Original Research Article

ISOLATION AND ANTIMICROBIAL RESISTANCE TO *SALMONELLA* INFECTION IN DIARRHOEIC DOGS PRESENTING TO CLINICS IN NORTHWESTERN NIGERIA

ABSTRACT

Salmonella is one of the most common food-borne bacterial incriminated in gastroenteritis in dogs. The emergence of multidrug resistance (MDR) genes raises spectre of intractable disease and constitute significant public health risk. Already, there are various reports of resistance to several antimicrobials by *Salmonella* isolates from canine patients. This study was therefore conceived to investigate for the first time in the studied region, *Salmonella* isolates circulating in canine population in Sokoto State, northwestern Nigeria. Presenting canine out-patients and those admitted for emergencies with overt symptoms clinical signs for Salmonellosis were recruited for the study. *Salmonella* was isolated from blood and faecal samples using standard laboratory techniques as required by the Clinical and Laboratory Standards Institute (CLSI). The Gram stain, *Salmonella-Shigella* agar (SS agar), Brilliant Green *Salmonella* agar (BGSA) and finally the xylose lysine deoxycholate agar (XLD) at specified temperatures and carbon dioxide adjustments were all applied on samples to isolate *Salmonella* as pure culture. The Kirby Bauer Disk diffusion method was used to test for antimicrobial resistance. Overall, a prevalence of 19.7% was recorded with pyrexia (18.5%) and diarrhea (13.7%) showing statistically significant ($P<0.05$) non-random association with *Salmonella* isolation in patients. The puppy age groups from day one to five months recording prevalence of 31(21.2%) followed by middle-age dogs 6-months to a year recording prevalence of 24(22.2%). Male dogs presented preponderance of cases compared to the females with prevalence of 48(76.1%). The Nigerian local dog was the highest in the number of cases with a statistically significant ($P<0.05$) prevalence of 23(36.5%), the German shepherd recorded 19(30.2%) that was also statistically significant ($P<0.05$). Various antimicrobials used recorded varying degree of resistance patterns when tested with isolates. A total of 19 isolates accounting for 43.2% were resistant to tetracycline, 15(35.7%) isolates recorded resistance for ceftriaxone, 13 isolates accounting for 38.2% presented resistance for metronidazole and 11(27.5%) recorded resistance for amoxicillin. Conversely, isolates were 100% susceptible to chloramphenicol and cotrimoxazole. It is imperative for small animal clinicians, diagnosticians, researchers and public health policy makers to have credible time-tested data on resistance status of *Salmonella* isolates in dogs as this is a roadmap to a healthy canine and human population.

Keywords: *Salmonella*, Out-patient, Zoonosis, Infection, Antimicrobials, Health

Introduction

Attention has focused on isolates of *Salmonella* circulating in canine populations in most regions of the world; this is due to evidence of rising mortality from Salmonellosis as the primary disease or secondary complications in most enteric canine out-patients. Salmonellosis is a broad term applied to enteric infections caused by a group of gram-negative motile, non-spore forming bacilli of the genus *Salmonella* belonging to the family *enterobacteriaceae* (Ojo and Adetosoye, 2009). Salmonellae are ubiquitous pathogens with a wide host range including dogs and cats (Green, 2012).

Salmonella from dogs have important public health significance because they are zoonotic and morbidity is high in kennels (Green, 2012). Most authors agree *Samonellae* belongs to a single species: *Salmonella enterica* based on agglutination reaction of flagella and somatic antigens, there are more than 2400 serovars of *S. enterica* (Green, 2012). The feces of nearly all-animal species including dogs may serve as a potential source of *Salmonella* transmission to other animals and humans and is used as primary samples for obtaining isolates (Jajere *et al.,* 2014). Several studies reported the isolation of *Salmonella* spp from apparently healthy as well as symptomatic dogs, with potential for transmission to humans (Akinyemi *et al.,* 2021).

Antimicrobial residues (whole drug or metabolite) are readily excreted into the environment by animals after exposure to antimicrobials in Nigeria (Alhaji and Isola, 2018). Antimicrobial consumption, use and abuse in livestock and the emergence of resistant genes have received low attention globally. The increase in antimicrobial resistance (AMR) in developing countries is compounded by minimal regulation and absence of effective legislation covering use and misuse (Lopez *et al.,* 2014). Global consumption of antimicrobials in animals is twice that of humans (Aarestrup, 2012). Previous studies in Nigeria have highlighted the contributions of food animals and the environment to AMR burdens (Sanya *et al.,* 2013). In a review of 42 documented surveys designed to test AMR from miscellaneous samples of humans, animals and the environment, 1139 isolates were resistant to 68 selected

commonly used antimicrobials for therapeutics in Nigeria (Sanya *et al.,* 2013). Five sentinel pathogens were reported generally as priority from collated surveys*: ESBL; Salmonella; Staphylococcus aureus; Klebsiella spp; Pseudomonas*.

This study was therefore conceived, to investigate, for the first time in the studied region, isolates of *Salmonella* circulating in symptomatic canine patients and test the isolates for AMR, it is hoped this will lead to better patient clinical and management outcomes by optimizing treatment protocols based on data borne out of this study.

