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Prevalence of canine distemper in dogs referred to Veterinary Hospital of Ferdowsi University of Mashhad, Mashhad, Iran

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Abstract

Canine distemper virus (CDV) is responsible for high morbidity and mortality in dogs worldwide. Epidemiological study of canine distemper can help to control and treat the disease in any area. This study aimed to investigate the prevalence of CDV in dogs referred to the Veterinary Hospital from September 23, 2018 to September 22, 2019. Dogs with at least two clinical signs of canine distemper underwent blood tests, rapid test kit from the eye and cerebrospinal fluid (CSF), and RT-PCR from whole blood and/or CSF samples. Out of 1212 referred dogs, 112 dogs were suspected to have canine distemper of which 90 underwent RT-PCR and rapid test kits. The disease prevalence was 4.04% (49/1212) and 7.44% (49/659) according to the total number of referring dogs and number of referring sick dogs, respectively. The distemper fatality rate was 69.57% (32/46). Seventy percent of distemper positive cases were under 12 months old and 52.08% were under 6 months old. Female dogs were more susceptible than males; however, the fatality rate of males was more than females. Of distemper positive dogs, 91.84% were unvaccinated. The highest prevalence (71.43%) of dogs diagnosed with CDV occurred during the cold seasons. It is concluded that canine distemper is endemic in the geographical area of Mashhad and its prevalence rate in dogs referred to the Veterinary Hospital of Ferdowsi University of Mashhad is 4.04% and its fatality rate is 69.57%. This indicates that a significant number of dogs may die if they develop distemper despite treatment.

Introduction

Canine distemper virus (CDV) is a member of the genus *Morbillivirus* of the family *Paramyxoviridae* and is closely related to other viruses of this family. This virus has a relatively large single-stranded RNA covered by a lipoprotein envelope incorporated viral glycoproteins H (attachment protein) and F (fusion protein). Despite slight genetic differences, CDV strains are serologically homogeneous; however, virus strains vary in their pathogenicity and this may affect the severity, extent, or type of clinical disease (respiratory, gastrointestinal, nervous, cutaneous, ophthalmic, etc.).[1] Although vaccination has greatly reduced the incidence of distemper, it still affects many dogs worldwide and causes the death in many cases; which may be due to the presence of a large number of non-vaccinated stray dogs that maintain the virus in the environment.[1,2] The survey of disease prevalence can help the diagnosis, treatment and prevention of the disease in any area and provide proper guidelines to veterinarians and government authorities, including the veterinary organization. Epidemiological studies around the world have shown that the overall prevalence of the canine distemper varies among countries.[2-11] For example, its prevalence has been reported 9.30% in Türkiye,[5] 7.50% in Nigeria,[10] 27.30% in Brazil,[4] and 8.86% in Iraq.[7] Studies have shown that climatic factors have an important role on the prevalence and transmission of distemper.[2,8,9] Also, disease screening methods might have a significant effect on the disease prevalence.[7,9] Different serological and molecular methods can be used for diagnosis of CDV, each with its advantages.[1] Molecular methods as a definitive diagnosis can detect the CDV in affected dogs; however, in animals that produce anti-viral antibodies, the virus may be cleared from the body and the test results may be negative. On the other hand, the neutralization test is the gold standard method for the diagnosis, but antibodies may be present in animals without symptoms, so presence

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of the disease in these animals is ambiguous and can indicate either past vaccination against CDV or past or present infection with CDV.[1] Several studies have reported the canine distemper prevalence in Iran. For example, its prevalence has been reported 73.00% and 55.60% in the north of Iran,[8,9] and 17.52% in clinically healthy dogs,[2] in Ahvaz (southwest of Iran). Molecular, serological, and rapid kit tests have been used in these studies. There have been many reports about dogs infected with distemper virus from clinics around Mashhad, but there is no precise data on the prevalence of the disease in this area. Thus, the objectives of this study were (1) to evaluate the prevalence of canine distemper in dogs referred to the Veterinary Hospital of Ferdowsi University of Mashhad and (2) to investigate the risk factors associated with the likelihood of the disease and (3) to compare the results of different diagnostic methods for diagnosis of distemper. To the best of our knowledge, this is the first study on the prevalence and risk factors of canine distemper carried out in Mashhad, Iran.

