



Veterinaria México OA

ISSN: 0301-5092

Universidad Nacional Autónoma de México, Facultad de
Medicina Veterinaria y Zootecnia

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Veterinaria México OA, vol. 9, e1044, 2022
Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia

DOI: <https://doi.org/10.22201/fmvz.24486760e.2022.1044>

Available in: <https://www.redalyc.org/articulo.oa?id=42375500020>

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Evaluation of the anti-Newcastle disease vaccine serum on dogs with canine distemper

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Abstract

Distemper is a contagious, highly lethal, and almost incurable viral disease in dogs and other terrestrial carnivores. This study evaluated a new distemper treatment for dogs. Two healthy male dogs of mixed breed were prepared. A 1 000-dose vial of Newcastle disease vaccine was then diluted with 6 mL normal saline and 3 mL of it was injected into the cephalic vein of each dog, collecting 20% of the blood volume 11 h later. Blood sera were separated and used as anti-Newcastle disease vaccine serum (ANDVS) to treat dogs suspected of having canine distemper. Dogs with confirmed distemper were grouped into the following seven treatment groups: 1-ANDVS; 2-ANDVS + Cotrimoxazole; 3-ANDVS + Penicillin-Gentamicin; 4-ANDVS + Cefazolin-Amikacin; 5-Cotrimoxazole; 6-Penicillin-Gentamycin; 7-Cefazolin-Amikacin. Then the fatality rate of dogs, association between distemper and sex, breed, age, and effects of distemper on hematological factors and vital signs were evaluated. The recovery rates in the ANDVS+ Cotrimoxazole and the ANDVS+ Cefazolin-Amikacin groups were higher than those in the ANDVS group ($P < 0.05$). Fatality rate was significantly different in distemper-positive and distemper-negative dogs. It is concluded that although ANDVS alone has no effect on the treatment of distemper, it can increase the recovery rate when combined with cotrimoxazole or cefazolin + amikacin as compared with the sole use of these antibiotics.

Keywords: Dog; distemper; anti-newcastle disease vaccine serum; RT-PCR; distemper treatment.

Submitted: 2022-01-14
Accepted: 2022-05-11
Published: 2022-11-15

Additional information and declarations
can be found on page 11

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Cite this as: Mohammad A, Ali Asghar S, Hadi M. Evaluation of the anti-Newcastle disease vaccine serum on dogs with canine distemper. *Veterinaria México OA*. 2022;9. doi: [10.22201/fmvz.24486760e.2022.1044](https://doi.org/10.22201/fmvz.24486760e.2022.1044)

Study contribution

Distemper is a fatal disease with a high mortality rate. Various methods are used to treat it, but there is no definitive cure yet. In this article, we evaluated a new treatment approach for canine distemper. Furthermore, a relatively accurate fatality rate of the disease has been presented, which has been reported in fewer articles on the distemper disease. The study provides new insights for veterinarians to treat this lethal disease.

Introduction

Canine distemper (CD) is a common fatal disease caused by the canine distemper virus (CDV).⁽¹⁾ It is the second most deadly viral disease after rabies in dogs.⁽²⁾ CDV belongs to the Morbillivirus genus from the Paramyxoviridae family⁽³⁾ and is antigenically related to human measles virus (HMV);^(1, 4) with the difference that it has more tropism to the nervous system⁽³⁾ and causes encephalomyelitis in half of the dogs.^(5, 6) Currently, there is not any specific antiviral treatment against CD⁽²⁾ and only supportive therapies including fluids, antibiotics and corticosteroids are available. Although the dog may recover with supportive treatment, the prognosis of nervous signs is poor, the symptoms are usually irreversible, and euthanasia is often recommended. Even in the absence of neurological symptoms, the owner should be warned about complications that may affect the animal in the future.⁽³⁾ As a result, it is necessary to find an effective treatment to reduce the death rate and its complications.

