ISSN: 2714-4674 (Online)
ISSN: 2714-4666 (Print)

Annals of Clinical and Experimental Medicine

(ACEMedicine)

This Journal Is A Publication of
ASSOCIATION OF SPECIALIST MEDICAL DOCTORS IN ACADEMICS SOKOTO STATE CHAPTER

Volume 1, No. 2, July - December 2020

In this issue

www.acemedicine.asmeda.org

Detection of Zoonotic Yersinia Infection in Symptomatic and Asymptomatic Dogs in Sokoto State Nigeria

Nicholas Nathaniel Pilau[1*], Shehu Zaid[1], Abubakar Sadiq Yakubu[2], Bashir Saidu[3], Umar Yakubu Dabai[4], Goodluck Godson Chidozie[1] and Adamu Jibril Bamaiyi[5]

[1]*Department of Veterinary Medicine*
[2]*Department of Veterinary Surgery and Radiology*
[3]*Department of Veterinary Physiology and Biochemistry*
[4]*Department of Veterinary Public Health and Preventive Medicine*
[5]*Department of Physiology, Faculty of Basic Medical Sciences*
Usmanu Danfodiyo University Sokoto, Nigeria

Corresponding author:

Nicholas Nathaniel Pilau
Department of Veterinary Medicine Usmanu Danfodiyo University Sokoto, Nigeria
Email: *nicholaspilau@gmail.com, nicholas.pilau@udusok.edu.ng*
Tel: *+2347032409535*

ACCESS TO THIS ARTICLE ONLINE

DOI: 10.47838/acem 26011977.127122020.asmeda.1.2

Website
http://www.acemedicine.asmeda.org

Abstract

Background: Zoonotic *Yersinia* infection has been previously reported in humans and animal hosts in Nigeria, occasionally with fulminant disease. Despite earlier evidence of *Yersinia* pathogen circulating in human and animal populations in Nigeria, studies and suspicion index to *Yersinia* is below an acceptable average amongst clinicians, diagnosticians, academics and health policy officers.

Methods: The Deoxycholate Citrate Agar (DCA) was used as selective media to culture *Yersinia* preceded by inoculation in MacConkay agar. Plates with evident growth in the differential media consistent with reported accounts for *Yersinia* were picked and inoculated in selective medium and left for 48 hours until growth was seen, other samples left until five days before being discarded as negative. Pure cultures were subjected to comprehensive biochemical tests standard and previously applied for diagnosis and discrimination of *Yersinia* species.

Result: This research recorded an overall microbial prevalence of 30%. Prevalence of *Yersinia enterocolitica* was 18.3% and *Y. pseudotuberculosis* 11.7%. Male dogs presented a relative prevalence of *Y. enterocolitica* 40.9% compared with 59.1% recorded by female dogs. Symptomatic dogs presented a relative prevalence of *Y. enterocolitica* of 86.4% and *Y. pseudotuberculosis* of 71.4%, symptoms was associated with isolation of *Yersinia* ($P<0.05$).

Conclusion: *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* is circulating in canine population that serve as asymptomatic carriers and in some cases as symptomatic dispersal hosts of the pathogen in Sokoto State.

Key words: *Yersinia*, symptoms, culture, zoonoses, diagnosis

Introduction

The term yersiniosis refers to infection caused by *Yersinia enterocolitica* or *Yersinia pseudotuberculosis* as well as the plague-borne *Yersinia pestis* which appears as enteritis and, sometimes as septicemia in human and animals (1). *Yersinia* is a foodborne pathogen causing a variety of symptoms in humans and wide spectrum of animal species (2), the symptoms cause wide ranges of diseases from mild diarrhea to immunological complications, with acute gastroenteritis and mesenteric lymphadenitis mimicking appendicitis in humans with potentially lethal septicemia (3,4). Infection with *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* has been associated with acute appendicitis in humans and gastroenteritis complex in animals (5). Swine tonsils are proven to be the major reservoir of pathogenic *Yersinia enterocolitica* (6). There is demonstrably, high similarity between pigs and human strains of *Yersinia enterocolitica* using DNA based methods (6).

