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Occurrence and Phenotypic Antimicrobial Resistance of *Escherichia Coli and Salmonella Enterica* as Well as Coliform Load Recovered from Healthy Dogs in Tamale Metropolis, Ghana

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ABSTRACT

Salmonella enterica and *Escherichia coli* are vital bacteria associated with infections in both humans and animals. Their presence in dogs expose humans to the risk of infections. This study determined the occurrence and phenotype antimicrobial resistance of *Salmonella enterica* and *Escherichia coli* recovered from healthy dogs in the Tamale Metropolis, Ghana. The study also examined coliform loads in these dogs. A total of 120 samples from five different parts (anus, mouth, nose, inner ear and outer ear) of apparently 24 healthy dogs were examined. Isolation and antimicrobial resistance of *Salmonella enterica* and *Escherichia coli* were determined according to the Bacteriological Analytical Manual and the Disc Diffusion methods, respectively. The occurrence of *Salmonella enterica* was highest at 41.7% and lowest at 8.33% while *Escherichia coli* in the dogs was highest at 62.5% and lowest at 16.7%. The coliform load was highest at 3.7 log cfu/cm^2 and lowest at 3.1 log cfu/cm^2. The *Salmonella enterica* isolates were highly resistant to teicoplanin (100%) and tetracycline (89.5%), but susceptible to gentamicin (68.4%). The MAR index ranged from 0.1 to 0.7 and the resistance pattern TecTeCCro (resistant to teicoplanin, tetracycline, chloramphenicol and ceftriaxone) was the most common. For *Escherichia coli*, they were highly resistant to teicoplanin (84.6%) and tetracycline (73.1%), but susceptible to gentamicin (80.8%), ceftriaxone (88.5%) and chloramphenicol (92.3%). The MAR index ranged from 0.0 to 0.6 and the resistannce pattern Tec (resistant to only tetracycline) was the most common. In conclusion, this showed that apparently healthy dogs were sources of *Salmonella enterica* and *Escherichia coli*. The *Salmonella enterica* and *Escherichia coli* isolates showed varied resistances to antibiotics.

Keywords: Antimicrobial resistance, Dogs, *Escherichia coli*, Ghana, *Salmonella enterica*, Tamale

INTRODUCTION

Escherichia and *Salmonella* species are a group of bacteria of the Enterobacteriaceae family that normally inhabit the intestines of animals and humans (Janda and Abbott, 2021). Thus, most animals including dogs, cats, rabbits, rodents, reptiles, birds and livestock could be carriers or be infected by *Escherichia* and *Salmonella* species of any kind (Adzitey *et al*., 2012; Bataller *et al*., 2020; Mustapha and Goel, 2020; Valat *et al*., 2020; Wei *et al*., 2022). Mustapha and Goel (2020) had observed *Escherichia coli* in the urine of dogs, while Bataller *et al*. (2020) found that apparently healthy dogs could be sources of *Salmonella* species. *Escherichia coli* and *Salmonella* infections have been recorded around the world and are essential public health concerns (Popa and Papa, 2021; World Health Organization (WHO), 2022). The centers for Disease Control and Prevention (2021) reported an outbreak due to *Escherichia coli* which was responsible for twenty-two illnesses, eleven hospitalizations and one death. *Salmonella* caused sixteen illnesses, two hospitalizations with no deaths (CDC, 2022). According to Popa and Papa (2021), *Salmonella* was the most common cause of foodborne outbreaks in Europe.

There is evidence of transmission of *Escherichia* and *Salmonella species* from dogs to humans and vice versa (Valat *et al*., 2020; Lei *et al*., 2021). According to Lei *et al*. (2021), extended-spectrum β-lactamase genes isolated from *Escherichia coli* of dog origin were similar to those found in their owners. Major *Salmonella* STs (ST372 and ST73) found in dogs were common to those considered as human-associated (Valat *et al*., 2020). The close association between man and dogs has the potential to increase the risk of human exposure to these pathogens (Benz-Schwarzburg *et al*., 2020).

