Comparison of Tissue and Fluid Samples for the Early Detection of Canine Distemper Virus in Experimentally Infected Dogs

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ABSTRACT. The clinical utility of various specimens was examined for the early diagnosis of canine distemper (CD). Seven healthy dogs at 17 weeks of age were experimentally infected with a field isolate of canine distemper virus. The RT-PCR was carried out to detect CDV NP gene. Dogs showed mild fever and leukopenia, however, typical clinical signs of CD were not seen throughout the experimental period. CDV amplicons were detected more, earlier and for longer period in the conjunctival swabs than in the other samples employed. These results suggested that conjunctival swab samples, which are easy to obtain and non-invasive, would be the most suitable and practical specimen for the early antemortem diagnosis of CDV infection.

KEY WORDS: canine distemper, conjunctival swab, early diagnosis.

Canine distemper (CD) is a contagious multisystemic disease caused by canine distemper virus (CDV), classified in the Morbillivirus genus of the Paramyxoviridae [4, 7, 8, 10]. Nonimmune dogs of any age are susceptible, however, CD is most common in puppies between the age of 3 and 6 months, the period when they have lost maternal antibody [7, 11]. Diagnosis of CD on clinical grounds is rarely possible in mild cases. In more severe cases, the combination of any of the principal signs: conjunctival inflammation, respiratory catarrh, diarrhea, illness of 3 weeks or longer and nervous signs suggests a presumptive diagnosis of CD [7, 8, 10]. Specimens that include conjunctival and vaginal imprints, urinary epithelial cells, skin and stomach biopsy specimens, cells from tracheal washings, blood smears, and cerebrospinal fluid (CSF) taps have been used for an etiological diagnosis [1, 2, 7, 9, 15]. In addition, virus isolation, immunofluorescence, immunocytochemistry, staining inclusions, or enzyme-linked immunosorbent assay have been used for detection of CDV infection. However, the majority of those methods are of limited value when they are applied to clinical specimens [5, 7]. Recently, CD infections have been definitively diagnosed by the reverse transcription-polymerase chain reaction (RT-PCR) by which the detection rates of CDV were relatively high [5, 13]. The early detection of CDV is essential for determining CDV infection and subsequent patient management. In this study, RT-PCR was utilized on various tissue and fluid samples in order to determine the most suitable one for the antemortem diagnosis of CDV infection.

Seven healthy mixed-breed dogs were placed in isolated individual cages and given commercial dog food and water ad libitum during the entire experimental period. Dogs were experimentally infected at 17 weeks of age, at which time all puppies’ serum neutralizing antibody titers against CDV were below the detection level (<2). A field strain of CDV was isolated from the cerebellum of a dog that died from natural case of distemper with neurological signs. Each puppy was sprayed intranasally with 1.0 × 10^4 median tissue culture infective dose (TCID50) of CDV which had been passaged twice in Vero cells. All experiments were carried out in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. Samples were collected every day from 2 days before the experimental infection to 14 days post infection. One ml of peripheral whole blood collected by venipuncture from the external jugular vein, conjunctival swab collected by swabbing with the sterilized cotton sticks and put in eppendorf tube with 0.5 ml of the sterilized 0.9% saline solution, 1.0 ml of nasal irrigation with the sterilized 0.9% saline solution, 1 ml of urine collected by urinary catheterization, and 0.5 ml of CSF collected by puncture of the atlanto-occipital joint, were obtained from each puppy. The RT-PCR was carried out by the previously described method [5] with a minor modification to detect CDV nucleocapsid protein (NP) gene from the collected samples. Total RNA was extracted with the RNA purification kit (OMEGA BIOTEK) according to manufacturer’s instruction. Oligonucleotide primers used for amplification of the NP gene sequence were a 21-mer (5’-ACAGGATTTGGAGGACCTAT-3’) and a 21-mer (5’-CAAGATACCATGTACGGTGTC-3’). The RT-PCR assay in this study detected viral RNA in samples containing 10^1.7 TCID50/ml. The identity of sequences between CDV used in challenge and amplicon amplified from samples collected after challenge was confirmed by direct sequencing. The percentage differences of positive proportion among samples were analyzed by use of the Friedman’s nonparametric repeated measures ANOVA. Percentage data were arcsine transformed and then analyzed for group and time effects. When differences with time and between groups were detected, pair-wise comparisons were made by use of chi-square test or Fisher’s exact test if appropriate. A P value of <0.05 was considered to be statistically significant.
significant.