Distribution of pathogenic species of *Yersinia* shows geographical variation, as does the incidence of classical gastroenteritis associated with different strains of *Yersinia*. There are proven difficulties associated with isolating *Y. enterocolitica* from clinical, experimental and naturally contaminated samples (6). Notably, the culture methods have limitations to do with low sensitivity, vulnerability to contaminations and limited ability to profile pathogenic from saprophytic *Yersinia* strains (5). Numerous virulence factors can be demonstrated using special media and methods but this is not required for routine diagnosis (7). For most studies, except those interested in elucidating virulence genes important for pathogenicity and evolution, the culture and biochemical characterization is adequate for detection of *Yersinia* species in clinical specimen (8).

Y. pseudotuberculosis occurs in water and in the environment as well as various wild and domesticated animals (1). *Yersinia* belongs to the *Enterobacteriaceae* family and contains 11 species with *Yersinia enterocolitica, Yersinia pseudotuberculosis* and *Y. pestis* documented to be pathogenic in humans and the former two documented as the commonest foodborne pathogens causing poisoning in dogs (8). Most strains of *Yersinia* are facultatively anaerobic, gram negative, non-spore forming rods or coccobacilli, they grow at about $37^{?}$C on MacConkey agar, species were catalase positive, oxidase negative and ferment glucose with production of acid (7).

In a comprehensive study, Smego *et al.* (7) described three pathogenic species of *Yersinia* namely: *Y pestis, Y pseudotuberculosis* and *Y enterocolitica*; while three others namely: *Y. intermedia*, *Y frederiksenii* and *Y kristensenii* are said to be saprophytic. Similarly, infection with the genus *Yersinia* has been categorized into the plague, caused by *Y. pestis* and non-plague infection associated with other species. There are vast scholarly research establishing baseline microbiological and biochemical characteristics of *Yersinia,* which has been referred for differentiation of species of *Yersinia* in samples.

Yersinia being an entero-invasive pathogen transmitted to humans by ingesting contaminated food, particularly pork poses a great danger to human and animal populace in Nigeria. This is because almost all of the 12 states of southern Nigeria consume pork and dogs along with about 40% of populace in the northern region.

Research and evidence-based data on *Yersinia* circulating in canine and human population is scarce in the region of Nigeria where this research was undertaken. Research is necessary to identify reservoirs of various species of *Yersinia* within the study region. Even for the well-known pathogenic *Yersinia enterocolitica*, a good understanding of the natural reservoirs and modes of transmissions to humans remains elusive in most developing countries (9,1). Most presenting cases in human and veterinary hospitals is speculative and treatment general. Various isolates of *Yersinia* in different species: cattle, pigs and humans have been done in other regions of Nigeria (10). While the risks of *Yersinia* infection in Nigeria have increased over the years with expanding population and cross border animal and meat trades, it has not been commensurately matched with research and preventative public health policies and programs. This research was therefore conceived to investigate the status of *Yersinia* infection and provide baseline data for reference to clinicians, diagnosticians, academics, public health authorities and other research enthusiasts. There was no previously reported prevalence of *Yersinia* in the study area, and no record of total canine population in Sokoto State. This study reports detection of zoonotic *Yersinia* infection in canine population in the northwestern part of Nigeria, located at the border with Niger Republic. This study leverages previously reported cultural isolation and biochemical characterization to detect pathogenic *Yersinia* in obtained clinical samples from dogs with potential for human transmission due to close

association of both species.

Materials and Methods

Study Area and Design

This research was conducted at Sokoto State, the northwester part of Nigeria located on longitudes 4? 8'E and 6? 54'E; latitudes 12? N and 13? 58'N (11), with rich human and animal population. It was a prospective investigation into detecting *Yersinia Enterocolitica* and *Y. pseudotuberculosis* species in presenting dogs within the study area. Every dog encountered is sampled in a randomized frame generated using zones that compartmentalized the 23 Local Government Area (LGA) in the state into four Agricultural zones which consists of Sokoto Zone (Kware, Wamakko, Sokoto North, Sokoto South, Bodinga, Dange Shuni and Tureta), Gwadabawa Zone (Ilella, Gada, Tangaza, Gudu, Binji, Silame), Tambuwal Zone (Kebbe, Shagari, Yabo, Tambuwal) and Isa Zone (Isa, Sabon Birni, Goronyo, Wurno, Rabah). A purposive sampling method was used to select two Local Government Areas for each zone, the total samples "N" calculated for canine samples divided by the number of LGA's in the state will give the relative sample size "n" for each LGA. However, most veterinary hospitals are located in Sokoto metropolis comprising all the LGAs in Sokoto zone. Symptomatic dogs were sampled from patients presenting in the government and private veterinary clinics located in this zone. Records with the veterinary authorities were used to locate clients keeping dogs as pets and for sampling.