MATERIALS AND METHODS

Study Area

The study was carried out in Sokoto State, geographically located in the North western part of Nigeria and sited along the Sahel. The State lies between latitudes 12°N and 13°58'N, Longitude 4°8' and 6°54'East. Sokoto State has a total of 23 Local Government Areas in four Agricultural zones namely: Sokoto Zone, Isa zone, Gwadabawa zone and Tambuwal zone. Population data on livestock is available for the State but no record on dog population in the State. Most dogs are kept in Local Governments that make up the Sokoto metropolis, these LGAs include, Sokoto South, Sokoto North, Wamakko, Kware and Dange Shuni. Elite clients reside mostly in the metropolis, the suburban territories and rural places are populated by native persons with often, stray dogs and hunting dogs in packs. The State covers a total land area of 25,973 square kilometers with an estimated human population of 4,244,399 (NPC, 2006). Annual rainfall is about 600mm with most of it falling in July and August (Mohammed and Baba, 2013).

Sample Size Determination

There is no published similar study on *Salmonella* infection amongst diarrheic dogs in Sokoto State. A prevalence of 31.8% reported by Jajare *et al.* (2014) in dogs was used to compute the required sample size using the formula $N=Z^2P(1-P)/d^2$ (Thrushfield, 2009). Where: N is the sample size to be calculated, Z (1.96) is standard error at 95% confidence interval, P is the prevalence (31.8%) reported from previous studies, d is the level of precision taken to be 5% for this study.

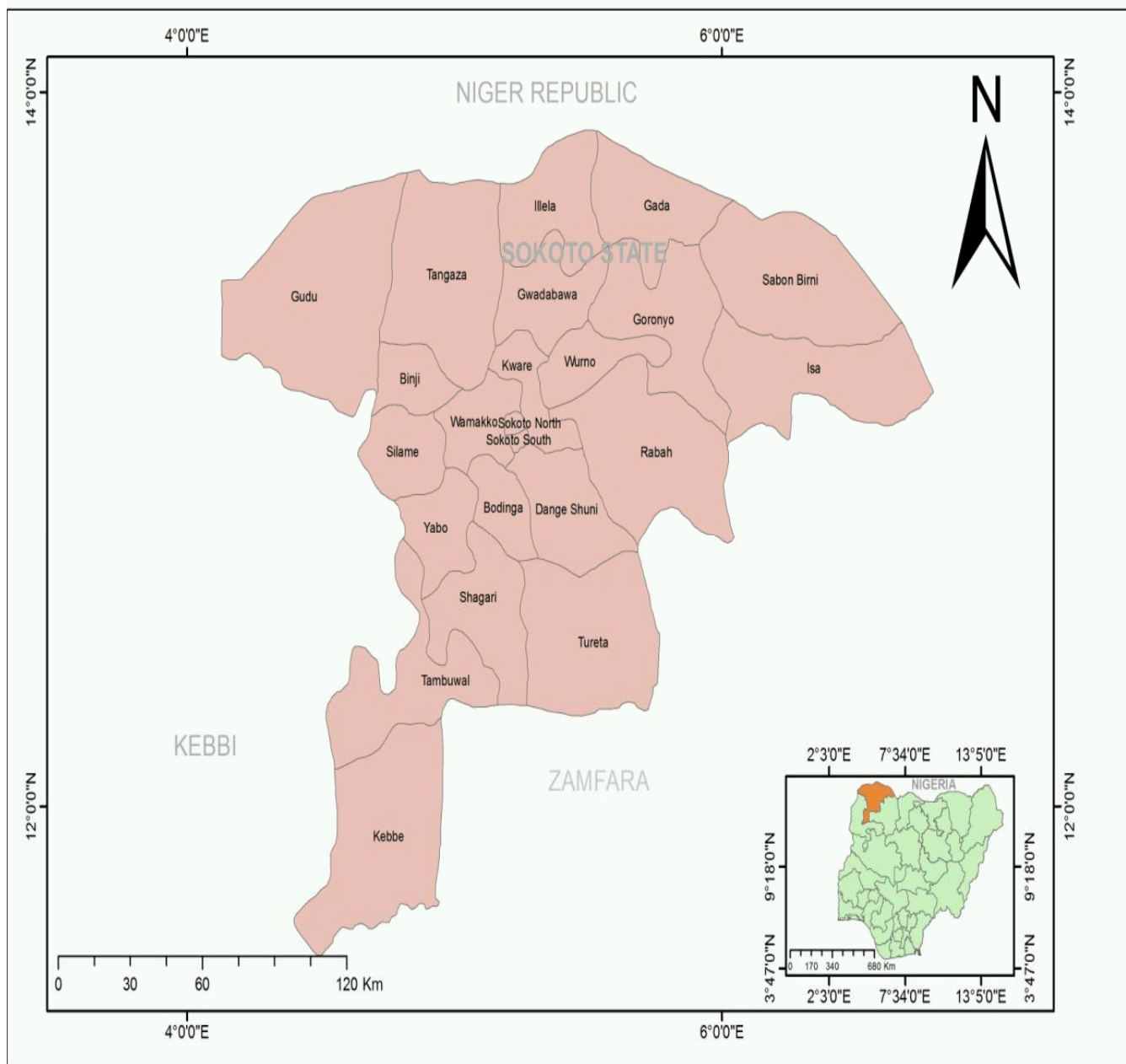Minimum sample size (N) for dogs calculated was 333 samples.

