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Short Communication

Distribution of Antigen Types of Canine Parvovirus Type 2 in Dogs with Hemorrhagic Enteritis in Turkey

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Abstract: The results of antigenic characterization of canine parvovirus type 2 (CPV2) isolated from the feces of 60 dogs with hemorrhagic enteritis are reported. Antigenic characterization was carried out by comparing the reactivity pattern of 4 monoclonal antibodies (mAbs) to CPV2 in the hemagglutination inhibition (HI) test. CPV2 was detected in 21 out of 60 dogs (35%) and antigenic typing was able to be performed for only 16 dogs with ≥1:8 titers. Nine samples were characterized as CPV2a and 7 as CPV2b, indicating that the CPV2a variant was more common than CPV2b in dog populations in Bursa province in Turkey.

Key Words: Canine parvovirus type 2, antigen types, hemorrhagic enteritis, dog

Türkiye'de Hemorajik Enteritisli Köpeklerde Canine Parvovirus Tip 2'nin Antijenik Tiplerinin Dağılımı

Özet: Bu çalışmada, hemorajik entreritisli 60 köpek dışkısından izole edilen canine parvovirus tip 2 (CPV2) izolatlarının antijenik karakterizasyonu ile ilgili sonuçları rapor edildi. Antijenik karakterizasyon hemagglutination inhibition (HI) testi ile CPV2'ye karşı dört monoklonal antikorda (mAbs) oluşan reaktiviteye göre değerlendirildi. CPV2, 60 köpeğin 21'inde (% 35) tespit edildi ve antijenik tiplendirme yalnız ≥1:8 titreye sahip 16 köpekte gerçekleştirilebildi. Dokuz örneğin CPV2a, 7 örneğin de CPV2b olarak karakterize edilmesi; Türkiye'de Bursa bölgesinde yaşayan köpeklerde CPV2a varyantının CPV2b'den daha yaygın olduğunu göstermektedir.

Anahtar Sözcükler: Canine parvovirus tip 2, antijen tipleri, hemorajik enteritis, köpek

Introduction

In the early 1970s, a new infectious disease of puppies characterized by either gastroenteritis or myocarditis was observed worldwide. A small, round, non-enveloped virus was observed using electron microscopy in stool specimens from affected animals. Subsequently, a novel parvovirus was isolated both in canine and feline cell cultures. The virus was named canine parvovirus type 2 (CPV2), to distinguish it from the previously described parvovirus [minute virus of canine (MVC) or CPV1], which is antigenically unrelated to CPV2 (1-3).

In 1979 and 1980, an antigenic variant of CPV was identified in several different countries using monoclonal antibodies (mAbs) and the variant was termed CPV type 2a (CPV2a). In the mid - 1980s, the virus underwent a further antigenic change, and the new variant was referred to as CPV type 2b (CPV2b) (4). Currently, the antigenic variants of CPV have completely replaced the original type 2 and are variously distributed in canine populations worldwide (1,2,5-11). Recently, sequence analysis of the capsid protein-encoding gene revealed 2 amino acid changes in 2 isolates of CPV2 collected from 2 dogs affected by severe hemorrhagic diarrhoea,

strongly suggesting the existence of a new variant of CPV2 (CPV2c) (2).

CPV2 is well known as a causative agent of a worldwide pandemic of severe hemorrhagic enteritis in dogs; however, to date, CPV2 and its antigenic variants have not yet been recognized in Turkey, except for in a case study by Özkul et al. (12), who reported that the DNA of CPV was detected in a diarrheic dog using polymerase chain reaction (PCR) technology. Therefore, the main objective of the present study was to antigenically characterize CPV2 strains detected in the feces of dogs affected by hemorrhagic gastroenteritis.

Sixty dogs, between 2 and 6 months old, admitted to the Small Animal Clinic of Internal Medicine, Veterinary Medicine Faculty, Uludağ University (Bursa, Turkey), from January to March, 2003, were used. Anatolian sheep dogs, German shepherds and Dobermans, were predominant. The most common clinical and hematological findings in the affected animals were vomiting, anorexia, depression, dehydration, bloody diarrhea, hypothermia or fever, marked thrombocytopenia, and leukopenia. Fecal samples were collected into plastic tubes and stored at -20 ºC until tested. All samples were sent for analysis of CPV to the Department of Health and Animal Welfare, Faculty of Veterinary Medicine, University of Bari (Bari, Italy).

