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Isolation of Canine Distemper Virus from Clinical Domestic Dogs in Addis Ababa Pet Clinics, Ethiopia

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Research Article

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Abbreviations: NAHDIC: National Animal Health Diagnostic and Investigation Center; CD: Canine Distemper; CDV: Canine Distemper Virus; CPE: Cytopathic Effect

ABSTRACT

Background: Canine Distemper is highly contagious viral disease of canine species with worldwide occurrence. However, previous studies have underreported canine distemper status in Ethiopia. Therefore, the objective of this study was to assess occurrence of CD on clinical dogs in association with other cases in Addis Ababa pet clinics.

Results: Out of 150 dogs that are brought to different pet clinics, 70 cases (46.66%) were identified to have signs suggestive of CDV infection. Of all the 70 suspected cases of CD, ocular and nasal discharge swab samples were collected and screened by fastest distemper strip kit for the presence of virus. Of the screened samples, 22.86% were positive for canine distemper. Screened positive samples were further confirmed using cell culture in Vero cell as gold standard technique. Out of the 16 screened positive samples inoculated in Vero cells, 12 specimen (75%) showed Cytopathic Effects (CPE) in the form of Syncytia formation within 24 and 48 hours of inoculation while the rest 4 negative samples were passaged in Vero cell three times before declaring negative. Uninoculated negative controls included in each run did not show any effects.

Conclusion: The present study revealed the existence of CD showing 16 positive dogs out of 70 cases do not received periodic immunization according to CD strains. Besides this, CD is chief disease of local dogs relative to exotic breed. Therefore, isolation of clinical domestic dogs from healthy dogs and routine immunization of animals according to circulating strains of CD is suggestive.

INTRODUCTION

Canine Distemper (CD) is a highly contagious worldwide occurring infectious disease of canine species caused by Canine Distemper Virus (CDV) under the genus *morbillivirus* of the family *Paramyxoviridae* [1]. Canine distemper virus is an enveloped, single stranded, negative-sense RNA virus which contains six structural proteins: two membrane glycoproteins, the Fusion (F) and Hemagglutinin (H), the envelope–associated Matrix (M) protein, the phosphoprotein (P), the Large polymerase (L) and the Nucleocapsid (N) protein [2]. Additional accessory genes, the C and V proteins are found mostly as extra transcriptional units, with in the P gene. The lipid envelope, surrounding the virion contains the two surface glycoproteins F and H, which mediate viral entry and exit from the host cell. Furthermore, the helical neoclocapsid core, containing the N, P, and L protein, initiates intracellular replication and is located within the envelope. The viral M protein connects the surface glycoproteins and nucleocapsid during viral maturation [3].

Host range of canine distemper virus encompasses all species of the families' *canidae* (Dog, Dingo, Fox, Jackal, Wolf), *procyonidae* (Raccon, Coatimundi, Panda), *mustelidae* (Weasel, Ferret, Fishers, Mink, Badger, Marten, Otter), the large members of the family *felidae* (Lions, Leopards, Cheetahs, Tigers) and the collared peccary (Tayassu Tajacu). At least seven distinct lineages of canine distemper are recognized worldwide, based on the sequence analysis of the H genes: Asia-1, Asia-2, American-1, American-2, and Arctic-like, European wild life, and Europe. Additional lineage probably will be identified in the future [4]. Genetic analysis of strains causing outbreak shows that CDV does not become more virulent and spread to new host species in a region, but the same strain circulates among susceptible animals of several host species in a given geographic area [5].

CD is highly infectious and frequently lethal disease in dogs and has a high mortality rate after rabies. The disease is transmitted through aerosol and the virus has high affinity for lymphocytes and macrophages. The duration and severity of the disease depends mainly on the ability of the infected animal to quickly mount an immune response to CDV. If the serum antibody titer high level within 8-9 days of infection, the virus disappears from the lymphatic and the other tissues and the infection remains subclinical or mild. However, if the immune response is weak or delayed, the virus disseminates too many tissues causing an acute or chronic disease with high mortality [6]. Immunologically naïve populations may experience high death rates. The mortality rates due to CDV infection vary among susceptible species and could be as high as 100% in ferrets [7,8]. Domestic dogs' mortality rates will largely depends on the immune status of the animal ranging up to 50% [9]. Outbreak in Africa wild dogs have led to mortality rate up to 95% [10].