The use of anti-Newcastle disease vaccine serum for treating distemper has been reported to be useful in empirical clinical practice and to increase the recovery rate of CD, even in dogs with neurological signs.^(7, 8) The precise mechanism of treatment with anti-Newcastle disease vaccine serum is unclear. Certainly, antibodies do not play a role in the treatment since they are not produced in the donor dog within 10 to 12 h after the injection of the vaccine. Instead, it takes about two weeks to appear in the blood. It is hypothesized that a series of unknown cytokines stimulate the immune system, triggering a rapid immune response and quickly destroying the distemper virus.⁽⁷⁾ Newcastle disease virus (NDV) vaccine has been used to express protective antigens against several pathogens such as CDV.⁽⁹⁾ Moreover, it is proven that infection with different NDV strains, such as Lasota and F48E9 can stimulate and modulate cytokine expression patterns.⁽¹⁰⁾ Therefore, it is possible for the NDV vaccine to produce some protective cytokines against CDV in the donor blood. Despite various empirical clinical reports, there is no definite scientific report on the treatment of distemper by anti-Newcastle disease vaccine serum. Therefore, this study investigated the efficacy of anti-Newcastle disease vaccine serum for treating canine distemper.

Materials and methods

Ethical statement

The study protocol was assessed by the Research Committee of the Faculty of Veterinary Medicine and approved by the Research Ethics Committee of Ferdowsi University of Mashhad (Approval ID: IR.UM.REC.1399.121).

Experimental design

Two 10- and 12-month-old mixed-breed dogs weighing 31 and 33 Kg without a history of vaccination were prepared and dewormed with Praziquantel Forte (Praziquantel, Pyrantel Pamoate, and Febantel, one tablet per 10 kg). The dogs were kept in College Hospital for two weeks and their health was assessed. A 1000-dose vial of Newcastle disease vaccine (Lasota strain, Razi Institute, Karaj, Iran) was dissolved in 6 mL normal saline. A catheter was placed in the cephalic vein of the dogs and 3 mL of the dissolved vaccine was injected into each dog. After 11 hours, the animals were anesthetized and 20 % of their blood volume was extracted. Blood was poured into 10 mL tubes without anticoagulant and centrifuged after clotting. Sera were separated and frozen in glass tubes in plastic bags. These sera were then tested to treat dogs affected by canine distemper referred to the Veterinary Teaching Hospital.

The dogs were randomly divided into seven groups (according to the order of the groups):

1. Anti-Newcastle disease vaccine serum (ANDVS)
2. ANDVS + Cotrimoxazole (ANDVS+CoTrim)
3. ANDVS + Penicillin-Gentamicin (ANDVS + PG)
4. ANDVS + Cefazolin-Amikacin (ANDVS + CA)
5. Cotrimoxazole (CoTrim)
6. Penicillin-Gentamicin (PG)
7. Cefazolin-Amikacin (CA)

The number of dogs in each group is given in [Table 1](#). The amount of serum in the ANDVS-receiving groups was 1 mL/dog + 2.2 mL/10 kg body weight subcutaneously every 12 h for three times.⁽⁷⁾ Therefore, for example, a 10 kg-dog received 3.2 mL ANDVS each time. The dose of cotrimoxazole (Co-Trimoxazole 24 %) was 15–30 mg/kg intramuscularly every 12 hours, cefazolin 20–30 mg/kg intravenously or intramuscularly every 8 hours, amikacin 15–30 mg/kg intravenously or intramuscularly every 24 hours, penicillin 20 000–40 000 IU/kg intramuscularly every 24 h, and gentamicin 6–8 mg/kg intravenously or intramuscularly every 24 hours. The antibiotics were administered for at least one week. If it was necessary, the dogs of all groups were also treated with supportive treatments (fluid therapy, B complex, antiemetics).

The treatment protocol was explained to the animal's owners and was initiated with their consent. A detailed history of each dog, including previous vaccination and antiparasitic treatments were recorded and treatment was performed only on dogs not receiving the distemper vaccine. After the observation of distemper symptoms (gastrointestinal, respiratory, cutaneous, neurological and systemic), a 2 mL whole blood sample was obtained from each dog for hematological evaluation,

while 6 mL of blood was obtained and stored in EDTA-treated tubes at -80 °C for definitive diagnosis of distemper by RT-PCR assay. The conjunctival swabs were also obtained for early diagnosis using rapid test kits (Anigen Rapid CDV Ag Test Kit, BioNote, Hwaseong, Korea). In this study, all statistical analyses were performed on RT-PCR positive samples.

