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Evaluation of infection with N protein-specific Immunoglobulin M and G in naturally occurring distemper in dogs

Hasbi Sait Saltik, Mehmet Kale*

Department of Virology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Turkey

**Corresponding author: hasbi.saltik@gmail.com*

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Abstract: In dogs, canine distemper has a worldwide distribution with high morbidity/mortality, despite the widespread usage of vaccines and has no specific treatment. In susceptible animals with the canine distemper virus, respiratory, gastrointestinal and nervous system disorders, immunosuppression and cutaneous lesions can also be seen. Especially puppies and unvaccinated dogs are prone to get the viral infection. IgM and IgG antibodies constitute the major component of the natural antibodies produced during the primary and secondary antibody response that have long been recognised to inhibit viral infections. In the present study, the presence of the viral N protein-specific IgM and IgG was investigated by indirect ELISA in naturally infected dogs. Moreover, the rate of outbreaks in naturally infected dogs was shown by the detection of new and re-infections. In the Western Mediterranean region, blood serum samples were collected from 50 unvaccinated dogs for the mentioned infection between 2015 and 2017. At 0–12 months, in the dogs with clinical symptoms, the indirect ELISA detected 4% acute, 54% early convalescent, 40% late convalescent and 2% no infections phases. The clinical manifestations were studied in four main groups follow as: respiratory, gastrointestinal, nervous and cutaneous symptoms. The evaluation showed that the canine distemper virus N protein-specific antibodies detection by the indirect ELISA is quick and safe in naturally infected dogs. In conclusion, the method is very useful for the pre-diagnosis of the disease when evaluated together with the clinical symptoms. It helps to distinguish acute and convalescent (early/late) phases. Distinguishing these phases of infection is important for monitoring the spread of the outbreaks and identifying the risk of severe forms of canine distemper.

Keywords: antibody; canine; convalescent; ELISA; morbillivirus; unvaccinated

One of the most important hosts of the canine distemper virus (CDV), which causes infection in many different animal species, is the dog (Appel and Summers 1999). CDV causes a highly contagious, infectious and fatal disease in dogs with a worldwide distribution (Martinez-Gutierrez and Ruiz-Saenz 2016). Puppies and unvaccinated dogs are especially reported to have a high rate of a CDV infection (Gray et al. 2012; Wyllie et al. 2016). The pathogenic viral agent of Canine distemper (CD) is an enveloped, single-stranded and negative-sense RNA virus, which belongs to the family *Paramyxoviridae* (Martella et al. 2008; MacLachlan and Dubovi 2016). The CDV that elicits the highest antibody response has been reported to be the N protein (von Messling et al. 1999). CDV may result in multi-systemic symptoms such as respiratory, gastrointestinal and central nervous system (CNS) issues (Beineke et al. 2009). The most common ones are fever, coughing, oculonasal discharge, diarrhoea, lymphopenia, hyperpla-

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sia of the skin, tremors and paralysis (Appel and Summers 1999). In some cases, it is may be difficult to distinguish the CD from other infections which have similar symptoms. A 4-fold increase in the antibodies between the paired samples is strongly associated with a CDV infection (Lan et al. 2005; Sawatsky et al. 2011). However, secondary sampling is difficult in places where there are no control programmes for free-roaming dogs. ELISA (enzyme-linked immunosorbent assay) is currently the most commonly used method for the serological diagnosis. Antigens prepared from CDV-infected cells or recombinant CDV proteins identify the specific Immunoglobulin M (IgM) and Immunoglobulin G (IgG). Detection of the CDV-specific IgM and IgG is important for indicating exposure to CD especially of the unvaccinated dogs. Capture-ELISA, developed for the detection of the CDV-specific IgM in the blood serum is useful for diagnosing the acute phase of the CD in the unvaccinated dogs (von Messling et al. 1999). The detection of the specific IgGs in the convalescent period plays an important role in the immunity or disease exposure. The CDV infections can be evaluated by comparison of the IgM and IgG status for each dog with the clinical symptoms. According to the seroconversion results of the IgM and IgG, the infection phases can be interpreted (Boivin et al. 2017). Investigation of the CDV-specific IgM and IgG status in unvaccinated dogs is useful for analysing the acute, convalescent (early/late) and non-infection infection phases in the unvaccinated dogs (Appel and Summers 1999). As a result, it helps to distinguish the acute and convalescent (early/late) phases of the CDV infection. The diagnosis, by distinguishing between the phases of the CDV infection, is important not only for monitoring the spread of the epidemic, but also for identifying the risk of severe forms of CD.

