Diagnosis of canine coronavirus infection using nested-Polymerase Chain Reaction (n-PCR) (14-Aug-1999)

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Abstract
Canine coronavirus (CCV) is a single-stranded RNA virus belonging to the *Coronaviridae* family and is responsible for generally mild gastroenteritis in pups. Infected dogs shed CCV in feces for 6-9 days, but shedding can be prolonged in some pups. The virus content of feces is very high at the time of clinical signs. Electron microscopic (EM) examinations of fecal suspensions or isolation in tissue cultures are the most commonly used techniques for the diagnosis of the infection in dogs. However, both the methods may be carried out only in specialized laboratories, and they are difficult and time-consuming.

Recently we developed a nested-Polymerase Chain Reaction (n-PCR) assay for the diagnosis of CCV infection that was highly sensitive and specific and we report here the results of n-PCR in comparison with EM and virus isolation.

DNA sequence analysis of the 409bp PCR products showed the fragment of strain tested, to have 94% nucleotide sequence identity to CCV Insavc (reference strain). Of the samples examined, only 14/71 (19.71%) were positive in the PCR assay; however, 30/71 (42.25%) of the samples were positive in the n-PCR assay. CCV was isolated in cell culture from only 3 of the 30 n-PCR positive samples.

EM examinations revealed CCV-like viral particles in only 3/X (X%) of the samples examined. Two of those samples resulted positive in assay isolations, while the third was negative by both EM examination and virus isolation.

Of the several methods used for the detection of CCV, EM, using negatively stained fecal samples, appears to be an essential diagnostic tool; however, the common presence of coronavirus-like particles in feces requires confirmation by other diagnostic methods. Nested-PCR, on the other hand, allows the rapid and more sensitive diagnosis of CCV infection and would be especially valuable for the diagnosis of CCV in fecal samples where CCV is inactivated, or when the number of virions is less than 10^6/gr of feces and cannot detected by EM examination or viral isolation, which is tedious and time-consuming. The results of the present study also suggest that the role of CCV as an enteric pathogen may be underestimated since fecal samples from pups with gastroenteritis might be negative for both viral isolation and EM examinations, yet positive by n-PCR. Nested-PCR results revealed that CCV is widespread in the dog populations and, therefore, might be responsible for a higher frequency of enteritis in pups than previously reported.

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