Materials and Methods

This study was approved by the Research Ethics Committee of Ferdowsi University of Mashhad (Approval ID: IR.UM.REC.1399.123). Over a one-year period, from September 23, 2018 to September 22, 2019, a total number of 1212 dogs referred to the Veterinary Hospital of Ferdowsi University of Mashhad were visited and those with at least two clinical signs of canine distemper were included in the study. According to reference textbook clinical signs of canine distemper were; nausea, vomiting, diarrhea, nasal and ocular discharges, sneezing, coughing, dyspnea and tachypnea, myoclonus, seizures, para or tetraparesis/plegia and lameness, impetigo, and nasal or foot pad hyperkeratosis.[1] A detailed history (including age, gender, breed, vaccination status, date of onset of clinical signs and appetite status) was obtained and a thorough physical examination was performed for each dog. Then whole blood was collected from cephalic vein for determining the following parameters: number of red blood cells (RBC) and white blood cells (WBC), hematocrit, and differential count of lymphocytes, neutrophils, monocytes, eosinophils, and band cells.

The CDV was detected from conjunctival swab and CSF samples by rapid diagnostic kits (Anigen Rapid CDV Ag Test Kit, BioNote, Hwaseong, South Korea). Moreover, whole blood and/or CSF samples were collected by routine procedures and frozen at – 80.00 ˚C for reverse transcriptase polymerase chain reaction (RT-PCR) analysis.

For molecular detection of CDV by RT-PCR assay, the RNA was extracted from 2ml whole blood sample using the Blood RNA isolation kit (DENA Zist Asia, Mashhad, Iran) according to manufacturer's instruction. The RNA quantity and quality were analyzed by spectrophotometric method (NanoDrop; Thermo Scientific, Waltham, USA) and electrophoresis in 1.50% agarose gel, respectively. The total amount of isolated RNA was between 20.00 to 1,000 ng mL^{-1}. Immediately after RNA extraction, the cDNA was synthesized from RNA using a DNA synthesis kit (Parstous, Mashhad, Iran) according to manufacturer's instruction. The RT-PCR was performed using the three oligonucleotide primers as shown in Table 1 for amplification of the CDV nucleoprotein (NP) gene sequences.[12] RNA integrity was ensured by using oligonucleotide primer pairs for amplification of a sequence from a housekeeping gene that encodes glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (primers were provided by Humanizing Genomic Macrogen, Seoul, South Korea), (Table1). The PCR products were analyzed by electrophoresis in a 1.50% agarose gel stained with green viewer in 1x Tris borate EDTA buffer and visualized under UV light (Fig. 1).

Sequence analysis of PCR products. The identities of the RT-PCR amplicons were confirmed by Sanger nucleotide sequencing protocol (Bioneer, Daejeon, South Korea) of PCR products obtained from one infected dog to distemper using the sense primer pairs one and two and the sense and anti-sense of primer pairs three. The sequences determined in the study have been registered at the GenBank® and their accession numbers are shown in the results. The quality of each nucleotide sequence was analyzed with SnapGene Software (version 3.2.1; GSL Biotech, Chicago, USA) without editing and the similarity of each sequence checked against sequences deposited in the NCBI GenBank® using BLAST program (https://blast. ncbi.nlm.nih.gov/Blast.cgi).

Table 1. Nucleotide sequence and position of primer pairs (S: sense; AS: antisense) used for RT-PCR.

Primer	Sequence (5′-3′)	Nucleotide position	Fragment size (bp)
Primer pair 1	S: ACA GGA TTG CTG AGG ACC TAT	769 - 789	286
	AS: CAA GAT AAC CAT GTA CGG TGC	1,055 – 1,035	
Primer pair 2	S: AAC TAT GTA TCC GGC TCT TGG	941 - 961	259
	AS: CGA GTC TGA AGT AAG CTG GGT	1,200 – 1,180	
Primer pair 3	S: CAA AGA CGT GTG GTC GGA GAA	711 - 731	899
	AS: CTT AGT AAG CAT CCT CAT CTT GGC	1,610 – 1,587	
GAPDH	S: GCC AAA AGG GTC ATC ATC TC	Not available	229
	AS: GGC CAT CCA CAG TCT TCT	Not available	