Presenting symptoms used as inclusion criteria include fever, diarrhea, lymphadenopathy, dehydration, emaciation, anemia (evinced from pale mucous membranes). Some or all of these symptoms presenting were considered as basis. Demographic data (age, sex, breed, ongoing medication) were carefully labeled for each dog sampled.

Sample Size Determination

A prevalence of 7.5% reported by Okwori *et al.* (1) in dogs in Jos, Plateau State was used to compute the required sample size using the formula $N=Z^2P\ (1-P)/d^2$ (12). Where: N is the sample size to be calculated, Z (1.96) is standard error at 95% confidence interval, P is the prevalence (7.5%) reported from previous studies, d is the level of precision taken to be 5% for this study. Total Sample size N calculated was 106.6, this was rounded to 120.

Sample Collection and Preservation

For each dog encountered, fecal samples were collected using sterile swabs (Micropoint Diagnostics? Lot number 151101) which was then inserted into 10mls of phosphate buffer in a 25mls falcon tubes (Eppendorf) adjusted at PH 7.4 using the PH meter as earlier described by Shayegani *et al,* (13). Sample was then transported to the Microbiology Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria, preserved at 4? C until inoculation for culture and identification. Detailed demographic data was documented for each dog sampled, the age, sex, breed, vaccination status and presenting symptoms if any, were all recorded into prepared field record charts.

Media Preparation and Sample Inoculation

Yersinia selective media: deoxycholate citrate agar (DCA) with 0.5 % sucrose supplement was used as basal media for isolation. Measured 29g of the *Yersinia* selective media was dissolved in 500mls of double distilled water Gibco? and the mixture stirred using autoclaved stirrer. The resultant medium was then autoclaved at 121? C for 15minutes, the cork was then paraffin sealed after cooling and labeling done in level 2 biosafety cabinet at a different but proximate laboratory: Central Research Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto. Field samples were first subjected to cold enrichment. This was achieved by dissolving 2g of faecal sample from each dog sampled into a 10m phosphate buffered saline (PBS) solution and the product vortexed for

2minutes, each tube was then incubated at 4° C for three weeks. Samples from the cold enrichment was subsequently cultured on MacConkey agar for 30 hours and incubated at temperature of 20° C. Colonies were picked and plated on the petri dishes labeled with the *Yersinia* selective medium, left for 48hours and then up to 5days before being considered negative. The incubated streaked colonies were subjected to Gram stain after 48 hours. Colonies were later dissolved in buffered saline and viewed using Illumina light microscope at 40 and 60 magnifications.

Identification and Biochemical Tests: Small, raised and round translucent Gram negative colonies, reddish in colour because of the sucrose fermentation in DCA indicated growth of *Yersinia* species. Isolates with the colonial morphology were then transferred to Triple Sugar Iron (TSI) agar (Oxoid, UK). Isolates then formed acid-like ring at the slant and or butt of the TSI. Isolates were then selected and subjected to batteries of biochemical tests.

The motility, sucrose and Voges Proskauer were performed at 25° C according to earlier method demonstrated by Shayegani *et al.* (13) and Kwaga *et al.* (10) while the indole, urease, sorbitol, raffinose and rhamnose tests were carried out at 35 to 37° C accordingly as described by Shayageni *et al.* (14). Species of *Yersinia* were identified based on published and adopted taxonomy by Brenner (15) and Manual of *Yersinia* species differentiation by Darland *et al.* (16).

Statistical Analysis

Data generated was imported into an Statistical Package for Social Sciences (SPSS), version 2011, non significant and significant critical association between infection and predisposing variables was analyzed using Pearson's Chi Squared Test to test association between occurrence of disease and various epidemiological variables; age, sex and presenting symptoms with level of significance at $P<0.05$.