Antimicrobial resistance occurs when antibiotics are no effective in eradicating the bacteria to which they previously susceptible (Adzitey, 2015). Antimicrobial resistance is a growing public health challenge worldwide, due to the therapeutic challenges in managing humans and animals microorganisms. *Escherichia* and *Salmonella species* isolated from dogs were demonstrated to be resistant to antimicrobials such as tetracycline, chloramphenicol among others (Wei *et al*., 2020; Mustapha and Goel, 2020).

Pets including dogs are domesticated animals kept by man for companionship or pleasure (Benz-Schwarzburg *et al*., 2020). Solomon *et al*. (2019) described dogs as 'man's best friend' because they fit in with human life. Dogs are used for security, guiding the visually impaired, hunting, herding and other security purposes (Solomon *et at*., 2019; Benz-Schwarzburg *et al*., 2020). In addition, they can be trained to use their sense of smell for disease detection in human body, or illegal drugs, in the airport, crime and accident scene to help police identify or locate victims (Solomon *et al*., 2019).

In Ghana, more people keep dogs for security reasons and as pets (Adzitey *et al*., 2022). Nonetheless, data on the occurrence of Escherichia and *Salmonella species* in dogs and their antimicrobial resistance patterns in Ghana is scarce. Howbeit, the close association of dogs to their keepers exposes both to the risk of *Escherichia* and *Salmonella* infections. This study was therefore designed to determine the occurrence and phenotypic antimicrobial resistance of *Escherichia coli* and *Salmonella enterica* recovered from apparently healthy dogs in the Tamale Metropolis, Ghana. Also, the coliform loads of the various parts of the dogs were determined.

MATERIALS AND METHODS

Study area

The study was conducted in the Tamale Metropolis, Ghana. The Metropolis occupies an estimated land size of 64,690,180 sqkm and lies between latitude 9˚16 and 9˚ 34 North and longitudes 0˚ 36 and 0˚ 57

West (Ghana Statistical Service, (GSS) 2013). It has a population of 233,252 (GSS, 2013).

Sample collection

A total of 120 samples were collected from 24 apparently healthy dogs from different locations in the Tamale metropolis. Sterile cotton swabs were used to take five samples each from the anus, mouth, nose, inner ear and outer ear of each dog. The swabbed samples were placed in Coleman box containing ice blocks and subsequently transported to the Spanish Laboratory, at the University for Development Studies, Nyankpala, for microbial analysis.

Enumeration of coliform bacteria count

This was done using a slightly modified procedure of Maturin and Peeler (2001) and Adzitey *et al*. (2020). The samples were dipped in 10 ml of 1% buffered peptone water (BPW, Oxoid Limited, Basingstoke, UK) and serial dilutions from 10^{-1} to 10^{-5} were made. Serially diluted samples were spread plated (0.1 ml) onto MacConkey agar and incubated at overnight at 37°C. Colonies were then counted and expressed in colony forming units.

Isolation and identification of *E. coli*

Swabs were dipped in 9ml of BPW and incubated overnight at 37℃. Overnight aliquots were streaked onto Levine Eosin Methylene Blue agar (LEMB Oxoid Limited, Basingstoke, UK) and incubated at 37℃ for 18-24 hours (Feng *et al*., 2020). Presumptive *E. coli* isolates forms colonies with a green metallic sheen and a dark nucleated core on LEMB. Such isolates were purified and confirmed using Gram stain and *E. coli* latex agglutination test by following the manufacturer's instructions.