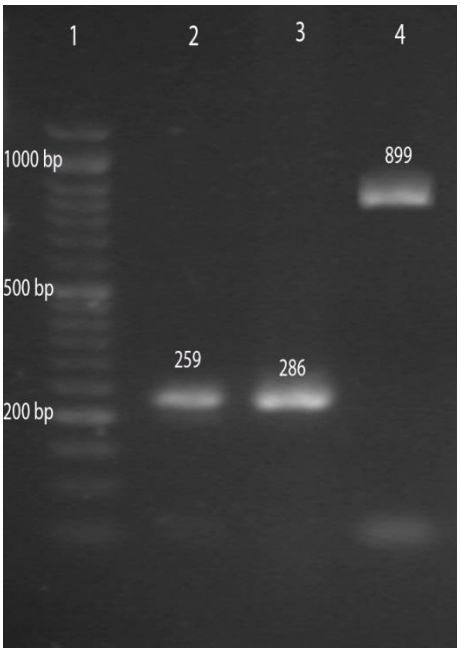

Fig. 1. The RT-PCR products in a referred distemper positive dog in the present study; Lane 1: ladder 50 bp; Lane 2: primer pair 1 (259 bp); Lane 3: primer pair 2 (286 bp); Lane 4: primer pair 3 (899 bp).

Statistical analysis. Statistical analyses were conducted using SPSS (version 24.00; IBM, Armonk, USA). The prevalence in the population of referred animals was defined as the percentage of dogs with confirmed disease. Proportion of distemper positive dogs to the total sick dogs was also calculated. The fatality rate of distemper was calculated as the percentage of dogs succumbed to disease to the total distemper positive dogs. Correlations of prevalence and fatality rates (as dependent variables) with age, sex, breed (in cases that there were at least three dogs in each group; as independent variables) were evaluated by chi-square test followed by logistic regression analysis. Univariate logistic regression was used to calculate odds ratios (OR) and 95.00% confidence intervals (CI). A value of $p < 0.05$ was considered significant. Correlation of prevalence with season, vaccination and age groups in distemper positive dogs were evaluated by Chi-square goodness of fit test. The independent t-test was used to compare ages of distemper positive and negative dogs. The CBC and vital signs were compared in distemper positive and negative dogs by independent t-test if they were normally distributed and by Mann-Whitney U test if they were not normally distributed. Kruskal-Wallis test and subsequent U-Mann-Whitney test were used to compare the CBC and vital signs between the three groups of "onset of distemper signs to blood sample collecting" and between different age groups. The difference between the observed clinical signs in distemper positive and distemper negative dogs was compared with Chi-square test. A p value less than 0.05 was considered significant.

Results

Sequencing, molecular and disease prevalence results. The sequencing results of all primers except antisense of PP-I and PP-II compared with other disposal sequences showed the highest nucleotide similarity level to canine distemper virus strain HL N (GenBank® accession number EU489475.1) and canine morbillivirus strain PT61/Pt 2004 (GenBank® accession number KX774415.1). The determined sequences in this study have been registered at the GenBank® and the accession numbers are as follows: MZ707910 for PP-I, MZ798146 for PP-II and MZ802994 for PP-III. The sensitivity of PP-I, PP-II, and PP-III for detection of CDV was 65.00%, 88.00%, and 41.00%, respectively. During the one-year period, 1212 dogs were referred to the Veterinary Hospital, of which 112 (9.24%) dogs were suspected to have distemper based on history and clinical signs. Of these, 83 dogs underwent the RT-PCR, 55 of which were also tested by rapid diagnostic kits. In addition, seven other dogs were tested only with the rapid diagnostic kit (without performing RT-PCR). Thus of the suspected dogs, 62 cases were tested with rapid diagnostic kits on ocular and/or CSF specimens. Out of 83 dogs that underwent RT-PCR, 45 were positive and 38 were negative. In addition, in three dogs that did not undergo RT-PCR, the rapid test kits were positive from the conjunctiva (one dog) and CSF (two dogs), and in one dog with a negative RT-PCR result, the rapid test kit was positive from the CSF. Thus, the results of 49 out of 90 cases were positive by one of the rapid test kits or RT-PCR assays; therefore, the prevalence of the disease in dogs referred to the Veterinary Hospital was 4.04% (49 out of 1212 cases). Out of 1212 dogs, 553 (45.63%) had been referred for checkup or vaccination; and 659 out of 1212 (54.37%) dogs referred for various diseases. Thus, the proportion of distemper in sick dogs was 7.44% (49 out of 659 cases). On the other hand, out of 62 dogs that underwent rapid test kits from the conjunctival and/or CSF samples, 31 were positive and 31 were negative. Out Of 31 cases that were positive using rapid diagnostic kits, three blood samples were lost for RT-PCR. Out of the remaining 28 cases, 27 (96.43%) were positive by RT-PCR assay. Out of 31 cases which rapid test kits were negative, four cases did not have blood samples for RT-PCR analysis; out of remaining 27 cases, RT-PCR was negative in 20 (74.07%) and positive in seven (25.93%) dogs. So, in this study, the sensitivity and specificity of the rapid test kits were obtained 96.43% and 74.07%, respectively. The agreement (Kappa statistics) was found to be 0.71 between two tests ($p < 0.001$).