Canine distemper virus is spread by the respiratory route with initial viral replication in respiratory epithelium and alveolar macrophages [11]. The initial infection is in epithelial cells and lymphoid tissue in the nasopharynx [12]. After multiplication in regional lymph nodes, the virus enters the blood stream, where it circulates with in infected B and T-cells. Primary viremia is synchronous with the first bout of fever and virus that is spread to lymphoid tissue throughout the body; circulating gut- associated lymphoid tissues, and fixed tissue macrophages such as kuffer cells in the liver. Virions formed in these sites are carried by blood mononuclear cells during second viremia that coincides with the second peak of fever. Epithelium cells do not possess CD150 (SLAM) and the receptor that facilitates virus entry in to epithelial cells is yet to be defined [7].

Canine distemper virus may be shed in virtually all body secretions and excretions depending on the stage of infection. Transmission most commonly occurs through inhalation of air borne virus or direct contact between susceptible and actively infected dogs. Fomite or environmental transmission of canine distemper virus is also

possible, but the virus does not remain infectious for more than hours to a few days depending on the ambient temperature and other conditions. Fomites and environmental contamination is therefore of less importance for disease transmission than for a hardier virus such as canine parvovirus. Because the virus does not persist long in the environment, mildly affected and recovering animals plays an important role in maintaining transmission cycles in shelters [13].

In dogs, canine distemper virus infection may result in subclinical infection or clinical disease. It is estimated that 75% of the infections occur as subclinical infections. The clinical disease has been characterized by systemic signs (dermatological, respiratory and gastrointestinal) with frequent nervous disfunction. A transient fever usually occurs 3-6 days after infection and there may be a leucopenia (especially lymphopenia) at this time but these signs may go unnoticed. The fever subsides for several days before a second fever occurs, which lasts<1 weeks. This may be accompanied by serous nasal discharge, mucoprulent ocular discharge, and anorexia. Hyperkeratosis of foot pads (hard pad disease) and epithelium of the nasal plane may be seen [14]. Canine distemper virus affects both white and gray matter in the central nervous system. Thus, various neurological signs may be observed including behavioral changes, seizures, cerebellar and vestibular signs, visual deficits, paresis, paralysis, limb weakness, tremors, and myoclonus (is a gray matter sign characterized as a rhythmic jerking of single muscle or muscle groups) [15]. Seizure and myoclonus are typically gray matter signs, while visual deficits and different motor impairment are mainly signs of white matter dysfunction. Signs of leptomeningitis, such as cervical rigidity and generalized hyperesthesia, may also occur [16].

In suspected cases of distemper, a complete blood count and thoracic radiographs used to access leukocyte responses and pneumonia respectively. In dogs presenting with neurologic disease suspected to be due to CDV, routine Cerebrospinal Fluid (CSF) analysis carried out to distinguish CDV infection from other diseases. The presence of CDV- specific antibody in CSF can confirm the diagnosis but requires special laboratory [17]. Routine diagnosis of canine distemper virus by Immuno Flourescence (IF) is applied to various specimens, including conjuctival, nasal, and vaginal smears, using polyclonal or monoclonal antibodies. This test is not sensitive and can detect canine distemper virus antigens only within 3 weeks after infection, when the virus is still present in the epithelial cells. Serological methods such as ELISA and Sero Neutralization (SN) assays have a little diagnostic value because high titers of antibodies to canine distemper virus may be the result of previous vaccination or subclinical or clinical infection [18]. Definitive diagnosis of canine distemper by virus isolation on canine cells is fastidious and time consuming, taking several days to weeks, notably when applied to clinical specimen [19]. During life, clinical diagnosis can be confirmed by finding typical cytoplasmic and intranuclear inclusion bodies in the smears of cells of the respiratory epithelium and peripheral blood. Unfortunately, these inclusions are not present in all cases; hence their absence does not preclude the diagnosis of distemper [11]. Distemper inclusions in canine erythrocytes are irregular to round to ring shaped and stains magenta with Romanowsky and Diff-Quik stains (the inclusions may stain with other rapid blood stains). Distemper inclusions are transient [20]. These inclusions appear as homogeneous, red to purplish, red or pale blue, pleomorphic but homogeneous cytoplasmic inclusions; they are found in neutrophils, monocytes, lymphocytes and erythrocytes. They probably occur in the early viremic stage and before clinical illness [21]. RT-PCR has been applied successfully to diagnosis of canine distemper [22,23].