Fig 1. Map of Sokoto State with the Local Governments divided into Agricultural zones. Source: (Mohammed and Baba, 2013)

Sample Frame

The Agricultural zones, namely: Sokoto Zone comprising the following Local Government Area (Kware, Wamakko, Sokoto North, Sokoto South, Bodinga, Dange Shuni and Tureta), Gwadabawa Zone comprising the following LGA (Ilella, Gada, Tangaza, Gudu, Binji, Silame), Tambuwal Zone comprising the following LGA (Kebbe, Shagari, Yabo, Tambuwal) and Isa Zone comprising the following LGA (Isa, Sabon Birni, Goronyo, Wurno, Rabah) was used as a sampling frame. A purposive random sampling was used to select the Sokoto Zone with its constituent LGAs for samples. Veterinary clinics in Sokoto Zone were used to sample canine patients presenting as in-patients and out-patients. Inclusion criteria were any dog with overt clinical signs of salmonellosis: Diarrhea, pyrexia, vomiting, dehydration, and emaciation. Patients presenting any combination of diarrhea with any or all of this signs were sampled. Identified clinics were: Veterinary Teaching Hospital (VTH), Usmanu Danfodiyo University Sokoto; Sokoto State Clinic, Aliyu Jodi; Wammako Clinic; remote Underground Dog Slaughter Centers: Mammy Markets, Army and Police Barracks.

Physical Examination

An overt physical examination for selected clinical signs included as criteria for suspicion of *Salmonella* infection. Every presenting patient is examined for pyrexia, emaciation, dehydration, lymphadenopathy, diarrhoea and vomiting. Any patient presenting any or all of the clinical signs ruled in inclusion criteria is sampled

Ethical Clearance

The study is a two part study on spatial epidemiology of *Salmonella* and *Leptospira* with common ethical clearance obtained from Institutional Animal Care and Use Committee with approval number UDUS/IACUC/2014/AUP/R0-11

Sample Collection and Inoculation

From each dog, 5 ml of blood was collected through recurrent tarsal venipuncture according to methods described by Gatley (2009). Each blood sample was collected aseptically and emptied into a labeled bijou bottle (Becton Dickinson[R]). Rectal swab was collected aseptically using sterile swab stick (Micropoint Diagnostics[R], Michigan, USA) then it was dipped into a falcon tube (Eppendorp) containing 5 ml Selenite Broth (Oxoid, UK) for enrichment. Detailed data (sex, breed, age, medications) on each dog sampled was recorded.

The samples were transported in iced styrofoam box, packed with cotton wool and sealed to make airtight. Samples were preserved at -20°C at the Microbiology Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, until the following day when they were inoculated in batches.

Bacterial Culture and Sensitivity

Each broth was sub-cultured by streaking into MacConkey Agar (Oxoid, UK), *Salmonellar-Shigella* agar (Oxoid, UK), Xylose-Lysine Deoxycholate (XLD) agar (Oxoid, CM0469) and Brilliant Green *Salmonella* agar (Oxoid, CM1092) at 37°C for 48h as earlier used by Jajere *et al.* (2014). Cultured plates were examined in Biosafety Cabinets (BSC) 2 for colonial morphological characteristics. Tentative *Salmonella* colonies presenting colorless, non-lactose fermenting growth on MacConkey agar, dome-shaped non-lactose fermenting colonies with black center on XLD-agar, and pinkish raised colonies on *Salmonella-Shigella* agar was recorded as positive for any sample. The light microscope (Illumina®) was used at Mag 40, Mag 60 and Mag 100 to further view cultures after gram stain.

Identification of Isolates, Gram Staining and Biochemical characterization

Gram staining was carried out to observe the gram reaction, size, shape, and arrangement of the isolates according to standards of CLSI and as previously reported by Yang *et al.,* (2020).

Isolates from pure cultures were subjected to a number of biochemical tests, these include: hydrogen sulphide (H$_2$S) production test, motility test, indole test and citrate utilization test all according to updated CLSI updated protcols. Briefly, hydrogen sulphide (H$_2$S) production and motility test was done using the S.I.M (Sulphide, Indole, Motility) (Oxoid, CM0435) medium. This was prepared in test tubes as directed by the manufacturer, then slanted overnight. The isolates were sub-cultured on Nutrient agar (Oxoid, CM0003) by streaking method, incubated aerobically at 37°C for 24h. After 24h, the growth on Nutrient agar was inoculated on SIM medium slant using inoculation needle. Inoculation was made by stabbing the centre of the medium to a depth of 0.5 inch. Then incubated aerobically at 37°C for 24hours. Finally, observed for H$_2$S production and motility after 24h of incubation. Indole test was carried out by streaking overnight culture on nutrient agar on Simmons citrate with Kovacs reagent added to denote colour changes: pink indicating indole positive and yellow indole negative as previously reported by Saltzman *et al.* (1996). Citrate production was tested according methods described by Naveena and Joy (2014)

Preservation of Bacteria Isolate

Cultured *Salmonella* were sub-cultured on Nutrient agar slant, using sterilized inoculating loop. The slant bottles were sealed tightly to avoid contamination, then stored in refrigerator at -8°C for further bacteriological analysis. Typical colonies based on the morphological characteristics and biochemical test (to indicate presence of *Salmonella* isolates) were picked and used for antimicrobial resistance study.