Samples	Pathogen	Motility	Sorbitol	Sucrose	Urease	Raffinose	Voges Proskauer	Indole	Rhamnose
D1	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D3	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D4	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D5	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D8	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D11	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D14	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D16	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D24	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D31	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D34	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D38	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D41	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D43	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D47	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D56	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D61	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D63	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D65	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D66	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D67	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D69	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D79	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D81	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D85	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D88	*Y.pseudotuberculosis*	+	-	-	+	+	-	+	+
D91	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D97	*Y. enterocolitica*	+	+	+	+	-	-	-	-
D98	*Y. enterocolitica*	+	+	+	+	-	-	-	-

Interpreted Based on the Report of Fukushima *et al.* (17)

Keys: + Positive for the element

\- Negative for the tested element

Yersinia Species	Number Positive	Number Negative	Relative Prevalence %	Total
Y. enterocolitica	22	98	18.3	120
Y. pseudotuberculosis	14	106	11.7	120

Table 3. Prevalence of *Yersinia* Species Based on Selected Demographic Data

Parameters	*Yersinia* Species *Y. enterocolitica*	*Yersinia* Species *Y.pseudotuberculosis*	Relative Prevalence %		X^2(DF)	P-Value
			Y. enterocolitica	*Y. pseudotuberculosis*		
Male	9	5	40.9	35.7	0.4256	0.5142
Female	13	9	59.1	64.3	0.3254	0.2113
Young (8Mths)	6	5	27.3	35.7	0.1234	0.4335
Adult (9Mths)	16	9	72.7	64.3	0.067	0.7971
Symptomatic	19	10	86.4	71.4	0.2531	0.0481*
Asymptomatic	3	4	13.6	28.6	0.3632	0.1738

Discussion

Yersinia species can be diagnosed presumptively from clinical samples using direct microscopy (5). However, gold standard and definitive diagnosis requires culture of clinical samples at precise temperatures in a variety of routine culture media: blood agar and brain heart infusion, triple sugar iron (TSI) and deoxycholate agars (7). This research leveraged culture media previously used for isolation of *Yersinia* in samples to detect the pathogen circulating in canine population in Sokoto State.

In a four-year study of *Yersinia* infection in humans, animals and the environment to elucidate linear or triangular relationship between infection, ecology and disease, Shayegani *et al.* (13) determined that isolates of *Yersinia enterocolitica* possessing same characteristics biochemically, phage types and serogrouping were found in human, animals and the environment, suggesting transmission from the environment to animals and then animals to humans. This underscores the significance of *Yersinia* isolation to determine possible reservoir and the dynamics shaping transmission and disease manifestation. In the present study, evident isolation of two zoonotic pathogenic *Yersinia* species using standard culture isolation previously used by numerous diagnosticians and, biochemical tests adopted and interpreted based on the reports of Fukushima *et al.* (17), suggests or indicates the potential for transmissions to other animals and or humans. Studies on *Yersinia* infection in Nigeria is scanty, a few traces is the study of Kwaga *et al.* (10) where evidence of *Yersinia enterocolitica* was demonstrated using TSI culture and serotyping. In the study, agglutinins to serotype *enterocolitica* 0:3 and 0:8 was reported raising the possibility and fear of human infection and, possible epidemic in the future in Nigeria. Okwori *et al.* (1) also undertook an experimental infection study in Nigeria, *Y. pseudotuberculosis* used for the infection was isolated from the feces of animals in Nigeria, post infection, fulminant disease resulted when experimental animals were subjected to immunosuppressive factors. These studies provided informative and clinical pathology data, but for the most part, over a decade old and may not be reflective of current situation in Nigeria. In this study, overall prevalence of *Yersinia* was 30%. Prevalence of *Yersinia enterocolitica* was 18.3% and *Y. pseudotuberculosis* 11.7% (Table 2). Okwori *et al.,* (1) reported an overall prevalence of 7.5% to *Yersinia* in Jos using culture isolation in same medium used in this research. This varied prevalence is likely due to varying climatic and demographic factors between the two study areas, and the two referred studies span a period of over a decade which would have affected the dynamics of disease transmission and infection spread amongst reservoirs.

A number of studies have reported isolation of *Yersinia* pathogen in feverish, debilitated and diarrheic animals, children and human adults, most of these studies reported consistent symptoms. This study presented statistically significant association ($P<0.05$) of set of symptoms with the isolation of *Yersinia* in canine patients presenting in sampled clinics within Sokoto State. The symptoms in the inclusion criteria were overt and previously reported for most cases of *Yersinia* infection. It is important for clinicians to suspect *Yersinia* in presenting canine patients manifesting the inclusive symptoms of fever, lymphadenopathy, anemia and diarrhea manifesting in part or in all amongst patients. However, this observation needs to be repetitive with studies with higher samples to see reproducible and predictive pattern before claims of consistence, as pathognomonic signs be included in clinical protocol. The relative prevalence of 40.9% to *Yersinia enterocolitica* and 35.7% to *Yersinia pseudotuberculosis* amongst male dogs recorded in this study is similar to relative prevalence of 49.9% to *Yersinia enterocolitica* and 35.4% to *Yersinia pseudotuberculosis* reported by Okwori *et al.* (1).