MATERIAL AND METHODS

Samples

Between the years 2015–2017, serum samples of stray dogs were collected from the Western Mediterranean region in Turkey. The samples were obtained from 0–12 month old stray dogs with clinical symptoms. All the samples were stored at –80 °C (Haier, Qingdao, P.R. China) until tested. The blood serum samples were centrifuged at 1 500 *g* for 5 minutes. The blood serum samples were transferred to 2 ml sterile microtubes. The samples were stored at –80 °C until the study was performed. All the serum samples were inactivated at 56 °C for 30 min just before testing.

Indirect ELISA

The CDV-specific IgM and IgG were investigated in the blood serum samples. A commercially available CDV indirect ELISA (Agrolabo, Scarmagno, Italy) was applied for the detection of the CDV-specific IgM and IgG. The test was performed according to the manufacturer's instructions. The infections were evaluated by comparison of the IgM and IgG status for each dog with the clinical symptoms.

Clinical manifestation

A total of 50 free-roaming, unvaccinated, 0–12 month old dogs which were living in animal shelters or in urban areas were selected. The clinical manifestations were classified in four groups as follows; respiratory (coughing, oculo-nasal discharge, sneezing, etc.), gastrointestinal (vomiting, diarrhoea, dehydration, etc.), nervous (seizures, tremors, paralysis, etc.) and cutaneous (red rashes, hyperkeratosis of the nostril and footpad, etc.) symptoms (Figure 1).

Statistical analysis

The data were analysed using IBM SPSS® Statistics v23.0 software. The statistical evaluation was performed using the Chi-squared and Spearman's correlation test. *P* values less than 0.001 were considered statistically significant.

RESULTS

In the study, 47 (94%) IgG and 29 (58%) IgM positive samples were detected by the CDV-specific indirect ELISA in the 50 serum samples. Only the IgM was detected in 2 (4%) cases and only the IgG was detected in 20 (40%) cases. Although there was a case

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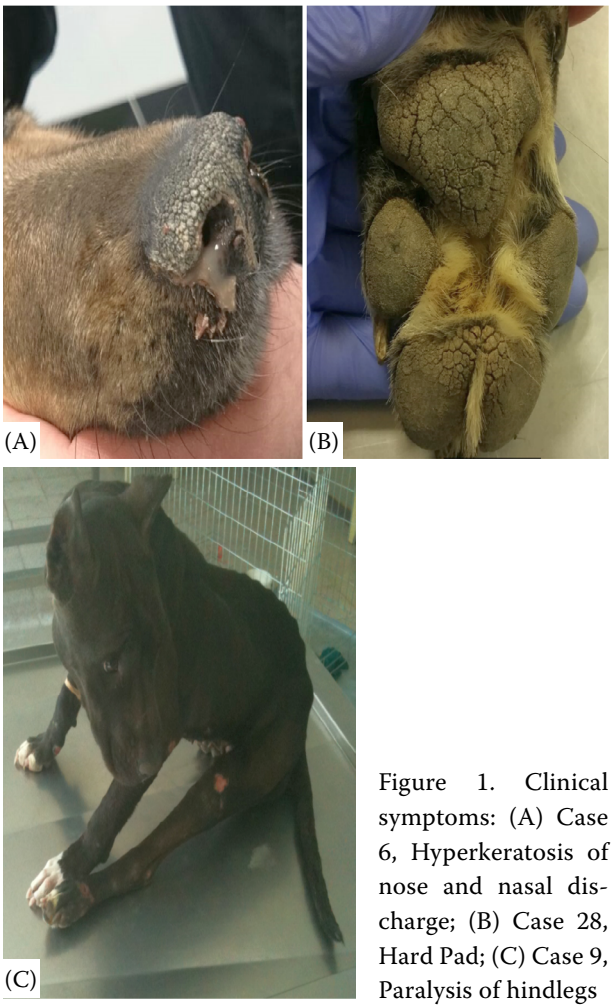

Figure 1. Clinical symptoms: (A) Case 6, Hyperkeratosis of nose and nasal discharge; (B) Case 28, Hard Pad; (C) Case 9, Paralysis of hindlegs

with only respiratory symptoms, no antibody was detected. For each case, the result of the CDV specific indirect ELISA (IgM-IgG) and clinical manifestation observed in the dogs are shown in Table 1. The results have been classified into two groups as follows: age under and above 6 months old. Under 6 months old, 19/33 (57.57%) and 30/33 (90.90%) positive results were found for the IgM and IgG, respectively. Above 6 months old, 10/17 (58.82%) and 17/17 (100%) positive results were found for the IgM and IgG, respectively. The results have also been evaluated according to an interpretation described previously (Boivin et al. 2017). The interpretation of the results for the CDV-specific antibodies showed that 4%, 54% and 40% of the dogs were in the acute, early convalescent and late convalescent phase of the CDV infection, respectively (Table 2). The respiratory symptoms, gastrointestinal symptoms and nervous symptoms were more common in the early and late convalescent phases of the CDV infection.