Antibiogram Determination Using the Kirby Bauer Disk Diffusion Method

Antibiogram was carried out using standard Direct Sensitivity Testing (DST) as reported by Jajere *et al.* (2014) with Kirby-Bauer disk diffusion method, labeled against Mueller-Hinton Agar commercially procured from Oxoid, UK. Log Phase culture was serially diluted and graded concentrations plated on sensitivity disc to study intensity of inhibition after 48-hours incubation at 37^oC and 3% CO_2 adjustment, this was used to determine the Minimum Inhibitory Concentration (MIC) starting at 30μg/ml, serially diluted to 20μg/ml, 10μg/ml, 5μg/ml and 0. Antimicrobials plated on the disk for determination of antibiogram include the following panel: Tetracycline 10μg; Amoxicillin 5μg; Gentamycin 10μg; Metronidazole 30μg; Chloramphenicol 100μg; Ampicillin 25μg; Cotrimoxazole 100μg; Ciprofloxacin 15μg; Ceftriaxone 20μg; Nalidixic acid 30μg. Disks were placed on petri dishes bearing live colonies of *Salmonella* and left incubated for 48-hours aerobically at room temperature. Zones of inhibitions determined thereafter and resistance and susceptibilities calculated.

Data Collation and Statistical Analyses

Data generated from the study was imported into STATA version 2011, non significant and significant critical association between infection and predisposing variables was analyzed using correlation regression matrix, to test relationships between occurrence of disease and various epidemiological variables; age, sex, breed and presenting symptoms with level of significance at $P<0.05$.

RESULTS

Table 1 shows the association of salmonellosis with various symptoms previously reported in literatures and included in the study. A total of 35 presenting patients with pyrexia recorded positive isolation accounting 18.5% of all positive cases. This is followed by 19 patients having diarrhea recording positive isolation of *Salmonella* accounting for 13.7% of all positive cases. Other symptoms and isolation rates included vomiting 28(33.3%), lymphadenopathy 11(8.7%) and dehydration 17(16%).

Table 2. Presents the four categories of ages used and corresponding relative prevalence of *Salmonella* Isolates in each category. Puppies aged day 1 to three months accounted for the highest isolation group with 31 patients recording positive isolation accounting for 21.2% of all positive cases. Canine patients aged 6 to 12 months had 24 positive cases accounting for 22.2% of all positive cases presented. Middle aged dogs between 6months and one year had 6 positive cases accounting for 12.2% of all positive cases and adult dogs, some of which were senile aged beyond 24months recorded 2 positive cases accounting for 11.8% of all positive isolation

Table 3 shows the distribution of cases of salmonellosis in presenting patients based on sex The male patients had a preponderance of isolation with 48 presenting patients testing positive for *Salmonella* accounting for 76.1% of all positive cases while the female patients sampled had 15 testing positive accounting for 23.8% of all positive cases.

Table 4. shows the distribution of salmonellosis amongst various breeds sampled. The mongrel or the Nigerian local dog had the highest representation with 23 testing positive for *Salmonella* accounting for 36.5% of all isolation. The German shepherd presented the second highest isolation rate with 19 patients testing positive accounting for 30.2% of all isolation. The Caucasian had 16 positive cases accounting for 25.4% and the Rottweiler had 2 accounting for 0.9% of all cases

Table 5 presents various antimicrobials tested in the study for resistance against isolates of *Salmonella* in diarrheic dogs. Most resistance antimicrobial was Nalidixic acid with 29 Isolates recording resistance accounting for 52.7%, next was tetracycline with 19 isolates recording resistance at 43.2%. A total of 13(38.2) isolates presented resistance for metronidazole and so does 15 (35.7) isolates for ceftriazone from presenting dogs. Isolates were 100% susceptible to chloramphenicol and cotrimoxazole and, 90% susceptible to gentamycin

Table 1. Association of *Salmonella* Infection With Symptoms Seen In Canine Patients In Sokoto State Nigeria

Variables	(N)	C+Ve(N=63) (%)	OR (95% CI)	P
Pyrexia	189	35(18.5)	0.037 [4.521, 6.753]	0.01*
Lymphadenopathy	127	11(8.7)	0.134 [0.211, 2.334]	0.31
Diarrhoea	138	19(13.7)	7.531[3.275, 11.542]	0.00*
Vomiting	76	28(33.3)	0.351[0.415, 2.127]	0.27
Dehydration[a]	106	17(16.0)	1.00	

