



Epidemiological Dynamics of Canine Morbillivirus in Resident Dogs of Makurdi Metropolis, Benue State, Nigeria

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ABSTRACT

Canine distemper is an endemic viral disease of dogs in Nigeria. Knowledge of the carrier status of dogs is key to successful control of the disease. In this study, the carrier status of apparently healthy dogs' resident in parts of Makurdi metropolis was determined using the immunochromatographic rapid antigen assay kit for qualitative detection of canine morbillivirus antigens in ante-mortem samples. Ocular-, nasal-, and rectal- swabs, as well as serum were taken from each of 204 dogs bringing a total of 816 samples. The results showed that 26.96 % of the dogs sampled were positive for Canine morbillivirus antigens. Viral antigens were detected in 9.8%, 11.27%, and 4.41% of nasal, ocular, and rectal swaps respectively, and in 6.37% of the serum samples collected. An age-related susceptibility was observed as viral antigens detection rate was higher in younger dogs compared to older ones. Similarly, 40.43% of vaccinated and 22.93% of unvaccinated dogs were positive for canine morbillivirus antigens. Of significant importance is the prevalence rate in unvaccinated population of dogs. In terms of breed-related detection rate, 23.93% of Nigerian local dogs and 40.63% of exotic breeds tested positive for viral antigens, and it was observed that 25.54% of dogs which had history of contact with other dogs and 40% of dogs which had no such history carried canine morbillivirus antigens. The significance of this study is that it details the current epidemiological dynamics of canine morbillivirus in resident dogs of Makurdi metropolitan area, and the findings are discussed.

Subject Areas: Animal infectious disease epidemiology.

Key words: canine distemper, canine morbillivirus antigens, antigen detection rate, carrier status, immunochromatographic assay, epidemiological dynamics.

INTRODUCTION

Canine morbillivirus, formerly known as canine distemper virus (CDV), is a member of the *Morbillivirus* genus of the *Paramyxoviridae* family of public health , and economically important viruses (De Vries et al., 2015). Morbilliviruses are considered to be one of the most infectious group of viruses known, and associated morbidity and mortality rates of 90–95% are not surprising in immunologically naive populations (Wilkes, 2023). These viruses, including human measles virus, Newcastle disease virus, canine morbillivirus, small ruminants morbillivirus, and rinderpest virus etc. have caused devastating outbreaks for centuries, posing a threat to human health, food security, and endangered species (Uhl et al., 2019).

Canine morbillivirus is a single-stranded non-segmented enveloped negative-sense RNA virus, and it is the etiological agent of Canine distemper (CD) (Mansour & Hasso, 2021). It is a monotypic virus, as defined by polyclonal antisera, although a variety of biotypes exist that differ in their pathogenic patterns (Martella et al., 2008a). The virus is pantropic, often described as a multi-cell pathogen based on tissue tropism (Rendon-Marin et al., 2019; Wilkes, 2023). The genome encodes six structural and two non-structural proteins (Wilkes, 2023). The structural proteins include a single-envelope-associated protein (M), two glycoproteins (the hemagglutinin H and the fusion protein F), two transcriptase-associated proteins (the phosphoprotein P and the large protein L), and the nucleocapsid protein (N) that encapsulates the viral RNA (Martella et al., 2008a). The Viral non-structural proteins include the V (virulence factor) and C proteins

(polymerase cofactor) which possess immunomodulatory functions (Wilkes, 2023). Canine distemper is a highly contagious and fatal viral disease of dogs, other canids, and a wide range of other animals (Kennedy et al., 2019). The disease is predominantly that of terrestrial carnivores, but many other species, including seals, ferrets, skunks, badgers, porpoises, and exotic *Felidae*, have been infected by either canine morbillivirus or related viruses (Rendon-Marin et al., 2019). Canine morbillivirus causes a multi-systemic disease (Anis et al., 2018) with symptoms depictive of gastrointestinal, respiratory, endocrine, urinary, lymphatic, cutaneous, skeletal, immunosuppressive, hematopoietic-, and nervous involvement (Lempp et al., 2014). Both clinical disease, and subclinical infection are associated with canine distemper, with the latter constituting an important epidemiological niche known as “carrier state” or “reservoir”. Infected dogs shed virus in their nasal and ocular secretions, and infection usually is transmitted by aerosol or by direct contact leading to respiratory infection of susceptible animals (Martella et al., 2008b). Based on clinical presentation, canine distemper occurs in two forms - acute and chronic, with the acute form sharing striking similarity to measles infection in humans (Uhl et al., 2019; Wilkes, 2023). Signs accompanying the acute form of the disease include a combination of diphasic fever, conjunctivitis, ocular, and nasal discharges, anorexia, depression, vomiting, diarrhea, dehydration, leukopenia, and pneumonia. The occurrence of hard pad disease and the encephalitic distemper is linked to chronicity of infection, however the latter has been shown to affect puppies in the acute stage (GÜLERSOY et al., 2023; Martella et al., 2008a). The clinical outcome associated with this disease depends on:

the host: morbidity and mortality vary greatly between different host species (Wilkes, 2023); the immune status of the infected animal; the age of infected dogs dictates the mortality rate which is usually highest in puppies, and the virus tropism and virulence (Rendon-Marin et al., 2019).

