Eco-epidemiology and microbiological evaluation of poultry salmonellosis in North Central Nigeria, and socio-economic and public health impact

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Eco-epidemiology and microbiological evaluation of poultry salmonellosis in North Central Nigeria, and socio-economic and public health impact

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DECLARATION

I, the undersigned, declare that the thesis hereby submitted to the University of Pretoria for the degree PhD (Veterinary Science) and the work contained herein is my original work and has not previously, in its entirety or part, been submitted to any university for a degree. I further declare that all sources cited are acknowledged using a list of references.

Signed _____ this __04th__ day of _____July__ 2024.

QUOTES

“No one will reap except what they sow “– Quran 6:164

“Taking pains to remove the pains of others is the true essence of generosity”- Abu Bakr (RA)

“Sometimes the best things in life are unplanned.

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LIST OF SUPPLEMENTARY MATERIALS AND APPENDICES

LIST OF SYMBOLS AND ABBREVIATIONS

Acronyms	***Full meaning***
ASFH	Amo Farm Sieberer Hatchery Ltd.
AG GDP	Agriculture Value Added- Gross Domestic Product
AIC	Akaike Information Criterion
ASL2050	Africa Sustainable Livestock 2050
AMP10	Ampicillin
AMR	Antimicrobial Resistance
AOR	Adjusted Odds Ratio
AUROC	Area Under the Receiver Operating Characteristic Curve

BCA	Benefit - Cost Analysis
BCR	Benefit – Cost Ratio
BSA	Brilliance *Salmonella* Agar
C30	Chloramphenicol
CAZ10	Ceftazidime
CDC	Centers for Disease Control and Prevention
CDSs	Coding Sequences
CEA	Cost Effectiveness Analysis
CHE	Current Health Expenditure
$\chi 2$	Chi square
CIs	Confidential Intervals
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CMLE ORs	Conditional maximum likelihood estimates of Odds Ratio
CN10	Gentamicin
$CNTS_{nOH}$	Total cost of non-One Health interventions against NTS
$CNTS_{OH}$	Total cost of One Health interventions against NTS
CTX30	Cefotaxime
DALY	Disability-Adjusted Life Year
DCs	Data Collectors
DNA	Deoxyribonucleic Acid
DOT	US Department of transportation
ECDC	ECDC
EFSA	EFSA
ELISA	Enzyme Linked Immuno-Solvent Assay
ESBL	Extended-Spectrum Beta-Lactamase
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FCT	Federal Capital Territory
FMARD	Federal Ministry of Agriculture and Rural Development
FMoH	Federal Ministry of Health
F/SMoH	Federal/ State Ministries of Health
GDP	Gross Domestic Product
GIT	Gastrointestinal Tract
GMP	Good farm Management Practice
HIV	Human Immunodeficiency Virus
HPAI	Highly Pathogenic Avian Influenza
HRAs	Hired Research Assistants
ICER	Incremental Cost-Effectiveness Ratio
iNTS	Invasive non-typhoidal *Salmonella* (salmonellosis)
IQR	Interquartile range
K30	Kanamycin
KOD	Kogi Dust sample
KWD	Kwara Dust sample

LGAs	Local Government Areas
LMICs	Low- and Middle-Income Countries
MDR	Multi Drug-Resistant
N	Naira
NA	Nutrient Agar
NA	Not Applicable
NA30	Nalidixic Acid
NCDC	Nigeria Center for Disease Prevention and Control
NCN	North Central Nigeria
NEARLS	National Agricultural Extension and Research Liaison Services
NPHCDA	National Primary Health Care Development Agency
NPIP	National Poultry Improvement Plan
NTS	Nontyphoidal salmonellosis
OCT	Outbreak Costing Tool
OH	One Health
PCR	Polymerase Chain Reaction
Ph	Potential of Hydrogen
PPP	Purchasing Power Parity
PSA	Probabilistic Sensitivity Analysis
PDR	Pandrug-Resistant Bacteria
QALY	Quality-Adjusted Life Year
REC	Research Ethics Committee
Ref	Reference
ROC	Receiver Operating Characteristics
RTI	Research Triangle Institute
RV	Rappaport-Vassiliadis
RVS	Rappaport-Vassiliadis Soy Peptone
S3’300	Sulphonamides
S.	*Salmonella*
SAG	*Salmonella*-Associated Gastroenteritis
SE	Standard Error
SORMAS	Surveillance Outbreak Response Management and Analysis System
Spp.	Species
TE30	Tetracycline
USA	United State of America
USDA	United State Department of Agriculture
UDUS	Usmanu Danfodiyo University
VA	Value Added
VSL	Value of Statistical Life
VSLY	Value of Statistical Life Year
VTH	Veterinary Teaching Hospital
W5	Trimethoprim
WHO	World Health Organization
WOAH	World Organisation for Animal Health

XDR	Extensively Drug-Resistant
XLD	Xylose Lysine Desoxycholate
YLD	Years of healthy Life lost due to Disability
YLL	Years of Life Loss

Glossary of Terms

AIC	The Akaike information criterion (AIC) is an estimator of prediction error and thereby relative quality of statistical models for a given set of data.
BCR	The benefit-cost ratio (BCR) is a ratio that attempts to identify the relationship between the cost and benefits of a proposed project.
CMLE ORs	It is the Odds ratio estimated for the parameters of an assumed probability distribution, given some observed data estimates obtained by fitting the no-three-factor-interaction model in log-linear and logit analyses
DALY	Disability-adjusted life years are a measure of overall disease burden, expressed as the number of years lost due to ill-health, disability, or early death.
ECDC	The European Centre for Disease Prevention and Control (ECDC) is a public health agency of the European Union (EU), operational since 2005.
EFSA	an agency of the European Union set up in 2002 to serve as an impartial source of scientific advice to risk managers and to communicate on risks associated with the food chain.
OCT	An Excel®-based tool to calculate the cost of animal disease and its control in a country/region.
VSL	It represents aggregate demand for widespread, but individually very small, reductions in mortality risk, i.e. how much individuals are willing to pay for a very small reduction in the probability of death, paid for by forgoing the consumption of other goods and services.
VSLY	It is an estimate of the value society places on a year of life (VSL) defined above.
YLD	The number of years of what could have been a healthy life that were instead spent in states of less than full health. YLD represent non-fatal burden.
YLL	The number of years of life lost due to premature death, defined as dying before the ideal life span.

LIST OF PUBLICATIONS AND CONFERENCE CONTRIBUTIONS

PEER REVIEWED PUBLICATIONS

1. Sanni, A. O., Onyango, J., Usman, A., Abdulkarim, L. O., Jonker, A., Fasina, F. O. (2022). Risk Factors for Persistent Infection of Non-Typhoidal *Salmonella* in Poultry Farms, North Central Nigeria. ***Antibiotics***, 11(8), 1121. https://doi.org/10.3390/antibiotics11081121.
2. Sanni, A. O., Onyango, J., Rota, A. F., Mikecz, O., Usman, A., PicaCiamarra, U., & Fasina, F. O. (2023). Underestimated economic and social burdens of non-Typhoidal *Salmonella* infections: The One Health perspective from Nigeria. ***One Health***, 16, 100546. https://doi.org/10.1016/j.onehlt.2023.100546.
3. Sanni A. O., Jonker, A., Were, V., Fasanmi, O. G., Adebowale, O. O., Shittu, A., Jibril, A. H., Fasina, F. O. (2024). Cost-effectiveness of One Health intervention to reduce risk of human exposure and infection with Non-Typhoidal Salmonellosis (NTS) in Nigeria. ***One Health*** 2024, 100703, https://doi.org/10.1016/j.onehlt.2024.100703.
4. Sanni A. O., Jibril, A. H., Fasanmi, O. G., Adebowale, O. O., Jambalang, A. R., Shittu, A., Jonker, A., Abdulkarim L. O., Fasina, F. O. Non-Typhoidal *Salmonella* in Nigeria: Do Outcomes of Multisectoral Surveillance, Treatment and Control justify the intervention costs? ***International Journal of Veterinary Science and Medicine***. Accepted, https://doi.org/10.1080/23144599.2024.2365567.
5. *Salmonella enterica* isolates from poultry, North-Central Nigeria reveal multi-drug resistant patterns and risk of contamination to the human food chain. To be submitted to ***Frontiers in Microbiology***.
6. Spatial distribution and predictive risk of perpetuation of non-typhoidal Salmonellosis in poultry farms and human communities, Nigeria. Submitted to ***Geospatial Health***.

CONFERENCE PRESENTATION

1. Sanni, A. O., Jonker, A., Fasina, F. O. (2023). *Economic and social burdens of Non-Typhoidal Salmonella infections in Nigeria*. 20th Annual Congress of the Southern African Society for Veterinary Epidemiology and Preventive Medicine (SASVEPM), 20th Annual Congress of the Southern African Society for Veterinary Epidemiology and Preventive Medicine (SASVEPM), 23-25 August 2023, Gaborone Botswana (oral presentation).