[a] Reference category, * Significant, OR = Odd Ratio, CI = Confidence Interval, C+ve Culture Positive, C-Ve Culture negative

**Table 2. Distribution of *Salmonella* Infection in Canine Patients Based on Age
In Sokoto State Nigeria**

Age(s)	No. Sampled	C+ve (%)	OR (95% CI)	P
0-5Mths	146	31(21.2)	0.215 [3.781, 6.251]	0.04*
6-12Mths	108	24(22.2)	0.428[0.613, 0.912]	0.27
12-24Mths	49	6(12.2)	0.661[0.331, 0.431]	0.19
> 24Mths[a]	17	2(11.8)	1.00	
Total	320	63(19.7)		

[a] Reference category, * Significant, OR = Odd Ratio, CI = Confidence Interval, C+ve
Culture Positive, C-Ve Culture negative

Table 3. Distribution of *Salmonella* Infection In Canine Patients Based on Sex in Sokoto State Nigeria

Variables	(N)	C+Ve(N=63) (%)	OR (95% CI)	P
Male	271	48(76.1)	0.021[0.213, 1.342]	0.71
Female[a]	49	15(23.8)	0.356 [0.174, 2.269]	0.21
Total	320	63(19.7)	1.00	

[a] Reference category, [*] Significant, OR = Odd Ratio, CI = Confidence Interval, C+ve Culture Positive, C-Ve Culture negative

Table 4. Distribution of *Salmonella* Infection In Different Breeds of Canine Patients In Sokoto State Nigeria

Variables	(N)	C+Ve(N=63) (%)	OR (95% CI)	P
Mongrel	162	23(36.5)	0.752 [0.378, 1.498]	0.49
Caucasian	80	16(25.4)	0.048 [0.015, 0.161]	0.01*
German Shepherd	65	19(30.2)	9.661[4.132, 22.632]	0.01*
Rotweiller	6	2(0.03)	0.351[0.415, 2.127]	0.27
Mastiff/Mongrel	4	2(0.03)	0.126 [0.186, 3.121]	0.31
Boerboel[a]	3	1(0.01)	1.00	
Total	320	63(19.7)		

[a] Reference category, * Significant, OR = Odd Ratio, CI = Confidence Interval, C+ve
Culture Positive, C-Ve Culture negative

Table 5. Antimicrobial Resistance in *Salmonella* Isolates in Canine Patients in Sokoto State

Antimicrobials	%Susceptibility	% Resistance	MIC(μg/ml)
	N-Susceptible/N tested	N-Resistant/N-Tested	
Chloramphenicol 20μg	100(51/51)	0(0/51)	0.5
Ampicillin 25μg	80(38/47)	9(19.1)	0.5
Gentamycin 10μg	90(45/59)	14(23.7)	1.0
Cotrimoxazole 100μg	100(38/38)	0(0/38)	0.2
Ciprofloxacin 15μg	85(39/46)	7(15.2)	5.0
Tetracycline 10μg	57(25/44)	19(43.2)	8.0
Metronidazole 30μg	62(21/34)	13(38.2)	2.4
Nalidixic Acid 30μg	46(25/54)	29(53.7)	5.0
Ceftriaxone 20μg	64(27/42)	15(35.7)	2.0
Amoxicillin 5μg	70(29/40)	11(27.5)	10.0

Discussion

Salmonellosis is a bacterial disease affecting both humans and animals and Nigeria is not an exception to its incidence (Fagbamila *et al.,* 2017). Some of the reported cases of salmonellosis in human and canine patients in Nigeria, presents mild to highly fulminant disease with life threatening emergencies in some cases (Raufu *et al.,* 2013). Invasive non typhoidal *Salmonella* commonly cause infections amongst infants, senile patients, canine patients and immune-compromised individuals worldwide and especially, in African countries where, the disease is driven in part by co-infection with malaria and human immunodeficiency virus (Ao *et al.,* 2015). Most studies and case reports of salmonellosis: typhoidal and non-typhoidal salmonellosis in Nigeria involves humans and avian species. Notable amongst them is the comprehensive studies of Jajere *et al.* (2014) and extensive prospective studies of Fagbamila *et al.* (2017) both studies in serovars of *Salmonella* in poultry in Nigeria. Both culture isolation, biochemical identification and molecular analysis targeting previously reported highly conserved genes in isolates of *Salmonella*. Globally, cultural isolation using specific selective medium for *Salmonella* is adequate for confirmation. Generally, few African countries, especially Nigeria report *Salmonella* surveillance data, consequentially, disease burdens, incidence in different regions of Africa and ecology of *Salmonella* is barely known. There is therefore, a paucity of evidenced based studies on Salmonellosis in different species of animals in Nigeria.