Isolation and identification of *Salmonella enterica*

A slightly modified procedure of Andrews *et al*. (2014) was used. Overnight aliquots in BPW incubated at 37ºC were enriched in Rappaport-Vassiliadis broth, Oxoid Limited, Basingstoke, UK (incubated at 42°C for 24h) and Selenite Cystine broth, Oxoid Limited, Basingstoke, UK (incubated at 37°C for 24h). After which, the aliquots were streaked on Xylose Lysine Deoxycholate and Brilliant Green agar. Presumptive *Salmonella enterica* were purified and confirmed using gram stain, triple sugar iron agar, lysine iron agar, and *Salmonella* latex agglutination test by following the manufacturer's instructions.

PCR for the confirmation of *E. coli* and *Salmonella enterica* isolates

DNA template was prepared by lysing colonies of *E. coli* and *Salmonella enterica* in 30µl DNase/RNase free water at 99℃ for 30min (Kichana *et al*., 2022). Conventional PCR was carried out to amplify partial Invasion A (*invA*) gene of *S. enterica* by following the procedures of Bej *et al*. (1999) and partial Beta-D-glucuronidase (*uidA*) gene of *E. coli* by following the procedures of Malorny *et al*. (2002) in a 50µl reactions. Briefly, the reaction consisted of 10µM each of respective primer pairs (F: AAA ACG GCA AGA AAA AGC AG, R: ACG CGT GGT TAC AGT CTT GCG for *E. coli* and F: GTG AAA TTA TCG CCA CGT TCG GGC AA, R: TCA TCG CAC CGT CAA AGG AAC C for *Salmonella enterica*), 1.8mM $MgCl_2$, 20mM Tris-HCl (pH 8.9 at 25°C), 22mM NH_4Cl, 0.2mM dNTPs, 5% glycerol, 0.06% IGEPAL® CA-630, 0.05% Tween-20, xylene Cyanol FF, Tartrazine, 1.25U One Taq® DNA polymerase (New England Biolabs® Inc) and 4µl DNA template. The cycling conditions were initial denaturation at 95℃ for 5 min, followed by 35 cycles of denaturation at 95℃ for 30 s, annealing at 57℃ for 30s and extension at 72℃ for 5min 30 s. The PCR amplicons were resolved on a 2% (w/v)

agarose gel stained with EtBr and fragment size estimated by comparing with FastRuler™ Middle Range DNA Ladder [10mM Tris-HCl (pH 7.6), 10mM EDTA, 0.005% bromophenol blue and 10% glycerol].

Antimicrobial resistance of *E. coli* and *Salmonella enterica*

Antimicrobial resistance test was done according to Bauer *et al*. (1966). The isolates were examined against; amoxicillin/clavulanic acid (Amc) 30 µg, azithromycin (Azm) 15 µg, ceftriaxone (Cro) 30 µg, chloramphenicol (C) 30 µg, ciprofloxacin (Cip) 5 µg, gentamicin (Cn) 10 µg, sulfamethoxazole/trimethoprim (Sxt) 22 µg, teicoplainin (Tec) 30 µg and tetracycline (Te) 30 µg purchased from Oxoid Limited, Basingstoke, UK. The cultures were incubated in tryptic soy broth at 37ºC for 18 h and the concentration adjusted to 0.5 McFarland turbidity. It was then spread plated on Mueller Hinton agar, Oxoid Limited, Basingstoke, UK (MH) and incubated at 37°C for 24 h. The inhibition zones were measured and the results were interpreted according to Clinical Laboratory Standard Institute (2022). The Multiple Antibiotic resistance (MAR) index was calculated and interpreted according to Krumperman (1983) using the formula: a/b, where 'a' represents the number of antibiotics to which a particular isolate was resistant and 'b' the total number of antibiotics tested.

Statistical analysis

Coliform count was analyzed using ANOVA of GenStat 12.1 Edition. The means were separated at 5% significant level. Occurrence data was analyzed using binary logistic of IBM Statistical Package for the Social Sciences (SPSS) Version 17. Test for statistical difference was done using wald chi-square at 5% significance level.