For RT-PCR, RNA was extracted from whole blood by blood RNA isolation kit (DENAzist Asia, Ferdowsi University of Mashhad, Iran) according to the manufacturer's instructions. For negative control, diethylpyrocarbonate (DEPC)-treated water was used. RNA quantity and quality were analyzed by NanoDrop spectrophotometry and electrophoresis in 1 % agarose gel using loading buffers 6x after staining with DNA green viewer.

Immediately after RNA extraction, the cDNA was synthesized using Pars Tous DNA synthesis kit (Mashhad, Iran) according to the manufacturer's instruction. PCR was performed using the oligonucleotides primer pairs; I (sense [5'-ACA GGA TTG CTG AGG ACC TAT-3'] and anti-sense [5'-CAA GAT AAC CAT GTA CGG TGC-3']), II (sense [5'-AAC TAT GTA TCC GGC TCT TGG-3'] and anti-sense [5'-CGA GTC TGA AGT AAG CTG GGT-3']), III (sense [5'-CAA AGA CGT GTG GTC GGA GAA-3'] and anti-sense [5'-CTT AGT AAG CAT CCT CAT CTT GGC-3']); designed to amplify amplicons of 286, 259, and 899 bp of the CDV nucleoprotein (NP) gene.⁽¹¹⁾ The PCR amplification process was conducted as follows: one step of denaturation at 94 °C/1 min, 40 cycles of denaturation at 94 °C/1 min, annealing at 59 °C/2 min, extension at 72 °C/1 min, and the final extension at 72 °C/5 min. DEPC-treated water and a sample whose PCR products were proved distemper by sequencing were included as negative and positive controls, respectively. The PCR products were analyzed by electrophoresis in 1.5 % agarose gel in 1X TBE buffer after staining with the green viewer and visualizing under ultra-violet light.

Dogs under the following conditions were included in the design:

- At least two symptoms of distemper
- RT-PCR results were positive
- Did not received any antibiotics before the visit
- Completed the course of treatment

Attempts were made to perform differential diagnosis of CDV from canine parvovirus (CPV), salmonellosis, and lead poisoning. For that purpose, we used physical examination, complete blood count (CBC) profile panel evaluation, obtaining history, and in a few cases with bloody diarrhea, by using rapid CPV antigen test kits. Finally, with appropriate follow-up, the total recovery (and fatality) rate of the distemper, as well as the effects of distemper on hematological profiles, vital signs, and the association of the disease with sex, breed, and age were evaluated. The improvement in symptoms was recorded daily until the end of the medication period. Moreover, one and three months after treatment, the status of the dogs was monitored for the recurrence of the symptoms or becoming a nervous form.

Statistical analysis

The total recovery (and fatality) rate of CD was reported as frequency rate. The efficacy of each drug group and the comparison between treatment groups, association of distemper with sex, age and breed of dogs (in breeds that contain at least three dogs) was evaluated by the Chi-square test. The comparison of hematological indices and vital signs between groups was evaluated by one way analysis of variance. The level of significance was set at $P < 0.05$.

Results

The number of dogs, and the recovery and fatality rates in each group are presented in Table 1. Totally, 120 dogs suspected of having distemper participated in this study. Sixty of 120 dogs recovered, and 60 dogs died. RT-PCR was performed on 113 dogs, which were positive in 66 dogs. Out of 66 dogs, 22 (33.3 %) recovered and 44 dogs (66.7 %) died (Table 1). Chi-square comparison showed that there was a significant difference between fatality (and recovery) rate of distemper positive and negative dogs ($P < 0.0001$).

The results also showed a significant difference in the fatality rate of distemper-positive dogs among treatment groups ($P = 0.042$). Paired comparison between treatment groups showed that the fatality rate in group 1 (ANDVS) was higher than that of ANDVS + CoTrim ($P = 0.007$) and ANDVS + CA ($P = 0.036$) groups. None of the distemper positive dogs in the ANDVS group (Group 1) recovered. Conversely, 8 of 13 dogs in group 2 (ANDVS + CoTrim) recovered (61.5 %), which is significantly higher than dogs in group 1 that received only ANDVS ($P = 0.007$). However, although the recovery rate in dogs treated with ANDVS + CoTrim (Group 2) was higher than that of dogs treated with CoTrim (Group 5), this difference was not significant but approached the borderline of significance ($P = 0.063$). In the other groups that received a combination of antibiotics and ANDVS, in Group 4 (ANDVS + CA), the recovery rate was 50 % (5 out of 10), which is higher than that of CA (28.6 %) group. In group 3 (ANDVS + PG), the recovery rate was 25 %, which is higher than that of Group 1 (0 %) but it does not differ much from Group 6 (PG), where the recovery rate was 28.6 % (Figure 1).

