIPV), a closely related virus to CCV, is reported to be more labile (Stoddart, 1985). The survival of CCV at various temperatures and the effect of routine laboratory handling and storage on the infectivity of CCV has not previously been described. Thus, the aim of this paper is to investigate the survival of CCV under various conditions and to determine optimal storage conditions for field and experimental samples.

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## 2. Materials and methods

In the first experiment, three separate four ml aliquots of CCV-C54 ( $10^{6.3}$  TCID<sub>50</sub>/ml) (Tennant et al., 1991b) were mixed with 36 ml of either Wellcome growth media (Gibco ltd), virus transport medium (VTM) (Hanks balanced salt solution, Gibco ltd) or virus free 30% w/v canine faecal suspension in phosphate buffered saline. All solutions also contained 10% foetal calf serum, 0.067 mg/ml benzyl penicillin, 0.11 mg/ml streptomycin sulphate and 0.0022 mg/ml amphotericin B. One ml aliquots made from each of these samples were stored at either 20°C, 4°C, –20°C or –70°C, whilst one ml aliquots of CCV in growth media were also stored at 37°C, for variable periods of time. Three bijou containing virus in growth media were subject to one, three or six freeze thaw cycles (frozen at –70°C and thawed at 37°C). A second experiment was performed to investigate the effect of dilution of faecal samples on the survival of CCV. Thus, CCV-C54 was mixed with a faecal suspension as described above. Ten-fold dilutions, of the CCV-C54 infected faecal suspension, were made in Wellcome growth media. Duplicate dilutions were stored at either 4°C or 20°C for variable periods of time. The viral titres in all aliquots were then determined as previously described (Tennant et al., 1991b).

## 3. Results

The results of CCV-C54 survival in various media at different temperatures are shown in Figs. 1a–e. The pattern of virus survival was similar for growth medium and VTM at all temperatures although the former tended to be marginally superior. The lower the temperature of storage then the greater the retention of infectivity. Viral titre was rapidly lost at 37°C in growth medium, with a drop in virus infectivity from the initial titre of  $10^{5.3}$  TCID<sub>50</sub>/ml to zero by 13 hours (Fig. 1a). At 20°C, in growth medium and VTM, virus infectivity declined more slowly, but there was at least a  $10^2$  reduction in titre by 48 hours (Fig. 1b). At 4°C little loss in titre was seen in either growth medium or VTM up to 72 hours (Fig. 1c). Virus stored in faecal suspension rapidly lost infectivity at 20°C (Fig. 1b) and 4°C (Fig. 1c) such that after 24 hours there was an approximate  $10^3$  or more reduction in viral titre. Frozen virus in A-72 growth medium or in VTM retained significant titres at –20°C (Fig. 1d) or –70°C (Fig. 1e) over a two year period though –70°C was preferable. Storage of virus in frozen faecal material resulted in complete loss of infectivity at either –20°C (Fig. 1d) or –70°C (Fig. 1e) by 3 months. Almost complete retention of viral infectivity was demonstrated following six consecutive freeze-thaw cycles. The CCV titre decreased from  $10^{5.3}$  TCID<sub>50</sub>/ml initially to  $10^5$  TCID<sub>50</sub>/ml after the sixth freeze-thaw cycle (Fig. 2).

The data from the second experiment show a decreased rate of virus loss at 4°C where the faecal suspension was diluted with growth medium (Fig. 3a). Viral titre was rapidly lost in the 1:1 dilution with a  $10^3$  log<sub>10</sub> reduction by 24 hours, whereas with the 1:10 dilution a similar drop in titre was only seen after 72 hours. Dilution at 1:1000 appeared to preserve virus infectivity completely over this time period. At 20°C the results were less clear cut. Dilution with growth media appeared to reduce virus loss only in the first 12 hours, after which time virus infectivity decreased rapidly (Fig. 3b).