Table 1. The ELISA results and clinical manifestations for each case

No	Months	Sex	IgG	IgM	RS	GS	CS	NS
1	2	♀	+	+	✓	✓	x	x
2	2	♂	+	+	✓	✓	x	x
3	1.5	♀	+	+	✓	✓	✓	✓
4	1.5	♂	–	+	✓	x	✓	✓
5	1	♀	+	+	✓	x	✓	✓
6	1.5	♂	+	+	✓	✓	✓	✓
7	5	♀	+	+	✓	✓	✓	✓
8	2	♂	–	+	✓	✓	✓	✓
9	2	♀	+	+	✓	✓	✓	✓
10	3	♀	+	+	✓	✓	x	✓
11	9	♂	+	+	✓	✓	x	x
12	4	♂	+	+	✓	✓	✓	x
13	8	♀	+	+	✓	✓	✓	x
14	1	♀	+	+	✓	x	x	x
15	3	♀	–	–	✓	x	x	x
16	5	♂	+	–	✓	✓	x	x
17	7	♀	+	+	x	✓	x	✓
18	10	♀	+	–	✓	x	x	x
19	3.5	♀	+	–	✓	x	x	x
20	10	♂	+	+	✓	✓	✓	✓
21	4	♂	+	+	x	✓	x	✓
22	7	♀	+	–	✓	x	x	✓
23	11	♀	+	+	x	x	x	✓
24	1	♀	+	+	✓	✓	x	✓
25	6	♂	+	–	x	✓	✓	x
26	3	♀	+	–	✓	x	x	x
27	8	♂	+	+	✓	✓	✓	✓
28	5	♂	+	+	x	✓	✓	x
29	4	♂	+	+	x	✓	x	x
30	4	♀	+	+	x	x	x	✓
31	2	♂	+	–	✓	✓	✓	✓
32	1	♂	+	–	x	✓	✓	✓
33	2	♂	+	–	✓	x	x	✓
34	8	♀	+	–	x	x	✓	x
35	2.5	♀	+	–	✓	x	x	x
36	5	♂	+	+	x	x	✓	x
37	9	♀	+	+	x	x	✓	✓
38	12	♂	+	–	x	x	✓	x
39	12	♀	+	+	x	x	x	✓
40	11	♂	+	+	x	x	x	✓
41	7	♀	+	–	x	x	✓	x
42	11	♀	+	+	x	x	x	✓
43	6	♂	+	–	x	✓	✓	✓
44	1.5	♀	+	–	✓	✓	✓	✓
45	10	♀	+	–	x	x	✓	✓
46	9	♀	+	–	✓	x	✓	✓
47	4	♀	+	+	✓	x	✓	✓
48	3	♂	+	–	✓	✓	✓	✓
49	3	♂	+	–	✓	✓	x	x
50	3	♀	+	–	✓	x	x	x

CS = cutaneous symptoms; GS = gastrointestinal symptoms; NS = nervous symptoms; RS = respiratory symptoms

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Table 2. Interpretation of the results for the CDV-specific IgM and IgG

EIA		Numbers (*N*)	Rate (%)	Infection phase
IgG	IgM			
(–)	(+)	2	4	acute infection
(+)	(+)	27	54	early convalescent
(+)	(–)	20	40	late convalescent*
(–)	(–)	1	2	no infection

*Current or recent infection

DISCUSSION

Between 2015 and 2017, samples were collected from 50 unvaccinated dogs in the Western Mediterranean region. The CD which is one of the most infectious diseases of domestic and wild dogs, has high morbidity and mortality all over the world (Martinez-Gutierrez and Ruiz-Saenz 2016). It is widely seen in both owned and stray dogs in Turkey. In particular, dogs up to 1 year of age are more likely to be exposed to a CDV infection. Vaccination programmes for owned dogs are widely used in private veterinary clinics in Turkey. However, since no vaccination programme is applied to free-roaming dogs, all the vaccinated or unvaccinated dogs are at risk of the disease (Lorusso and Savini 2014). In recent years, there have been studies on respiratory and digestive tract infections in dogs living in shelters, but studies on the CD are limited in Turkey (Timurkan and Oguzoglu 2015; Aydin et al. 2018; Timurkan et al. 2018).