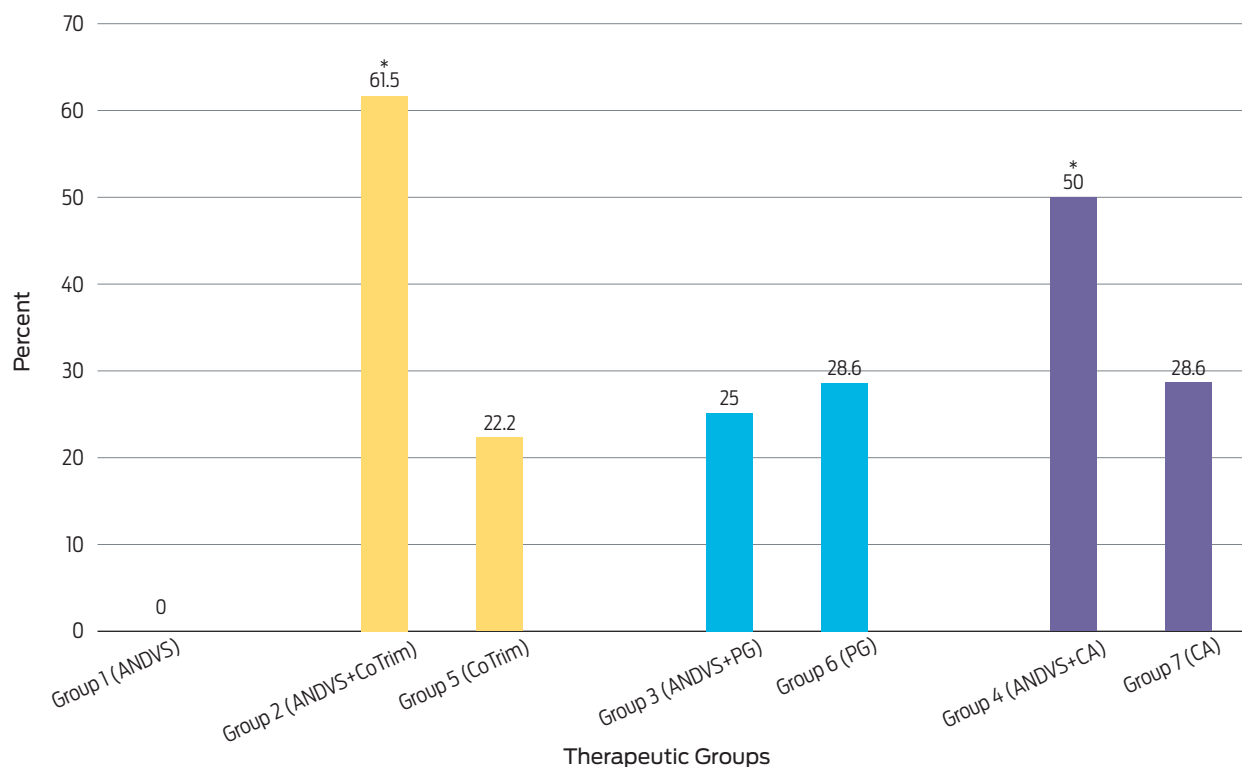
These results revealed that dogs receiving ANDVS with cotrimoxazole had the highest improvement (61.5 %), followed by dogs receiving ANDVS with ceftazolin-amikacin (50 %). These results also showed that the fatality rate of distemper in dogs confirmed by RT-PCR is high (44 out of 66; 66.7 %). Of the 66 cases whose distemper was confirmed, 39 (59.1 %) dogs were under six months old, 20 (30.3 %) were between 6 and 12 months, and 7 (10.6 %) were between 1 and 5 years old. Moreover, 27 of 39 (69.2 %) dogs with distemper that were less than 6 months old died. In dogs between 6 and 12 months, 11 dogs out of 20 (55 %) and in dogs between 1 and 5 years, six out of 7 (85.7 %) died. There was no significant difference in the fatality rates between different ages (Table 2).