Eco-Epidemiology and Microbiological Evaluation of Poultry Salmonellosis in North Central Nigeria, and its Socioeconomics and Public Health Impacts

By

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EXECUTIVE SUMMARY

Nigeria is a country with a mid-2020 human population of approximately 209 million, and the poultry industry in Nigeria has rapidly expanded in recent years despite many health and economic challenges. Poultry production in the different agro-ecological zones of Nigeria are characterized by generalized and specific production and health-related challenges principal among which are: 1) low level of production, 2) inadequate scaling up and specialization, 3) antimicrobial use and resistance, and 4) a poor level of biosecurity implementation. Hence, there are a number of poultry-related zoonoses can be found in humans and animals in Nigeria. The *Salmonella* spp. is a Gram-negative enteric pathogen (bacterium), with potentials to infect almost all animals including humans. Though, only two species have been identified in this genus, vis the *enterica* and *bongori*, almost 2,700 serotypes (serovars) have been listed with approximately 10% isolated from birds. Most serotypes of *Salmonella* can infect several animal species including humans, such as *Salmonella* Typhimurium and *Salmonella* Enteritidis, which are primarily poultry-associated. Salmonellosis, as a bacterial zoonosis, causes an array of health conditions in humans and animals, and the non-typhoidal salmonellosis (NTS) is prevalent with substantial under-appreciated public health impacts. This work was set out with the objectives of conducting microbiological evaluation of NTS in North Central zone of Nigeria (NCN) using classical and molecular methods; conducting a comprehensive re-analysis of risk of introduction of NTS to poultry farms, determining the epidemiology of foodborne *Salmonella* among poultry farmers and consumers, determining the economic burden of food borne salmonellosis in humans and poultry, demonstrating the benefit of disease control measures against salmonellosis in poultry using validated tools, and map spatial heterogeneity of habitat suitability for salmonellosis in poultry farms in Nigeria to aid evidence-based support to decision makers.

Using field sampling, laboratory methods and a semi-structured questionnaire (n = 1000) in poultry farms in NCN, the incidence and risk factors for the persistence of NTS infection in poultry were explored. Approximately 41.6% (95%CI: 38.58 to 44.68) of the farms had experienced NTS over the last 18 months and the awareness of salmonellosis was moderate. A number of risk factors for increased odds of NTS infection in poultry including increasing stock in smallholder farms, self-mixing of concentrate on the farm, usage of stream water, pen odour, non-adherence

and partial adherence of farms to recommended poultry vaccination against pullorum and fowl typhoid and lack of and non-adherence to biosecurity were identified. Overall, 66 isolates including *Salmonella enterica*, *Salmonella arizonae*, *S. paratyphi*, and *S. typhi*, with 94.5% mixed infections with *Klebsiella pneumoniae* and *Lactobacillus bulgarius*, were obtained, and irrational antibiotic-use practice remains a major problem in the industry. Specifically, the study obtained a number of multi-drug resistant isolates, with likelihood of passing such resistant organisms through the human food chain. To evaluate the economic and social burden of NTS, poultry and human populations, economic and epidemiological data were retrieved from various sources and validated. A customized and validated Microsoft Excel® tool was utilized to conduct the economic analysis for the reference year 2020. The burden of NTS was 325,731 human cases and a total of 1,043 human deaths per year, at a disability-adjusted life year (DALYs) of 37,321. The cost associated with infection in humans was US$ 473,982,068, and for poultry, US$ 456,905,311 (the direct value of animal loss, US$ 224,236,769, loss from salvage slaughter and culling, US$ 220,386,556, and value of foregone production, US$ 12,281,987). Using Outbreak Costing Tool (OCT), the benefit-cost of multisectoral intervention against NTS was estimated. Approximately 4,835 technical officers and 3,700 non-technical staff (n = 8,535) with an annual investment of over 2.2 million work hours, and at a total cost of US$ 53,854,660.87 are needed for an annual NTS control programme in humans and animal. The non-labour-related cost was 89.21% of the total intervention costs, and major costs were incurred in medical countermeasures, travel and transports, and laboratory support. The overall intervention's investment was 374.15% of the estimated national and subnational systems' annual budget for diarrhoeal diseases, and the outbreak response period incurred the highest costs (53%) of the total intervention. The benefit–cost ratio (BCR) of intervention was 17.29. Through a cohort study, the cost-effectiveness of NTS in humans in Nigeria for the year 2020 was determined. Specifically, an Excel-based cost-effectiveness analysis tool was developed to compare structured (One Health) and unstructured (episodic intervention against NTS), with input data from various sources. The non-complicated and complicated cases were 309,444 (95%) and 16,287 (5%) respectively, and the overall programme cost was US$ 31,375,434.38. The current non-systematic episodic intervention costed US$ 14,913,480.36, indicating an additional US$ 16,461,954 to introduce the proposed

intervention. The intervention averted 4,036.98 NTS DALYs in a single year. The non-complicated NTS case was US$ 60/person with significant rise in complicated cases. The cumulative costs of NTS with and without complications far outweighed the program cost for One Health intervention with an incremental cost-effectiveness ratio (ICER) of -US$ 221.30). The suitability map for continued infection in humans and poultry indicated that the disease would remain prevalent unless significant behavioural change communication is undertaken and intense control for NTS challenges are implemented.

The identified risk practices must be mitigated intentionally, and biosecurity and hygiene must be improved to reduce the burden of NTS. Since the utilization of One Health approach to intervention is cost-effective and cost-beneficial, and they carry additional mitigative benefits for other diseases, multisectoral investigation and response against NTS in Nigeria is advocated. The health system should re-focus and re-prioritize, with coordinated collaborations and through the utilization of a more decentralized approach. Anticipatory planning, adequate resource allocation and more intense outbreak investigations to reduce critical response time are warranted. Identified limitations in this study must be improved to optimize benefits associated and to facilitate policy discussions. The outcomes of this work provide empirical evidence to support informed decisions and investments in the control and eradication of human and poultry salmonellosis (NTS) in Nigeria.

Key words

Economic analysis; infectious disease; Nigeria; non-typhoidal *Salmonella*; One Health; Zoonoses.

THESIS OUTLINE

The thesis is presented in manuscript format for publication in suitable journals. The **Chapters One, Two, Nine** and **Ten** are the Introduction, General Literature Review, General Conclusion, and General References respectively, and have been elaborated in the document without the summaries in this section. The remainder of the thesis comprises of the chapters as shown below:

Chapter 3: Economic Burdens of Persistent Infection of Non-Typhoidal *Salmonella* in Poultry Farms, North Central Nigeria. (Manuscript 1, published).

This chapter briefly evaluated and described the impact of NTS in social and economic terms. Relevant population, economic and epidemiological data were retrieved and used for economic analysis in a purpose-built validated Microsoft Excel® tool, using the year 2020 reference point. The burden of NTS was 325,731 cases and a total of 1,043 human deaths, at a disability-adjusted life year (DALYs) of 37,321. The cost associated with infection in humans was US$ 473,982,068. A total loss of US$ 456,905,311 was estimated in poultry including the direct value of animal loss, US$ 224,236,769, loss from salvage slaughter and culling, US$ 220,386,556, and value of foregone production, US$ 12,281,987. The chapter's outcomes provide empirical evidence to support informed decisions and investments in the control and eradication of human and poultry salmonellosis (NTS) in Nigeria.

Chapter 4: Cost Effectiveness Analysis of Intervention Against Non-Typhoidal *Salmonella* in Nigeria. (Manuscript 2, published).

The chapter utilized details from the economic burden above to build an economic case (policy discussions and resource allocation) for investment in intervention against the pathogens at the human and animal interfaces. Basically, the cost-effectiveness of intervention against Non-typhoidal *Salmonella* infection (NTS) was conducted using a customized Excel-based cost-effectiveness analysis tool, and by partitioning the measures into structured (One Health) and unstructured (episodic intervention against NTS) intervention. The non-complicated and complicated cases were 309,444 (95%) and 16,287 (5%) respectively, and the overall programme cost was US$ 31,375,434.38. The current non-systematic episodic intervention costed

US$ 14,913,480.36, indicating an additional US$ 16,461,954 to introduce the proposed intervention. The intervention averted 4,036.98 NTS DALYs in a single year. The non-complicated NTS case was US$ 60/person with significant rise in complicated cases. The cumulative costs of NTS with and without complications far outweighed the program cost for One Health intervention with an incremental cost-effectiveness ratio (ICER) of -US$ 221.30). Utilising structured One Health intervention is cost-effective against NTS in Nigeria and carries additional mitigative benefits for other diseases. It is also less costly and more effective, indicative of a superior health system approach.

Chapter 5: Benefit-Cost Analysis of Intervention Against Non-Typhoidal *Salmonella* in Nigeria. (Manuscript 3, in-review).