Moreover, due to the lack of a comprehensive study of the disease in wild animals in Turkey, the risk of CD transmission from wildlife is not known. The low prevalence of CDV was reported when vaccination and control programmes were performed properly (Chappuis 1995; Temilade et al. 2015; MacLachlan and Dubovi 2016). In Turkey, the presence of the CDV-specific IgM and IgG in dog serum samples were investigated, where 30/47 (63.82%) and 19/47 (40.42%) positive results were detected, respectively (Caliskan and Burgu 2007). The presence of the CDV-specific IgG in dog serum samples were investigated, where 70/116 (60.34%) positive results were detected (Esin 2013). In northwest Zimbabwe, McRee et al. (2014) reported that 75/225 (34%) positive results were detected for the CDV-specific IgG in the dog serum samples. Serological methods can be preferred in the diagnosis of some infectious diseases (Nelson and Couto 2014; Boivin et al. 2017). In this study, the presence of the specific IgM and IgG for the N protein of the CDV in the blood serum samples of 50 unvaccinated dogs was investigated by indirect ELISA.

The results of the specific immunoglobulins were compared for each case (Table 2). Detection of the CDV specific IgM allows for the elimination of the disease with similar clinical symptoms with the CD. Moreover, it is reported that the CDV specific IgMs detected by ELISA may help to evaluate the infection phase (Waner et al. 2003). Since the N protein has a strong antibody response, it has been reported that N protein is more immunologically dominant than all other proteins (von Messling et al. 1999). This indicates that N protein-specific antibodies are more likely to be detected in periods of reduced humoral immunity. It has been reported that in some CD cases, where the nervous system is affected, the immune response to N proteins is not relatively impaired (Krakowka et al. 1975; Rima et al. 1991). The IgM shows up from 5 weeks to 3 months in infected dogs depending on the virus strain and host immune status (Appel and Summers 1999).

However, since the immune system can be suppressed in the CDV infections, it is difficult to diagnose the disease with only the IgM findings. A seroconversion showing the presence of the virus-specific IgM with the negative IgG response indicates the diagnosis of a primary viral infection. Detection of the CDV-specific IgG in an unvaccinated dog's blood serum sample indicates that it has been exposed to a virus in the past (Boivin et al. 2017).

In this study, an infection phases table was created with the presence of the CDV N protein-specific IgM and IgG results in the blood serum samples. In the present study, the acute and early/late convalescent phases of the CD were determined and the infection was interpreted. As a result, it was determined that the dogs, in which the specific immunoglobulins were detected in their blood serum samples, were infected. A VNT (virus neutrality test) is widely used in serological diagnosis. However, a VNT is expensive and disadvantageous for routine diagnostic laboratories because the application is time-consuming (at least four days). The use of N-protein based tests may be more advantageous than the VNT. Therefore, ELISA is

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an alternative and effective method to the VNT (von Messling et al. 1999; Elia et al. 2015). In routine diagnostic laboratories, the high-sensitivity and specificity ELISA are preferred to the VNT for the detection of the neutralising antibodies. The CDV-specific antibodies detection by the indirect ELISA method is much quicker and cheaper than the VNT. ELISA is also useful for the prediagnosis of the CDV infection when the method is evaluated together with the clinical symptoms. In the current study, the IgM and IgG results were compared between two groups as follows: age under and above 6 months old.

As a result of this comparison, no significant difference was observed between the groups. However, severe infections caused by CDV, especially in young and unvaccinated dogs, have been reported in the immunoglobulin findings in previous studies (Jozwik and Frymus 2002; Martella et al. 2008; Gray et al. 2012; Wyllie et al. 2016). Many viral diseases can be diagnosed by serological methods. However, diagnosis becomes difficult when the CDV-specific antibodies are not formed due to immunosuppression (Kubo et al. 2008; Elia et al. 2015; Boivin et al. 2017). Therefore, the clinical manifestations and serologic results were evaluated together in the current study (Table 1). Furthermore, CDV is a pathogen that has widespread host species in wildlife. It is known that the virus is propagated through the body secreta/excreta of the CDV-infected animals. Interactions between domestic and wild animals cause the disease to spread easily (Di Sabatino et al. 2015). Therefore, comprehensive studies are also needed to demonstrate the transmission of CD in Turkey's wildlife. The detection of CDV specific antibodies by indirect ELISA would be a useful method to demonstrate the role of animal interactions in the CD.

In the conclusion, the co-evaluation of the presence of the CDV-specific IgM and IgG in non-vaccinated dogs is useful for the pre-diagnosis of an infection. It is appropriate to evaluate the serological results together with the clinical symptoms. The hygiene, quarantine and disinfection procedures should be considered in which the susceptible dogs live. Vaccination should be included in routine prevention and control programmes applied in animal shelters to reduce the risk of CD transmission between infected and susceptible dogs. In Turkey, it is necessary to carry out studies showing the existence of CD in the wild.

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Conflict of interest

The authors declare no conflict of interest.

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