Control of canine distemper virus infection is based on adequate diagnosis, quarantine, sanitation, and vaccination with attenuated canine distemper virus vaccines. The virus is very fragile, and susceptible to standard disinfectants. Thorough disinfection of premises, however, can be very challenging. For treatment, hyper immune serum or

immune globulin can be used prophylactically immediately after exposure. Antibiotic therapy generally has a beneficial effect by lessening the effect of secondary opportunistic bacterial infections [3]. Canine distemper virus is monotypic virus as defined by polyclonal antisera and a single exposure to the virus normally confers long- lasting immunity in dogs. In general, the introduction of live attenuated canine distemper virus vaccines in the 1950s and their extensive use drastically reduced the incidence of the canine distemper in dogs [24]. Therefore, previous underreporting of canine distemper in Ethiopia together with its strain makes this study mandatory. The principal objectives of the present study were to assess the occurrence of canine distemper relative to other contagious infectious and non-infectious disease of dogs in Addis Ababa, and to isolate CDV from clinical cases of canine distemper.

MATERIALS AND METHODS

Study area

The study was conducted from November 2018 to April 2019 in Addis Ababa pet clinics (Akaki kality, Sholla, Kera) in most clinical dogs which were suggestive to canineS distemper. Addis Ababa is located at the elevation of 2000-3000 m a.s.i. The mean annual rain fall is 1800 mm with bimodal pattern. There are alternating dry and rainy seasons in the area. The long rainy season extending from June to September contributes about 84% of the annual rainfall while dry season last from October to February. The short rainy season lasts from March to May. The mean annual minimum and maximum temperature are 14°C and 21°C respectively with an overall average of 17°C. The mean relative humidity is 61.37 [25].

Study animal

The study was carried out on local and exotic breeds of dogs that brought to Addis Ababa small animal clinics (Akaki-Kality, Sholla, and Kera).

Study design

Purposive study conducted for isolation and characterization of canine distemper from clinical dogs from November, 2018 to April, 2019 by collecting swab specimen from ocular and nasal discharge of clinical dogs on events associated with canine distemper virus in different pet clinics that found in Addis Ababa.

Clinical examination of animal

Domestic dogs that were brought to the different clinics were diagnosed using the client history and methods clinical examination. Those animals showing diarrhea, ocular and nasal discharge, coughing, vomition, digital hyperkeratosis, biphasic fever and central nervous system signs in either single or mixed form were considered as suspected clinical cases of canine distemper and subjected to screening by fastest distemper strip kit test.

Sample collection and transportation

Clinical specimens (ocular and nasal swabs) were collected from suspected cases of canine distemper on clinical examination of animals. Swab samples were collected aseptically from ocular and nasal discharge of clinical dogs screened by fastest distemper strip kit test and was chilled in ice pack immediately after collection and specimens collected were transported in Viral Transport Medium (VTM) with antibiotics. Antibiotics and antimycotics were added to prevent microbial growth.