Evaluation of the fatality (and recovery) rate of the distemper disease. Out of 49 dogs with a final diagnosis of distemper, the status of three dogs was not determined. Out of remaining 46 dogs, 32 (69.57%) died and 14 (30.43%) survived. On the other hand, out of 38 dogs

whose distemper were negative, the outcome of two dogs was not determined. Out of remaining 36 dogs, 13 (36.11%) died and 23 (63.89%) survived. There was a significant difference in fatality and recovery rate between distemper positive and negative dogs (p = 0.003). The mean age of the positive and negative groups was 10.6 ± 11.2 and 18.3 ± 23.4 months respectively, with no significant difference between them (p = 0.067).

To investigate the correlation of age with the prevalence of distemper, patients were divided into four groups: under 6 months, 6 - 12 months, 1 - 5 years and over 5 years.[13] Out of 49 distemper positive dogs, the age of one dog was not recorded. Out of the remaining 48 dogs, 25 (52.08%) were under 6 months old and 36 (75.00%) were less than one year old. Therefore, most of the patients were under 6 months of age, which was significantly different from patients aged 6 - 12 months and over one year (p = 0.02, p = 0.03, respectively).

The fatality rate of distemper are also assessed at different ages. Out of 49 dogs with a final diagnosis of distemper, the age of one dog was not recorded, and the outcome of the three dogs was unknown. Out of the remaining 45 dogs, 24 were under 6 months, 11 were between 6 - 12 months and 10 were between 1-5 years old. The fatality rate was 75.00% (18/24) in dogs under the age of 6 months, 45.45% (5/11) between the ages of 6 - 12 months and 80.00% (8/10) between the ages of 1 - 5 years. The Chi-square comparison and univariate logistic regression analyses showed that there was no significant difference in the fatality rate between different ages in distemper positive dogs (Table 2). The prevalence of distemper in male and female dogs is depicted in Table 3. Chi-square comparison and logistic regression analysis showed that the prevalence of distemper in female dogs was significantly higher than male dogs (p = 0.003, OR = 4.02, 95.00% CI, 1.60 - 10.20). However, the fatality rate of male dogs was significantly higher than females (p = 0.024, OR = 5.40, 95.00% CI, 1.20 - 23.00), (Table 2).

All 112 suspected patients in the present study consisted of nine breeds. However, since the six breeds contain more than three patients, only these six breeds participated in statistical comparisons. The mortality and fatality rates of distemper in each breed are shown in Table 4. The Most patients with distemper disease were in the mixed breed and the least patients in the terrier breed. The Chi-square and logistic regression analyses showed no significant difference in the fatality rate of distemper in different breeds (Table 2).

Out of 49 distemper positive dogs, 45 patients (91.84%) had not received the distemper vaccine or their vaccination status were incomplete or unclear, and four patients (8.16%) however had received the vaccine but developed distemper symptoms. The Chi-square goodness of fit test showed that distemper incidence is significantly higher in unvaccinated dogs (p < 0.001).

The most observed clinical signs in distemper positive dogs were: Anorexia (n = 27), diarrhea (n = 26), lethargy (n = 26), fever (n = 70), ocular discharge (n = 13), foot pad hyperkeratosis (n = 7), nasal hyperkeratosis (n = 6), nasal discharge (n = 6), conjunctival hyperemia (n = 5), hypothermia

Table 2. Results of univariate logistic regression analysis of factors potentially associated with outcome of 49 dogs with confirmed canine distemper.