This is the first attempt to investigate the occurrence of *Salmonella* in canine patients presenting with a list of included symptoms in Sokoto State north-western Nigeria. An overall prevalence of 19.7% was obtained using culture isolation standard method as reported by the Clinical and Laboratory Standard Institute (CLSI), This is considerably lower compared to the prevalence of 33.1% reported by Jajere *et al.,* (2014) in northeastern Nigeria and environs. It is reported by other *Salmonella* researchers that the prevalence in dogs is decreasing in different regions of developing countries because there is increased awareness and importation of canned canine food with associated hygiene awareness. Several climatic, demographic, socioeconomic, ecological and temporal factors may account for the variations even though same technique was used for isolation but in two different ecologically and geographically distinct regions. The studies in northeastern Nigeria took an extended period because season was a variable under consideration, therefore minimum of two replicates data was required to statistically identify season as a variable significantly predisposing to *Salmonella* infection, more samples were therefore collected. This is different in the present study where season was not a variable under study. Sources of pets and management practices may be another significant outlier accounting for variations in prevalence between the two studies in two different regions. Akwuobu *et al.* (2018) reported 5.5% with just 11 dogs testing positive for *Salmonella* of 200 dogs sampled and Ojo and Adetosoye (2009) reported lower isolation rate of canine *Salmonella* infection of 3.7% in Zaria another research region in Nigeria further confirming a systematic decline from previously reported prevalence in the region.

Symptoms recorded for most presenting canine patients included in the studies were: pyrexia, diarrhoea, lymphadenopathy, emaciation, dehydration and vomiting. Many studies have reported these sets of symptoms at different stages of the diseases in dogs as well (Jajere *et al.,* 2020). Pyrexia and diarrhea present a statistically significant association with isolation of *Salmonella* in presenting patients at P<0.05. The authors stopped short of associating any set of symptoms as classical for presenting cases of salmonellosis in dogs based on the synthesized data. This data is not adequate to suggest the symptoms as being classical, but it raises suspicion index amongst clinicians and diagnosticians on *Salmonella* infection in dogs. Given the non-random association of pyrexia and diarrhea with isolation of *Salmonella,* further studies are warranted to investigate if these symptoms could be classical or pathognomonic for canine in studied regions. Table 4 presents list of symptoms studied and outcome of statistical association with isolation. Clinicians should develop a management protocol for presenting acute and chronic cases of canine salmonellosis using the studied symptoms as guide. In Table 2 puppies presented the highest incidence to the disease with 31 diseased of a total of 63 sampled accounting for 21.2% incidence. Literature is scarce on prevalence of *Salmonella* based on age in dogs. The puppies' categories consisting of dogs aged less than 5months recorded a statistically significant association with isolation of *Salmonella* at P<0.05. A putative reason may be the highly vulnerable puppies interacting with all sources of fomites few weeks and months after birth, given that salmonellosis is not vaccinated against in Nigeria, there is a chance most puppies will be challenged with the pathogen in early life. Other age categories with previous exposure to isolates of *Salmonella* may have developed immunity for common serovars and therefore will record very low numbers of the pathogens difficult to isolate.

Dogs aged 6-12 months had incidence of 22.2% with 24 presenting cases out of a total of 63 sampled.
The variation of isolation amongst male and female dogs in Table 3 was inferred in sketchy details in the literature as is the age-wise variation in *Salmonella* infection in dogs. Male dogs recorded a high prevalence of 76% with 45 presenting cases testing positive for isolates from a total of 63 samples collected compared with 23.8% in females with 15 dogs testing positive for isolation from a total of 63 dogs testing positive. The preponderance of male dogs with *Salmonella* may not be unconnected with increased outdoor activities of males compared to females. Okewole and Ayoola (2009) reported increased prevalence for *Leptospira* and *Salmonella* for dogs in south-western Nigeria using culture isolation studies. Most male adult dogs roam like stray dogs in search of food or mating mates, they

seek association with other dogs readily when the owners are not with them. In-contact association is one predisposing factor for *Salmonella* infection. In a recent study, Akwuobu *et al.* (2018) reported a lower isolation rate for mongrel (Nigerian local dog) of 2.4% compared to the high of 36.5% recorded in this report in Table 4. The high prevalence recorded in our study is not unconnected with the poor sanitary conditions mongrels are found coupled with the high incidence of straying amongst this breed. There was therefore a pattern of straying from urban to suburban environments in search of food and contacts with dogs of undetermined medical history as well as fomites. This may have accounted for the high prevalence compared to other breeds like Caucasian with a prevalence of 25.4%, as well as German shepherd with a prevalence of 30.2%.

Sanchez *et al.* (2002) have highlighted the danger of zoonoses of *Salmonella* because of the unbreakable association of humans with dogs. Preventing zoonosis logically follows therefore from keeping a healthy canine population unable to shed off pathogenic *Salmonella* serovars to the environment or directly to children. It is imperative further research is carried to screen pets of *Salmonella* to decrease transmission to immune-compromised humans hosts.