RESULTS

Coliform counts of healthy dogs in the Tamale metropolis

Table I shows the coliform counts of the anus, mouth, nose, inner ear and outer ear of the apparently healthy dogs in the Tamale metropolis. The coliform count was 3.7 log cfu/cm^2, 3.3 log cfu/cm^2, 3.3 log cfu/cm^2, 3.2 log cfu/cm^2 and 3.1 log cfu/cm^2 for anus, mouth, nose, inner ear and outer ear, respectively. There were no significant differences p value 0.139 in coliform counts among the anus, mouth, nose, inner ear and outer ear samples examined.

TABLE I: Coliform count of samples from dogs

Source	Coliform count (log cfu/cm^2)
Anus	3.7
Mouth	3.3
Nose	3.3
Inner ear	3.2
Outer ear	3.1
Sed	0.179
P-value	0.139

Incidence of *Escherichia coli* in apparently healthy dogs

The incidence of *Escherichia coli* in the apparently healthy dogs in the Tamale Metropolis is presented in Table II. The overall incidence of *Escherichia coli* in the dogs was 45% (54/120). *Escherichia coli* was most common in the mouth (62.5%), followed by anus (58.3%), nose (45.8%), outer ear (41.7%) and inner ear (16.7%). Significant differences (*P<0.05*) occurred among the incidence of *Escherichia coli* in the dog samples analyzed. Mouth, anus, nose, and outer ear samples positive for *Escherichia coli* did not differ significantly

(*P>0.05*) from each other, but were significantly higher *(P<0.05)* than those from inner ear samples.

TABLE II: Incidence of *Escherichia coli* in apparently healthy dogs

Sources	Number of samples tested	Positive samples	Incidence %
Anus	24	14	58.3
Mouth	24	15	62.5
Nose	24	11	45.8
Inner ear	24	4	16.7
Outer ear	24	10	41.7
Overall	120	54	45.0

Incidence of *Salmonella enterica* in apparently healthy dogs

The incidence of *Salmonella enterica* in apparently healthy dogs in the Tamale metropolis is shown in Table III. The overall incidence of *Salmonella enterica* in healthy dogs was 24.2% (29/120). *Salmonella enterica* was isolated from the anus (41.7%), mouth (33.3%), nose (25.0%), outer ear (12.5%) and inner ear (8.3%). Significant differences (*P<0.05*) occurred among the incidence of *Salmonella enterica* in the dog samples analyzed. Anus samples positive for *Salmonella enterica* did not differ (*P>0.05*) from that of mouth and nose, but were significantly higher (*P<0.05*) than those obtained from the outer and inner ears. Mouth samples positive for *Salmonella enterica* were significantly higher (*P<0.05*) than those obtained from the inner ear, but not nose and outer ear samples. Nose, inner and outer ears positive for positive for *Salmonella enterica* did not differ (*P>0.05*) from each other.

TABLE III: Incidence of *Salmonella enterica* in apparently healthy dogs

Source	Number of samples tested	No. positive	Incidence %
Anus	24	10	41.7
Mouth	24	8	33.3
Nose	24	6	25.0
Inner Ear	24	2	8.3
Outer Ear	24	3	12.5
Total	120	29	24.2

Antimicrobial susceptibility of *Escherichia coli* isolated from dogs in Tamale

The antimicrobial resistance of *Escherichia coli* isolated from dogs are presented in Table IV. The overall resistant, intermediate resistant and susceptible was 29.9%, 11.5% and 58.6%, respectively. The highest resistance occurred for teicoplanin (84.6%), followed by tetracycline (73.1%). The isolates showed high susceptibility to chloramphenicol (92.3%), ceftriaxone (88.5%) and gentamicin (80.8%). Relatively higher intermediate resistances were observed for ciprofloxacin (30.8%), azithromycin (23.1%), gentamicin (19.2%) and tetracycline (19.2%). Intermediate resistance *Escherichia coli* isolates are those that are neither completely resistant nor susceptible and can be difficult to treat when they are involved in infections (Lorian, 2005; Adzitey *et al*., 2020).