For the isolation of CPV, fecal samples were suspended (20% w/v) in Dulbecco-Minimal Essential Medium (D-MEM). After centrifugation, at 6000 x g for 20 min, the supernatant of each sample was filtered (0.22 µm) and inoculated onto cell cultures immediately after trypsinization. A canine cell line (A72), grown in D-MEM containing 10% bovine fetal serum (BFS), was used. Viral growth was monitored using the hemagglutination (HA) test and the indirect fluorescent antibody test (IFAT) (3,9). The HA test was carried out on the supernatant of the infected cell cultures, using a 1% suspension of pig red blood cells at +4 °C (3,9). The IFAT was carried out on cells, grown on slides, 48 h after infection using a canine serum positive to CPV and a fluorescein-labeled anti-dog IgG (Sigma).

Antigenic characterization, as a CPV2a or CPV2b variant, was carried out by comparing the reactivity pattern of 4 monoclonal antibodies (mAbs) to CPV2, kindly supplied by Dr. Parrish (Cornell University, Ithaca, New York, USA). The criolysate of the third passage on A-72 cells of each isolate was tested with the 4 mAbs, in a hemagglutination-inhibition (HI) test using 6 HA units of virus (3,9). Twenty-one samples examined induced a moderate cytopathic effect at the first passage on the A-72 cell line and, furthermore, all the isolates agglutinated pig erythrocytes and gave positive results by IFAT using dog serum (9).

The results showed that CPV2 was positive at 1:4 to 1:8 titers from 5 fecal samples and ≥ 1:8 titers from 16 fecal samples (Table). Antigenic typing was able to be performed on only 16 dogs with ≥ 1:8 titers. Nine samples were characterized as CPV2a and 7 as CPV2b.

In this study, CPV2 was detected in 21 out of 60 dogs tested (35%), suggesting that CPV2 infection is currently a common clinical problem among young dogs in the Bursa province of Turkey. Nine samples (60%) were characterized as CPV2a and 7 (40%) as CPV2b, indicating that the CPV2a variant is more common than CPV2b in dog populations in Turkey. Currently, the prevalences of CPV2a and CPV2b are at varying levels in different countries (5,6,13). CPV2b is the predominant antigenic type in dogs in the United States (7), South Africa (14) and Japan (10), whereas CPV2a is more common than CPV2b in Italy and other European countries (1,3,5,8). It is interesting to note that CPV2 was also detected in 11 dogs that were vaccinated once at least 9 days beforehand (Table) with a polyvalent vaccine containing attenuated CPV2 strains. This is probably related to poor immunization, as a consequence of the use of a polyvalent vaccine or, more commonly, to the use in the field of a CPV2 vaccine that induces relatively low antibody titers against heterologous viruses (CPV2a and CPV2b) (13).

These results showed that CPV2 infection was a common clinical problem and that the CPV2a variant was more common than CPV2b in dog populations in Bursa province in Turkey.

Table. Results of antigenic analysis of CPV2 strains using mAbs.

Sample No.	Breed	Age months	HA test	CPV2a/2b	CPV2* Vaccine
1	Anatolian sheep dog	6	1:4–1:8	--------	-
5	Anatolian sheep dog	3.5	1:4–1:8	--------	-
30	Anatolian sheep dog	6	1:4	--------	-
31	Anatolian sheep dog	6	1:4–1:8	--------	-
42	Irish Setter	2	1:4–1:8	--------	-
7	Husky	6	1:1024	CPV-2a	-
11	German Shepherd	4	1:32000	CPV-2b	+
12	German Shepherd	4	1:16000	CPV-2b	+
21	German Shepherd	4	1:2048	CPV-2b	+
25	German Shepherd	4	1:32000	CPV-2b	+
28	German Shepherd	4	1:512	CPV-2b	+
38	German Shepherd	3.5	1:256	CPV-2b	+
43	Rottweiler	2	1:256	CPV-2a	-
46	Doberman	6	1:1024	CPV-2a	+
49	Boxer	4	1:1024	CPV-2a	-
52	Boxer	3.5	1:2048.	CPV-2a	+
53	Doberman	2.5	1:32	CPV-2a	-
54	Cocker Spaniel	4	1:128	CPV-2a	+
55	Terrier	6	1:4000	CPV-2a	+
57	Doberman	2	1:1024	CPV-2b	-
60	Rottweiler	5.5	1:128	CPV-2a	+

* Polyvalent Vaccine: Nobivac DHPPi (Inter-Vet, Holland) or Adenomune 7 (Ege-Vet, Turkey)

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