Canine morbillivirus has a worldwide distribution and remains endemic in many areas despite the widespread use of vaccines that are highly effective in preventing the disease caused by the virus (Viana et al., 2015). In tandem with the epidemiological scenario in other parts of the world, canine distemper has remained endemic in Nigeria despite sustained vaccination efforts, and the incidence in dogs appears to have increased in recent years. Such observation could be due to several reasons including: immunological window or immunity gap; a situation whereby unprotective lactogenic (maternal) immunity interferes with vaccine efficacy i.e. a period during which the maternal antibody level is too low to be protective but high enough that it inhibits sufficient response to a vaccine which drives age-related susceptibility (Martella et al., 2008a). Vaccine failure and vaccine break. The possibility of vaccine reversion to virulence, and vaccine-induced immunosuppression (Sawatsky & von Messling, 2010; Wimsatt et al., 2006). The sylvatic (wild life) cycle involvement, as the virus appears to be maintained by a metareservoir rather than a single species, requiring the need to vaccinate the wildlife species at risk (Wilkes, 2023). The inter-communal dog movement and associated risks, and the large population of free roaming local dogs. The last two points underscore the description of canine distemper as a “crowd” disease, generally displaying a “boom and bust” infection cycle requiring interacting

populations of animals to maintain the virus in an enzootic state (Wilkes, 2023).

Due to its wide host range, tissue tropism, and the propensity for cross-switching, the continuous and uninterrupted circulation of the Canine morbillivirus in its reservoirs pose a threat to dog population, companionship, dogs’ associated health benefits to humans, and endangered animal species. The paucity of data as regards the epidemiological dynamics of canine morbillivirus in Makurdi metropolis compelled this study. Identification of carrier dogs is imperative for a successful control of this disease as reservoir dogs serve as a constant source of the virus leading to clinical disease in susceptible dogs. In this study, the virus was identified in carrier dogs of Makurdi metropolitan area of Benue State, Nigeria, using a rapid immunochromatographic assay kit for canine morbillivirus antigens qualitative detection in serum, nasal-, ocular-, and rectal- swabs. It is hoped that this study, with more sample size, and sample heterogeneity, will substantiate current data (Mlanga et al., 2018), bridge information gaps thereby enhancing the development of guidelines for CDV surveillance by relevant authorities and response systems, and initiate proactive measures in order to achieve elimination of this disease.

MATERIALS AND METHODS

Study Area

The Makurdi metropolitan area lies within Latitude 7.74° North and Longitude 8.51° East with an elevation of 104 meters above sea level. Sampling sites were selected randomly at various localities within the metropolis.

Sample Size Determination

Based on an initial reported prevalence rate of 8.6% (Mlanga et al., 2018), the estimate of appropriate sample size for this study was calculated using the formula stated in Thrustfield textbook of veterinary epidemiology (Thrusfield, 2004) by employing a defined confidence level and precision of 99% and 5% respectively.

$$n = \frac{Z^2 \times P_{exp} \times (1 - P_{exp})}{d \times d}$$

where: n = required sample size;

Z = z-score

P_{exp} = expected prevalence;

d = desired absolute precision.

P_{exp} = 8.6%

d = 5%

Z value for 99% confidence level = 2.576

$$n = \frac{2.576^2 \times P_{exp} \times (1 - P_{exp})}{d \times d}$$

$$n = \frac{2.576^2 \times 0.086 \times (1 - 0.086)}{0.05 \times 0.05}$$

$$n = 209$$

The calculated sample size for the study, ideally, should be 209 dogs. However, we were only able to sample 204 dogs within the study period. Ocular-, nasal-, and rectal- swabs, as well as serum were taken from each of 204 dogs bringing a total of 816 samples.

Questionnaires Administration

Structured questionnaires were administered by visiting Veterinarians to dog owners in households within study localities of Makurdi metropolis. The localities that constituted the study area are: Wadata, High level, Otukpo road, Wurukum, Gboko road, and North bank areas.

Samples

Sample Types

Ocular-, nasal-, and rectal- swabs, as well as serum were taken from each of 204 dogs bringing a total of 816 samples.

