

## REVIEWED COMPARATIVE ANALYSIS OF THREE CASE-STUDIES USING RT-PCR TO DETECT CANINE DISTEMPER VIRUS

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### Abstract

*An important canine virus that are spread among the dog population is the canine distemper virus (CDV) which affects domestic and wild animals all over the world, particularly due to the fact that it passes the species. Canine distemper virus is an important veterinary issue in areas with a high number of dogs that are not vaccinated against the virus and also where the disease is present in wild animals. A useful tulle in diagnosis of this disease is the real time PCR that can detect the viral genome from oral swabs, ocular swabs, LCR (cerebrospinal fluid), feces or urine, depending on the clinical stage of the disease. This paper presents a reviewed analysis of three case-studies conducted by the Universities of Bari (Italy), Liège (Belgium) and Pécs (Hungary) concerning clinical manifestations of the infection with the Canine Distemper Virus and the efficiency of reverse transcription polymerase chain reaction tests in the detection of viral RNA. The emergence of CDV cases in wildlife is also explored, as is the opportunity of vaccination against CDV infection as a means of protecting domestic dogs in shelters and homes.*

Keywords: canine distemper virus, RT-PCR tests, viral RNA, wildlife, vaccination

### 1. INTRODUCTION

An important canine virus that are spread among the dog population is the canine distemper virus (CDV) which affects domestic and wild animals all over the world, particularly due to the fact that it passes the species. Widespread systemic CDV infection occurs in a range of wild animals belonging to various genera, from viverrids such as genets and civets to mustelids such as minks or ferrets, ursids like giant pandas and black bears and even ailurids and procyonids. Not only have terrestrial carnivores been suspected as vectors for the infectious agent, but non-carnivore species infected lethally have proven the extraordinary ability of the pathogen to cross barriers between species. The findings of numerous studies point to the fact that disease eradication is considerably hindered by the broad and extending range of CDV and its persistence within the wildlife reservoir.

Canine distemper virus is an important veterinary issue in areas with a high number of dogs that are not vaccinated against the virus and also where the disease is present in wild animals. Young animals are more receptive to the disease but the infection can produce at any age. The transmission route can be direct from one animal to other and also by indirect route through the body fluids, like respiratory secretions, saliva, urine and feces. Because the disease is very contagious it is mandatory the quarantine because of the transmission to others dogs in order to avoid the infections from one sick animals to one health animals. The infection has different stages of evolution: it can affect the nervous system, and in this case the prognostic is bad

but if the animal develops an strong immune response the recovery can be possible but with some sequels (Elia G. et al. [4], MSD Veterinary manual [6]).

Canine distemper virus have different clinical signs, from gastrointestinal signs like vomiting, diarrhea, to respiratory signs and neurological signs which are the most dangerous and can lead to death.

A useful tulle in diagnosis of this disease is the real time PCR that can detect the viral genome from oral swabs, ocular swabs, LCR(cerebrospinal fluid), feces or urine, depending on the clinical stage of the disease.

The current study explores the way in which RT-PCR diagnosis tests have proved to be among the most efficient in detecting CDV RNA in the early stages of the disease. Also, the studies performed have outlined the importance of vaccination as one of the most rapidly protective measures against the disease.

CDV infection usually debuts with brief fever episodes in the initial 3-6 days, which may be accompanied by leukopenia or lymphopenia. In some cases, anorexia also occurs, but other than that the symptoms might easily elude suspicion. The fever can persist for a few days, being followed by another bout, with nasal secretions, ocular mucopurulent secretion, lethargy and anorexia.

These may be followed by gastrointestinal and respiratory signs that can be followed by second bacterial infections; associated encephalomyelitis may occur, or follow the systemic disease, or even be manifest in the absence of systemic symptoms. Dogs that survive the acute phase of the disease may display hyperkeratosis of the footpads and epithelium of the nasal planum, as well as enamel hypoplasia

in incompletely erupted teeth. As a rule, neurologic signs tend to appear at a later stage of the illness, although it cannot be anticipated whether an infected dog will develop neurologic manifestations or not.

Neurologic symptoms include: myoclonus, chorea, flexor spasm, hyperkinesia, convulsions, salivation and chewing. Other neurologic symptoms are circling, head tilt, nystagmus, paresis to paralysis, seizures (Lanszki Z. et al. [3]).

## 2. MATERIALS AND METHODS

Two case studies stress the importance of early accurate CDV diagnosis. They were both admitted at the Teaching Veterinary Hospital of the University of Bari, Italy, in May 2013. Both dogs came from a kennel with an outbreak of distemper disease that affects mixed breed animals between 2,5 months and 1 year. The dogs had not been vaccinated upon admission in the shelter and had clinical signs that suggested CDV infection, which was subsequently confirmed. Not willing to agree to euthanasia, the owners of the first dog, a puppy aged 3 months old, accepted to let their dog be included in an observational study and receive medical care for the disease.

### CASE 1

The 3-month age dog had respiratory signs like ocular and nasal secretions and coughing. Its overall health condition was good, and still have present the appetite. After few days the dog developed fever, 39.5–40.0 °C, anorexia, vomiting and hemorrhagic diarrhea. These signs persisted for 20 days, and a slight prostration still present after recovery. One month after hospitalization appear neurological symptoms like ataxia, paresis and paralysis, accompanied by fever. After 9 days the animal died.

### CASE 2

The second case was a 8 months old dog which have systemic disease, with fever (39.4 °C), lethargy, nasal secretions, conjunctivitis and hyperkeratosis of the nose. The dog recovered very rapidly within one week.