Often, the question of whether to invest in mitigation or not occurs at farm and organizational level. In this Chapter, the Outbreak Costing Tool (OCT) and decision tree were utilized to answer the question. Using multiple data sources, and the determined burdens in the above chapters, approximately 4,835 technical officers and 3,700 non-technical staff (n = 8,535) and an investment of over 2.2 million work hours is needed to intervene annually against NTS. These interventions may cost up to US$ 53,854,660.87, with the non-labour-related cost being 89.21% of the total intervention costs. The overall intervention's investment was 374.15% of the current budget available for similar programme at national and subnational levels. The benefit–cost ratio (BCR) of intervention was 17.29, adjustable upward or downward depending on the prevailing scenarios. Multisectoral investigation and response against NTS in Nigeria would benefit from health re-focusing and re-prioritization. A decentralized framework with a sub-national focus and empowerment for rapid investigation, response, control, data collection, and analyses will improve understanding of underestimated outbreaks. Anticipatory planning will also benefit outbreak investigation and reduce critical response time to intervention.

Chapter 6: Risk Factors for Persistent Infection of Non-Typhoidal Salmonella in Poultry Farms, North Central Nigeria. (Manuscript 4, published).

Farm-level practices and knowledge affect the farm infection and prevalence of non-typhoidal salmonellosis in poultry farms in Nigeria. Using field sampling, laboratory methods and a semi-structured questionnaire for 1000 poultry farms in NCN, the incidence and risk factors for the persistence of NTS infection in poultry was explored. Approximately 41.6% (95%CI: 38.58 to 44.68) of the farms had experienced NTS over the last 18 months. Increasing stock in smallholder farms, self-mixing of concentrate on the farm, usage of stream water, pen odour, non-adherence and partial adherence of farms to recommended poultry vaccination against pullorum and fowl typhoid and lack of and non-adherence to biosecurity were identified risk factors that increased the odds of NTS infection in poultry. Antibiotic use practice may have reduced the isolation rate of NTS, yet NTS continues to challenge poultry farms in Nigeria. Identified risk practices must be mitigated intentionally and biosecurity and hygiene must be improved to reduce the burden of NTS.

Chapter 7. Molecular Epidemiology and Antimicrobial Resistance Patterns of Non-Typhoidal *Salmonella* spp. found in Poultry Farms, North Central Nigeria. (Manuscript 5, drafted).

This chapter dug deeper at the farm-level non-typhoidal salmonellosis (NTS), manifesting as fowl cholera and fowl typhoid, and considered the environmental components of infections. Six hundred (600) faecal and dust samples were collected from the North Central States and the federal capital territory, processed using standard bacterial culture and invA-based PCR method. Isolates obtained were tested against 11 most used antimicrobials in poultry using Kirby–Bauer disk diffusion methods. An overall prevalence rate of 18.7% (95%CI: 15.8 to 22.0) (112/600). Prevalence in dusts and faeces were 20.5% (95%CI: 16.3 to 25.5) and 17.1% (95%CI: 12.1 to 23.5) respectively. Prevalence was lower in battery cages than in deep litter system, in flock > 1,000 birds compared to those less than 1,000, in older birds (> 52 weeks) versus younger birds, and in layer farms compared to in broiler farms. The odds of infection with non-typhoidal *Salmonella* spp. are at least 2 folds higher in younger birds, and in Niger state compared to other states. Isolates were most resistant to commonly used antimicrobials: tetracycline (73.8%), nalidixic acid (59.5%), sulphonamides (54.8%), and ciprofloxacin (47.6%), and most sensitive to ceftazidime (88.1%) and cefotaxime (78.6%). We observed single-resistant, multidrug-resistant, extensively

drug-resistant and a pandrug-resistant isolate. The poultry industry use and abuse of antimicrobial is a key driver that increases risk of AMR. Animal health authority must implement stricter control on access to antimicrobials to mitigate AMR pathogens, likely to enter and complicate human food chain with health and economic implications.

Chapter 8. Spatial Distribution and Predictive Risk of Perpetuation of Non-Typhoidal Salmonellosis in Poultry Farms and Human Communities, Nigeria. (Manuscript 6, drafted).

Salmonellosis in poultry and non-typhoidal salmonellosis (NTS) in humans are pathogenic bacterial zoonosis, a widely prevalent disease in Nigeria. The historical perspective of the disease and the prevalence were determined with a view to propose mitigation against the continued risk of salmonellosis in poultry and NTS in humans. Spatial and temporal distribution of prevalence and hotspots for risks of Salmonella were mapped, particularly the poultry-associated ones. Using peer reviewed data, hospital record, laboratory data and District Health Information Software (DHIS) – 2 data, metaanalysis was conducted and the national and subnational-levelprevalence were determined. Correlation analyses of NTS in humans and poultry were done using prevalence and diarrhoea data to determine association as predictors of infection. Overall, salmonellosis prevalence in poultry was 31.6% (95%CI: 9.2 to 64.2) with state-level prevalence ranging from 8.0% (95%CI: 7.8 to 23.8) in Ekiti to 70.2% (95%CI: 55.9 to 84.6) in Ogun state. Regionally, the North-West, South-West and South-South regions of Nigeria have the highest regional level prevalence of 38.5% (95%CI: 35.5 to 41.6), 36.9% (95%CI: 34.0 to 40.0) and 33.6% (95%CI: 30.7 to 36.6) respectively. Thirteen (13) states have higher than the national average prevalence (31.6%) (95%CI: 9.2 to 64.2), and spatially, the correlation analyses indicated that prevalence of NTS in humans negatively predicted salmonellosis in poultry, but prevalence of diarrhoea in humans positively predicted salmonellosis in poultry. In addition, prevalence of NTS in humans negatively predicted diarrhoea in humans, while prevalence of NTS in poultry was positively predicted by poultry populations. In conclusion, this work pointed out some health data gaps, and result of the humans NTS – poultry salmonellosis correlation was counterfactual to logic and plausibility based on empirical evaluation. Outcome may be influenced by underreporting linked

to self-treatment, under-testing in the laboratory, and lack of uniform primary healthcare services in the underserved areas of Nigeria.

Chapter 9: General Conclusion

This chapter summarised the notable findings of the study and discussed the strengths and limitations of the research. Recommendations for future research were made based on findings from this study.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Nigeria is a country with a mid-2020 human population of approximately 209 million, a projected population growth rate of 2.62% and an estimated GDP of $496.122 billion (WPR, 2020). Based on the GDP and purchasing power parity (PPP), Nigeria is ranked number 24th and the 26th largest economy among the comity of nations (Anon, 2020). Unarguably, Nigeria is the largest economy and market in sub-Saharan Africa. The country's agriculture industry accounts for over 38% (range: 20-42%) of non-oil foreign earnings and employing 70% of the labour force (FAO, 2006; Olajide et al., 2012; Olomola & Nwafor, 2018). Poultry is any domesticated bird used for food. Varieties include chicken, turkey, goose, duck, Rock Cornish hens, and game birds such as pheasant, squab and guinea fowl. Also included are huge birds such as ostrich, emu and rhea (ratites) (USDA, 2024). The poultry industry in Nigeria has rapidly expanded in recent years despite many health and economic challenges (Fagbamila et al., 2017). Adene and Oguntade (FAO, 2006) have robustly described the Nigerian poultry industry in an earlier report, and it consists of some 180 million chickens, producing 650 000 tonnes of eggs and 300 000 tonnes of poultry meat in 2013 alone (FAO, 2018). The different zones in Nigeria have its poultry characterized by generalized and specific production and health-related challenges including the following among others: 1) low level of production, 2) inadequate scaling up and specialization, 3) antimicrobial use and resistance, and a poor level of biosecurity implementation (FAO, 2008; Fasanmi et al., 2016; Fagbamila et al., 2017; Oloso et al., 2018, 2019).

Poultry is a preferred form of livestock because a large majority of farmers is changing their preference from ruminants to monogastric animal farming due to the scarcity of resources like land. For instance, while monogastric animals like chickens and pigs may be raised in a small area and with minimal capital investment, ruminant livestock (cattle, sheep, goats, etc.) would need large areas of land for grazing and other inputs. It is difficult to estimate the total number of poultry farms in Nigeria, yet, the Federal Ministry of Agriculture and Rural Development has attempted to create a database of all poultry farms following the challenges of Highly Pathogenic Avian influenza in Nigeria in 2006-2008. To date, large and small, egg and meat-type poultry farms are scattered all over the country, with predominant concentrations around the major urban and peri-urban centres (FAO, 2006; Oloso, 2019, 2021).