Collection Process

Swabs

Packs of sterile swab sticks were purchased from an equipment shop within Makurdi metropolis. Just before application, each swab stick was torn open and the cotton bud wetted with sterile normal saline. The wet bud was inserted into and twirled at the sample site to obtain some specimen on the swab. Each swab was placed into a sterile test tube and corked. Each test tube was kept in food flasks containing ice packs. A total of 204 apparently healthy dogs were sampled.

Serum

Blood samples for sera were obtained by sterile cephalic venipuncture of each dog using 21G needle fixed unto a 5ml syringe. The blood was placed in a sampling bottle and allowed to clot. The clear serum was separated into micronic sampling tubes following centrifugation at 1,500 RPM for 5 minutes.

Diagnostic Assay

Laboratory investigations were carried out in the Veterinary Microbiology Laboratory, Department of Veterinary Microbiology, College of Veterinary Medicine, University of Agriculture Makurdi.

Sample Analysis

The samples collected were analyzed for canine morbillivirus antigens using the immunochromatographic rapid antigen assay kit. The test kit was inscribed with the letter's "T" and

“C” denoting **Test line** and **Control line** on the surface of the device. Both the test and the control lines in the result window were not visible before the application of samples. The control line was used as a procedural control for each test. The control line must always appear in every test procedure to show that the test is conducted properly and the test reagents are working.

Test procedure

The test procedure was carried out according to the manufacturer’s instructions. Two to three drops of the centrifuged swab material or serum was added unto the specimen tube containing 300ul of assay diluent. The specimen in diluent was mixed thoroughly to the extract well. Four drops of the mixed sample in diluent was added drop-wise slowly to the sample hole using the dropper. As the test began to work, a purple colour moved across the result window in the centre of the test device. If the migration did not appear after one minute, more drops of mixed sample was added to the sample hole.

Reading of the test result

The test result was read visually after 5-10 minutes. The appearance of two purple colour bands across the result window for “C” and “T” was an indication of a positive result. On the other hand, the appearance of only one band across the result window was an indication of a negative result. If the purple colour band was not visible within the result window after performing the test, the result was considered invalid. In the latter case, the test protocol may not have been followed correctly. To obtain a valid result from such sample, the test was repeated.

Data Analysis

The results of this study were statistically analyzed using percentages and chi square

distribution analysis test. The data were presented using tables and figures with the aid of Microsoft Excel 2016 version, and Microsoft Power BI Desktop respectively.

RESULTS

Prevalence of canine morbillivirus antigens in resident dogs of Makurdi metropolis: The study shows that 26.96 % (55) of the 204 dogs sampled as shown in table 1, both vaccinated (47) and unvaccinated (157), were positive for canine morbillivirus antigens.

Table I: Canine morbillivirus overall antigens detection rate, and detection rate by vaccine history of resident dogs in Makurdi metropolitan area

<i>Vaccination History</i>	<i>No. of Dogs Sampled</i>	<i>No. of CmVags Positive Dogs</i>	<i>Percentage CmVags Positive Dogs</i>
Vaccinated	47	19	40.43%
Unvaccinated	157	36	22.93%
Total	204	55	26.96%

Chi square dif. = 5.6231; P value = 0.0177; Statistical significance = Yes

Vaccine uptake rate for canine distemper among dog owners that participated in the study: Fig. 1 depicts that 76.96% of dog owners that participated in the study had dogs that were unvaccinated

Vaccination status and canine morbillivirus antigens detection rate in resident dogs of Makurdi metropolis: Fig. 2 depicts the relationship between antigens detection rate and vaccination history of dogs that participated in the

study. There was statistical difference ($P < 0.05$) between groups based on Chi square distribution analysis (table 1).

Canine morbillivirus antigens detection rate based on age distribution of resident dogs in Makurdi metropolis: Fig. 3, reveals that 7 out of 21 dogs aged 0 – 3 months (33.33%) and 14 out of 50 dogs aged 4 months –1 year (28%) were carriers of viral antigens. Of the 69 dogs (aged 1-2 yrs), and 64 dogs (aged >2 years) sampled, the antigen detection rate were 24.64% and 26.56% respectively.

Canine morbillivirus antigens detection rate based on breed distribution of resident dogs in Makurdi metropolis: Fig. 4 shows that 39 (23.93%) out of the 163 Nigerian local dogs (NLD) sampled tested positive for viral antigens. 40.63% (13) out of 32 exotic breeds were positive for canine morbillivirus antigens (CmVags), and 33.33 % (3) out of the 9 crossbreeds of dogs carried CmVags. The specific breed related antigens detection rate is shown in table 2.

