Viral aetiology of diarrhoea in puppies from a same shelter in Turkey: presence of mixed infections

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SUMMARY

This study was conducted to evaluate the prevalence of viral agents that cause enteritis in puppies. Prevalences of canine distemper virus (CDV), canine parvovirus (CPV) and canine rotavirus (CRV) were established using RT-PCR, PCR and polyacrylamide gel electrophoresis (PAGE) techniques respectively, in faecal samples from 1 to 2-month-old diarrheic puppies (n = 34) stemming from a same shelter. Twenty-nine samples (85.3%) were found positive for at least one enteritis virus: the prevalences of CDV and CPV infections were 44.1% and 76.5% respectively while no CRV was evidenced in any sample tested. On the other hand, 12 samples (35.3%) were positive for both CDV and CPV infections. These results demonstrate that the prevalence of viral infections in diarrheic puppies is strong and also that CDV and CPV infections, which may be simultaneously present with a high rate, should not be under-estimated particularly in shelters with a high animal population.

Keywords: CDV, CPV, CRV, mixed infection, PCR, PAGE.

RÉSUMÉ

Etiologie virale des diarrhées chez des chiots d’un même élevage en Turquie : présence d’infections mixtes

Cette étude a été entreprise afin d’évaluer la prévalence des virus responsables d’enterites chez les chiots. Les prévalences du virus de la maladie de Carré (CDV), de celui de la parvovirose canine (CPV) et du rotavirus canin (CRV) ont été établies en appliquant respectivement les techniques de RT-PCR, de PCR et d’électrophorèse en gel de polyacrylamide sur les prélèvements fécaux de 34 chiots diarrhéiques âgées de 1 à 2 mois issus d’un même élevage. Vingt-neuf échantillons (85.3%) se sont révélés positifs pour au moins un virus entéritique : les prévalences des infections virales par le CDV et le CPV ont été respectivement de 44.1% et de 76.5% tandis que le CRV n’a été détecté dans aucun des prélèvements. En outre, la présence simultanée du CDV et du CPV a été démontrée pour 12 échantillons (35.3%). Ces résultats montrent que la prévalence des infections virales chez les chiots diarrhéiques est forte et que l’incidence des infections par le CDV et le CPV (qui peuvent être conjointes avec une fréquence élevée) ne devrait pas être sous-estimée particulièrement dans les élevages de grande taille.

Mots-clés : CDV, CPV, CRV, infection mixte, PCR, PAGE.

Introduction

Canine distemper virus (CDV) is a highly contagious Morbillivirus that causes infection in the digestive, respiratory and nervous systems and is seen commonly throughout the world. When it leads to an infection in the nervous system, CDV is fatal, and mortality is very high in areas where the disease is endemic [2]. The most sensitive and rapid test for detecting CDV in serum, whole blood, cerebrospinal fluid, urine and stool is the RT-PCR technique [6, 17].

Canine parvovirus (CPV) is a virus of the Paroviridae family that causes hemorrhagic enteritis and myocarditis especially in young dogs and puppies and that leads to infection in the digestive system after being transmitted by oronasal route. The highest risk CPV infection risk is observed in 6-12 weeks old puppies, for which maternal antibodies may not provide sufficient protection against the disease [10]. CPV was first isolated in the USA in 1978 [1] and was later determined to be a main viral agent responsible for diarrhoea in puppies in all over the world. Analysis of CPV isolates with monoclonal antibodies and restriction enzymes revealed that the most common antigenic strains were CPV 2a and CPV 2b types [15, 19].

The group A rotaviruses are the major agents found in neonatal diarrhoea in humans and animals. Rotaviruses, which have 11 segments, are of two genotypes referred as G and P, which regroup many subtypes. Although they affect neonates of many animal species, only few studies have reported rotaviruses isolations from dogs [7, 11].

This study was carried out to evaluate the prevalence of the main infectious viruses (CPV, CDV and CRV) in diarrheic puppies stemming from the same dog shelter.
Material and Methods

1. ANIMALS

This study included 34, 1-2 months old stray crossbreed dogs. All animals were reared in the same local dog shelter located outside the Kayseri city center in Turkey and exhibited diarrhoea at the time of sampling. Faecal samples were collected from animal using rectal swabs. In addition, the dogs involved the study were clinically examined to determine body temperatures, dehydration conditions, respiration rate and heart beats.

