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https://dx.doi.org/10.4314/nvj.v39i1.5 **ORIGINAL ARTICLE**

Epidemiology of Canine Distemper in Makurdi, Nigeria

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SUMMARY

Canine distemper (CD), a major disease of dogs all over the world, hitherto controlled by extensive vaccination of dogs around the world, appears to be persisting and even re-emerging in many parts of the world. It is thought that wild life reservoir hosts contribute to the emergence of CD, but in areas of the world with minimal wildlife contact with domestic dogs, it is thought that cyclical infection between clinically normal dogs and susceptible neonatal animals may be responsible for maintaining the canine distemper virus (CDV) in the canine population. We decided to examine clinically normal dogs in the Makurdi metropolis for evidence of infection with CDV, to determine if such dogs may act as sources of persistence of the CDV in the canine population. We tested 70 unvaccinated, clinically normal dogs for evidence of distemper virus using a rapid CDV antigen (Ag) chromatographic assay test kit designed for the qualitative detection of canine distemper virus antigens in urine, conjunctiva, serum or plasma. We found six (6 or 8.6%) of the 70 dogs positive for distemper antigen; three (3) of the dogs were under one (1) year of age, whereas three were 5 years or more. We conclude that the CDV is circulating among clinically normal dogs in Makurdi, and that a cyclical infection between infected but clinically normal adult dogs and puppies may be responsible for maintaining the disease in the canine population in Makurdi. Further studies are necessary to elucidate the role of vaccination and the possibilities of emergence of new antigenic strains of CDV in the epidemiology of CD in the Makurdi area.

Key words: Canine Distemper, Vaccination, Laboratory diagnosis, maintenance in population, Makurdi, Nigeria.

INTRODUCTION

Canine Distemper (CD) is a pantropic worldwide infectious disease caused by canine distemper virus (CDV), a member of the genus *Morbillivirus* within the family *Paramyxoviridae*. CDV is an enveloped virus and has a non-segmented negative-stranded RNA genome (Del PuertoI *et. al.*, 2010). Clinically, CD is characterised by

diphasic fever, leukopenia, gastrointestinal and respiratory catarrh, frequently pneumonia and often, neurologic complications. The disease occurs in a wide range of domestic and wild animal species, including *Carnivores* (dogs, foxes, and wolves), *Mustelidae* (ferret, mink, and skunk), *Procyonidae* (raccoon, coatimundi) *Ailuridae* (red panda), *Ursidae* (bear) *Elephantidae* (Asian elephant), *primates* (Japanese monkey) *Felidae* and some *Viveridae* (binturong) (Gaskin, 1974; Greene and Appel 1990; Appel and Montali 1994; Appel *et. al.,* 1994; Cook and Wilcox 1981; Deem *et. al*., 2000; Qui and Mainka 1993). Many of these wild animals serve as reservoirs for maintaining the virus in the canine population where there is direct or indirect contact with domestic dogs, however, it is puzzling how the disease continues to persist in domestic dogs in urban communities where there is high level of vaccination and little contact with potential wildlife reservoir hosts. Despite extensive vaccination in many regions, canine distemper remains a major disease of dogs worldwide. Epidemics have occurred in dog populations in isolated areas where the disease had been absent for several years (Greene and Appel, 2006). One possibility is that the virus is maintained in the canine population through a constant supply of puppies that provides susceptible populations for infection (Greene and Appel, 2006). The other possibility is that dogs imported (or straying) from other communities bring the virus.

Vaccination has been used widely for the control of CD, however, during the last decade**,** sporadic reports of reemergence of CDV has become commonplace, and there is anecdotal evidence that the number of CD cases has increased as much as four- to fivefold in dogs in the last few years despite extensive vaccination (Kapil *et. al*., 2008). It is estimated that 25% to 75% of dogs susceptible to CD are infected sub-clinically and are transmitting the virus without showing clinical sign of disease (Greene and Apple 2006). Such asymptomatic dogs are not diagnosed and may be important CDV reservoirs. It is therefore essential to investigate canine distemper virus occurrence in asymptomatic dog populations, especially as it is thought that contact among clinically or sub-clinically infected dogs may be the main method of maintaining the virus within the dog population (Greene and Apple 2006), the objective of the study described here is to evaluate normal dogs in the Makurdi area for evidence of infection with canine distemper virus.

