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Evaluation of Newcastle disease virus vaccine effectiveness in dogs with neurological signs of canine distemper

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Abstract

Canine Distemper Virus (CDV) is the cause of a highly lethal infectious disease affecting a broad range of carnivores. Despite using various treatments, there is still no effective treatment, especially in the neurological form of distemper. The aim of this study was to evaluate the therapeutic effect of injecting Newcastle disease vaccine into the subarachnoid space of dogs with neurological form of distemper. The dogs that had symptoms of nervous distemper, particularly myoclonus, were included in the plan. After anesthetizing of dogs, 0.10 to 1.00 mL of cerebrospinal fluid (CSF) were removed and, 0.10 to 0.50 mL of the prepared Newcastle solution were injected into their subarachnoid space. Another 0.50 to 1.00 mL of normal saline was then injected to remove the needle from the vaccine. The live attenuated LaSota or B1 vaccine was used in this study. Rapid kit tests and reverse transcription polymerase chain reaction (RT-PCR) assays were used to diagnose of the disease. Dogs were monitored for up to 3 to 24 months during that time they were evaluated for improvement or worsening of clinical symptoms. Out of nine dogs in which distemper were diagnosed with different tests, one dog recovered completely and another dog recovered greatly. Therefore, the overall recovery rate was 22.20%. It is concluded that administration of Newcastle vaccine into the subarachnoid space of dogs with nervous distemper causes at least 22.20% improvement and does not cause specific side effects and can be used to treat affected dogs.

Introduction

Canine distemper virus (CDV) along with Newcastle disease virus, Rinderpest virus and Measles virus are members of the genus *Morbillivirus* of the *Paramyxoviridae* family.[1,2] The CDV is a vastly infectious, highly fatal and immunosuppressive agent which cause multi-systemic disorders in its hosts.[3,4] The development of vaccination has greatly reduced the prevalence of the disease, however, it may still occur in non-vaccinated and sporadically in vaccinated dogs.[5,6] Its clinical symptoms vary depending on the virulence of virus, environmental conditions, age and immune status of the animal.[7,8] Most distemper infections are subclinical or cause mild involvement of the upper respiratory tract with mild symptoms that resolve without treatment, however, unvaccinated or immuno-suppressed dogs may show severe systemic signs of distemper.[9]

Progressive systemic infection of distemper affects mostly unvaccinated pups aged 12 to 16 weeks. Presence of maternal-derived antibodies (MDAs) in puppies under 12 weeks of age prevents disease in colostrum-fed cubs. On the other hand, the MDAs against distemper received in utero and in colostrum in puppies blocks the vaccination with distemper vaccine. The human measles vaccine can be used to vaccinate these puppies against canine distemper, however, neither the measles vaccine nor the Newcastle disease vaccine has been used to treat dogs with distemper.[10] Neurological signs usually begin 1 - 3 weeks after recovery from systemic symptoms and may include: dementia, disorientation, seizures, circling, cerebellar or vestibular signs, tetraparesis, ataxia[10,11] and myoclonus which is the most common of them.[12] There is still no reliable method to treat the neurological form of the disease worldwide.[13] Certain corticosteroids and anti-

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convulsants have been mentioned in some texts, however, these treatments are usually not effective and the rate of recovery is negligible.[9,10] In the 1970s, an invention was proposed by a veterinarian residing in Lancaster, California, in dogs with nervous form of distemper using a Newcastle vaccine solution. Since then, some dogs have been treated sporadically, however, there were no scientific reports (other than the clinical cases reported by him and several others) to confirm this claim.[14] On the other hand, symptomatic treatment with mexiletine has been proposed in dogs with myoclonic contractions.[15] Treatment with this medication is temporary and signs return after treatment discontinuation. Thus, the aim of this study was to investigate the effect of Newcastle disease vaccine on the treatment of neurological signs of distemper.

Materials and Methods

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. 120). This study was performed on 13 dogs referred to the Veterinary Teaching Hospital of Ferdowsi University of Mashhad, Mashhad, Iran. The treatment protocol was explained to the animal's owners and was started with their consent. A detailed history of each dog including previous vaccinations, anti-parasitic deworming program and previous treatments was recorded. The dogs with symptoms of nervous distemper, particularly myoclonus were included in the plan. For injection of Newcastle disease vaccine, dogs were anesthetized by intravenous injection of 10.00 mg kg^{-1} ketamine hydrochloride (Bremer Pharma GmbH, Warburg, Germany) and 1.00 mg kg^{-1} diazepam (Caspian Tamin Pharmaceutical Co., Tehran, Iran). The skin of occipital area was shaved and disinfected. After aseptically removal of 0.10 to 1.00 mL of cerebrospinal fluid (CSF) (depending on the size of the dog), 0.10 to 0.50 mL of the prepared Newcastle vaccine (Razi Institute, Karaj, Iran) solution were injected into their subarachnoid space. Another 0.50 to 1.00 mL of normal saline was then injected to remove the needle from the vaccine. The live attenuated LaSota or B1 Newcastle vaccine (Razi Institute, Karaj, Iran) was used by adding 6.00 mL of vaccine solvent or 6.00 mL of normal saline to a 1,000-dose vial of vaccine.[14] Rapid diagnostic kit tests (Anigen Rapid CDV Ag Test Kit; BioNote, Hwaseong, Korea) were performed on samples of CSF immediately after fluid collection and the remaining fluid was frozen at – 80.00 ˚C for Reverse transcription polymerase chain reaction (RT-PCR) analyses.