Fastest distemper strip kit test

Samples collected from dogs with clinical signs suggestive of canine distemper were tested by sensitive, reliable and specific immunochromatographic assay, Fastest distemper strip, designed to detect canine distemper virus

antigen in nasal and ocular discharge. This test is a rapid immunochromatographic screening test for the detection of canine distemper virus antigen in nasal and ocular discharge. The method employs a unique combination of a specific antibody binding protein, which is conjugated on dye particles, and a second monoclonal antibody which is immobilized to the solid phase membrane. As the test samples flows laterally across the membrane, the specific binding antibody protein–dye conjugate bind to canine distemper antigens in the sample. Then, if the sample contains any antigen to canine distemper, the complex binds to the antibody on the solid phase in the test zone producing a pink/purple band. In the absence of canine distemper antigen, there is no line in the test zone. The liquid continues to migrate along the membrane and produces a pink/purple band in the control zone demonstrating that the reagents are functioning properly and that the next test has been carried out correct. The test components include specimen tubes; dipsticks distemper tests in foil pouches, sample collection swabs, dropper bottle containing 5.0 ml buffer diluents.

Virus isolation

Each swab specimen positive with screening immunochromatographic assay in the field was individually inoculated on sub cultured monolayer Vero cells after centrifugation of specimen at 4000 rpm for 20 minutes in tissue culture flask. The flask incubated at 37°C for 60 minutes to allow the virus to absorb on to the cell culture in humidified incubator and examined daily for Cytopathic Effect (CPE) which is observed as gaint multinucleated syncytium formation and detachment of cells. Each sample was inoculated in Vero cells and uninoculated flasks used as negative controls were included in each run. Each sample in the virus isolation passaged in cell culture three times before declaring specimen negative.

Data management and analysis

The raw data was collected and entered in a computer Microsoft-excel spread sheet then was analyzed by using STATA, version 11 software. Chi-square (x^2) test was used to determine the association of different risk factors and p-value of less than 0.05 was taken as statistically significant difference.

RESULTS

Result of clinical examination

Out of the total of 150 dogs clinically examined, 70 domestic dogs were identified as suspected cases of canine distemper based on the client history and clinical examination. These identified cases other than CD show the relative importance of canine distemper while comparing it with other types of disease during study time (Table 1).

Table 1. Major diseases of domestic dogs clinically diagnosed in Addis Ababa pet clinics tentatively diagnosed cases in domestic dogs.

Diagnosed disease	Frequency	Percent (%)
Rabies	6	4
Ascaris	8	5.33
Babesiosis	13	8.67
Dermatophytosis	6	4
Canine distemper	70	46.67
Ecto-parasitism	6	4
Kennel cough	7	4.67
Parvovirus	13	8.67
Miscellaneous	13	13.35

Screening of samples by fastest distemper strip kit test

Out of 70 clinically suspected case of CD and tested using fastest distemper strip kit test, 16 (22.9%) were positives. This is based on pink/purple band line formation in both test and control zoned formation in the test zone, negative samples. From the 16 dogs tested by the screening test 12 (75%) were aged less than one year while 4 (25%) were greater than twelve months old. Eleven (68.75%) of the positives dogs were males while the rest 5 (32.25%) were females. There was no statistical significant difference in the percentage of infection among the sexes and different age groups of domestic dogs. Out of the 16 positive samples 2 (12.5%) were exotic and the rest 14 (87.5%) were local breeds. There is statistical significant ($p<0.05$) difference in infection rate in different breed. All sixty positive dogs had a history of no prior vaccination to CDV (Table 2).

Table 2. FASTest DISTEMPER Strip kit test result with associated risk factors.

Risk factors	No examined	No positives	Prevalence (%)	Chi-value(x^2)	p-value
Sex					
Male	51	11	21.57		
Female	19	5	26.32	0.1769	0.674
Age					
Puppy	48	12	25		
Adult	22	4	18	0.3977	0.528
Breed					
Exotic	24	2	8.33		
Local	46	14	30.43	4.3691	0.037

Virus isolation in cell culture

The 16 FASTest DISTEMPER Strip kit test positive specimens were processed and inoculated in tissue culture for isolation of CDV. The 12 out of 16 (75%) screened positive samples were showed Cytopathic Effect (CPE) seen as giant multinucleated Syncytium formation observed in positive specimens between 24 and 48 hours of inoculation while rest of 4 negative samples were passaged in cell culture three times before declaring as negative. Uninoculated negative controls included in each run did not show any effects.

