

Molecular characterization of canine distemper virus circulating in Ethiopia

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Abstract

High mortality and morbidity of canine were reported from Addis Ababa city administration and Bedele and Nekemte zonal towns of western Ethiopia in 2010. A team from National Animal Health and Investigation Center (NAHDIC) was assigned to investigate what caused the death of dogs. Varying ranges of clinical signs were observed which include febrile condition, body temperature of 39.5 to 41.5°C, Runny eyes, nasal discharges, vomiting, diarrhea, various neurologic disorders and hyperkeratosis of foot pad “hard pad disease”. Death occurred in all ages of dogs but most death was occurred in puppies. Some owners reported that they lost all the puppies they had at time of the outbreak. About 200 dogs were died only in Nekemte while number of death in Bedele (Ilubabore) and Addis Ababa were not exactly known. Eye swab from live animals (10 from Addis Ababa 5 from Nekemte and 5 from Bedele) and tissue samples (liver, kidney, lymph node and brain) from Nekemte and Bedele) were collected and tested using antigen detection fast kit. Virus isolation and molecular characterization was carried on these canine distemper positive samples and also brain tissues were tested for rabies virus (lyssaa virus) and found negative with molecular test. The investigation result showed that canine distemper virus was responsible for the outbreak canine disease in the areas. Sequencing of positive samples from all distantly located area indicates that Asia-1 lineage canine distemper virus is circulating in outbreak. Vaccination of the dogs against canine distemper virus is required in order to control the disease.

Keywords: Antigen detection, Canine distemper virus, Dog

Introduction

Canine distemper is a highly contagious systemic viral disease of canidae (dogs, foxes and wolves), Mustelidae (ferret, mink, skunk, wolverine, marten and badger), most Procyonidae (eg, raccoon, coatimundi), some Viveridae (binturong), red pandas (Ailuridae), Elephantidae (Asian elephant), primates (Japanese monkey) and large Felidae and distributed worldwide (Martella *et al.*, 2008). Canine distemper virus belongs to the genus *Morbillivirus* in the *Paramyxoviridae* family, possesses a single-stranded negative RNA encoding six non overlapping transcriptional units which produce eight proteins (Herder and Adme, 1997; Griffin, 2007; Van Regenmortel *et al.*, 2000). There is one serotype and seven different genotypes have been described (Asia-1, Asia-2, Europe, European wildlife, Arctic-like, America-1 and America-2).

Canine distemper virus transmitted from domestic dogs to wildlife and vice versa (Kapil and Yeary, 2013). The domestic dog has largely been responsible for introducing canine distemper to previously unexposed wildlife and now causes a serious conservation threat to many species of carnivores and some species of marsupials. It played a considerable role in the extinction of the thylacine (Tasmanian tiger) and recurrently causes mortality among African wild dogs (McCarthy *et al.*, 2007). In 1991, the lion population in Serengeti, Tanzania, experienced a 20% decline as a result of canine distemper (Assessment, 2005). Outbreaks of canine distemper continue to occur throughout the United States and elsewhere in world, and are caused by many factors. These factors include the over population of dogs and the irresponsibility of pet owners (ASPCA, 2012).

Canine distemper is sometimes also called hard pad disease because certain strains of the virus can cause an abnormal enlargement or thickening of the pads of an animal's feet. Clinically, it is characterized by a diphasic fever, leukopenia, gastro-intestinal tract and respiratory catarrh and frequently pneumonic and neurologic complications with canine distemper virus outbreak. The objective of this investigation was to identify canine distemper disease and characterize the virus circulating in dog population in Addis Ababa and western Ethiopia.

Material and Methods

Study area

Addis Ababa is the capital city for Federal democratic Republic of Ethiopia and residence to many diplomats including African Union. Bedele a town found in Ilubabor zone while Nekemte is zonal city of eastern Wellega zone of Oromia regional state.