TABLE IV: Antimicrobial susceptibility of *Escherichia coli* isolated from dogs in Tamale

Antimicrobials	% Resistant	% Intermediate resistant	% Susceptibility
Amoxicillin/clavulanic acid 30 µg	42.3	0.0	57.7
Azithromycin 15 µg	15.4	23.1	61.5
Ceftriaxone 30 µg	0.0	11.5	88.5
Chloramphenicol 30 µg	7.7	0.0	92.3
Ciprofloxacin 5 µg	3.8	30.8	65.4
Gentamicin 10 µg	0.0	19.2	80.8
Teicoplanin 30 µg	84.6	0.0	15.4
Tetracycline 30 µg	73.1	19.2	7.7
Sulphamethoxazole/trimethoprim 22 µg	42.3	0.0	57.7
Overall	29.9	11.5	58.6

Key: Amoxicillin/clavulanic acid (Amc) 30 µg, Chloramphenicol (C) 30 µg, Gentamicin (Cn) 10 µg, Ceftriaxone (Cro) 30 µg, Ciprofloxacin (Cip) 5 µg, Azithromycin (Azm) 15 µg, sulfamethoxazole/trimethoprim (Sxt) 22 µg, Tetracycline (Te) 30 µg and Teicoplainin (Tec) 30 µg.

Antimicrobial susceptibility of *Salmonella enterica* isolated from dogs in Tamale

From Table V, *Salmonella enterica* isolates from the dog samples exhibited an overall resistance, intermediate resistance and susceptibility of 49.1%, 11.8% and 39.1%, respectively. The *Salmonella enterica* isolates were highly resistance to teicoplanin (100%), followed by tetracycline (89.5%). Gentamicin, ciprofloxacin and sulfamethoxazole/trimethoprim had the highest susceptibility of 68.4%, 63.2% and 63.2%, respectively. Intermediate resistance was prominent for gentamicin (26.3%), ciprofloxacin (26.3%) and ceftriaxone (21.8%). Intermediate resistant *Salmonella enterica* isolates can alter treatment patterns when they are involved in infections. Lorian, 2005; Adzitey *et al*., 2020).

Antimicrobial resistance profile and multiple antibiotic resistance (MAR) index of individual *Escherichia coli* from apparently healthy dogs' origin

The antimicrobial resistance profile and MAR index of *Escherichia coli* isolated from the apparently healthy dogs can be seen in Table VI. Two, five and eleven *Escherichia coli* isolates were resistant to five, four and three different antimicrobials, respectively. Therefore, multidrug resistance (resistant to 3 or more different antimicrobials) was recorded for 18 isolates (69.2%). In this study, 5 (19.2%), 4 (15.4%), 3 (11.5%), 2 (7.7%), 1 (3.8%) and 0 (0.0%) showed resistance to two, five, eleven, one, one and one antimicrobials. Multiple antibiotic resistance (MAR) ranged from 0 to 0.56, that is, resistant to 0 and 5 antimicrobials, respectively.

TABLE V: Antimicrobial susceptibility of *Salmonella enterica* isolated from dogs in Tamale

Antimicrobial	R%	I%	S%
Amoxicillin/clavulanic acid 30µg (Amc)	42.1	0.0	57.9
Azithromycin 15µg (Azm)	63.2	15.8	21.0
Ceftriaxone 30µg (Cro)	47.4	21.8	30.8
Chloramphenicol 30µg(C)	52.6	0.0	47.4
Ciprofloxacin 5µg (Cip)	10.5	26.3	63.2
Gentamicin10µg (Cn)	5.3	26.3	68.4
Teicoplanin 30 µg (Tec)	100	0.0	0.0
Tetracycline 30µg (Te)	89.5	10.5	0.0
Sulfamethoxazole/trimethoprim (Sxt)	31.6	5.2	63.2
Overall%	49.1	11.8	39.1