Of the 66 dogs positive RT-PCR test, the sex of one dog was unknown. Of the remaining 65 cases, 36 (55.4 %) were female and 29 (44.6 %) were male (Table 3). Fifteen out of 36 female dogs recovered (41.7 %), while the recovery rate of male dogs was 24.1 % (7 out of 29). This difference was not statistically significant ($P = 0.138$).

Table 1. The number of dogs recovered and died in treated groups

RT-PCR result			Groups ¹							Total
			ANDVS	ANDVS + CoTrim	ANDVS + PG	ANDVS + CA	CoTrim	PG	CA	
Positive	Outcome	Live	0	8	3	5	2	2	2	22
		Dead	8	5	9	5	7	5	5	44
	Total		8	13*	12	10*	9	7	7	66
Negative	Outcome	Live	0	0	1	3	6	8	15	33
		Dead	3	2	1	1	3	0	4	14
	Total		3	2	2	4	9	8	19	47
Total	Outcome	Live	0	8	4	8	8	10	17	55
		Dead	11	7	10	6	10	5	9	58
	Total		11	15	14	14	18	15	26	113

¹ANDVS, Anti-Newcastle disease vaccine serum; CoTrim, Cotrimoxazole; PG, Penicillin-Gentamicin; CA, Cefazolin-Amikacin.

**Figure 1.** Distemper recovery rate across treatment groups.

*The recovery rate was significantly higher in groups 2, and 4 compared with group 1 ($P = 0.007$ and $P = 0.036$, respectively).

Table 2. Number of dogs (percentage) live or dead according to diagnostic results of distemper and age

RT-PCR			Age (months)				Total
			<6	6-12	12-60	>60	
Positive	Outcome	Live	12 (30.8)	9 (45.0)	1 (14.3)		22 (33.3)
		Dead	27 (69.2)	11 (55.0)	6 (85.7)		44 (66.7)
	Total		39 (59.1)	20 (30.3)	7 (10.6)		66 (100.0)
Negative	Outcome	Live	15 (57.7)	4 (66.7)	13 (92.9)	1 (100.0)	33 (70.2)
		Dead	11 (42.3)	2 (33.3)	1 (7.1)	0 (0.0)	14 (29.8)
	Total		26 (55.3)	6 (12.8)	14 (29.8)	1 (2.1)	47 (100.0)
Total	Outcome	Live	27 (41.5)	13 (50.0)	14 (66.7)	1 (100.0)	55 (48.7)
		Dead	38 (58.5)	13 (50.0)	7 (33.3)	0 (0.0)	58 (51.3)
	Total		65 (57.5)	26 (23.0)	21 (18.6)	1 (0.9)	113 (100.0)

Table 3. The distemper positivity in male and female dogs

		Sex		Total
		Female (%)	Male (%)	
Outcome	Live	15 (41.7)	7 (24.1)	22 (33.8)
	Dead	21 (58.3)	22 (75.9)	43 (66.2)
	Total	36 (55.4)	29 (44.6)	65 (100)

Table 4. Fatality and recovery rates of different breeds in distemper positive dogs

RT-PCR			breeds					
			Afghan	German Shepherd	Husky	Mixed	Spitz	Terrier
Positive	Outcome	Live	0	2	1	14	0	2
		Dead	6	1	3	21	6	2
	Total		6	3 ¹	4	35	6	4

¹ The fatality rates in German Shepherd breed was lower than other breeds. The highest fatality rate was in the Afghan and Spitz breeds (P = 0.030).

Of the 66 dogs whose distemper was positive, the breed of one dog was not recorded. The remaining 65 dogs were from 11 breeds. Of these, only Afghan (a local breed), German Shepherd, Husky, Mixed, Spitz and Terrier breeds had more than three patients and were statistically analyzed (Table 4). The results showed a significant difference in the fatality (and recovery) rate between breeds (P = 0.030). Accordingly, the number of distemper-related deaths in the German Shepherd breed was lower than in other breeds, and the highest fatality rate was in the Afghan and Spitz breeds, in which all six infected dogs died.

The mean heart rate, respiratory rate, body temperature, erythrocytes and white blood cells are listed in Table 5. Hematocrit (28.83 ± 6.98) and red blood cells (RBC) count (4.80 ± 1.05) decreased significantly in distemper-positive dogs compared to distemper negative dogs (P = 0.012 and P = 0.015, respectively). Other hematological indices and vital signs were not significantly different between distemper-positive and distemper-negative dogs (P > 0.05).