MATERIAL AND METHODS

Samples were collected from clinically normal dogs that had no history of vaccination against canine distemper. Sampling was done over a period of 6 months (August, 2012 to January, 2013). The area sampled included six (6) districts of the Makurdi municipality: (i) Judge's Quarters, (ii) New GRA, (iii) Wadata, (iv) Ankpa Quarters, (v) Lobi2 Quarters and (vi) High Level (Figures 1 and 2). Demographic information obtained on the dogs included: the age (estimated), breed, sex, apparent health status, hydration status, character of mucous membrane, body condition, vaccination status and obvious gross abnormalities. Blood (2-5mls) was collected from each dog by routine venipuncture of the cephalic vein, deposited into plain (no anticoagulant) sample bottles, and immediately transported to the laboratory where the serum was separated by centrifugation at 1500 rpm for 10 minutes. The serum was stored at -20^{o}C until analysed.

Figure 1: Map of Benue state

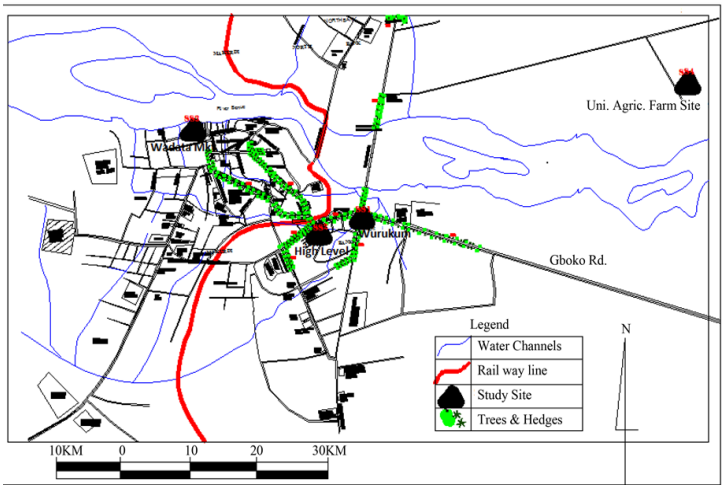


Figure 2: Map of Makurdi showing the area sampled

Laboratory assay

A rapid CDV antigen (Ag) chromatographic essay test kit for the qualitative detection of canine distemper virus antigens in urine, conjunctiva, serum or plasma, was used to determine presence or absence of canine distemper antigen in the sera. The rapid canine distemper virus Ag Test Kit includes internal control lines to validate each test (Figure 3 below) The specially selected canine distemper rims (monoclonal) antibodies are used in test band as both capture and detector materials. The rims antibody allows the rapid CDV Ag Kit to identify canine distemper rims antigen in conjunctiva, urine, serum or plasma with a high degree of accuracy. The test system is described below.

Rapid CDV Ag Test Kit

The Rapid CDV Ag Test Kit is chromatographic immunoassay for the qualitative detective of Canine Distemper virus antigen in conjunctiva, urine, serum or plasma.

Principles

The rapid Canine Distemper virus Ag Test Kit has a letter of "T" and "C" as test line and control line on the surface of the device. Both the test line and control line in result window are not visible before applying any samples. The control line is used for procedural control. Control line should be always appeared. If the test procedure is performed properly and the test reagents of control line are working. A purple test will be visible in the specimen.

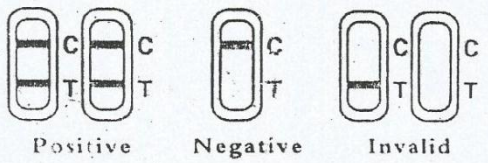
The specially selected Canine Distemper virus antibodies are used in test band as both capture and detector materials. These enable the Rapid CDV Ag Kit to identify Canine Distemper virus antigen in conjunctiva, urine, serum or plasma with a high degree of accuracy.

Materials provides

1) Rapid CDV Ag Test Kit
2) Specimen tubes containing assay diluent buffer
3) Disposable droppers
4) Instruction for use

drops of serum or plasma into the specimen tube containg of 300µl of assay diluent using the dropper.

3) Mix the swab samples with assay diluent to extract well.
4) Remove the test device from the foil pouch and place it on a flat and dry surface.
5) Add four (4) droppers of the mixed sample into the sample hole using the dropper, drop by drop and solely.
6) As the test begins to work, you will see purple color move across the result window in the centre of the test device. If the migration has not appeared after 1 min, add one more drop of mixed sample to the sample well.
7) Interpret test results at 5-10min.