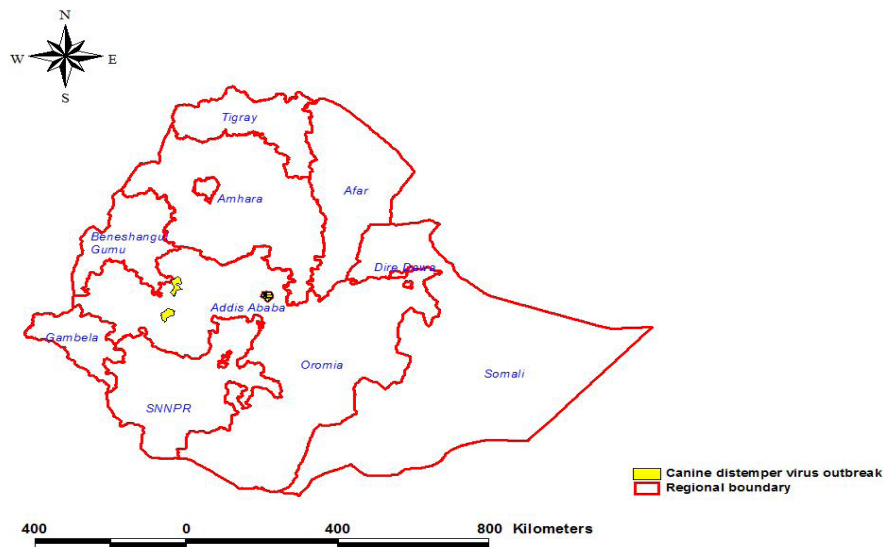


Figure 1: Map showing canine distemper virus outbreak areas

Study design

Canine distemper virus outbreak was reported from 2010 to 2013 Bedele, Nekemte zonal town and Addis Ababa city. NAHDIC involved in investigation outbreak in canine population. The study design was a combination of clinical examination and active disease investigation in response to an outbreak reported by district animal health professionals.

Sample collection

Dogs manifesting the clinical signs of canine distemper were thoroughly examined and ocular discharge was collected by clean sterile swab and placed in tube. Identification number was given to each sample including date of collection, places of collection, history of vaccination for rabies and canine distemper diseases. For those samples collected in Addis Ababa city swabs were tested at spot using commercial antigen detection test and those positive samples were stored at virus transport medium (VTM) in falcon tube and transported to NAHDIC. Those samples collected from Bedele and Nekemte were tissue samples (liver, lung, spleen, and heart and brain tissue) from animal dead by the diseases. These samples were preserved in virus transport medium (VTM) and transported in cold-chain to National Animal Health Diagnoses and Investigation Center (NAHDIC) and kept in -70°C until test.

Clinical examination

In Bedele and Nekemte the investigation included clinical and postmortem examination and swabs and tissue samples were collected. While in Addis Ababa those dogs that were brought for clinical examination or vaccination were examined for canine distemper disease for showing any febrile condition by rectal thermometer (to check febrile condition) and external symptoms (runny eyes, nasal discharge, vomiting, diarrhea, various neurologic disorders, hyperkeratosis of foot pad “hard pad disease” were examined and the result recorded on data record sheet.

Laboratory test

Canine distemper antigen detection test used to detect canine distemper virus from swab and tissue samples. The test was done using Fast Antigen Detection Test-Kit (Produced by Diagnostic MegaCor, Austria) in accordance to instruction in kit. Ten drops of buffer diluents were placed in test tubes and then allowed to mix well in diluents. The swab was squeezed to get back all fluids in the specimen tube. The dipstick was then dropped in to the specimen tube for 1 minute. Then the dipstick was removed from the specimen tube and placed on flat dry surface. Result was red after ten minutes. The test was considered positive when the test zone and control zone show a pink/purple band and negative when only the control zone shows a pink/purple line. If there was no pink/purple band neither in the test nor control zone it was considered conclu-

sive and the test repeated with the new dipstick. Twenty swabs from clinically sick dogs 10 from Addis Ababa and 5 swabs from each Bedele and Nekemte towns were tested by antigen detection. Post mortem samples were collected from two puppies one from Bedele and the other from Nekemte respectively. Liver, kidney, lymph nodes and brain tissues were also tested as above.