Previous studies in other continents did not clearly indicate female animals are likely to have higher prevalence. There is a chance not enough samples were collected during the study, or that female dogs being more social may have had higher contact to in-contact infected dogs than the male dogs, the preponderance of cases amongst females is an observation that needs to be studied repeatedly with higher sample size and with questionnaire to ascertain basis for variation in exposure and infection. The limitations to the study were clear, uneven distribution of dogs across the sampling frame generated, ensued security challenges in some of the areas within the sample frame and within Sokoto Sate and lack of complete panels for use during biochemical characterization as well as inability to obtain antisera to *Yersinia* to serotype earlier reported non-plague intermediate *Yersinia* strains, these were some of the regrettable difficulties.

Y. enterocolitica has a broad spectrum animal reservoir including rabbits, dogs, rodents and birds (18). It is important to epidemiologically identify reservoir populations of *Yersinia* to understand the occurrence and progress of disease. Identifying the source makes public health protection possible and prevention easy. Establishing causal relationship between animal infections and human transmission is significant in control and eradication of yersiniosis. Wang *et al.* (18) demonstrated human infection from animal reservoirs in China, the data reported homologous patterns of porcine and human isolates after doing pulse field electrophoresis. Although this study was not molecular and human samples not involved, evidence of infection in canine population is demonstrated which is the first warranted step in disease investigation. This is significant with far reaching implications because of the proximity of humans and dogs. This age-long inter-species social bond and ecology of serotypes of *Yersinia* isolated from dogs and the environment may be determining factors in the occurrence of epidemics in the future. Okwori *et al.* (1) reported that consumption of pork and dog meat common in Nigerian cities is a major predisposing factor to *Yersinia* infection, this conclusion was borne out of a comprehensive survey o*f Yersinia* serotypes circulating in diarrheic children in north-central states of Nigeria. Association of humans with dogs as pets and consumption of the former in some cultures in Nigeria is a double edge risks to infection and dispersal of *Yersinia.* To assure preparedness and keep track of microbial ecosystems with spectre for epidemics, it is imperative that surveys are repetitive, reservoirs identified and causal relationship clearly delineated. Isolation and treatment, prophylaxis and reservoir-hosts interruption limit the chance of epidemics previously recorded on European continent with devastating consequence.

In a published paper, Smego *et al.* (7) reported that plague and non-plague yersiniosis has a global distribution and, marking the 100th anniversary since the great "Black Death" that threatened to wipe out European population, the paper reported that between 1979 and 1993, 56% of all reported cases of yersiniosis in humans, and 75% of deaths were on African countries. It is therefore imperative and expedient for different regions to conduct evidence-based surveys to detect circulating *Yersinia* in symptomatic human or animal populations, the environment as well as identify reservoir of the pathogen. This study clearly showed *Yersinia enterocolitica* and *Y. pseudotuberculosis* are circulating in apparently healthy (asymptomatic) as well as the symptomatic canine population within the study region. A more detailed and wider study is called for to investigate on a regional or national scale, evidence of *Yersinia* infections in humans and animals and possibly, delineate the genes mediating virulence, adaptation and evolution of the pathogen in Nigeria. This study provides a reference data of a pathogen rarely studied nor considered in the referred region in Nigeria but it only scratched the surface of the unknown on the current epidemiology of *Yersinia* in animal and human populations in northwestern Nigeria.

Conclusion

This research established the first diagnosed case of yersiniosis in dogs presenting to clinics in Sokoto State. Most importantly, this study showed evidence of *Yersinia* species in apparently healthy dogs that may serve as shedders to in-contact animal and human hosts. An overall microbial prevalence of 30% to *Yersinia* species in symptomatic and apparently healthy dogs was recorded. The study established relative prevalence of *Yersinia enterocolitica* to be 18.3% and *Y. pseudotuberculosis* 11.7%. Symptom was significantly associated with isolation of *Yersinia i*n sick dogs ($P<0.05$). This is important with direct application to increase suspicion index and prioritize surveillance for zoonotic *Yersinia* reservoirs.