Dogs experimentally infected with CDV showed mild fever and leukopenia on 3 and 4 days post infection. However, typical clinical signs of CD were not seen in the remaining experimental period. In the various samples collected from dogs during 2 days before the experimental infection, RT-PCR results were all negative (data not shown). The RT-PCR conducted with various samples resulted in amplicons of the expected length for the primer pair. However, CDV amplicon was not detected in any CSF samples throughout the experimental period (Fig. 1). In peripheral whole blood samples, CDV amplicons were detected in 2 dogs out of 7 experimental dogs from 3 days post infection, and detected in all 7 dogs from 6 to 9 days post infection. After that CDV amplicons were detected only in 3 or 5 out of 7 experimental dogs (Fig. 2). In conjunctival swab samples, CDV amplicons were detected from 1 to 14 days post infection, and in all 7 dogs CDV amplicons were detected from 3 to 14 days post infection. In nasal irrigation samples, CDV amplicons were detected from 3 to 14 days post infection.

Fig. 1. RT-PCR results of various samples collected from a dog (no. 5) on 7 days after experimental infection with a field strain of canine distemper virus. Lane M, 100-base pair (bp) molecular weight marker; lane 1, peripheral whole blood; lane 2, conjunctival swab; lane 3, nasal irrigation; lane 4, urine; lane 5, cerebrospinal fluid samples; lane 6, positive control; lane 7, negative control. The expected size of RT-PCR product is 287-bp.

Fig. 2. Comparison of detection rates of canine distemper virus (CDV) amplicons among samples (peripheral whole blood, conjunctival swab, nasal irrigation, and urine) on each day after experimental infection of a field strain of CDV. Data about cerebrospinal fluid samples are excluded in this figure because there was no positive sample during 14 days of sampling period. The different letters on each day represent statistically significant ($P<0.05$).
days post infection. However, CDV amplicons were detected in only 1 to 4 out of 7 experimental dogs throughout the experimental period. In urine samples, CDV amplicons were detected from 5 to 14 days post infection. Only on 6 days post infection, CDV amplicons were detected in all 7 dogs. From 7 to 12 days post infection, CDV amplicons were detected in 6 dogs and the number of positive dogs was reduced to 2 dogs on 14 days post infection. Detection rate of CDV amplicons for conjunctival swab samples was significantly higher than those of the other samples during experimental period with the exception from 6 to 9 days post infection (p<0.05).

The various samples including peripheral whole blood, conjunctival swab, nasal irrigation, urine, CSF, and swab from vagina, lung, stomach, intestinal and bladder tissues have been used for the diagnosis of CD [2, 5, 7, 9, 15]. Among these samples, whole blood or serum is commonly used for virus detection or antibody determination [5, 6, 13]. In this study, CDV in whole blood were detected later and eliminated rapidly than those in conjunctival specimens. Thus, the whole blood specimen is not fully satisfied for the early diagnosis of CD. The conjunctiva and eye probably become infected at the time of generalized viremia in the early course of the disease [2, 14]. However, there are possibilities of aero-exposure of CDV in conjunctiva while challenging and of contamination through lacrimal duct. We suppose the CDV in conjunctiva was not subjected to rapid elimination by immune system, but replicated in the conjunctival sac or orbital cavity. Summers et al. [15] had demonstrated a more widespread ocular involvement, with CDV-infected cells in the conjunctival epithelium, corneal endothelium, and iris in dogs with subacute fulminating canine distemper encephalomyelitis. In this study, CDV amplicons in conjunctival swab samples were detected before viremia and virus detection in conjunctival swab samples lasted for longer period than the other samples. Also, conjunctival swab samples had higher detection rates than the other specimens (p<0.05). Shen et al. [12] observed that viruria in some dogs experimentally exposed to CDV was demonstrable from days 6 through 22 after exposure, however, CDV was detected in all infected dogs only from days 6 to 8 after exposure. The former result coincides with our results, showing that CDV amplicons were detected from 5 to 14 days post-infection and CDV amplicons were detected in all dogs only on 6 days post infection. In this study, CDV amplicon was not demonstrated in CSF samples during the entire experimental period. It is tempting to speculate that puppies experimentally infected with CDV would develop antibodies rapidly and then these antibodies eliminated virus from the circulation [3, 4]. With findings in this study, the conjunctival swab sample is thought to be the most suitable specimen in the early diagnosis of CD, which had higher detection rate than the other specimens and is easy to obtain and non-invasive procedure.

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REFERENCES