Key: Amoxicillin/clavulanic acid (Amc) 30 µg, Chloramphenicol (C) 30 µg, Gentamicin (Cn) 10 µg, Ceftriaxone (Cro) 30 µg, Ciprofloxacin (Cip) 5 µg, Azithromycin (Azm) 15 µg, Sulfamethoxazole/trimethoprim (Sxt) 22 µg, Tetracycline (Te) 30 µg and Teicoplainin (Tec) 30 µg.

TABLE VI: Antimicrobial resistannce profile and multiple antibiotic resistant (MAR) index of individual *Escherichia coli*

Sources	No. of Antibiotics	Antibiotic resistance profile	MAR index
Outer ear	5	CipAmcTecTeSxt	0.56
Outer ear	5	AmcTecTeCSxt	0.56
Anus	4	AmcTecTeSxt	0.44
Mouth	4	AzmCnTeSxt	0.44
Nose	4	AmcTecTeSxt	0.44
Outer ear	4	TecTeCSxt	0.44
Outer ear	4	AmcTecTeSxt	0.44
Anus	3	AmcTecTe	0.33
Anus	3	AmcTecTe	0.33
Mouth	3	AmcTecTe	0.33
Mouth	3	AmcAzmTec	0.33
Mouth	3	TecTeSxt	0.33
Nose	3	AmcTecTe	0.33
Nose	3	AzmTecTe	0.33
Nose	3	TecTeSxt	0.33

Outer ear	3	TecTeSxt	0.33
Outer ear	3	TecTeSxt	0.33
Inner ear	3	AzmTecTe	0.33
Inner ear	2	TecTe	0.2
Anus	1	Tec	0.11
Anus	1	Te	0.11
Anus	1	Tec	0.11
Mouth	1	Tec	0.11
Mouth	1	Amc	0.11
Nose	1	Tec	0.11
Inner ear	0	All susceptible	0.00

Key: Amoxicillin/clavulanic acid (Amc) 30 µg, Chloramphenicol (C) 30 µg, Gentamicin (Cn) 10 µg, Ceftriaxone (Cro) 30 µg, Ciprofloxacin (Cip) 5 µg, Azithromycin (Azm) 15 µg, Sulfamethoxazole/trimethoprim (Sxt) 22 µg, Tetracycline (Te) 30 µg and Teicoplainin (Tec) 30 µg.

TABLE VII: Antimicrobial resistance profile and multiple antibiotic resistance (MAR) index of individual *Salmonella enterica*

Source	No. of Antibiotics	Antibiotic resistant profile	MAR index
Nose	6	CipAzmTecTeCSxt	0.67
Mouth	6	AmcAzmTecTeCCro	0.67
Mouth	6	AzmTecTeCCroSxt	0.67
Anus	6	AmcTecTeCCroSxt	0.67
Anus	6	CipAzmTecTeCSxt	0.67
Mouth	5	AzmTecCnTeCro	0.56
Inner ear	5	AmcAzmTecTeCro	0.56
Anus	5	AmcAzmTecTeC	0.56
Anus	5	AzmTecTeCSxt	0.56
Anus	5	AmcAzmTecTeC	0.56
Anus	5	AmcAzmTecTeSxt	0.56
Nose	4	TecTeCCro	0.44
Inner ear	4	TecTeCCro	0.44
Anus	4	TecTeCCro	0.44
Anus	4	AmcTecTeCro	0.44
Mouth	3	AzmTecTe	0.33
Mouth	3	AzmTecTe	0.33
Inner ear	3	AmcTecTe	0.33
Outer ear	1	Tec	0.11

Key: Amoxicillin/clavulanic acid (Amc) 30 µg, Chloramphenicol (C) 30 µg, Gentamicin (Cn) 10 µg, Ceftriaxone (Cro) 30 µg, Ciprofloxacin (Cip) 5 µg, Azithromycin (Azm) 15 µg, sulfamethoxazole/trimethoprim (Sxt) 22 µg, Tetracycline (Te) 30 µg and Teicoplainin (Tec) 30 µg.