Acknowledgements

The authors wish to thank technologists in the Veterinary Microbiology Laboratory, Usmanu Danfodiyo University, especially Mr. Yakubu Umar Dabai as well as authorities of the Army Barracks that gave us access to sample numerous dogs kept there.

Grants and Conflict of Interest

The authors sponsored this work as an independent university-based research. The authors have no conflict of interest to declare

References

1. *Okwori AEJ, Agina SE, Odugbo MO, Olabode AO, Lombin LH. Experimental Yersinia pseudotuberculosis Enteritis In Laboratory Animals. Afri J Biotech. 2007: 2411-2414.*
2. *Van Damme I, Habib I, Zutter LD. Yersinia enterocolitica in Slaughtered Pig Tonsils: Enumeration and Detection by Enrichment Versus Direct Plating Methods. Food Microbiol. 2010: 158-161.*
3. *Bottone EJ. Yersinia Enterocolitica: The Charisma Continues. Clin Microbiol Rev. 1997: 257-276.*
4. *Bottone EJ. Yersinia enterocolitica: An Overview and Epidemiologic Correlates, Microb Infect. 1999: 323-333.*
5. *Hetem DJ, Pekelharing M, Thijsen SFT. Probable Transmission of Yersinia enterocolitica from a pet dog with diarrhea to a 1-year–old infant. BMJ Case Rep. 2013: 1136-1146*
6. *Ahoma MF, Stolle A, Stephen R. Prevalence of Pathogenic Yersinia enterocolitica in Pigs Slaughtered at Swiss Abattoir. Inter J Food Microbiol. 2007: 207-212.*
7. *Smego RA, Frean J, Koornhof HJ. Yersiniosis: Microbiological and Clinico-epidemiological Aspects of Plague and Non-Plague Yersinia Infections. Eur J Clin Microbiol Infect Dis. 1999:1-15.*
8. *Linde HJ, Neubauer H, Hermann M, Aleksic S, Lehn N. Identification of Yersinia species by the Vitek GNI Crad. J Clin Microbiol. 1999: 211-214.*
9. *Shayegani M, De-Forge I, McGlynn DM, Timothy R. Characteristics of Yersinia enterocolitica and Related Species Isolated from Humans, Animals and Environmental Sources. J Clin Microbiol. 1981: 304-312.*
10. *Kwaga JP, Adesiyun AA, Lombin LH, Mijinyawa SM, Agbonlahor DE. Cultural and Serological Studies of Yersiniosis in Camels in Nigeria. Can Vet J. 1987: 1-2.*
11. *NPC. Population Census figures, National Population Commission, Abuja, Nigeria, 2006. pp 1-40.*
12. *Thrushfield M. Veterinary Epidemiology. 3rd ed, Oxford, UK: Blackwell Science Limited, 2009. p 228-238.*
13. *Shayegani M, De-Forge I, McGlynn DM, Timothy R. Characteristics of Yersinia enterocolitica and Related Species Isolated from Humans, Animals and Environmental Sources. J Clin Microbiol. 1981: 304-312.*
14. *Shayegani M, Menegio EJ, McGlynn DM, Gaafar A. Yersinia enterocolitica in Oneida County, New York. Cont Microbiol Immunol. 1979: 196-205.*
15. *Brenner DJ. Speciation in Yersinia In: Carter PB, Lafleur L, Toms S. editors, Contributions to Microbiology and Immunology. 2nd ed. 1979. p 33-43.*
16. *Darland G, Ewing WH, Davis, BR. The Biochemical Characteristics of Yersinia enterocolitica and Yersinia pseudotuberculosis, A Center for Disease Control Manual of United States Department of Health Education and Welfare, 1974. Atlanta, p11-24*
17. *Fukushima H, Shimizu S, Inatsu Y. Yersinia enterocolitica and Yersinia pseudotuberculosis Detection in Foods. J Patho. 2011: 1-10.*
18. *Wang X, Cui Z, Jin D, Tang L, Xia S, Wang H, Xiao Y, Qiu Q, Hao Q, Kan B, Xu J, Jing H. Distribution of pathogenic Yersinia enterocolitica in China. J Clin Microbiol Infect Dis. 2009: 1237-1244.*