In Nigeria, poultry meat and eggs are the major sources of animal protein in Nigeria, as in many other developing countries, they are affordable and widely acceptable (Bettridge et al., 2014; Fagbamila et al., 2017). This source is threatened by poultry diseases among which are salmonellosis, avian influenza, and Newcastle disease (FAO, 2006; Fasanmi et al., 2018). Salmonellosis is an important bacterial disease affecting both humans and animals globally (Raufu et al., 2013; Kagambèga et al., 2013; Ao et al., 2015; Magwedere et al., 2015). In poultry, *Salmonella Gallinarum* biovar *Pullorum* (*S*. Pullorum) causes extremely high mortality in growing broilers and commercial laying birds. *Salmonella Gallinarum* biovar *Gallinarum* (*S*. Gallinarum) may also affect adult birds significantly causing systemic disease or at times inapparent illnesses where some gastrointestinal tracts zoonotic serovars occur (Mamman et al., 2014). While the more adapted *Salmonella enterica subspecies enterica* serovar Typhi causes typhoid fever only in humans, its other serotypes named the non-typhoidal *Salmonella* (NTS) is zoonotic (animal to

human transmission and vice versa) (Su et al., 2004). Foodborne diseases caused by NTS are also a global health concern despite the global attempts in the advancement of sanitary measures, water treatment and food safety standards. NTS are infections by serovars of *Salmonella enterica* ssp *enterica* other than Typhi and Paratyphi A (Magwedere et al., 2015). The main source of NTS transmission are foods of animal origin (Magwedere et al., 2015, WHO, 2015).

Infected poultry manifests and shed infections for long period as latent carrier with occasional faecal shedding, thus contaminating feed, water and the environment (Magwedere et al., 2015). To date, the comprehensive sources and modes of transmission of non-typhoidal *Salmonella* are poorly understood in Africa where the lack of coordinated national epidemiological surveillance systems for the infections exist (Kagambèga et al., 2013; Magwedere et al., 2015). However, in food-producing animals and especially in poultry, *Salmonella* remains one of the leading causes of infection, with direct impact on marketing, food production, yields and burden of diseases (Magwedere et al., 2015). The host-adapted serovars of *Salmonella* continue to serve as major constraints to poultry production in Nigeria (Fagbamila et al., 2017; Oloso et al., 2017, 2019, 2021). Such constrains may include increased mortality, morbidity, reduced productivity, increased control costs among others. *Salmonella* serovars are excreted in faeces by apparently healthy birds and these may contaminate raw foods, anywhere along the farm to fork continuum, thereby posing a health risk to consumers of contaminated foods (Sanchez et al., 2002; Oloso, 2018).

Animal-sourced foods contaminated by *Salmonella* predispose humans to a risk of food-borne illnesses expressed by the microbial toxins or the pathogens. Salmonellosis is characterized by mild gastroenteritis in humans, but may present with life-threatening systemic infections

particularly in high-risk individuals (aged people, infants and children), persons with underlying conditions and immunocompromised individuals (Raufu et al., 2013; WHO 2015). Invasive NTS have been reported among infants, children, elderly and immunocompromised individuals worldwide and in African countries, where these infections are worsened by co-infection with malaria or human immunodeficiency virus (HIV) (Ao et al., 2015). Globally, the non-typhoidal *Salmonella* cause an estimated 93.8 million illnesses with 80.3 million being foodborne and up to 155,000 deaths in humans (Majowicz et al., 2010). Significant proportion of the affected population are resident in Africa (Majowicz et al., 2010; WHO, 2015).

In Nigeria, recent independent surveillances for salmonellosis have yielded a number of serovars. These activities have been carried out to cover the North-East (Raufu et al., 2013), the South-West (Oloso et al., 2017, 2019, 2021; Mshelbwala et al., 2017), North and South (Fagbamila et al., 2017), North (Jibril et al., 2020), North Central (Ahmed et al., 2019). In addition, the Nigerian federal government has supported the surveillance of *Salmonella* in commercial poultry farms, through the competitive agricultural research scheme in 2013. The aim of the project was to create a baseline data to aid on the development of the *Salmonella* control program (Fagbamila et al., 2017). This project considered the twelve states with the highest number of registered poultry farms, excluding Kogi state and Abuja, the Federal Capital Territory (FCT). It can therefore be inferred that part of the central belt of the country have not been studied enough in term of *Salmonella* surveillance in poultry. Based on these previous evaluations, drawbacks that have been observed include limited number of samples, lack of representativeness, lack of access to serotyping facilities, restricted geographical coverage and limitation of resources (Fagbamila et al., 2017). Therefore, there is a need to implement studies in previously understudied

geographical areas of Nigeria in terms of microbiology, socioeconomics and public health aspects of poultry salmonellosis to aid the understanding of the disease in Nigeria. It should also assist in informing comprehensive policy decision on salmonellosis and how behavioural change communication can be implemented to reduce the burden of salmonellosis.

1.2 Problem Statement

Salmonella, an enteric pathogen is a Gram-negative bacterium, with potentials to infect almost all animals including humans. There are only two species in this genus, *enterica* and *bongori* (Lin-Hui and Cheng-Hsun, 2007), but almost 2,700 serotypes (serovars), of which around 10% have been isolated from birds. Most serotypes of *Salmonella* can infect several animal species (Gast, 2008), such as *Salmonella* Typhimurium and *Salmonella* Enteritidis. Poultry has been linked as a source of salmonellosis in humans and food safety is paramount in commercial poultry production. The main concern related to *Salmonella* is that poultry meat and eggs are the most common sources of human infection (food poisoning and foodborne diseases). Poultry can also be infected with non-typhoidal *Salmonella* and show no signs of disease. It is enormously difficult to eradicate completely, *Salmonella* from poultry production. However, a combination of good farm management practices (GMP), understanding of the risks, biosecurity and proper vaccination protocols. In Nigeria, *Salmonella* is prevalent in poultry farm (prevalence of 43.6 – 47.9%; Fagbamila et al., 2017; Jibril et al., 2020) and the following isolates were among the ones identified to date: *S*. Gallinarum, *S*. Typhimurium, *S*. Enteritidis, *S*. Heidelberg, *S*. Montevideo, *S*. Infantis, *S*. Mbandaka, *S*. Kentucky, *S*. Javiana, *S*. Newport, S. Abadina, S. Aberdeen, S. Alachua, S. Agama, S. Birmingham, S. Bradford, S. Chester, S. Chomeday, S. Colindale, S. Corvalis, S. Cotham,

S. Elizabethville, S. Esen, S. Give, S. Graz, S. Isangi, S. Ituri, S. Larochelle, S. Liverpool, S. Menston, S. Muenster, S. Poona, S. Schwarzengrund, S. SaintPaul, S. Poona, S. Takoradi, S. Telelkebir, S. Virchow, S. Waycross, *Salmonella*-:z13,z28:I,z13,z28. Among these serotypes, a large variability exists because some are more intestinal adapted while other affect other organs (colonization of liver and spleen) and may get into the blood stream. In addition, while some survive longer in the environment, others do not. These pathogens might be passed from poultry to humans.

Most humans with *Salmonella* infection may have a combination of diarrhoea, fever, and stomach cramps which may last for 4 – 7 days. In aggravated situation, the symptoms may last much longer. It should be noted that some *Salmonella* strains may cause infection in urine, blood, bones, joints, or the nervous system (spinal fluid and brain) and can cause severe disease. The attendant hospitalization, disability, man-hour lost, and other human costs may be enormous. To date, no comprehensive assessment of these costs has been done in Nigeria.

1.3 Hypotheses

a. The prevalence of *Salmonella* spp. is high in poultry farms in the North-Central States and the Federal Capital Territory (FCT) (Figure 1.1).
b. The poultry value chain, marketing and distribution systems and farm management practices predisposed poultry birds and human to higher risks of infection, and the *Salmonella* spp. contaminating poultry are diverse within the North Central area.
c. Antimicrobial resistance of *Salmonella* spp. in the field has complicated responses to treatment against salmonellosis.
d. Day-old chicks and poultry feeds are important sources of introduction of *Salmonella* to poultry farms in the province.

e. Management systems affect environmental contamination and infection of broiler at the farm level and contamination of carcasses at the abattoirs.

f. The economic burdens of food borne salmonellosis in humans and poultry in huge in Nigeria, and disease control measures against salmonellosis would be cost beneficial in Nigeria.

g. Salmonellosis in poultry farms in Nigeria is spatially and temporally diverse heterogeneity.

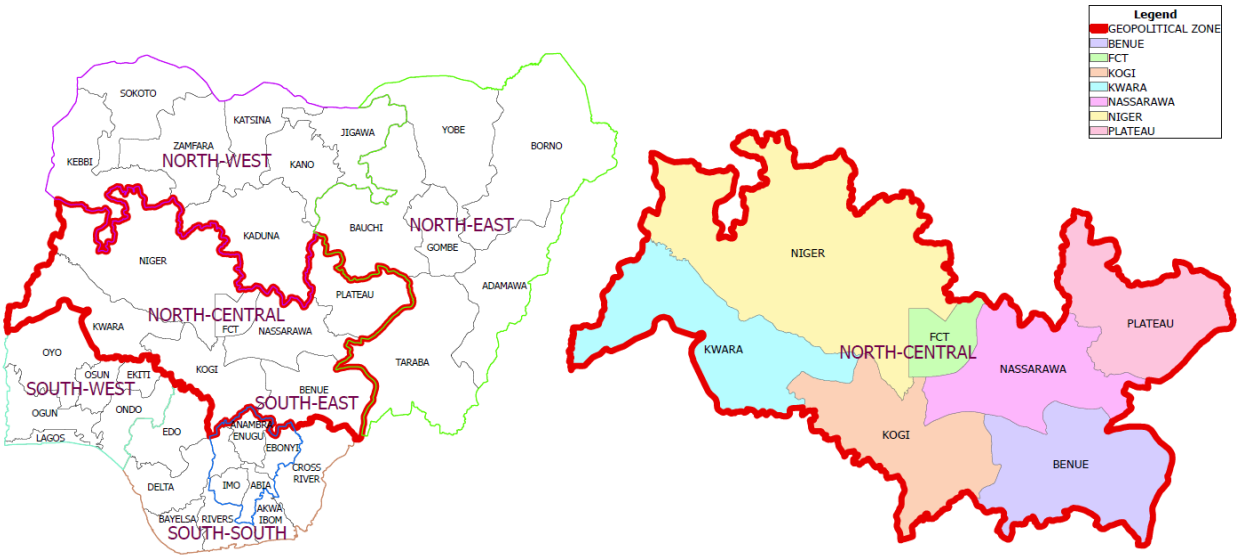

Figure 1.1. Map of Nigeria with a call-out map of the North Central zone.