Factor	Category	No. of positive cases	No. of deaths	Prevalence (%)	OR	95.00% CI	*p*-value
Age	1 - 5 year	10	8	80.00	Referent	NA	NA
	< 6 months	24	18	75.00	0.75	0.12 - 4.60	0.75
	6 - 12 months	11	5	45.45	0.21	0.03 - 1.50	0.11
Sex	Female	24	13	54.17	Referent	NA	NA
	Male	22	19	86.36	5.40	1.20 - 23.00	0.02
Breed	Afghan	5	4	80.00	Referent	NA	NA
	German Shepherd	4	2	50.00	2.70	0.60 - 45.10	0.49
	Husky	4	3	75.00	0.70	0.05 - 9.50	0.76
	Mixed	20	14	70.00	2.00	0.11 - 35.80	0.63
	Spitz	5	4	80.00	1.60	0.21 - 11.80	0.66
	Terrier	5	3	60.00	2.70	0.16 - 45.1	0.49

CI: Confidence interval. NA: Not applicable. OR: Odds ratio.

Table 3. Prevalence and fatality rates of male and females in distemper positive and negative dogs.

Distemper	Outcome	Female (%)	Male (%)	Total
Positive	Live	11 (78.57)	3 (21.40)	14
	Dead	13 (40.63)	19 (59.40)	32
	Unknown	2	1	3
	Total	26 (53.10)	23 (46.90)	49
Negative	Live	5 (21.70)	18 (78.30)	23
	Dead	4 (30.80)	9 (69.20)	13
	Unknown	0	5	5
	Total	9 (22.00)	32 (78.00)	41

(n = 6) and myoclonus (n = 2). In distemper negative dogs, diarrhea (n = 20), ocular discharge (n = 15), conjunctival hyperemia (n = 14), lethargy (n = 12), Anorexia (n = 11), foot pad hyperkeratosis (n = 11) and myoclonus (n = 9) were more common than other signs. Among these symptoms, anorexia and lethargy were significantly higher in the distemper positive dogs than the distemper negative dogs (p = 0.007 and p = 0.037, respectively), while conjunctival hyperemia and myoclonus were more observed in the distemper negative dogs than distemper positive dogs (p = 0.006 and p = 0.010 respectively).

In the present study, 15 out of 49 distemper confirmed dogs (30.61%) were referred in autumn, 20 (40.82%) in winter, nine (18.37%) in spring and five (10.20%) in summer. In other words, 35 dogs (71.43%) were referred in the cold seasons of the year (autumn and winter) and 14 dogs (28.57%) in the warm seasons (spring and summer). The Chi-square goodness of fit test showed that these differences were also significant (p < 0.001), so that in cold seasons was more than warm seasons (p = 0.003).

The association of distemper with vital signs and CBC parameters. The Mean ± SD of vital signs and CBC parameters in distemper positive and negative dogs are presented in Table 5. The results projected that, only the decrease in heart rate and hematocrit in distemper positive dogs were statistically significant (p = 0.001 and p = 0.046, respectively). However, further evaluation at different ages revealed that the decrease in heart rate in the age group of under 6 months was significant in distemper positive dogs compared to distemper negative dogs (p = 0.028). Furthermore, in dogs aged 6 - 12 months, increase in body temperature, decrease in hematocrit and RBC count were significant compared with distemper negative dogs (p = 0.012, p = 0.025 and p = 0.005, respectively). Based on the onset of distemper signs to blood sample collecting, patients were divided into three different groups: Less than two days (group 1, n = 5), two - four days (group 2, n = 5) and more than four days (group 3, n = 14). The median of WBC (4200, range: 1200 - 11100) was decreased in group 2 compared with groups 1 (6200, range: 3400 - 21900) and 3 (9100, range: 2600 - 24500); however, this decrease was only significant compared with group 3 (Mann-Whitney U test, p = 0.033).

Table 4. The total number of referred breeds and the mortality and fatality rates in each breed dog within 1-year study period.