Clinical features of CD in clinical domestic dogs

Of the 12 dogs that were positive in virus isolation through cell culture and screening test, two showed systemic signs only (vomition, diarrhea, conjunctivitis, ocular discharge, depression, anorexia). Nine dogs showed combination of systemic and respiratory (nine dogs) and one dog displayed combination of cutaneous (digital hyperkeratosis), systemic and respiratory form of the disease. There were no dogs which showed nervous and respiratory signs only (Table 3).

Table 3. Description of clinical presentation of CD in positive animals by screening test and cell culture.

S.N	Sex	Age	Breed	Clinical sign	Vaccination history
1	Male	Puppy	Local	Ocular, nasal discharge, coughing, hemorrhagic diarrhea, depression, fever, conjunctivitis	No
2	Male	Puppy	Local	Coughing, vomition, diarrhea, nasal discharge, conjunctivitis, anorexia	No
3	Male	Puppy	Local	Hemorrhagic diarrhea, fever, coughing, ocular and nasal	No
4	Male	Puppy	Local	Coughing, ocular discharge, conjunctivitis, Diarrhea, depression	No
5	Male	Adult	Exotic	Coughing, diarrhea, depression, conjunctivitis, ocular discharge	No
6	Male	Adult	Local	Anorexia, diarrhea, depression, vomition, ocular discharge	No
7	Male	Adult	Local	Anorexia, diarrhea, vomition, ocular, nasal discharge	N
8	Female	Puppy	Local	Diarrhea, depression, anorexia, conjunctivitis, ocular discharge	No
9	Female	Puppy	Local	Ocular, nasal discharge, anorexia, depression, weakness,	No
10	Male	Puppy	Local	Coughing, diarrhea, conjunctivitis, weakness, fever	No
11	Male	Puppy	Local	Coughing, hemorrhagic, diarrhea, ocular and nasal discharge	No
12	Male	Puppy	Local	Diarrhea, ocular, nasal discharge, conjunctivitis	No

DISCUSSION

Canine distemper is endemic in Africa as in other parts of the world. Under reporting is a characteristic of the almost every infectious disease in most developing countries [26]. Several approaches have been used for the diagnosis of canine distemper [27]. In this study, CDV screened by fastest distemper strip kit test and isolated by cell culture in Vero cells from clinically sick domestic dogs. Out of the 70 samples collected from suspected cases of CD, 16 (22.85%) were found to positive with Fastest distemper Strip kit test. This result is much lower than the findings of Latha and Srinikasan [28]. WHO reported 70% over all prevalence based on screening of the conjuctival samples by Dot-ELISA in India. This may be due to variation in global distribution of the CDV strains with its virulence, severity and the season that the samples were collected. Severity of canine distemper depends on the virulence of the virus, immune competence and age of the affected dogs.

In this study, there was no statistical significant difference among age groups of domestic dogs except numerical difference. Thus, puppies (less than 1 year) as well as adult (greater than 1 year) were equally susceptible to CDV infection. This finding is different from the other reports where the dogs of 3-6 months of age more susceptible [29]. According to Latha and Srinikasa, dogs of 1-5 years of age, which means in our study Adult, were susceptible to CDV infection because of the interference of maternal antibodies during primary immunization or poor storage and handling of the vaccines or immune status of the animal, which would result in quick depletion of vaccine antibodies and the lack of the routine vaccination of dogs. The equal susceptibility of the puppy and Adult age groups to CD infection in this study could be because of lack of routine vaccination of dogs. For thus, there were no any maternal derived distemper antibodies that passed to puppies to develop immunity makes equal susceptibility of both age groups to CD. Improved vaccination has reduced the frequency and magnitude of canine distemper outbreaks demonstrate that the lack of vaccination against distemper was associated with several hundred-fold in the risk of the disease [30,31]. However, 16 (22.85) CDV positive dogs in our study had been unvaccinated against CDV rather than rabies.