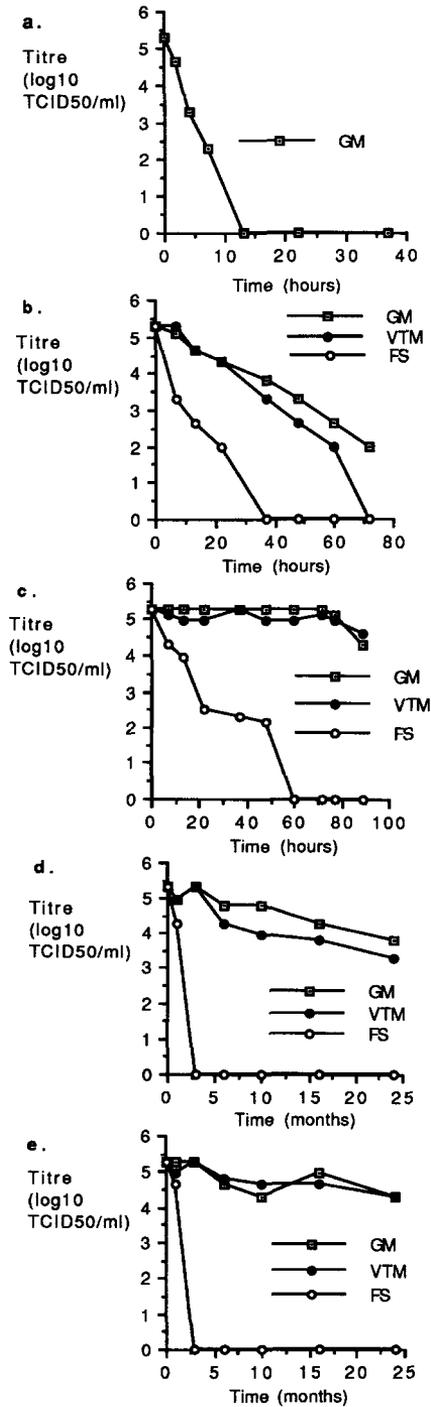


Fig. 1. Survival of CCV-C54 in growth medium (GM), virus transport medium (VTM and faecal suspension (FS) at: a, 37°C; b, 20°C; c, 4°C; d, -20°C and e, -70°C.

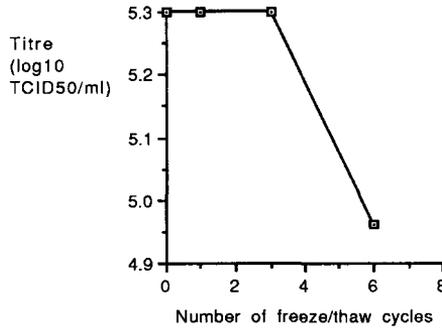


Fig. 2. Survival of CCV-C54 in growth medium (GM), following successive freeze/thaw cycles.

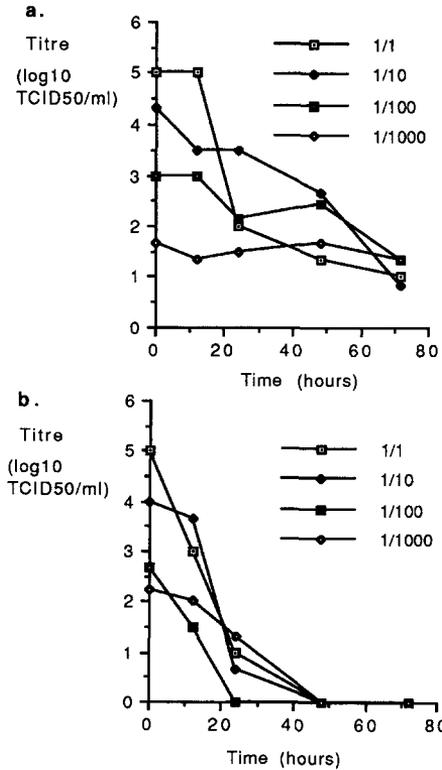


Fig. 3. Survival of CCV-C54 in different dilutions of faecal suspension in growth medium at: a. 4°C and b. 20°C.

#### 4. Discussion

The stability of coronaviruses at various temperatures appears to be dependent on the nature of the environmental conditions. In general it appears that coronaviruses are inactivated at 56°C in 10–15 minutes, at 37°C in several days and at 4°C in several months whilst virus frozen at –60°C survives for many years without loss of infectivity (McIntosh, 1974; Pensaert and Callebaut, 1978; Siddell et al., 1983). The effect of routine laboratory handling

and storage on the infectivity of CCV has not been previously described, although anecdotal reports suggest a longer survival of faecal CCV in the winter months (Carmichael and Binn, 1981), presumably due to lower ambient temperatures.

Our initial experiments showed that CCV survived significantly better in growth medium or VTM, compared to undiluted faecal suspension and confirmed that in the short term, CCV survived for longer at lower temperatures. Thus, infectivity was retained after several days at 4°C, and for at least 2 years at –20°C or –70°C, when stored in growth media or VTM. However, the virus tended to lose infectivity rapidly at 37°C and over several days at room temperature. The longer survival of CCV in A-72 media and VTM, compared with faecal suspension, is probably attributable in part to the pH or presence of ions and colloids in faeces which may inactivate the virus. The inclusion of foetal calf serum in the faecal suspension appeared not to substantially affect virus survival.

This work suggested that in the field, it may be better to dilute faecal samples in growth medium or VTM in order to preserve CCV infectivity. This hypothesis was examined in the second experiment, when it was shown that in general, dilution of the faecal suspension in growth media significantly reduced the rate of loss of viral infectivity, particularly at 4°C. Thus, it is suggested that diagnostic faecal material should be diluted 1:10 (w/v) with growth medium and examined at the earliest opportunity.

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