Breed (No.)	No. of positive cases (%)	Mortality rate (%)	Fatality rate (%)
Mixed (255)	22 (8.63%)	5.49	14/20* (70.00)
Husky (59)	4 (6.78%)	5.08	3/4 (75.00)
Afghan (96)	6 (6.25%)	4.17	4/5* (80.00)
Spitz (149)	5 (3.36%)	2.68	4/5 (80.00)
German Shepherd (133)	4 (3.01%)	1.50	2/4 (50.00)
Terrier (173)	5 (2.89%)	1.73	3/5 (60.00)
Other (347)	3 (0.86%)	0.58	2/3 (66.67)
Total (1212)	49 (4.04%)	2.64	32/46 (69.57)

The logistic regression analysis showed no significant difference in the fatality rate of distemper in different breeds (p > 0.05).
* Two mixed breed dogs and one Afghan dog excluded from the fatality analysis because outcome of them were unknown.

Table 5. Mean ± standard deviation (SD) of vital signs and hematological parameters in canine distemper (CD) positive and negative dogs.

Vital signs / hematological parameters	CD result (No.)	Mean ± SD	p-value
Heart rate (beats per min)	Positive (32)	108.80 ± 30.40	0.001
	Negative (27)	144.30 ± 46.40	
Respiratory rate (breaths per min)	Positive (25)	47.80 ± 22.20	0.79
	Negative (19)	45.90 ± 21.0	
Temperature (°C)	Positive (35)	39.10 ± 1.30	0.09
	Negative (33)	38.50 ± 1.10	
Hematocrit (%)	Positive (42)	31.00 ± 7.40	0.04
	Negative (38)	34.50 ±8.10	
WBC ($\times 10^3$ μL^{-1})	Positive (42)	11.45 ± 8.81	0.85
	Negative (38)	11.79 ± 7.36	
RBC ($\times 10^6$ μL^{-1})	Positive (42)	5.10 ± 1.10	0.06
	Negative (38)	5.60 ± 1.20	
Neutrophil ($\times 10^3$ μL^{-1})	Positive (40)	10.05 ± 7.99	0.74
	Negative (38)	9.50 ± 6.88	
Lymphocyte ($\times 10^3$ μL^{-1})	Positive (40)	0.84 ± 1.15	0.10
	Negative (38)	1.22 ± 0.90	
Monocyte ($\times 10^3$ μL^{-1})	Positive (40)	0.72 ± 0.70	0.89
	Negative (38)	0.70 ± 0.66	
Band ($\times 10^3$ μL^{-1})	Positive (40)	0.16 ± 0.29	0.86
	Negative (38)	0.16 ± 0.31	

Discussion

Canine distemper is a contagious and deadly infectious disease that mainly causes systemic, respiratory, gastro-intestinal and neurological involvement.[1,14] Like other parts of the world, CDV is endemic in Iran. There are several ways to diagnose canine distemper. Hematological and biochemical methods are nonspecific and not all patients show the same changes,[1,14] however, serological and molecular techniques are specific methods. Therefore, selecting the best sample and diagnostic method is very important. Blood and conjunctival sampling are non-invasive and can be taken much easier than CSF. As a result, in patients with systemic symptoms of distemper, it is recommended to use conjunctival and blood samples for accurate diagnosis with rapid test kits and RT-PCR assays. However, if the results are ambiguous, or there are only neurological symptoms, CSF specimens are preferred for diagnosis. Various studies have been conducted to investigate the prevalence of distemper worldwide. Depending on the geographical area, climate and the diagnostic methods, different prevalence rates have been reported.[2-11,13-16] In the present study, the prevalence and case fatality rates of distemper have been determined with relatively high accuracy, so that in dogs whose distemper was confirmed by RT-PCR and rapid test kits, the prevalence rate was 4.00% and the fatality rate was 69.60% which is relatively high. RT-PCR is currently one of the best available and preferred standard method to diagnose distemper ante mortem.[17-19] To the best of our knowledge, there is no study that accurately determines the prevalence and the fatality rate of canine distemper on the distemper positive dogs because in most epidemiological reports, the disease prevalence has been reported based on the presence of anti-distemper antibodies in dogs. Only a few studies have reported the death rate of distemper. For example, a study by Headly and Graça on necropsied dogs reported that 11.70% of deaths were due to distemper.[6] Other studies have reported deaths up to 50.00%.[1,20] Therefore, this study is one of the few studies in which the fatality rate of distemper has been accurately evaluated based on RT-PCR and rapid test kit assays, and accurate follow-up of patients.