1.4 Rationale and motivation

In view of the enormous human, animal, environmental and material costs associated with typhoidal and non-typhoidal salmonellosis and considering that the past and current research on salmonellosis in Nigeria have gaps, the current proposal is justified to fill some of the gaps.

Although, this research will also focus largely on the commercial poultry sector, similar to most previous research, it should have ramifications for rural and indigenous poultry, particularly the niche modelling, costing and risk evaluation aspects of the study. It should be understood that the majority of eggs and poultry products still originate from the commercial poultry sector despite the huge potential that the traditional and indigenous poultry hold. Therefore, information obtained from this research should inform policy change for the poultry value chain and poultry salmonellosis control in Nigeria. It may also assist in calibrating risk evaluations across the country in order to designate relatively free and infected zones. Behavioural change communication may also arise from the information obtained from this research as obvious risk practices may be mitigated against in view of the findings. Finally, a number of peer-reviewed manuscripts would be published in international journals.

1.5 Aims and objectives

1.5.1 Aim of the study

The primary aim of the project is to estimate the prevalence of non-typhoidal *Salmonella* in parts of Nigeria and determine the economic burden of salmonellosis in humans and poultry in Nigeria.

1.5.2 Specific Objectives

a) To conduct a microbiological evaluation of non-typhoidal salmonellosis in North Central zone of Nigeria using classical and molecular methods.

b) To conduct a comprehensive re-analysis of risk of introduction of non-typhoidal *Salmonella* into poultry farms in Nigeria.

c) To determine the epidemiology of foodborne *Salmonella* among poultry farmers and consumers.

d) To determine the economic burden of food borne salmonellosis in humans and poultry using validated tools of analysis and experts' opinion.

e) To demonstrate the benefit of disease control measures against salmonellosis using the modified benefit-cost model to evaluate the cost of comprehensive control and of not taking any action against salmonellosis in poultry.

f) To map spatial heterogeneity of habitat suitability for salmonellosis in poultry farms in Nigeria using spatial analysis or other suitable approach.

1.6 Research questions

- Is Non Typhoidal *Salmonella* (NTS) prevalent in poultry farms in North Central zone of Nigeria, and if yes, what is the level of prevalence?
- What serovars of NTS are present in the study area?
- If the level of NTS significant what is its economic burden in humans and poultry in the study area, where are these variables and can a comprehensive evaluation be carried out using available data and experts' opinion?
- What are the risk factors that influence the introduction of NTS organisms to poultry farms in the study?
- Is an investment in a control programme against NTS in poultry worthwhile? If yes, are there cheaper alternatives or best model for controlling the disease in poultry?

- Do various eco-climatic variables, value chains, marketing systems, environmental and anthropogenic factors influence the transmission of poultry salmonellosis in Nigeria farms?

1.7 Significance and expected outcome of the study

Overall, the study would add to the body of knowledge on poultry salmonellosis in Nigeria. Specifically, it should lead to the:

(a) Provision of data on areas where NTS is most prevalent in North Central Zones that will facilitate targeted control measures and appropriate policy formulation.

(b) Provision of data on the most prevalent serovars(s) of NTS in the north central zone for further exploration by scientific community.

(c) Provision of data on the farm management practices that influence the contamination and spread of *Salmonella* at different levels of the poultry production value chain.

(d) Fill the information gap on typhoidal and NTS in poultry farms in North-Central Nigeria,

(e) Evaluation and quantification of the economic and public health burdens of the disease with an aim to guide policy formulation on salmonellosis.

(f) Expected recommendations on effective intervention control measures will reduce or eliminate contamination by NTS at poultry farms in Nigeria. This will eventually become valuable in building a sustainable poultry health care, public health and environment interface.

(g) Contribution of valuable data to the existing database on the surveillance of NTS thereby providing information to regulatory authorities in Nigeria to improve and sustain the facilities for monitoring the process of production chain from farm to the consumer, and.

(h) Assistance in the development of targeted behavioural change communication which promotes good farming practices that should reduce the burden of NTS and encourage biosecurity practices.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Nigeria's Poultry Industry

The growth of poultry industry has been made possible by the introduction of intensive production systems utilizing new technologies and successful disease control has been a necessary tool to the efficiency of such systems. Thus, recognition, prevention and treatment of disease are of crucial importance and are the subject of much investigation and research (Jordan and Pattison, 1996). According to Okonkwo and Akubuo (2001), the importance of poultry to Nigeria's national economy cannot be overemphasized, where it has shown a significant effect on the national economy with about 10% of the Nigerians engaged in poultry production, mostly on subsistence and small or medium-sized farms. It has also become a popular industry for the small holders which have contributed sparingly to the economy of Nigeria by assuming greater importance in improving employment opportunities and animal feed production in Nigeria (Rahman and Yakubu 2005; FAO, 2000). Investment in poultry enterprises has become attractive because of low production cost per unit compared to other types of livestock and relatively short production cycle in some cases (e.g., broiler production) (Nwajiuba and Nwoke, 2000). High demand for poultry products, success of exotic breeds and the ease of mastering the techniques of poultry production among other factors has made it developed to the status of agribusiness in Nigeria as distinct from subsistence production (Nwajiuba and Nwoke, 2000; Sani *et al*., 2000). In any good poultry production system and enterprise, it is essential that a high degree of biosecurity exists, and monitoring carried out to prove freedom from common pathogens such as *Mycoplasma* species and *Salmonella* species (Jordan and Pattison, 1996). Major poultry

diseases are of worldwide occurrence, but some are restricted by distribution of vectors while others tend to be localized for unknown reasons. In developed countries however, coccidiosis, infectious bronchitis, infectious bursa disease, Marek`s disease, and mycoplasmosis are the major infectious diseases while in developing countries. These and other diseases such as fowl cholera, fowl pox, infectious coryza, infectious laryngotracheitis, Newcastle diseases, pullorum disease, and spirochaetosis have also been recorded (Briggs,1982). There is a greater incidence of infectious diseases in developing countries where management systems are less intensive and are less controlled hence the need for emphasis on preventive medicine (Jordan and Pattison, 1996).

2.2 The Chicken House Environment

The chicken house especially the litter, represents an ideal environment for microbial growth (temperature, moisture, and nutrient content well within the range for microbial proliferation) and the microbial population can be as high as 109 to 1010 cells per gram of litter (Lovanh *et al.,* 2007; Rothrock *et al.,* 2008) which is responsible for both beneficial (carbon mineralization, competitive exclusion, etc.) and detrimental (pathogen persistence) effects of litter. Several recent studies have characterized the microbial population in chicken litter using modern molecular methodologies (Lu *et al.,* 2003; Entiknap *et al.,* 2006; Lovanh *et al.,* 2007) but most of the work has been based on measures of culturable cells, which often represent only a fraction of the population (Nodar *et al*., 1990; Martin *et al*., 1998; Terzich *et al*., 2000; Fries *et al.*, 2005). During a broiler growth cycle for example, a constant influx of nutrients and intestinal micro-organisms results in a complex litter micro biota (Cressman *et al.,* 2010) and with continued re-

use, the litter environment becomes more complex, and may have a profound impact on flock growth, performance and health. The family *Enterobacteriaceae* found commonly in the litter, consists of gram negative aerobic or facultative anaerobic, asporogenous rod-shaped bacteria that grow well on artificial media and comprises of large number of antigenically related and biochemically similar bacteria that include *Salmonella, Escherichia, Shigella, Citrobacter, Klebsiella,* and *Proteus* (Jordan and Pattison, 1996).

Several methods have been developed to collect samples from the environment as an indirect indication of flock infection such as collection of samples from nests or floor litters, bulk litter samples, and drag swabs (Curtis and Drummond, 1982) and even directly as fecal material.