Antimicrobial resistance profile and multiple antibiotic resistance (MAR) index of individual *Salmonella enterica* from apparently healthy dogs' origin

The antimicrobial resistance profile and MAR index of *Salmonella enterica* isolated from the apparently healthy dogs can be seen in Table VII. Five, six, four and three *Salmonella enterica* isolates were resistant to six, five, four and three different antimicrobials, respectively. Thus, multidrug resistance (resistant to 3 or more different antimicrobials) was recorded for 18 isolates (94.7%). Therefore, 94.7% of the *Salmonella enterica* isolates originated from sources where they were frequently exposed to antimicrobials as indicated by Rotchell *et al*. (2016). Multiple antibiotic resistance (MAR) ranged from 0.11 to 0.67, indicating, resistance to 1 and 6 antimicrobials, respectively.

DISCUSSION

This study provides significant insights into the epidemiology of canine morbillivirus in Makurdi, highlighting the importance of vaccination and the role of various risk factors, including age, breed, and contact history, in the spread of the virus.

The vaccination uptake rate for canine distemper is alarmingly low, with 76.96% of participating dog owners having unvaccinated dogs. 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 the disease spread.

A prevalence rate of 26.96% was also recorded among apparently healthy dogs, both vaccinated and unvaccinated. 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 dogs' population for canine morbillivirus is 22.93%. Such is comparably higher than the 8.6% dogs and 7.5 % reported by (Mlanga et al., 2018) and (Temilade et al., 2015) in Makurdi metropolis and Abeokuta in 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.

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 (40.43%) than in unvaccinated dogs (22.93%), and it is statistically significant. 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 in this study could be due to the detection of vaccine antigen 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 was also a significant factor in antigen detection, with the highest prevalence (33.33%) observed in puppies aged 0-3 months. The reports for age related seroprevalence for canine morbillivirus is however conflicting with some reports (Avize et al., 2007, and supported by others (Kamalla et al., 2023; Temilade et al., 2015) and others. Generally, age has been a risk factor for various diseases. It is even more so in immunologically naive dog populations. A significant percentage of dogs used for this study (79.9%) are Nigerian local dogs with poor vaccine adoption rate among owners. 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.

In terms of breed-related antigen detection, exotic dogs showed higher prevalence rates (40.63%) than Nigerian local breeds (23.93%), although this difference was not statistically significant. There is also no significant difference for detection rate between crossbreeds and 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 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. 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.

Contact history also played a role in the detection of canine morbillivirus antigens. 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 CmVags 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 dogs that had contact with other 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

Finally, the study assessed antigen detection rates across various sample types. Sample type-related difference was noted in this study, and such was statistically significant. 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

The anus, mouth, nose and ear of dogs are potential sources of coliforms, *Escherichia coli* and *Salmonella enterica*. The *Escherichia coli* and *Salmonella enterica* isolates exhibited varying level of resistance and susceptibility to antimicrobials. To the best of our knowledge this study reports for the first time on the occurrence and phenotypic antimicrobial resistance of *Escherichia coli* and *Salmonella enterica* recovered from healthy dogs in the Tamale Metropolis, Ghana. The incidence of resistant *Escherichia coli* and *Salmonella enterica* in dogs in the study area means that humans are exposed to the risk of contracting infections that will be difficult to treat from these pathogens which is a threat to public health. Therefore, dog owners should keep their dogs under hygienic environmental conditions, including providing dogs with safe foods, preventing dogs from scavenging or roaming for food and regularly checking their pets' health status/vaccinations. Also, thorough hand washing with soap and water after touching pets is recommended.

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