The emergence of multidrug resistance (MDR) including colistin resistance amongst isolates of *enterobacteriaceae* including *Salmonella* poses a serious public health risks because of the potential for transmission of these resistant variants to humans through close contacts and via the food chain (Jajere *et al.,* 2020), the specter of increasing antibiotic resistance is here confirmed by data of resistant isolates to commonly used antimicrobials in the clinics in Table 5. The pattern of resistance with corresponding zones of inhibition measured suggests resistance has been gradual and is continuous, putative causes may be mutant variants of the isolates differentiating by possible addition or substitution of genes. Horizontal gene transfer is proven to occur amongst microbial niches and is continuous as the pathogens interact. Continuous interaction with the surrounding microbial community, the environment and response to antimicrobials indiscriminately used for pets may have accounted over the years, for the phenomenon of antimicrobial resistance detected in this study. Isolates presented moderate to high resistance to antimicrobials tested against, a calculated 19(43.2%) isolates presented resistance for tetracycline, 15(35.7%) resistant to ceftriaxone, 11(27.5%) resistant to amoxicillin, 13(38.2%) resistant to metronidazole and 14(23.7%) resistant to gentamycin. These antimicrobials are commonly used for management of enteric and septicemic bacterial infections and considered drugs of choices in clinic protocol. Chloramphenicol was amongst first line drugs used for cases of salmonellosis in humans and animals, strains resistant to this drug emerged in the late 70s associated with self-transferable *IncHi* plasmids (Lopez *et al.,* 2014). Trimethoprim-sulfamethoxazole and ampicillin were then employed but these soon recorded resistant strains in the early 90s leading to fluoroquinolones and ceftriaxones and cefotaxime as newest drugs but with rapidly acquiring resistant genes amongst isolates of *Salmonellae* (Lopez *et al.,* 2014). The withdrawal of chloramphenicol for decades with the emergence of newer antimicrobials may have minimized acquisition and horizontal sharing of resistance genes to chloramphenicol as seen in 100% susceptibility of isolates.

The resistant patterns raises concern for further studies and reassessment of management protocols. An extensive cross sectional questionnaire based studies reported 70% of animals owners in Nigeria, as pastoralists and pet owners in the urban and sub-urban environments are uninformed or do not give attention about antimicrobial resistance and associated dangers of indiscriminate self prescription and or administration of antibiotics to animals for prophylactic, therapeutic and growth promoting purposes (Alhaji and Isola, 2018). Antimicrobial use and misuse in livestock and pets has received low attention globally. Developed nations are changing the narrative based on evidence-based studies that is directing policy of zero use and misuse od unprescribed drugs in animals and humans, in contrasts, not enough studies, absence of political will and poorly enforced legislations against drug use is promoting resistance to antimicrobials in Nigeria. Aerestrup (2012) reported global consumption of antibiotics in animals is twice that in humans. It is important research data serve as a wake up call to Nigeria scientists and public health policy administrators of the potential danger of the future.

Dog keeping as pets is rapidly increasing amongst elite clients in the studied region and general parts of Nigeria with the resultant rise in canine population which has to meet corresponding systematic policies and plans for deceasing contagions, ensuring vaccination and treating diseases. The scale of the problems in AMR is not commensurate to policies in place in Nigeria. In a review of 42 documented surveys designed to test AMR from miscellaneous samples of humans, animals and the environment, 1,139 isolates were resistant to 68 selected commonly used antimicrobials in Nigeria (Sanya *et al.,* 2013). Five sentinel pathogens were reported for the study including *Salmonella, Stapphylococcus, Klebsiella, Pseudomonas* and *ESBL*. There are few studies on dogs and cats on AMR in the extreme northwestern States; the few available have sketchy conclusions about AMR status in dogs. The United Nations General Assembly (UNGA) declaration of 2016, called on member states to generate annual situation analysis of AMR and develop an action plan. In fulfillment of this demand, fact sheets of AMR in pets in Nigeria need to be developed for all regions, where few exist, it is often flawed and obsolete. This underscores the significance of data generated in this research about AMR in sick dogs presenting in Sokoto, northwestern Nigeria

for a given period. More studies on AMR in dogs is warranted to create a time-tested true situation of AMR status in apparently healthy and symptomatic pets and how that constitute public health danger in the complexities of pets-human interactions.

Conclusion

The data showed evidence of Salmonella as primary cause or secondary complication in presenting cases of diarrhea amongst dogs in the study region with an incidence of 19.7%. The research presents evidence of subtle but progressive antimicrobial resistance amongst isolates obtained. Isolates were most resistance to metronidazole with 38.2% presented resistance whilst 100% were susceptible to chloramphenicol.

References

1. Ojo, O.E. and Adetosoye, A.I. (2009). *Salmonella typhimurium* infection in diarrheic and non-diarrheic dogs in Ibadan, Nigeria. *Veterinarski arhiv*. 2009:79: 371-377.

2. Green, C.E. (2012). Enteric Bacterial Infections, In: *Infectious Diseases of the Dogs and Cats*, Craig, E. Green (Eds), Fourth Edition, Elsevier Publishers, pp 383-385.

3. Jajare, S.M., Onyilokwu, A.S., Adamu, N.B., ATsanda, N.N.,Saidu, A.S.,Adamu, S.G. and Mustapha, F.B. (2014). Prevalence of *Salmonella* Infection In Dogs in Maiduguri, Northeastern Nigeria. *International Journal of Microbiology.* 10: 1-5.