Interpretation

Figure 3: Summary of the CDV Rapid Antigen Test system

RESULTS and DISCUSSION

Six (6 or 8.6%) of the 70 samples collected were positive for distemper antigen (Table 1) Three (3) of the dogs were under one year of age, whereas the other three were aged 5 years or more. All the positive dogs came from 2 of the 6 districts surveyed,

suggesting that these two districts were experiencing

TABLE I: Summary of the characteristics of the distemper positive dogs

Date bled	Age	Breed	Sex	Health Status Indicators	Location
18/10/12	> 5 yrs.	Mongrel	M	Pale mucous membranes	New GRA
20/10/12	3 months	Rottweiler	M	Healthy	New GRA
22/10/12	7 yrs.	Mongrel	M	Emaciated, pale mucous membrane	New GRA
27/10/12	5 yrs.	Mongrel	M	Healthy	Ankpa Quarters
30/10/12	8 months	Mongrel	F	Healthy	Ankpa Quarters
31/10/12	7 months	German Shepherd	M	Healthy	Ankpa Quarters

Mongrel = mixed local breed dog

distemper outbreaks during the period of the survey. None of the dogs showed clinical signs of distemper, suggesting that they were either undergoing recent infection (especially the young dogs) or were recently recovered and were still shedding the virus, or were undergoing subclinical infection.

The results indicate that CDV is circulating among clinically normal dogs in Makurdi, albeit at a low level. It is estimated that 25% to 75% of dogs susceptible to CD are sub-clinically infected and are transmitting the virus without showing clinical sign of disease (Greene & Apple 2006) The low level detected is probably because of the low sensitivity of the test system used. Several laboratory tests are available to confirm clinical CDV infection; however, most of the commonly used tests may not be sufficiently sensitive to detect subclinical infection. Immunofluorescence (IF) on conjunctival, nasal, and vaginal smears can detect CDV antigens only within 3 weeks after infection, when the virus is still present in the epithelial cells (Appel and Jones 1967). Virus has also been demonstrated in external epithelia of recently vaccinated as well as sick dogs using polymerase chain reaction (PCR) (Kapil and Neel, 20i5). Immunofluorescence is thought to have low sensitivity and can generate false negative (Del PuertoI *et al*. 2010). Studies using real time PCR showed that 54.5% of dogs with asymptomatic canine distemper were positive for CDV (Del PuertoI *et al*., 2010). So far, there is no data reporting the percentage of CDV infected dogs with subclinical CD in Nigeria. A sensitive assay, such as Real Time PCR would be required for such epidemiological studies to determine the level of subclinical infection that may contribute to the persistence and transmission of CDV in our canine population (Del PuertoI *et al*., 2010 Kapil, *et al*, 2008).

Reports of reemergence and increased incidence of CDV on several continents suggests that there are problems with the vaccines in use around the world (Appel and Jones, 1967; Greene and Appel, 1990; Kapil *et. al*., 2008; Del PuertoI *et al.*, 2010). Most vaccine strains of CDV were isolated between 1930 and the 1950s. These vaccine strains (Ondersteport, Snyder Hill, and Lederle strains) were used in CDV vaccines worldwide. The wild-type strains of CDV related to the vaccine strains are no longer detected in the domestic canine populations in the United States (Norris *et. al* 2006). Wild-type CDV isolates identified in a recent study (Kapil, *et. al.,* 2008) were genetically and phylogenetically distinct from the vaccine strains of CDV, and showed less than 90% identity in H gene sequence with commercial vaccine CDV isolates.

It may well be that the vaccines currently in use in Nigeria are not effectively protecting

the vaccinated dogs from CDV strains currently circulating in Nigeria. In recent studies in South Africa by Woma and collaborators (Woma and van Vurren, 2009, Woma *et al*., 2010) the H gene of vaccines in use in South Africa was sequenced and compared. The sequences obtained from the sick dogs showed 100% nucleotide identity and was different from the H genes found in virus strains used in vaccines, as well as in virus isolates from other parts of the world as documented in in GenBank. The results suggest that a novel CDV lineage may be present in South Africa and the researchers concluded that a recent reversion of vaccine virus to virulence was not the cause of the clinical signs seen in dogs with a previous history of vaccination.

Further studies are needed to characterize distemper viruses isolated in Nigeria to determine presence of new strains circulating which may contribute to the epidemiology of the disease in Nigeria. It may be instructive to evaluate serum samples from vaccinated and unvaccinated dogs to determine the efficacy of dog vaccination in Nigeria. False-negative results may occur if samples are taken late in the course of infection, because antibody produced by the dog may coat the viral antigen and produce a false-negative result. (Guy, 1986). It is therefore likely that the actual extent of infection in clinically normal dogs may be higher than the 9% recorded in this study.

Future studies will aim at collecting more samples at various periods of the year, and also to conduct phylogenetic studies on the viruses isolated in the study, to compare with vaccine viruses and isolated from other parts of the world.

Conclusion

We conclude that distemper virus is circulating in clinically normal dogs in the Makurdi area, and that the virus is maintained in the canine population by circulating between young, presumably susceptible puppies and dogs undergoing subclinical infection or clinically infected and recovering from the disease.

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