2.3. Genus *Salmonella*

The genus *Salmonella* is divided into three different species, *S. bongori*, *S. enterica*, and *S. subterranea* (Brenner FW. 1998, (Su LH. 2007). Previously, the genus was broken up into many more species, with each individual serotype being considered its own species. The genus was subsequently divided into seven subgenera (I, II, IIIa, IIIb, IV, V, and VI) based on biochemical and genetic properties (Brenner FW. 1998). As more advanced genetic techniques such as DNA-DNA hybridization were used to analyze the members of the genus, it was discovered that many of the serotypes shared a high degree of genetic similarity. Consequently, the genus was divided into two species, *S*. enterica and *S*. bongori, with S. bongori containing the members of subgenus V and S. enterica containing the members of the remaining six subgenera (Brenner FW. 1998). *S*. subterranea was described as a species in 2005 (Su LH. 2007). Serotypes in what is now *S*. *enterica*

subspecies *enterica* (subspecies I) are the predominant pathogens associated with birds and mammals (Brenner FW. 1998).

Within subspecies I, there is a diversity of *Salmonella* serotypes that infect different animal hosts. The ability of different *Salmonella* serotypes to survive and thrive in different host environments involves a number of interconnected factors, including differences in host environments (pH, temperature, and sites of attachment, etc.), the host immune system and its response to different serotypes, the commensal organisms present, and the genetics of the pathogen itself (Foley et.al, 2011, Goto et al, 2011)

Salmonella infections are a worldwide major public health concern; Salmonellosis is caused by non-typhoidal *Salmonella* enterica serotypes (serotypes other than *S*. Typhi and *S*. Paratyphi) and is typically characterized by a self-limiting gastroenteritis syndrome (manifested as diarrhoea, fever and abdominal pain), with an incubation period between 4 and 72 h and mortality being rare [Donnenberg MS, 2000, Tsolis et.al, 2008, Guerrant et.al, 2005]. In healthy humans, the infectious dose is generally 106 to 108 colony forming units or cells? but lower bacterial counts can cause disease in certain conditions, as well as in infants and the elderly [Tsolis et.al, 2008]. Although uncommon, life-threatening invasive infections with bacteremia (5%–10% of infected persons) and/or other extra-intestinal infections may occur, affecting especially the risk groups (infants, young children, older people and immune-compromised patients) [Donnenberg MS, 2000, Tsolis et.al, 2008, Guerrant et.al, 2005]. In severe cases, effective antimicrobial agents are essential, so the emergence of *Salmonella* that are resistant to critical antibiotics is of concern [Donnenberg MS, 2000, Tsolis et.al, 2008]. In industrialized countries, the main reservoir of non-typhoidal *Salmonella* is the intestinal tract of food-producing animals, which readily leads to

contamination of diverse foodstuffs [Guerrant et.al, 2005, Herrington et.al 1988, Nataro 1998]. The primary route of infection in humans and animals is through fecal-oral transmission of *Salmonella*.

Salmonella pathogenesis has been studied mostly as it relates to human infections, while there is more limited information about the mechanisms of colonization and pathogenesis in food animals such as chickens. In general, when food contaminated with *Salmonella* is ingested, the bacteria have to pass through the alimentary system and survive the acidic environment of the stomach. *Salmonella* has been found to respond to the acidic environment through a complex adaptive system, called the acid tolerance response, which requires the synthesis of over 50 acid shock proteins, including the RpoS -factor, PhoPQ, Ada, and Fur ST. (Bearson et al., 1998; Bearson et al., 2006). Bearson et al. (2006) reported that *S*. Typhimurium RpoS and PhoPQ provided protection against inorganic acids, while RpoS and FurR offered protection against organic acids (Bearson et al., 1998)

Those *Salmonella* organisms that survive the low-pH environment proceed to the lumen of gastrointestinal tract (GIT) organs, including the small intestine, colon, and cecum (in poultry). Epithelial and immune cells lining these GIT organs provide the initial protective barrier against *Salmonella* in the gut. *Salmonella* competes with the gut microflora to make the initial contact with enterocytes or M cells in order to colonize the GIT (Ruby et.al, 2012, Velge et.al, 2012). Adhesion to the GIT epithelium by *Salmonella* is facilitated by flagella and fimbriae present on the bacterial cell surface (Darwin and Miller, 1999; Van Austen and Van Dilk, 2015).

Studies have shown that *Salmonella* serovars employ both conserved and host-specific factors that facilitate colonization in the host GIT (Stevens et al., 2009). Signature-tagged mutagenesis

studies have reported the ability of multiple *S*. Typhimurium transposon mutants to colonize intestinal tracts of mice, calves (Tsolis et al., 2008), chickens, and pigs (Carnell et al., 2007; Morgan et al., 2004). In addition to the oral-GIT route of invasion, *Salmonella* bacteria have been reported to invade and disseminate in swine and cattle following uptake in the tonsils and respiratory system (De Jong and Ekdahi, 1965; Fedora et al., 1995).

2.3.1 Survival in macrophages and dendritic cells

In a small percentage of cases, *Salmonella* cells are able to replicate within host cells, evade immune responses, and develop invasive and systemic infections (Tsolis et al., 2008; Ruby et al., 2012). These severe manifestations of salmonellosis usually occur when *Salmonella* cells invade macrophages or dendritic (migratory phagocyte) cells. *Salmonella* cells have been shown to be able to multiply within macrophages (Henkel et al., 1998) but do not appear to replicate within dendritic cells, even though they remain viable (Tierrez and Garcia-del Portillo, 2005). The exact mechanisms for the differences in *Salmonella* responses within different immune cell types are not entirely clear, as several pathogen and host factors may play roles (Bueno et.al, 2012). Dendritic cells and macrophages are widely distributed in the lymphoid and nonlymphoid tissues and can rapidly facilitate the spread of *Salmonella* cells to various organs of the host body (Sundquist et.al, 2004, Epelman et.al, 2014). Researchers have shown that the SPI-2 T3SS in *Salmonella* harbors genes that can suppress antigenic presentation in dendritic cells, which limits the host immune response to infected cells (Waterman et.al, 2003). In general, the ability of *Salmonella* to cause an infection in humans or animals depends on the innate ability of the bacteria to encode and express a set (or combination) of virulence genes that can evade and

neutralize host defenses. These factors are associated with pathogenicity islands, virulence plasmids, toxins, fimbriae, and flagella (Foley et.al, 2008, Van Asten et.al, 2005, Garai et.al, 2012, Lahiri et.al, 2010, Foley et.al, 2008). Classification of *Salmonella* has been controversial for many years but according to nomenclature which reflects recent advances in *Salmonella* taxonomy, the genus *Salmonella* consists of only two species: *Salmonella enterica* and *Salmonella bognori. Salmonella enterica* is divided into six sub-species which are distinguishable by certain biochemical characteristics, some of which corresponds to previous sub-genera (Jordan and Pattison, 1996).

2.3.2. *Salmonella* Pathogenicity Islands

The ability of *Salmonella* to efficiently colonize the host has been attributed to gene clusters, such as SPIs, encoding virulence factors that are distributed in the *Salmonella* genome (Foley et al., 2008). Several major pathogenicity islands have been reported for different serovars, with SPI-1 to -5 being present in most serovars and others being less widely distributed (Foley et al., 2008; , Foley and Lynne, 2008; Leung et al., 2011Marcus et al., 2000). In general, SPI-1 is required for invasion of host cells and induction of macrophage apoptosis, SPI-2 is required for systemic infection and replication within macrophages, SPI-3 is required for survival in macrophages and the ability of *Salmonella* to grow in low-magnesium environments, SPI-4 is required for intramacrophage survival and harbors genes for toxin secretion and apoptosis, SPI-5 has been found to cluster genes that encode multiple T3SS effector proteins, and SPI-6 has been found to transport proteins into the environment or host cells in response to external stimuli (Foley et al., 2008; Van Asten and Van Dijk, 2005; Stevens et al., 2009; Foley and Lynne, 2008; Leung et al.,

2011; Bingle et al., 2008; Amavisit et al., 2003). Amavisit et al. reported genetic variations among SPI-1, -3, and -5, while SPI-2 and -4 were well conserved among 13 different *Salmonella* serovars isolated from warm-blooded animals (bovine, porcine, avian, and equine), the environment, and human patients in their study (Amavisit et al., 2003). Those authors found that, with the exception of *S*. Typhimurium, all isolates within the same serovar were identical with regard to the five SPIs that were tested. SPI-1 and SPI-2 have been found to play a role in *Salmonella* persistence and enteritis in chickens (Morgan et al., 2004; Dieye et al., 2009), cattle (Zhang et al., 2003; Zhang et al., 2002; Coombes et al., 2005), pigs (Carnell et al., 2007), and humans (Ruby et al., 2012). Fibronectin-binding proteins, encoded by SPI-3, facilitate host-specific *Salmonella* colonization. For example, MisL contributes to *Salmonella* colonization in mice, chickens, and pigs but does not play a significant role in calves (Carnell et al., 2007; Morgan et al., 2004), while ShdA influenced *S*. Typhimurium persistence in mice but not in pigs (Bowen et al., 2006; Kingsley et al., 2002). SPI-4 has been reported to mediate adherence to and invasion of bovine ileal mucosa, possibly in combination with the SPI-1 T3SS, but not in chickens and pigs (Stevens et al., 2009; Morgan et al., 2004; Garlach et al., 2008).