Figure 1: Vaccine uptake rate among dog owners in Makurdi Metropolis, Benue State, Nigeria

Vaccination Status ● Unvaccinated ● Vaccinated

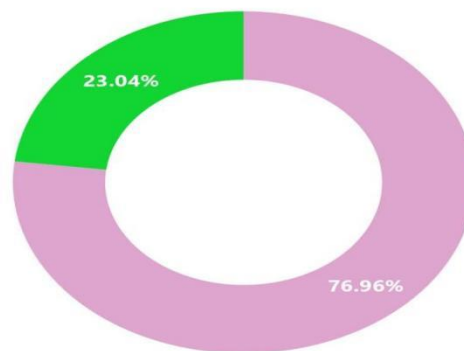


Figure 2: Relationship between vaccination status and canine morbillivirus antigens detection rate in resident dogs of Makurdi metropolis

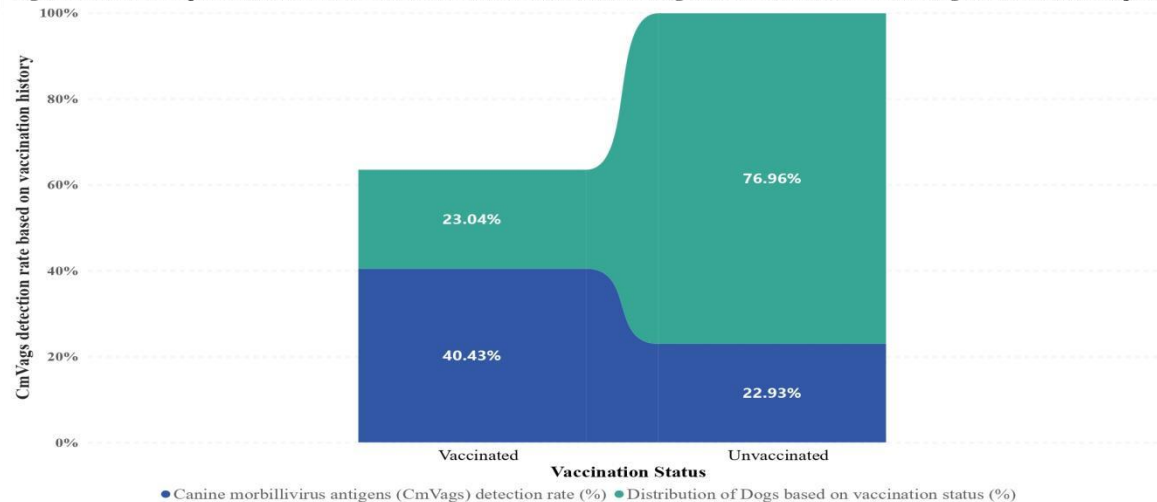


Figure 3: Canine morbillivirus detection rate and dynamics based on age of dogs and percentage age distribution in resident dogs of Makurdi metropolis

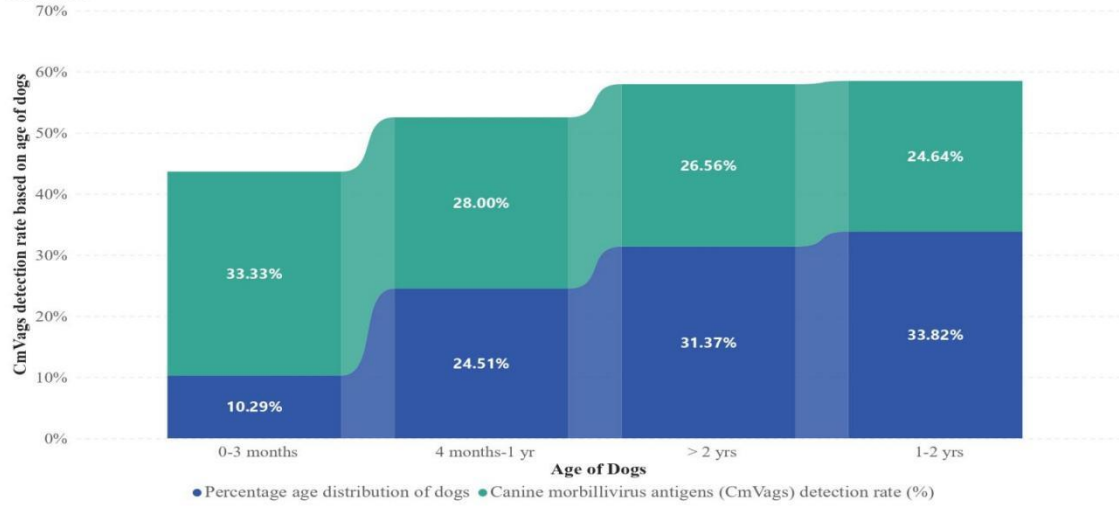


Figure 4: Canine morbillivirus detection rate and dynamics based on breed and percentage breed distribution in resident dogs of Makurdi metropolis