Dogs were monitored for up to 3 - 24 months and were evaluated for improving or worsening of clinical signs. Any changes were recorded weekly by examination of the animal or observation the submitted videos by the owners.

For CDV detection by RT-PCR assay, the RNA was extracted from 250 µL CSF with the RiboEx LS RNA isolation kit (GeneAll Biotechnology Co., Ltd., Seoul, Korea) according to manufacturer's instruction.

The cDNA was made from RNA using AccuPower® CycleScript RT PreMix, lyophilized (Bioneer, Daejeon, South Korea) according to manufacturer's instruction. The PCR was performed by adding 10.00 µL master mixture, 3.00 µL distilled water, 1.00 µL of each of the forward and reverse primers and 5.00 µL of cDNA followed by denaturation at 94.00 ˚C for 5 min and 35 cycles consisting of denaturation at 94.00 ˚C for 1 min, annealing at 59.00 ˚C for 2 min, extension at 72.00 ˚C for 1 min and final extension at 72.00 ˚C for 5 min in a GeneAtlas thermocycler (Astec, Fukuoka, Japan). The PCR products were electrophoresed on a 1.50% agarose gel after staining with green viewer in 1x TBE buffer and analyzed by visualizing under UV light.

Sequence analysis of PCR products. The identities of the PCR amplicons were confirmed by nucleotide sequencing of PCR products (Bioneer) obtained from one infected dog to distemper using the sense primer pair 1.00 and 2.00 and sense and anti-sense of primer pair 3.00 (Macrogen, Seoul, Korea), (Table 1). The quality of each nucleotide sequence obtained was analyzed with SnapGene software (version 3.2.1; GSL Biotech, San Diego, USA) and the similarity of each sequence was checked in GenBank® using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi), which showed excellent homology (99.00%) to CDV strain HL N protein mRNA complete CDS (GenBank® accession no. EU489475.1).

Table 1. Nucleotide sequence and position of primer pairs used for RT-PCR.

Primer	Direction	Sequence (5′-3′)	Nucleotide position
Primer pair 1	Sense	ACA GGA TTG CTG AGG ACC TAT	769 - 789
	Anti-sense	CAA GAT AAC CAT GTA CGG TGC	1,055 - 1,035
Primer pair 2	Sense	AAC TAT GTA TCC GGC TCT TGG	941 - 961
	Anti-sense	CGA GTC TGA AGT AAG CTG GGT	1,200 - 1,180
Primer pair 3	Sense	CAA AGA CGT GTG GTC GGA GAA	711 - 731
	Anti-sense	CTT AGT AAG CAT CCT CAT CTT GGC	1,610 - 1,587
GAPDH	Sense	GCC AAA AGG GTC ATC ATC TC	-
	Anti-sense	GGC CAT CCA CAG TCT TCT	

Results

The average age of the six female and seven male dogs was 20.00 ± 24.80 months (age range, 2.50 to 96.00 months). Two dogs were vaccinated at 4 and 8 months ago, respectively. All dogs were returned to normal state after anesthesia and no abnormalities including seizures were observed during anesthesia and recovery time. The study period was 3 - 24 months after injection of Newcastle disease vaccine. During this time, the dogs were examined at least once a week, and where necessary, were filmed and compared to previous videos. The most important clinical and laboratory signs observed in the studied dogs are listed in Table 2. Myoclonus was present in 11 of the 13 dogs in different parts of the body. In two other cases, paralysis without myoclonus were observed. The number of positive cases with each of the diagnostic methods was as follows: the rapid test kit was positive in two conjunctival samples, seven CSF samples, two conjunctival and CSF samples, and seven conjunctival or CSF samples. Also, RT-PCR was positive in five blood samples, seven CSF samples, and in three samples in both blood and CSF. Therefore, in general, the Rapid Kit and/or RT-PCR test was positive in 9 dogs.