Real-time PCR confirmed the distemper virus infection in both animals. In the first case, all the collected samples tested positive, while in the second case the virus was detected only in urine and rectal swab. It was made the genotyping of the distemper virus and it was detected the Arctic genotype.

In the first case the virus was present in all the samples with higher viral quantity in urine, rectal, nasal and conjunctival swabs. Also, all the samples that were tested positive for distemper virus presence have a virus quantity higher than  $1.06 \times 10^4$  RNA copies/ $\mu$ l in 37 days after hospitalization (Elia G. et al. [1]).

In the second case which was also positive for distemper virus by RT-PCR in urine samples until 50 days after hospitalization. Increases in viral quantities were detected from 10 to 22 days after hospitalization with a mean titer of  $1.47 \times 10^4$  RNA copies/ $\mu$ l. In the remaining observation period, the mean viral quantity detected in urine was  $7.22 \times 10^2$  RNA copies/ $\mu$ l. Rectal swabs were positive from 1 to 36 days of hospitalization with the highest viral quantity  $5.71 \times 10^4$  RNA copies/ $\mu$ l, detected at day 10 (Elia G. et al. [1]).

### CASE 3

Another study examining the re-emergence of CDV in wildlife in Belgium pointed out the fact that throughout the 3-year period between 2014 and 2017, none of the 30-50 foxes, occasional badgers and racoons submitted for postmortem examination at the University of Liege tested positive for the canine distemper virus. However, in November 2017 a racoon with several neurological impairment, which had to be euthanized, tested positive for the presence of CDV RNA by real time PCR. Following these findings, a number of 13 foxes were tested postmortem, six of them having had a history of abnormal behaviour and collapse. The brain tissues in 10 of the foxes revealed typical neuropathological lesions, and all of the animals tested positive for CDV RNA in the RT PCR.

In order to rule out the possibility of a viral strain circulating in the area, a conventional RT-PCR test was used, followed by a BlastN analysis, which revealed that all cases belonged to a single strain, thus disproving the circulating strain theory (Garigliany M. et al. [2]).

## 3. RESULTS AND DISCUSSIONS

All the cases presented above explored to what extent dogs or wildlife such as foxes and badgers can resist to CDV infection and how efficient RT-PCR tests prove to be in detecting viral RNA. While in the first cases (the dogs in Bari) the 3-month-old puppy could not ultimately survive the disease, the 8-month-old dog recovered in a shorter period of time, which could also indicate that the viral charge might have been higher in the first case, and also that the 3-month-puppy's immune system must have been heavily weakened by the CDV infection.

The study carried out on the 1-year age dog in Hungary emphasized the length of time over which the CDV appeared in the animal's urine samples, covering a period of 17 months since the initial hospitalization, with improvements and relapses in-between. The scientists performing this study in 2019-2020 pointed out that it was the longest known case of CDV to have been recorded in a patient.

On the other hand, the RT-PCR tests performed on the foxes in Belgium confirmed the presence of the viral infection through necropsy. The wild animals had neurologic manifestations and some had to be euthanized. Although no large-scale viral circulating strain was detected, it was obvious that the CDV has the power to cross barriers between species and affect a wide range of wildlife.

With the exception of this outbreak in Southern Belgium, the previous lack of CDV detection indicates the low prevalence of the virus. Yet, the large number of foxes having contracted the disease signalled a re-emergence of the virus in a naïve population.

The study provided further arguments in support of domestic dog's vaccination against CDV.

In another scientific article was presented a case of a 1-year-old male dog detected with distemper virus infection from Hungary. The dog was raised in poor conditions, that why was confiscated from its owner and transported to an animal shelter. It was considered that the dog was infected with the distemper virus before of caring out to the shelter. There were no information's about the dog immunoprophylaxis scheme. When the dog came to the veterinary clinic was presenting severe diarrhea and difficulty breathing. The dog was also diagnosed the *Dirofilaria immitis* infection (Lanszki Z. et al. [3]). After one month the animal's health began to improve and the dog was brought to the shelter. After one week the animal was taken again to the clinic because of the nasal secretions, weight loss and cough. It was performed an RT-PCR from urine but the animal in few days became asymptomatic and was brought again to the shelter. After 6 months from the very first signs the animal was declared healthy by a negative RT-PCR from urine.

Also, the solution that emerges in order to stop the CDV infection from spreading within shelters and in domestic dogs is vaccination of the latter, since interactions with wildlife are not always easy to control, nor is the spread of the disease to various species, overspill and spillback having already occurred in numerous animal populations all over the world (MSD Veterinary manual [6]).

#### 4. CONCLUSIONS

Canine distemper is an important problem that affects dogs, with high mortality. Infection with CDV develops a large variety of clinical signs. Many dogs do not develop the classical clinical picture, thus making diagnosis difficult, in addition to variations due to immune system and virus strain (Canine Distemper (CDV) [7]).

The cases of canine distemper described in this study confirm the importance of choosing appropriate biological samples for diagnosis. Owing to the fact that the dogs under study were infected with CDV naturally, various biological samples could be collected throughout the period of infection.

In recent decades, many cases have been detected as belonging to the Arctic-like lineage, which is known to circulate within Southern and Central Europe among domestic dogs and wildlife. Studies point to the fact that it is of paramount importance to provide effective surveillance for CDV in order to be able to monitor the distribution of the virus.

In future, more complex studies need to be carried out in order to establish the background mechanisms of cross-species transmission events (Lanszki Z. et al. [3]).

RT-PCR testing remains one of the most effective means of detecting CDV infection, both in domestic dogs and in wildlife.

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