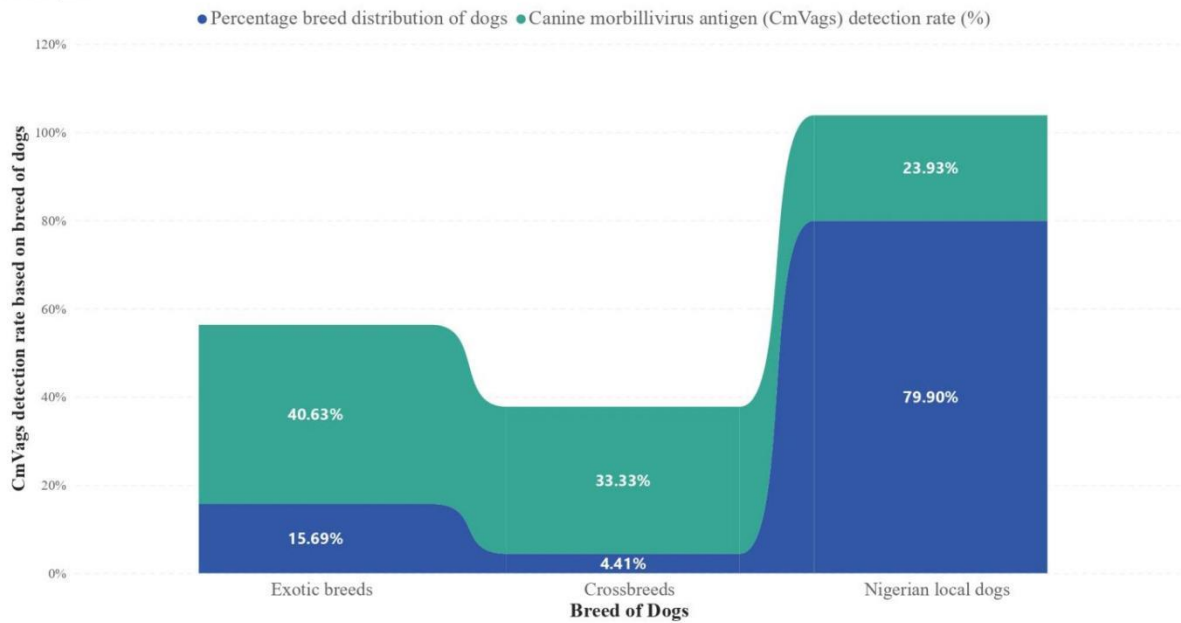


Table II: Canine morbillivirus antigens detection rate by breed of resident dogs in Makurdi metropolitan area.

Breed of Dogs	No. of Dogs Sampled	No. of CmVags Positive Dogs	Percentage CmVags Positive Dogs
NLD	163	39	23.93%
Alsatian	11	6	54.55%
Caucasian	7	0	0.00%
Rottweiler	3	3	100.00%
American Eskimo	3	3	100.00%
Boer Boel	2	0	0.00%
Mastiff	2	0	0.00%
Tibetan Terrier	1	0	0.00%
GS/BM Exotic Crossbreed	2	1	50.00%
AS/RW Exotic Crossbreed	1	0	0.00%
AS/NLD Crossbreed	2	0	0.00%
RS/NLD Crossbreed	2	0	0.00%
Unknown Crossbreed	5	3	60.00%
No. of Exotic breeds	32	13	40.63%
No. of Crossbreeds	9	3	33.33%
Total No. Dogs	204	55	26.96%