2.3.3. Virulence Plasmids

Strains from several *Salmonella* serovars have serotype-specific virulence plasmids (Rotger and Casadesus, 1999). These are low-copy-number plasmids (1 to 2 copies per cell) and range from 50 to 100 kb, depending on the serovar (Van Asten and Van Dijk, 2005). Each of the plasmids contains the *Salmonella* plasmid virulence (spv) locus, whose expression has been reported to be important for multiplication of *Salmonella* in the reticuloendothelial system, including the liver

and spleen (Van Asten and Van Dikko, 2005; Gerlach et al., 2008; Ahmer et al., 1999). Other plasmids, in addition to the serotype-associated virulence plasmids, also likely contribute to the observed resistance among *Salmonella* bacteria. Recent studies from our laboratories have identified several different plasmids that potentially contribute to virulence in serovars such as *S*. Heidelberg, *S*. Kentucky, and *S*. Typhimurium (Han et al., 2012; Johnson et al., 2010).

2.3.4. Toxins

Salmonella pathogenicity has also been attributed to the production of endo-and exotoxins. Endotoxins have been found to elicit a wide range of biological responses, while exotoxins, comprising cytotoxins and enterotoxins, have the ability to kill mammalian cells (Ashkenazi et al., 1988). Ashkenazi et al. reported that *Salmonella* serovars Choleraesuis, Enteritidis, and Typhi produced heat-labile, trypsin-sensitive cytotoxins with various molecular masses, including 56 kDa (*S*. Typhi), 70 kDa (*S*. Typhimurium), and 78 kDa (S. Choleraesuis) (Ashkenazi et al., 1988). A *Shigella dysenteriae* 1-like cytotoxin has been detected in *Salmonella* serovars Enteritidis, Kapemba, and Thompson (Ketyi et al., 1979). Two other types of exotoxins, salmolysin (encoded by the slyA gene) and *Salmonella* enterotoxin (Stn; encoded by the stn gene), have been identified in *Salmonella* serovars Typhi, Typhimurium, and Enteritidis (Prager et al., 1995; Libby et al., 1984).

2.3.5. Fimbriae

Fimbriae (pili) are filamentous surface structures that contribute to the colonization of the epithelium by *Salmonella* (Collinson et al., 1996). Each of the fimbrial operons contains multiple

genes (typically 8 to 11) that encode the structure and assembly of fimbrins (fimbria proteins) (Clouthier et.al, 1994). Several fimbrial operons, ranging from 7 to 9 kb in size, have been identified in *Salmonella*. The sequenced strain *S*. Enteritidis PT4 has 13 fimbrial operons (Betancor et al., 2012). Some examples of fimbrial operons include the agf and sef operons, which encode the *S*. Enteritidis fimbria SEF17 (White et al., 2003; Thorns et al 1996) the pil operon (located in SPI-7) in *S*. Typhi CT18 (Zhang et al., 2000) and the lpf (long polar fimbriae) and pef (plasmid-encoded fimbriae) operons in *S*. Typhimurium (Baumler and Hafferon 1995; Friedricg et al ., 1995) Friedrich et.al 1993)The SEF14 fimbriae have been found to be expressed by *S*. Enteritidis, *S*. Dublin, and poultry-associated *Salmonella* serovars Berta and Gallinarum, where they appear to be important for adhesion of these serovars to tissues of the reproductive tract (Turcotte and Woodward 1993; Doran et al., 1996). Type I fimbriae contribute to *Salmonella* colonization of pigs (Althouse et al., 2003), while 13 major fimbrial subunits of *S*. Enteritidis Phage Type 4 (PT4) have been found to play a role in adherence and colonization of the bacteria in chicken gut (De Buck al., 2005; Clayton et al., 2008); the loci on which these subunits were detected were conserved in S. Paratyphi and S. Gallinarum. A detailed distribution of fimbrial operons among *Salmonella* subspecies and serovars was highlighted in a review by van Asten and van Dijk (2005).

2.3.6. Flagella

The majority of *Salmonella* serovars possess up to 10 randomly positioned flagella on their cell surface, which confer motility to these bacteria (van Asten and van Dijk, 2005). The ability of certain serovars to display flagellin phase variation provides a potential means for the organisms

to minimize the host immune response by creating phenotypic heterogeneity of the flagellar antigens (van Asten and van Dijk, 2005). The fliC gene, encoding the phase 1 flagellin protein, has been found in *Salmonella* serovars Gallinarum and Enteritidis (Dauga et al., 1998). However, the exact role of flagella (motility and direction of rotation) in *Salmonella* pathogenesis and their possible role in adhesion and invasion of mammalian cells remain unclear (van Asten and van Dijk, 2005)

2.3.7. Other Factors affecting Pathogenicity of *Salmonella*

Some other virulence factors such as surface polysaccharides may also play a role in persistence of *Salmonella* in the intestinal tract. Multiple mutants affecting lipopolysaccharide (LPS) biosynthesis have been identified in *Salmonella* strains isolated from calves and chickens (Stevens et al., 2009; Carnell et al., 2007; Morgan et al., 2004; Turner et al., 1998). For example, the virulence of the LPS rfbK, dksA, hupA, sipC, and ptsC mutants and clpB and rfaY transductants was studied in 1-day-old chicks by Turner and coworkers (Turner et al., 1998). That study showed that all but the ptsC and rfaY mutants were attenuated for virulence in chickens. Signature-tagged mutagenesis showed that LPS S. Typhimurium mutants (rfaK, rfaB, rfaG, rfbP, rfbN, rfbU, rfbH, and rfbA) were unable to colonize calf intestines, suggesting a role of surface polysaccharides and cell envelope proteins as virulence factors contributing to *S*. Typhimurium colonization of calves (Morgan et al., 2004). LPS has been found to contribute to the ability of *S*. Enteritidis to survive in egg albumen (Gantois et al., 2006). That study showed that a mutant strain unable to produce LPS (rfbH) was not able to multiply in eggs at room temperature and did not survive in egg whites at 42°C. Those authors concluded that attenuation increased susceptibility of the rfb.

2.4. *Salmonella* in Chicken Host Serovars

Because the diversity of the *Salmonella* enterica species is quite expansive, this section focuses primarily on the host range adaptation of four particular serovars (S. Enteritidis, S. Heidelberg, S. Kentucky, and S. Gallinarum) that are associated very commonly with chickens and to various extents with other food animal species and human infections.

2.4.1. *Salmonella* Enteritidis

According to the CDC, in the United States, S. Enteritidis was the serovar most commonly implicated in human illness, overtaking *S*. Typhimurium as the most common serovar (Centers for disease control and prevention, 2011). Likewise, when data from the National Veterinary Services Laboratory of the USDA and from other studies examining the prevalence of *Salmonella* serovars were compared, *S*. Enteritidis was associated most commonly with chickens and eggs and to a much lesser extent with other food animal species (CDC 2009, Elson et al., 2005; Hennessy et al., 2004; Kuehen, 2010). *S*. Heidelberg is found in most of the major food animal species, eggs, and retail meat samples and is among the top five most common serotypes associated with human illnesses (Han et al., 2011; Hennessy et al 1996). Conversely, *Salmonella* serovars Kentucky and Gallinarum rarely cause human infections in the United States (although S. Kentucky is an emerging serovar in Europe and North Africa) (Le Hello et al., 2011). *Salmonella* serovar Gallinarum is a host-adapted serovar that is presently made up of two biovars, Gallinarum and Pullorum (which were previously considered two separate serotypes) (Eswarappa et al., 2011). This serotype was associated with severe losses to the poultry industry in the United States until it was targeted for eradication by the National Poultry Improvement Plan (NPIP)

starting in 1935 (USDA, 2010). After implementation of the NPIP, S. Gallinarum was eradicated from commercial poultry flocks in the United States by the mid-1960s (Foley et al., 2011).