Both sexes were equally affected (22:26%) by CDV infection in this study. This is the synonymous result [32]. In an epidemiological study of 250 cases in Brazil and in Texas, USA [33]. The percentage of the CDV infected cases was high in local breeds of domestic dogs according to this study while according to [27]. Brachycephalic dogs have been reported to have a lower prevalence of disease, sequelae and mortality compared with dolichocephalic breeds. This may be due to the variation of the immune status of both breeds (local and exotic) of dogs and also variability of handling local and exotic breeds of dogs in Ethiopia, management difference among two breeds.

The gold standard for the diagnosis of virus infection has for a long time been virus isolation in cell culture. Virus isolation is important not only to confirm a diagnosis and provide material for direct sequence analysis but also for investigation of the pathogenesis in animal experiments and vaccine improvements [34]. Thus, virus was not isolated from all submitted ocular and nasal swab specimens obtained from virus positive dogs. The 12 swab samples studied yielded CPE with in 24 and 48 hour while the rest 4 swab samples were negative from the total of 16 ocular and nasal swab samples positive by screening test. These 4 positive samples in screening test and becoming negative in cell culture is may be due to difference in sensitivity and specificity of the tests. It may also be due to problem while collecting, transporting the specimen through transport medium and storage of specimen, contamination when inoculating virus in sub cultured monolayer cells which support the statement that CDV survives in the environment for weeks at near-freezing (0°C-4°C). Isolate CDV using Vero-Dog SLAM cells in South Africa from brain, spleen and CSF [35]. Accordingly, two brain samples studied yielded CPE only after 48 hours while spleen samples from the same animals yielded CPE within 24 hours, Cerebrospinal fluid samples studied did not yield CPE.

In present study, animals displayed a variety of clinical signs characteristic of canine distemper. From 12 (7.5%) positive specimens in both test, two had systemic signs only, nine dogs had a combination of systemic and respiratory signs and one dog displayed combination of cutaneous (digital hyperkeratosis), systemic and respiratory form of the disease. There were no any nervous and respiratory signs only. These results were quite different from retrospective study undertaken in South Africa on 133 cerebrospinal fluid samples where 34 (25%) were positive, of which 23, 4, and 2 had only nervous, systemic and respiratory signs respectively. Four had a combination of nervous and systemic signs, one had both respiratory and systemic signs and no dogs were observed to have a combination of nervous and respiratory signs [36]. In a Finnish study, which reported 30% mortality, the majority showed classic respiratory signs and a few cases of digital hyperkeratosis. Lymphadenopathy has been reported among Nigerian dogs with distemper [37]. This difference could arise from distribution of the different strains in different parts of the world, breed difference in susceptibility of particular countries, vaccination status.

The rest negative samples in this study were mostly obtained from animals showing respiratory and systemic signs. In this investigation, many cases were incorrectly diagnosed with canine distemper when viewing other related clinical signs which may usually due to viral and/or bacterial agents which may show the difficulty of diagnosing CD based on clinical examination

CONCLUSION

Canine distemper is endemic disease in Africa and cause high mortality rates in the immunologically naïve population of dogs. The result of the present study revealed the existence of CD in the study area. Present study indicated that, sixty positive domestic dogs out of 70 cases do not received periodic immunization according to CD strains which mainly aggravates the disease occurrence. Besides this, the current study showed the presence of statistically significance difference in the percentage of infection between breeds and occurrence of CD indicating,

CD is chief disease of local domestic dogs. Since the disease is immunosuppressant and mainly associated with opportunistic infections, whole confirmed positive domestic dogs displayed systemic signs of CDV infection in current study. Therefore, isolation of clinical domestic dogs from healthy ones, good management practice, public awareness creation about the disease, routine immunization of animals according to circulating strains of CD is mandatory.

DECLARATIONS

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Availability of data and materials

All data are available up on request.

Authors' contribution

MS and ET were participated in the study design, sample collection, data analysis and write up of the draft and final version of the manuscript.

Ethical approval and consent to participate

The study protocol was approved by viral serology department of National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta. The purpose of study was explained to clients and verbal agreement was obtained before commencing to the study.

Consent for publication

Yet not established

Competing interests

The authors declare that they have no conflict interests

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