Table 5. Vital signs and hematological factors in distemper positive and negative dogs tested by RT-PCR

Variables	RT-PCR	n	Mean	Standard deviation	P-value
Heart rate (beats/min)	Positive	53	111.23	28.89	0.057
	Negative	39	126.59	42.81	
Respiratory rate (breaths/min)	Positive	46	42.50	20.33	0.968
	Negative	30	42.30	21.17	
Temperature (°C)	Positive	58	38.86	1.51	0.561
	Negative	44	38.71	1.06	
Hematocrit (%)	Positive	60	28.83	6.98	0.012
	Negative	44	32.72	8.53	
Red blood cell (count x 10 ⁶ /μL)	Positive	60	4.80	1.05	0.015
	Negative	44	5.37	1.29	
White blood cell (count/μL)	Positive	60	12581.67	9045.69	0.413
	Negative	44	14134.09	10114.53	
Neutrophil (count/μL)	Positive	59	10515.36	7767.19	0.607
	Negative	44	11389.25	9382.73	
Lymphocyte (count/μL)	Positive	56	1210.11	1499.69	0.302
	Negative	43	1499.21	1186.68	
Monocyte (count/μL)	Positive	56	973.93	1068.85	0.538
	Negative	43	853.93	785.41	
Band cell (count/μL)	Positive	26	416.38	379.99	0.888
	Negative	17	399.06	407.33	

Of the 60 cases whose RT-PCR was positive, rapid test kits were positive in 51 (85 %) dogs. Likewise, out of 36 cases with negative RT-PCR results, rapid test kits were negative in 29 (81 %) cases. Therefore, the sensitivity and specificity of rapid test kits were 85 % and 81 %, respectively. Thus, there is a strong significant relationship between the rapid test kit and RT-PCR assay ($P < 0.0001$, Phi test = 0.649).

Discussion

Some empirical clinical findings indicate that ANDVS produced in dogs is effective in treating distemper disease.⁽⁷⁾ However, to our knowledge, there is still no scientific report on the effect of ANDVS on CD in dogs. The results of this study showed that ANDVS alone (Group 1) was not effective to treat dogs with distemper since all puppies treated with this serum died. However, ANDVS along with cotrimoxazole caused a 61.5 % improvement in distemper-positive dogs, whereas only 22.2 % of dogs treated with cotrimoxazole alone (Group 5) recovered. Given these facts, it appears that the combination of ANDVS with cotrimoxazole is a promising approach to treat distemper. This synergistic effect was also observed for the combination of ANDVS with cefazolin-amikacin (Group 4). Because the cefazolin-amikacin (Group 7) caused a 28.6 % improvement, while the combination of ANDVS with cefazolin-amikacin resulted in a 50 % improvement in dogs.

Moreover, in dogs receiving penicillin-gentamicin, the ANDVS did not increase the recovery rate. Since these differences are not significant, it is impossible to judge the healing effect of the ANDVS.

The synergistic effect of antibiotics has been reported before, but since there is no study on the effect of ANDVS, the cause of its synergistic effect with some antibiotics is still unknown and more studies are needed in this field. In this study, although a relatively large number of dogs with distemper have been tested and treated, the number of dogs in each group was low due to the large number of groups. Hence, few cases in each group affected the final result. However, this result could give researchers insight that in the future they will work on more dogs to find a better approach.

Distemper is a fatal disease that is very difficult to treat. Many efforts have been made to treat distemper, but little success has been achieved. For example, the effects of EICAR (a ribavirin analogue), the silver nanoparticles, the essence of saint germain flower, and porcine anti-canine distemper virus IgG and F(ab')₂ antibody fragments as passive immunotherapy have been studied. Nevertheless, despite the beneficial effects in each of these methods, adverse effects have also been reported.^(12–15)

Numerous studies report the prevalence of the distemper in different countries.^(16–21) In most of these reports, the prevalence of the disease has been evaluated by serological methods, but there are few reports that reveal the accurate fatality and mortality rates of distemper in dogs. One study on necropsy cases reported that 11.7 % of patients were due to distemper.⁽²²⁾ In a study, the mortality rate of distemper in vaccinated dogs was estimated at 30 percent. In another article, the observed distemper mortality rate was 50 %.⁽²³⁾ In this study, a relatively high fatality rate was observed in treated dogs (66.7 %), suggesting mortality is likely higher in untreated dogs. In this study, in Group 1, in which ANDVS alone was used for treatment, the fatality rate was 100 %, despite using supportive therapies.