The results of treating dogs with injecting Newcastle disease vaccine into subarachnoid space were as follow: Six dogs died (two cases with no distemper related signs due to bleeding from skin lesion 19 days later and 2 months after uterine infection, respectively, one case was lost 2 days later due to the severity of the disease, one case died 9 months later without any changing in myoclonus, and two cases were euthanized at 14 and 42 days later), and three cases remained unchanged after 1 year. In one case the muscle tics were completely resolved and in two cases they were significantly declined. One of the dogs also went out of reach and the owner did not answer the phone. Therefore, in this study, out of nine dogs in which distemper was confirmed with various tests, one dog (dog No. 10) with mild myoclonus in the head and mandible improved completely and another dog (dog No. 9) with severe myoclonus in his hind limbs and diaphragm was improved significantly. Dog No. 8, which had severe myoclonus but whose tests were not confirmed, also was improved significantly. These last two dogs could walk but they could not bear their weight on the limbs involved. These dogs were recovered dramatically three months later, with some tremors in the affected limbs when raising from the ground, however, no myoclonus after standing or lying down. These dogs were not previously vaccinated and had previously shown respiratory symptoms of distemper and were fully recovered. Therefore, the overall recovery rate of nervous form of canine distemper is 22.20% (two out of nine confirmed distemper positive dogs).

Eleven out of the 13 infected dogs in the present study were not previously received the distemper vaccine, however, 2 of the infected dogs were previously vaccinated but were euthanized due to the severity of the disease and lack of response to treatment.

White blood cell counts were normal in most infected dogs. Two dogs with significant increase in white blood cell count had systemic form of the disease. Lymphopenia was observed only in three dogs (dog's number 2, 3 and 7). In addition, body temperature was normal in most dogs at the time of the examination (Table 2).

Discussion

In the present study, 11 out of 13 dogs showed myoclonus, however, not all of their tests were positive. Although myoclonus is one of the typical symptoms of nervous distemper,[16,17] it can also be caused by other diseases including lead poisoning and central nervous system lesions.[10] The history of dogs referred in this study showed that all of them had previously been infected with non-nervous distemper. In nine out of 13 dogs with neurological symptoms, distemper was confirmed at least by one of the diagnostic tests. There was a close correlation between the results of the rapid diagnostic kit and RT- PCR results on cerebrospinal fluid, so that all six dogs whose rapid diagnostic kits were positive, their RT-PCR results were also positive, and in five cases whose rapid diagnostic kits were negative, their RT-PCR results were also negative. Only in one case (dog number 11) in which the rapid diagnostic kit was negative, the RT-PCR result was positive. Therefore, the rapid diagnostic kit tests can be performed with high confidence in small animal clinics to check (confirm or rule out) the canine distemper. In four dogs with typical distemper symptoms, all tests were negative. In these four dogs, neurological symptoms had begun at least 3 months ago. This may be due to increased levels of anti-distemper antibodies in the blood and CNS. As the level of anti-viral antibodies in the blood and CNS increases, the presence of the virus in the blood, secretions and CNS decreases. Therefore, although the animal suffers from the effects of the virus, it may not be detected by these methods.[10]

Treatment of neurological symptoms of distemper by Newcastle disease vaccine was first proposed by Sears, a general veterinarian of Lancaster, USA.[14,18] He reported his experimental results, however, there were no scientific reports in this regard. His and some veterinarians empirical results showed that about 20.00% of dogs suffering from nervous distemper might recover after injecting of Newcastle disease vaccine into their subarachnoid space. It has been shown that NDV, a member of *Paramyxoviridae* family, is highly lethal in

Table 2. The results of tests and outcome of dogs contributed in the present study.