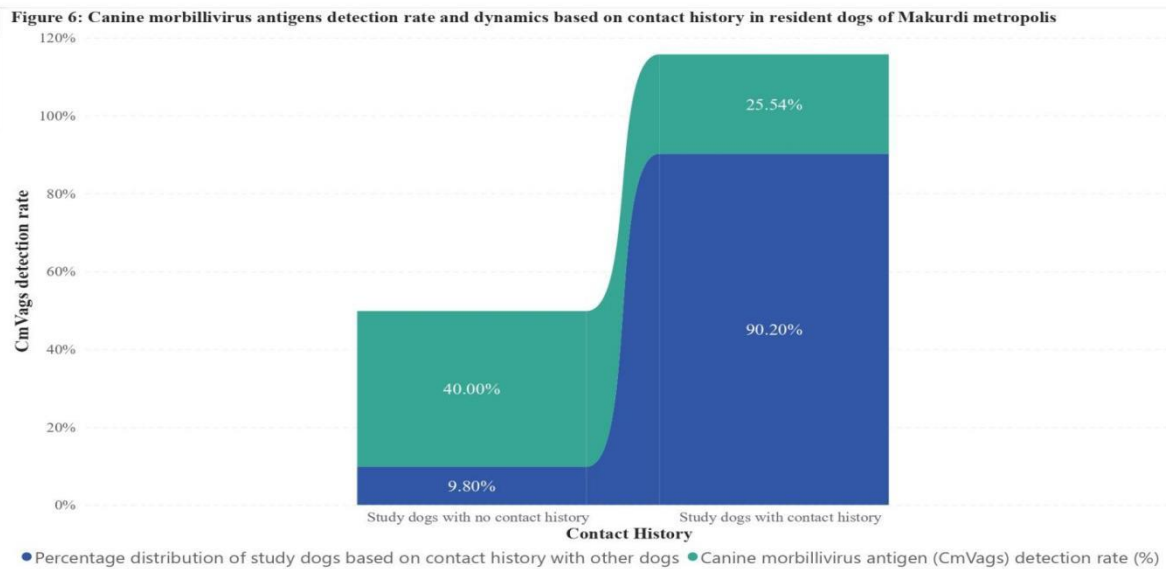
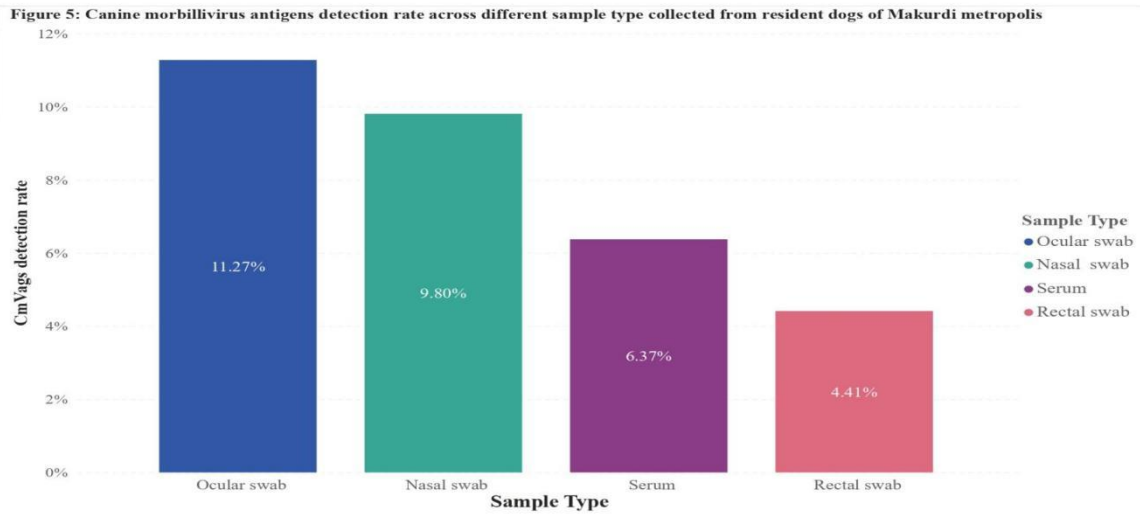
Key: GS = German Shepherd; BM = Bull Mastiff; AS = Alsatian; RW = Rottweiler Chi square dif. = 3.767; P value = 0.1521; Statistical significance = No

Canine morbillivirus antigens detection rate based on sample type: As shown in fig. 5, canine morbillivirus antigens were detected in 9.8%, 11.27%, and 4.41% of nasal-, ocular-, and rectal swabs respectively, and in 6.37% of the serum samples tested. There was statistical difference ($P < 0.05$) based on Chi square distribution analysis as shown in table 3.

Table III: Canine morbillivirus antigens detection rate by sample type in resident dogs of Makurdi metropolitan area

Sample Type	No. of Sample Tested	No. of CmVags Positive Samples	Percentage CmVags Positive Samples
Nasal swab	204	20	9.80%
Ocular swab	204	23	11.27%
Rectal swab	204	9	4.41%
Serum	204	13	6.37%

Chi square dif. = 8.208; P value = 0.0419; Statistical significance = Yes



Canine morbillivirus antigen detection rate based on the contact history of resident dogs in Makurdi metropolis: As for CmVags detection rate based on the history of contact (fig. 6) with other dogs, 47 out of 184 dogs (25.54%) which had history of contact with other dogs tested positive for CmVags. On the other hand, of the 20 dogs which had no history of contact with other dogs, 8 (40.00%) carried CmVags.