Those positive swabs and tissue samples by antigen detection test were subjected to cell culture on Vero cell lines for virus isolation. After the third passage the growing cultures were freeze and thawed then centrifuged at 3000 rpm. The supernatant was collected and retested again by using antigen detection to check whether the virus was canine distemper or not. Five tissue samples (Liver, kidney, lymph node, brain) six swabs and one virus isolate altogether 12 samples were sent to the OIE collaborating Center for diseases at Animal /Human Interface, FAO reference Center for rabies in Padua, Italy. Real time polymerase chain reaction (rT-PCR) and sequencing was carried out by reference laboratory.

Results

Clinical examination

Dogs showed the following clinical signs. They were in febrile condition, body temperature of 39.5 to 41.5°C were recorded. Runny eyes, nasal discharges, vomiting, diarrhea, various neurologic disorders and hyperkeratosis of foot pad “hard pad disease” were observed. Mucus membranes were congested in most of clinical cases. Death occurred in all ages of dogs but it was higher in puppies. About 200 dogs died only in Nekemte while the number of deaths in Bedele and Addis Ababa were not exactly known and it was suspected to be greater than this. It is only in Addis Ababa that different breeds of dogs are owned while in Bedele and Nekemte only local breed of dogs are owned.

Virus isolation

Canine distemper virus was isolated from 20 swabs which was previously positive by antigen detection and from tissues (Liver, Kidney and Lymph node) that were collected from 2 postmortem puppies at Bedele and Nekemte. The virus was grown on vero cell line and followed for cytopathic effect on second and third passages. The growth of virus on cell line was confirmed by antigen

detection kit. The canine distemper virus was recovered from all swabs and tissue samples.

Referral laboratory result

Among 12 samples that were sent to OIE collaborating Center for diseases at Animal /Human Interface, FAO reference Center for rabies in Padua, Italy, four tissues (liver, spleen, kidney and lymph node) all were positive for canine distemper virus by rRT-PCR and genetic analysis shows Asia-1 lineage. Brain tissue was tested for both canine distemper virus and Lyssaavirus by rRT-PCR and it was negative for Lyssaavirus and positive for canine distemper virus. Among the six swab samples and one virus Isolate only one swab was Negative by rRT-PCR all others were positive for canine distemper virus. Sequencing of rRT-PCR positive samples showed Asia-1 lineage of canine distemper virus.

Discussion

Canine distemper as it was observed from clinical examination a highly contagious systemic, viral disease of canidae and caused death to more than 200 dogs usually puppies with varying clinical signs of runny eyes, and nasal discharge, vomiting and diarrhea, various neurologic disorders and hyperkeratosis of foot pad “hard pad disease” and mucus membrane. It was seen that canine distemper was very important diseases for pet animal keepers of different breeds of dogs. It was only in Addis Ababa city administration that treatment and canine distemper virus vaccination for pet animals was practiced. Veterinary service for canine was very hardly done in Nekemte and Bedele where there is no facility to treat dogs. Canine distemper is the second pet diseases that seek veterinary services in the study areas. Handling and clinical examination of unvaccinated dogs for rabies imposes health risk for professionals.

Canine distemper virus transmitted from domestic dogs to wildlife and vice versa (Kapil and Yeary 2013). The disease affects wild canidae (foxes, wolves), Mustelidae (eg, ferret, mink, skunk, wolverine, marten, badger), most Procyonidae (eg, raccoon, coatimundi), some Viveridae (binturong), red pandas (Ailuridae), Elephantidae (Asian elephant), primates (Japanese monkey), and large Felidae and distributed worldwide (Martella *et al.*, 2008). Study done in South Korea in wild raccoon dogs showed that forty-five of the 102 animals (44.1%) were seropositive by antibody detection (Cha *et al.*, 2012). Canine distemper

played a considerable role in the extinction of the thylacine (Tasmanian tiger) and currently causes mortality among African wild dogs (McCarthy *et al.*, 2007). The lion population in Serengeti, Tanzania, experienced a 20% decline as a result of the disease (Assessment, 2005). The circulation of this virus in dog population will be a treat to our wild life.

Conclusion

In our study molecular characterization of the virus showed that Asia-1 lineage virus circulate in Addis Ababa, Bedele and Nekemte. From our investigation it can be recommended that vaccination of dog against canine distemper virus is required to safeguard our wild canidae.

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