These findings also highlight the importance of secondary bacterial infections in increasing the fatality rate of distemper. Especially, considering it is an immunosuppressive viral disease, which increases the risk of life-threatening bacterial infections.^(18, 24) The results of this study also show that the mortality rate caused by the virus itself is still high. Therefore, it is crucial to find treatments that effectively decrease the death rate caused by the virus. In this study, by producing anti-Newcastle disease vaccine serum and using it in animals with distemper, we tried to stimulate factors in the animal body to overcome these effects of the virus. In our opinion, this study could be a prelude to future studies so that scientists can find a definitive way to treat distemper.

In this research, 59 % of patients with the definitive diagnosis of distemper were under six months old. However, there was no significant difference in fatality rate at different ages. This indicates a high risk of death for dogs of any age. The cause of most infections at young ages is the lack of full development of the immune system in these dogs.^(25, 26) Due to the improper distribution of breeds in our experimental groups, the relationship between breed and distemper was not well evaluated. Other studies have reported that brachiocephalic dogs are generally more resistant and less likely to develop distemper.⁽²⁾ In our study, the opposite was true as German Shepherd breed was more resistant, which is a dolichocephalic

dog. Note that the Afghan breed, which is a strong, large and a local dog, is more sensitive to distemper.

Although most hematological variables were decreased in distemper-positive dogs, only hematocrit and total red blood cell count were significantly reduced compared with distemper-negative dogs. The effect of distemper on the hematological profile has been reported in various studies. Most studies have suggested that distemper decreases lymphocytes.^(3, 25, 27) However, our study showed that the decrease in lymphocytes was not statistically significant. One reason may be the wide range of lymphocytes in dogs with distemper. While some distemper-positive dogs may have an increased total number of leukocytes and lymphocytes, the opposite may also occur (i. e. decreased total leukocyte and lymphocyte counts). Hence, when the values of these dogs are averaged, they neutralize each other's effects to some extent.

We observed a strong correlation between results of RT-PCR and rapid conjunctival kits ($P < 0.0001$, Phi test = 0.649). The sensitivity and specificity of the rapid conjunctival kits compared to the RT-PCR were 85 % and 81 %, respectively. These results indicate that CD can be diagnosed in many patients (85 % of cases) referred to the clinic by performing a rapid conjunctival kit, which is 81 % correct if the test is negative. An et al. (2008) also reported that the sensitivity and specificity of rapid test kits are 100 %.⁽²⁸⁾ Since the rapid diagnosis of canine distemper can lead to better prevention and treatment, the use of these kits for rapid diagnosis is critical.

Conclusions

This study confirmed that distemper causes a high fatality rate in dogs. Although ANDVS or antibiotics alone did not improve the recovery rate of infected dogs, their combination increased their survival rate. Further research is needed on the role of ANDVS's cytokines and interferons to improve the effectiveness of this promising distemper treatment alternative.

Data availability

All relevant data are within the manuscript. The datasets used and analyzed in this study are available from the corresponding author on reasonable request.

Acknowledgments

The authors thank Dr. Fatemeh Fazel, Dr. Hesam Dehghani, Dr. Mehrdad Mohri and Dr. Pezhman Mirshokraei for their valuable contributions.

Funding statement

This research was funded by the research council of Ferdowsi University of Mashhad, grant number 47896, awarded to AA Sarchahi. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of interest

The authors have no conflict of interest to declare in regard to this publication.

Author contributions

Conceptualization: AA Sarchahi.

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Formal analysis: AA Sarchahi, M Arbabi.

Funding acquisition: AA Sarchahi.

Investigation: AA Sarchahi.

Methodology: AA Sarchahi, M Arbabi, H Mohebalian.

Project administration: AA Sarchahi.

Supervision: AA Sarchahi.

Validation: AA Sarchahi.

Visualization: AA Sarchahi, M Arbabi.

Writing-original draft: AA Sarchahi.

Writing-review and editing: AA Sarchahi, M Arbabi, H Mohebalian.

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