DISCUSSION

Poor vaccine uptake rate for canine distemper among dog owners in Makurdi metropolitan area:

As shown in fig. 1, 76.96% of dog owners that participated in the study had dogs that were unvaccinated. This shows poor vaccine uptake rate. Possible causes for this include; poor awareness levels as regards the importance of vaccination as a disease prevention strategy, poor socioeconomic status making some dog owners

just unable to afford getting their dogs vaccinated, and in rare cases, myth-driven vaccinophobia among dog owners. The implication of this is that increasing population of unvaccinated, free roaming, and possibly infected dogs, contribute to disease spread.

A significant epidemiological trend for canine morbillivirus in resident dogs of Makurdi metropolis:

The result obtained in this study shows the level of circulation of canine morbillivirus among apparently normal dogs sampled in Makurdi metropolis. A prevalence rate of 26.96% (table 1) is observed herewith. It is worthy of note that the prevalence of 26.96% is representative of the overall prevalence in both vaccinated and unvaccinated dogs. The exact prevalence, which is highly significant from an epidemiological perspective, in unvaccinated population of dogs for canine morbillivirus is 22.93% (figure 2). Such is comparably higher than the 8.6% and 7.5 % reported by (Mlanga et al., 2018) and (Temilade et al., 2015) in Makurdi metropolis and Abeokuta, Nigeria respectively. With more sample size and heterogeneity, the chances of antigen detection were maximized in this study. As such the prevalence rate of 22.93% reported is representative of the current canine morbillivirus epidemiological scenario in Makurdi metropolis.

Vaccination status is related to canine morbillivirus antigens detection rate in resident dogs of Makurdi metropolis:

Relationship between vaccination status and canine morbillivirus antigens detection rate in resident dogs of Makurdi metropolis is shown in fig. 2. In relation to vaccination history, viral antigens were detected in both vaccinated and

non-vaccinated dogs in the present study. Canine morbillivirus antigen prevalence appears to be higher in vaccinated dogs than in unvaccinated dogs, and there is statistical difference based on chi square distribution analysis. Such a finding is in tandem with previous studies in Abeokuta, Nigeria (Temilade et al., 2015) where the authors used the rapid antigen detection kit and reported a 3.7% antigen prevalence in immunized dogs. The high prevalence of canine morbillivirus antigens in vaccinated clinically normal dogs here could be due to vaccine virus shedding or as a result of natural infection. The presence of the latter, which is highly indicative of wild type virus presence and circulation, could pose a serious threat to unvaccinated susceptible population of puppies and adult dogs, as well as wild canids dwelling in the locality.

Age of dogs is related to canine morbillivirus antigen detection rate:

Canine morbillivirus detection rate and dynamics based on age of dogs and percentage age distribution in resident dogs of Makurdi metropolis is shown by fig. 3. In terms of age-related detection rate of viral antigens, in this study, the prevalence rate is higher in younger puppies than older puppies and mature adults. Puppies aged 0-3 months had the highest prevalence rate of 33.33%. The reports for age related seroprevalence for canine morbillivirus is however conflicting, with some in support (Kamalla et al., 2023; Temilade et al., 2015) and others against (Avizeh et al., 2007). Generally, age has been a risk factor for various diseases. It is even more so in immunologically naive dog populations as a significant percentage of dogs used for this study (79.9%) are Nigerian local dogs with poor vaccine adoption rate among

owners. More than that, since the diagnostic assay used is an immunochromatographic test, detection of maternally derived antibody (in puppies born to vaccinated bitches) could also be the reason for the high prevalence rate in this group of dogs.

Canine morbillivirus antigen detection rate in Nigerian local dogs is comparably low, but significantly important:

Canine morbillivirus detection rate and dynamics based on breed and percentage breed distribution in resident dogs of Makurdi metropolis is depicted by fig. 4. More information can be found in table 2. Exotic breeds of dogs had a higher prevalence compared to Nigerian local dogs. This shows that the viral antigen detection rate is higher in exotic breeds compared to the indigenous breeds. This difference is however not statistically significant. There is also no significant difference for detection rate between crossbreeds and the Nigerian local dogs. These findings are consistent with one study (Twark & Dodds, 2000) and at variance with another (Temilade et al., 2015). The later study observed a significant association between breeds and antigen presence. Vaccine uptake rate (VUR) by owners of exotic breeds is generally higher compared to owners of Nigerian local dogs, most dogs with limited contact to other dogs are exotic, and, generally, immunochromatographic tests cannot differentiate vaccinated from infected animals i.e., they lack DIVA compliance. Based on these factors, we speculate that the high viral antigen detection rate in “without contact”, vaccinated, exotic breed of dogs in this study is likely to be from vaccine antigens not wild type viral antigens. However, circulating wild type virus cannot be totally excluded as there have

been cases of infections despite vaccination, and lack of contact. This is as result of quite a number of factors including maternally-derived antibody interference, vaccine failure, vaccine break, and vaccine-induced immunosuppression (Martella et al., 2008a; Sawatsky & von Messling, 2010; Wimsatt et al., 2006). Since vaccine uptake rate for canine distemper is generally low in Nigerian local dogs, canine morbillivirus antigens detection in this group of dogs holds significant epidemiological importance as it is likely indicative of the wild type virus circulating in such dog population making them carriers and posing a threat to other susceptible canids, animals, and endangered species.