In the present study, 75.00% of distemper positive dogs were under one year old, of which 52.08% were less than 6 months old. Similar results have been reported in other studies. [6,14,21] In the present study, despite the higher prevalence in puppies less than 6 months of age, there was no significant difference in fatality rate between different ages in distemper positive dogs (Table 2). The cause of more involvement at puberty is probably the lack of immune system development. Puppies that receive enough maternally derived antibodies (MDA) are usually immune against CDV up to 3 months old. Therefore, the disease mainly affects puppies at the age of 3 - 6 months, but those that have not adequate MDA are susceptible to the CDV at birth.[1,22,23] Distemper is an immunosuppressive disease and may predispose dogs at any age to secondary viral and bacterial infections.[15,24]

In the present study, a comparison between gender and CDV revealed that the prevalence of distemper in the female dogs was significantly higher than male dogs, however, the fatality rate of disease in male dogs was higher than females. In other studies, contradictory results are reported between males and females, so that some studies similar to this study have reported that the prevalence rate in females is higher than males[10] and some concluded that there is no difference between male and female dogs.[9,16,25,26]

In this study, the effect of canine distemper on hematological parameters and vital signs was not quite evident. The change of the total WBC varies and depends on the time of blood samples are collected for CBC evaluation. Our results showed that a considerable decrease in WBC counts occurred two - four days after the onset of symptoms; then the WBC count increased again to the normal ranges. However, these CBC changes are not constant and many factors such as the disease severity and age can affect them. Thus, CBC profile panel evaluation is not a good method for CDV detection.

In Our study, distemper positive dogs were distributed in nine breeds and the highest prevalence were related to mixed breed (native dogs of the region) and the lowest prevalence were related to terriers, followed by German Shepherds (Table 4). There was no statistically significant difference in the fatality rate of distemper positive dogs between different breeds which may be due to the small number of distemper positive dogs in each breed. It has been reported that dolichocephalic breeds are more susceptible to CDV than brachiocephalic breeds.[13] In the present study, like other studies, mixed breeds (which are dolichocephalic dogs) were more affected.

In the current study, the most clinical symptom observed in distemper positive dogs was anorexia, followed by diarrhea and lethargy. All these three observed symptoms are non-specific for distemper and some other diseases such as canine parvovirus (CPV), food poisoning and intoxication can manifest these symptoms; therefore, CDV cannot be easily distinguished based on clinical signs. In contrast, some clinical signs, such as myoclonus, are highly specific to the distemper. Surprisingly, this symptom was observed more in the distemper negative dogs. This finding maybe largely related to removing of CDV from the blood and localization in the central nervous system (CNS);[1] Therefore, although the animal shows myoclonus, the virus cannot be detected in the blood or even in the CSF.

According to our results, 91.84% of distemper-positive dogs were unvaccinated. This result indicates the importance of vaccination for preventing the disease.

Other reports have also highlighted the importance of vaccination.[13] Besides, four (8.16%) vaccinated dogs, showed the canine distemper signs, of which three dogs (75.00%) died. Other reports suggesting that vaccinated dogs may also be infected with CDV.[15,23,27] Józwik and Frymus also reported that 22.00% of infected dogs had previously been vaccinated at least once against distemper.[27] The cause of infection in these dogs may be due to infection with the same and/or a new virus strain, incomplete vaccination, or inadequate immune system development.[28]

In the present study, most distemper-positive dogs (71.43%) were referred to the Hospital in the cold seasons of the year (autumn and winter). Since the CDV is an enveloped virus, it is sensitive to high temperatures and has a longer shelf life at low temperatures. Therefore, a high rate of suspected and confirmed referrals cases are expected to be in autumn and/or winter.[6,9]

Finally, this paper presents the first epidemiologic study on the prevalence and risk factors for canine distemper in a population of Iranian dogs referred to the Veterinary Teaching Hospital of Ferdowsi University of Mashhad in northeast of Iran. According to our results, it is concluded that canine distemper is endemic in the geographical area of Mashhad and its prevalence and fatality rate is 4.00% and 69.60%, respectively. This indicates that a significant number of dogs may die if they develop distemper despite treatment. Although the incidence of canine distemper is higher at younger ages, the mortality rate does not differ at different ages. The incidence of CD is higher in female dogs but the mortality rate is higher in male dogs.

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Conflict of interest

The authors declare no conflicts of interest.

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