2. NUCLEIC ACID PURIFICATION AND IN VITRO PCR AMPLIFICATION

Viral DNA (CPV) extracted by the phenol-chloroform-isooxy alcohol method and RNA (CDV) extracted by the guanidium thiocyanate-phenol-chloroform method were isolated from specimens as described by ÖZKUL et al. [13, 14]. For detection of CDV in faecal samples, purified RNAs were reverse transcribed to cDNA using MMLV reverse transcriptase (Fermentas, Lithuania) using random hexanucleotide primers. The resulting cDNAs were used as a template for PCR amplification. The P coding gene (UPPF [5'-ATGTT-TATGATCACAGCGG-3'] and UPPR [5'-ATTGGGTG-CACCACCTTGCT-3']) as well as the VP1-VP2 coding gene specific primer sets (CPV-P1: [5'-ATGGCACCTCCGCAAAGA-3'], CPV-P2: [5'-TTTCTAGG-TGCTAGTT-GAG-3']) were used for detection of CDV cDNA and CPV DNA respectively. DNA amplification was performed with following thermal cycling parameters consisting by an initial denaturation for 6 minutes at 94°C and by an amplification segment containing 40 cycles of 52°C for 2.5 min, 1 min at 72°C and 1 min at 94°C. The amplification was terminated by final extension step at 70°C for 10 min. Amplified DNA products were separated on 1.7% agarose gel containing ethidium bromide after electrophoresis at 80 V for 30 min in TBE (Tris-boric acid-EDTA) buffer. The DNA bands were visualized under UV light.

3. CRV DETECTION BY POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

The method reported by HERRING et al. [9] was used for detection of the CRV RNA segments in polyacrylamide gels. Viral RNA extraction from faecal samples has been carried out as described by BURGU et al. [3]. Isolated RNA samples were loaded onto gel slots in 2X loading buffer composed of 36 mM Tris-HCL, 30 mM Na2HPO4 and 1 mM EDTA (pH 7.8) and electrophoresis was carried out under non-denaturing conditions at 150 V for 2h. The gel was then analyzed after silver staining [18].

Results

Clinical examinations showed that the dogs’ mean body temperatures were 37.2°C (36.5 - 38.0°C), mean respiration and heart rates were 23 cycles/min (14 – 27 cycles/min) and 96 battements/min (90-112 battements/min) respectively. Two dogs had hemorrhagic diarrhoea, 2 paralysis and 3 exhibited serous then purulent ocular discharge and a mild to a severe dehydration.

By PCR amplification, P-gene specific 372 bp DNA product was observed indicating the presence of canine Distemper virus (Figure 1) and the DNA product of 2245 bp in size was observed for canine parovirus (Figure 2) in the agarose gels. Control reactions using live CDV and CPV vaccine strains gave the same DNA products. Twenty-nine samples (85.3%) were positive for CDV and/or CPV whereas CRV was never detected in any specimen (Table I). Fifteen samples (44.1%) were found positive for CDV and 26 samples (76.5%) for CPV. In addition, CDV and CPV were both detected in 12 samples (35.3%), indicating mixed infections in the same herd.

Discussion

The CPV and CDV are the main viral enteric agents causing diarrhoea in puppies, while the CRV is scarcely evidenced. The infection risk occurs in 6-12 weeks old puppies, because of the deficiency of maternal antibodies and failure of vaccination to provide sufficient protection against the infections [10].

Although many studies have been performed on each of the viral enteric agents, only few researches focused on the simultaneous occurrence of the infectious viruses (including CPV, CDV and CRV). In Japan, MOCHIZUKI et al. [12] reported that 2.1% rectal swap samples from 95 puppies were positive for CRV using reverse passive haemagglutination assay (RPHA), 23.4% for CPV using PCR amplification and 10.6% for CDV using the RT-PCR. However, very high CPV and CDV prevalences (76.5% by PCR and 44.1% by RT-PCR respectively) were observed in the present study performed in Turkey, while CRV was not detected by the PAGE technique. In Turkey, OZKUL et al. [14] have also found a high per-
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References