No.	Symptoms	Age (m*)	Sex	Breed	Last vac.	RKTc	RKT CSF	RT-PCR blood	RT-PCR CSF	Duration (d*)	RT (°C)	WBC (µL^{-1})	Neu. (µL^{-1})	Lym. (µL^{-1})	Mon. (µL^{-1})	RBC (×10^{6} µL^{-1})	PCV (%)	Outcome
1	Myoclonus in diaphragm Chewing gum LMN urinary incontinence Nose and pad hyperkeratosis	36.00	F	GSH	–	–	+	–	+	19	39.70	11,800	9,440	1,534	118	5.92	37.60	D
2	UMN paralysis of hind limbs Nose and pad hyperkeratosis	24.00	F	GSH	4 m* ago	–	+	+	+	14	38.70	6,400	4,800	832	384	4.56	29.50	E
3	Myoclonus in right hind limb	4.00	M	Mix	–	–	–	Unkn	–	90	38.40	12,500	9,250	1,875	500	6.25	39.90	U
4	Myoclonus in right neck and shoulder muscles and diaphragm Nose hyperkeratosis Enamel hypoplasia	5.00	M	Mix	–	–	+	–	+	406	38.70	10,600	5,936	3,498	848	5.58	31.00	U
5	Myoclonus in hind limbs Nose and pad hyperkeratosis	3.50	F	Dob	–	+	+	+	Unkn	2	-	119,000	109,480	2,380	5,950	3.07	19.30	D
6	Myoclonus in right hind limbs and diaphragm	12.00	M	Hus	8 m* ago	–	+	–	+	42	39.60	11,200	9,856	672	448	4.67	30.20	E
7	Myoclonus in right fore and hind limbs	2.50	F	Hus	–	+	+	+	+		41.80	6,600	5,412	528	594	4.86	29.70	Unkn
8	Severe myoclonus in right fore and hind limbs and diaphragm Mild myoclonus in left fore and hind limbs	18.00	M	Mix	–	Unkn	–	Unkn	–	92	38.70	8,100	5,184	2,025	405	5.27	33.20	MR
9	Severe myoclonus in hind limbs and diaphragm Mild myoclonus in fore limbs Enamel hypoplasia	12.00	M	Mix	–	Unkn	+	+	+	92	39.20	16,800	8,904	2,688	2,352	5.32	35.30	MR
10	Mild myoclonus on the head Chewing gum	24.00	M	Spitz	–	Unkn	-	+	-	92	38.50	10,500	7,665	1,890	630	5.28	37.60	R
11	UMN paralysis of hind limbs Urinary incontinence	96.00	F	GSH	–	Unkn	-	Unkn	+	60	-	22,500	18,450	2,250	1,350	4.78	28.00	D
12	Severe myoclonus in right forelimb and pectoral muscles Mild myoclonus in left forelimb and right hind limb	11.00	M	Mix	–	Unkn	–	–	–	270	38.20	NA	NA	NA	NA	NA	NA	D
13	Severe myoclonus in right limbs Mild myoclonus in left limbs and diaphragm Chewing gum	12.00	F	Spitz	–	Unkn	-	Unkn	–	90	-	NA	NA	NA	NA	NA	NA	U

D: died; d*: day; Dob: Doberman pincher: E: euthanized; F: Female; GSH: German Shepherd; Hus: Husky; LMN: Lower motor neuron; Lym.: Lymphocyte; M: Male; Mix: Mixed: Mon.: Monocyte; MR: Mostly recovered; m*: Month; NA: Not available; Neu.: Neutrophil; PCV: Packed cell volume; R: Recovered; RBC: Red blood cell; RKTc: Rapid kit test on conjunctiva; RKTcsf: Rapid kit test on CSF; RT: Rectal temperature; U: unchanged; WBC: White blood cell; Unkn: Unknown; UMN: Upper motor neuron Vac.: vaccination.

poultry, however, if used as a recombinant viral vector, it not only does not cause infection in dogs and other mammals but also can produce immunity against CDV.[19] The hypothesis of Sears protocol was that the Newcastle disease vaccine activated some immune pathways immediately after injection and resulted in the production of some unknown cytokines and/or interferons that suppressed the distemper virus.[18] Consequently, it may not be impossible that the Newcastle disease vaccine, can protect dogs against CDV in a way that is not yet known (producing unknown cytokines or interferons). The results of this study showed that only two out of nine dogs (22.20%) whose tests were positive for distemper recovered (If we do not take into account the third dog that recovered but its tests were negative) and was consistent with Sears' results. Although relatively small in number, given that nervous distemper does not have a good prognosis, this was a significant improvement and it was hoped that in the future, scientists would focus on this issue to increase the number of those recovering from the disease. Although it is more appropriate to use a larger statistical population for more accurate judgment, it should be noted that since this treatment is not a definitive method, and these animals must be anesthetized and an abnormal substance (such as a Newcastle vaccine) is injected into their subarachnoid space, many owners do not agree with this method, therefore it makes it difficult to use this treatment. In the present study, all 13 dogs tolerated Newcastle vaccine without any side effects.

Therefore, the results of this study could be promising that the initial side effects of the vaccine were negligible and might be welcomed by animal owners in the future.

As shown in Table 2, five out of the 13 dogs died or were euthanized due to the severity of the disease, and six of them either were recovered or not changed. Therefore, in similar patients, the owner of the animal can be advised to use this treatment in the animal, because even if this treatment does not cure his animal, it will not have adverse effects. Further studies are needed to confirm this claim. It is up to the animal owner to decide whether to keep the animal or euthanize it. It seems that if animal owners are explained that some animals with myoclonus can survive, more than 30.00% of animals, therefore, many dogs will not be euthanized.

Finally, it is concluded that administration of Newcastle disease vaccine into the subarachnoid space of dogs with neurological signs of distemper did not cause any specific side effects, however, it caused at least 22.20% improvement in symptoms, therefore, in the future, it could be used to evaluate its effectiveness in more dogs.

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Conflict of interest

The authors report no conflicts of interest.

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