Canine morbillivirus antigen detection rate in dogs with positive contact history is comparably low, but epidemiologically important:

Fig 6 depicts canine morbillivirus antigens detection rate and dynamics based on contact history in resident dogs of Makurdi metropolis. In this study, 25.54% of dogs which had a history of contact with other dogs carried canine morbillivirus antigens. This is significant in terms of the epidemiology of the disease as transmission of the virus is mostly by contact (Kamalla et al., 2023). On the other hand, viral antigen prevalence is higher in dogs (40.00%) that had no known history of contact with other dogs. Viral infection of this later group of dogs could have been possible through food, water, or fomites contaminated with infective body secretions or via vertical transmission (Martella et al., 2008a), or we speculate that vaccinated exotic breed of dogs constitute a significant percentage of this population, as such, possibly, the viral antigens circulating are of vaccine origin. There

is a relationship between breed of dogs, vaccination status, contact history, and CDVag detection rate in our study. Exotic breeds of dogs had a higher prevalence compared to Nigerian local dogs. So do vaccinated dogs compared to unvaccinated ones, and “without” contact dogs compared to dogs with a history of contact to other dogs. The 25.54% antigen detection rate in “without” contact dogs in this study is significant from an epidemiological perspective for the same reason that it is likely indicative of the wild type virus circulating in such dog population.

Ocular swab is the recommended sample of choice for canine distemper laboratory diagnosis in resident dogs of Makurdi metropolis:

Sample type-related difference was noted in this study (fig. 5), and such was statistically significant by Chi square distribution analysis. Canine morbillivirus antigens were detected in ocular, nasal, and rectal swaps, and in serum samples, with ocular swab yielding the highest antigen prevalence of 11.27 % and rectal swab yielding the least (4.41%). Such serves to underscore the pantropic nature of the virus as regards tissue tropism, and the associated pathological implications of canine morbillivirus as a multi-cell pathogen (Rendon-Marin et al., 2019). Since detection rate was higher, comparably, in ocular swaps, it is the recommended sample for clinical diagnosis followed by nasal swab, serum and rectal swab respectively.

CONCLUSION

In resource limited settings, the rapid immunochromatographic assay is an effective test for diagnosis of canine morbillivirus

infections, and an indispensable tool for cost-effective epidemiological studies. Canine morbillivirus infections are rising worldwide despite vaccination efforts, and being a “crowd” disease, free roaming carrier dogs are central to its epidemiology. We report the current epidemiological scenario and dynamics for canine morbillivirus in Makurdi metropolitan area in relation to vaccination status, age, breed, and contact history, detailing the implications of such relationship in disease spread over space and time. With poor vaccine adoption rate, especially in Nigerian local dogs, it is not surprising seeing an upward trend for canine morbillivirus as indicated by the sharp jump in prevalence reported herewith compared to previous studies. Free roaming carrier dogs serve as vehicles for this virus, ensuring its continuous distribution across significant distances posing exposure risk to naive dogs and other endangered species. The disease caused by canine morbillivirus generally displays a “boom and bust” infection cycle requiring interacting populations of animals to maintain the virus in an enzootic state. We hope that this study will serve as an indispensable guide for prevention and control programs aimed at the elimination of this important viral disease in the nearby future.

DECLARATIONS

➤ **Ethics Approval:** Ethical approval for the use of samples of animal origin was obtained from the Committee on Animal Ethics and Welfare of the College of Veterinary Medicine, Joseph Sarwuan Tarka University Makurdi. The reference number is **JOSTUM/CVM/ETHICS/2024/28**, and the link to the letter is <https://drive.google.com/file/d/1EC8w8zPsnWB>

[vFYVWVt7pIKLkUTsdTS2Q/view?usp=drive_1ink](https://doi.org/10.1186/s12917-024-0453-2)

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All authors have accepted the responsibility for the entire content of this manuscript, reviewed all the results and approved the final version.

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Dr. E. Mlanga: Contributed to manuscript writing

Dr. N. D. Rabo: Manuscript writing, data cleaning, analysis, and visualization.

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