4. Akinyemi, K.O., Ajosej, S.O. and Fakorede, C.O. (2021). A systematic review of literatures on human *Salmonella enterica* serovars in Nigeria (1999-2018). The journal of infection in developing countries. 15(9):1222-1235

5. Alhaji, N.B. and Isola, T.O. (2018). Antimicrobial Usage by Pastoralists in Food Animals in North-Central Nigeria: The Associated Socio-Cultural Drivers for Antimicrobial Misuse and Public Health Implications. *One Health,* 6: 41-47.

6. Lopez, J.J.G., Carrasco, F.S., Rodriguez, V., Montalva, A.S., Planes, A.M., Israel, M. and Larrosa, M.N. (2014). ESBL-Producing *Salmonella enterica* Serovar *Typhi* in Traveler Returning From Guatemala to Spain. *Emerging Infectious Disease,* 20: 1918-1920.

7. Aerestrup, F. (2012). The livestock reservoir for antimicrobial resistance: a personal view on changing patterns of risks, effects of interventions and the way forward. *Nature*13:465-466.

8. Sanya, T.E., Titilayo, O.F., Adisa, R. and Segun J.S. (2013). Use of Antibiotics among non-medical students in a Nigerian university. African Health Science 13: 1149-1155.

9. NPC (2006). Population Census figures, National Population Commission, Abuja, Nigeria, pp 1-40.

10. Mohammed, A. I. and Mohammed-Baba, T. A. (2013). Sokoto State-An overview. In. Mohammed, A.I. and Fada, A.G. (eds). The Impact of climate change on Sokoto State, Nigeria. UNDP-Sokoto State Government.

11. Thrushfield, M. (2009). *Veterinary Epidemiology*, 3rd Edition, Blackwell Science Limited, Blackwell Publishing Company, Oxford, UK, pp 228-238.

12. Gatley, J.M. (2009): The prevalence of *Leptospira* serovars causing infection in dogs in South Africa, MSc Thesis, University of Pretoria, pp 1-43.

13. Yang, C., Li, H., Zhang, T., Yitan, C., Zuo, J. and Chen, D. (2022). Study on antibiotic susceptibility of salmonella typhimurium L forms to the third and forth generation cephalosporins, Scientific reports. 10:3042http://doi.org/10.1038/s41598-02059456-8.

14. Saltzman, D.A., Heise, C.P., Hasz, D.E., Zebede, M., Kelly, S.M., Curtis, R., leonard, A.S. and Anderson, P.M. (1996). Attenuated *Salmonella typhimurium* containing interleukins 2 decreases MC-38 hepatic metastases: a novel anti-tumor agent. *Cancer. Bio. Rad.* 1102): 145-153.

15. Naveena ,V. and Joy, P.P. (2014). Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala. India.

16. Fagbamila, I.O., Barco, L., Mancin, M., Kwaga, J., Ngulukun, S.S., Zavagnin, P., Lettini, A.A. Lorenzetto, M., Paul, A.A., Kabir, J., Umoh, J., Ricci, A., Muhammed, M. (2017). *Salmonella* serovars and their distribution in Nigerian commercial chicken layer farms. *PLOS One* 12(3): e0173097.

17. Raufu, I., Bortolaia, V., Svendsen, C.A., Ameh, J.A., Ambali, A.G. andAerestrup, F.M. (2013). The firsts attempt of an active integrated laboratory-based *Salmonella* surveillance programme in the north eastern region of Nigeria. *J.App. Microbiol.* 115: 1059-1067.

18. Ao, TT.. Feasey, N.A., Gordon, M.A., Keddy, K.H., Angulo, F.J. and Crump, J.A. (2015). Global burdens of invasive non-typhoidal *Salmonella* disease. Emerg. Infect. Dis. PMCID:PMC 4451910

19. Akwuobu, C.A., Agbo, J.O. and Ofukwu, R.A.P. (2018). *Salmonella* Infection in clinically healthy dogs in Makurdi, Benue State, North-central Nigeria: A potential source of infection to humans . *J. Adv. Vet. Anim. Res.* 5(4): 405-408.

20. Jajere, S.M., Hassan, L., Jalila Z.Z. and Aziz, S.A. (2020). Antibiogram profiles and risk factors for multidrug resistance of *Salmonella enterica* recovered from village chickens (*Gallus gallus domesticus)* and other environmental sources in the central and southern peninsular Malaysia. *Antibiotics* 9:701-713.

21. Okewole, E.A. and Ayoola, M.O. (2009). Seroprevalence of *Leptospiral* serovars other than *canicola* and *icterohaemorrhagiae* in dogs in the Southwestern Nigeria. *Veterinarski Arhiv.* Vol 79, 2009, pp 87-96.

22. Sanchez, S., Hofacre, C.L., Lee, M.D., Maurer, J.J. and doylr, M.P. (2002). Animal Sources of Salmonellosis in Humans. *J. Am. Vet. Med. Ass.* 221: 492-497.