2.4.2. *Salmonella* Heidelberg

There is no strong association of subtypes with a particular food animal host and subsequent human infection. When S. Heidelberg isolates from human patients were compared to those of the major food animal species, there was extensive overlap in PFGE profiles, plasmid types, and antimicrobial susceptibility profiles, indicating a lack of host restriction among S. Heidelberg genotypes (Han et al., 2011; Mazurek et al., 2004; Kaldhone et al., 2008). In a study examining the core genomes of the population structure of many of the prominent serovars, it was concluded that the genomes of S. Heidelberg isolates were likely shaped by a high degree of horizontal genetic transfer (Lynne et al., 2008). Consequently, the S. Heidelberg strains resided in a lineage distinct from that of the avian-associated *Salmonella* serovars Enteritidis and Gallinarum, based on genomic comparison. Additionally, the members of S. Heidelberg fell outside the lineage containing *Salmonella* serovars Typhi-murium and Saintpaul, yet S. Heidelberg isolates shared a relatively high proportion of sequence similarity with the lineage (Lynne, 2008). S. Heidelberg isolates also often contain plasmids. While they lack a serotype-specific virulence plasmid, a common feature in serovars such as S. Typhimurium, S. Enteritidis, and S. Gallinarum (Rotger and Casadesus, 1999), they often contain plasmids with virulence genes (Han et al., 2012; Johnson et al., 2010). Interestingly, a number of S. Heidelberg strains isolated from poultry-associated sources were found to harbor IncFIB plasmids similar to those previously recognized as being important for extraintestinal survival in avian-pathogenic E. coli

(Han et al., 2012; Didelot et al., 2011; Johnson et al., 2006; Brenner et al., 2000). Similar plasmids have been found very commonly in S. Kentucky isolates from poultry as well. Many IncFIB plasmids contain genes for iron acquisition (aerobactin operon and Sit iron transport systems), colicin production, and serum survival, which likely play a role in increased fitness in the avian environment (Han et al., 2012). In many cases, these IncFIB plasmids also contain genes that encode resistance to multiple antimicrobials (often including resistance to tetracycline, streptomycin, chloramphenicol, and sulfonamide). This presents the possibility for coselective pressure, with antimicrobial use selecting for enhanced virulence, or conversely, the increased ability of these bacteria to survive iron-limited environments in the host could select for resistance to one or more antimicrobials (Han et al., 2012; Johnson et al 2012). S. Heidelberg plasmids also contain genes encoding disinfectant and heavy metal resistance, which may provide a selective advantage for survival in the avian production environment where pathogen control strategies are employed cc

Studies have shown that *Salmonella* serovars Gallinarum and Enteritidis are closely related in both their gene content and their antigenic formula (Thomson et al., 2008; Brenner et al., 2000; Porwollik et al., 2004; Porwollik et al., 2005) suggesting that these two serovars originated from a relatively recent common ancestor (Li et al., 1993; Olsen et al., 1996). Because of their close genetic similarity, they are valuable examples to explore host adaptation. Genomic comparisons of members of these two serovars have indicated that there is a high degree of genetic similarity between the two serotypes, with average nucleotide identities among orthologous genes being 99.7% (Thomson et al., 2008). The genomes of sequenced isolates shared 4,179 predicted coding sequences (CDSs). The differences in CDS content between the strains were associated primarily

with bacteriophages incorporated into the respective bacterial genomes (Thomson et al., 2008). The lineage of *S. enterica* that contains these serotypes tends to be one of the most conserved among the species, with an estimated 4% of their core genome sequences originating from recombination with genes from other *Salmonella* serovars (Didelot et al., 2011). Both serovars share the O1, O9, and O12 antigens (Brenner et al., 2000). However, even though S. Enteritidis and S. Gallinarum are relatively closely related on the genomic level, they are diverse in their numbers of pseudogenes. S. Gallinarum isolates are unable to carry out mannose-sensitive hemagglutination and do not express flagellar genes, leading to an observed lack of mobility, while the majority of S. Enteritidis strains are motile (Baumler et al., 1998). This lack of motility observed for S. Gallinarum isolates is in part associated with mutations in genes associated with flagellar biosynthesis and chemotaxis, including flhA, flhB, flgI, flgK, or cheM (Thomson et al., 2008). Mutations in fliC may also contribute to the lack of motility of isolates of this serovar (Li et al., 1993)

One study found that S. Enteritidis has 21 pseudogenes, compared to 147 for S. Gallinarum, but there are only 5 pseudogenes shared between the two serovars (Kuo and Ochman, 2010). Of these five pseudogenes, three are likely ancestral in origin, and the other two were likely independently acquired. Similarly, the genetically related S. Dublin had 212 identified pseudogenes, 177 of which are active genes in S. Enteritidis. Many of these functional genes encode surface structures or are involved in the central metabolism of *Salmonella*; thus, their inactivation in S. Dublin likely contributes to its host restriction in cattle (Betancor et al., 201). Likewise, certain pseudogenes are common to multiple host-restricted serotypes; for example, mglA and shdA are transcribed in S. Enteritidis but are present as pseudo-genes in S.

Choleraesuis, S. Dublin, S. Gallinarum, S. Paratyphi A, and S. Typhi (Betancor et al., 2012). MglA is a small GTP-binding protein subunit of a binding-protein-dependent galactose transport system (Richarme et al., 1993; Zhang et al., 2010) that likely plays a role as a virulence regulator in intracellular pathogens such as Francisella spp. (Nano and Schmerk, 2007) and motility in Myxococ-cus xanthus (Mauriello et al., 2010). ShdA is a protein that is involved in the colonization of Peyer's patches by S. Typhimurium and the shedding of the bacteria following infection (Kingsley et al., 2000; Kingsley et al., 2004). The presence of a larger number of functional genes in broader-host-range serovars such as S. Enteritidis likely contributed to the ability to colonize and infect a greater number of hosts (Wain et al., 2002).

There are also several differences in the numbers of the fimbrial genes between S. Gallinarum and S. Enteritidis. Several fimbrial operons, including lpf, bcf, stb, stc, std, and sth, are important for long-term carriage and shedding of *Salmonella* (Ruby et al., 2012). The S. Enter-itidis genome has 13 fimbrial operons, 12 of which are present in S. Gallinarum (Thomson et al., 2008). However, several of the CDSs within these common operons were identified as pseudogenes due to mutations that potentially prevent the expression of the functional gene product, which consequently impacts the overall function of the operons. These include mutations in stiC, stfF, safC, stbC, cheM, flhB, flhA, flgK, flgI, pegC, lpfC, sefC, sefD, sthE, sthB, and sthA (Thomson et al., 2008). Additionally, the virulence plasmids in the two serotypes contain fimbrial operons; however, they are unique, with the S. Enteritidis plasmids carrying the plasmid-borne fimbria (pef) operon, while the S. Gallinarum plasmids have fimbria genes similar to those of E. coli K88 (Van Asten and Van Dijk, 2005; Rotger and Casadessus, 1999, Rychlik et al., 2009, Thomson et al., 2008, Edwards et al., 2002).

2.4.3. *Salmonella* Kentucky

Salmonella Kentucky is currently one of the most common serotypes isolated from broiler chickens in the United States and is detected fairly often in dairy cattle as well (Centres for Disease Control and Prevention, 2011). The increase in the rate of isolation of S. Kentucky in broilers is likely related to a number of factors, including management practices, flock immunity, and genetic changes in the organism (Foley et al., 2011). When S. Kentucky was compared to other serovars with respect to virulence, it was found that the S. Kentucky isolates grew more rapidly than other serovars under moderately acidic conditions (pH 5.5) but worse under more highly acidic conditions (pH 2.5) (Joerger et al., 2009). The enhanced ability to grow under these moderately acidic conditions may provide an advantage over other serotypes in environments such as the chicken cecum. This serovar exhibited greater invasiveness in in vitro assays using chicken embryo hepatocytes than serovars such as S. Enteritidis and S. Typhimurium (Joerger et al., 2009). Many S. Kentucky isolates also have plasmids with factors such as those associated with antimicrobial and disinfectant resistance, iron acquisition, bacteriocin production, and complement resistance, which may enhance their abilities to survive in birds (Han et al., 2012).

It is interesting that S. Kentucky has not become a larger public health problem for consumers in the United States, as *Salmonella* from poultry is predicted to be the fourth most important pathogen-food combination associated with food-borne illnesses in the United States (Batz et al., 2011). It is quite probable that S. Kentucky strains most common in poultry are not overly virulent to humans. In analyzing the data from the CDC National *Salmonella* Surveillance Program (http://www.cdc.gov/ncidod/dbmd/phlisdata/*Salmonella*.htm), the states with the largest numbers of

human S. Kentucky infections were generally those associated with higher rates of dairy production rather than poultry production (10Centres for Disease Control and Prevention, 2009; Johnson et al, 2006). Likewise, several studies have utilized pulsed-field gel electrophoresis (PFGE) for the molecular characterization of S. Kentucky isolates from various sources (chickens, dairy cattle, and human infections); the most common profiles associated with human infections were similar/visually indistinguishable from common profiles from cattle but typically distinct from those of poultry (Le Hello, 2007; USDA, 1999). Likewise, a majority of S. Kentucky strains isolated from poultry-associated sources fall into the sequence type 152 (ST152) clonal complex, while the majority of those isolated from human patients were of ST198 (Le Hello, 2011). Therefore, a plausible explanation for S. Kentucky-associated human infections could be due to occupational exposure to cattle or due to consumption of contaminated products such as raw (unpasteurized) milk or raw-milk cheeses. S. Kentucky has been one of the most commonly detected serotypes isolated from prepasteurized milk in the United States (Van Jessel et al., 2011), and consumption of raw milk and raw-milk cheese has been associated with multiple outbreaks of salmonellosis (Van Kessel et al., 2011; Centres for Disease Control and Prevention, 2011).

2.4.4. *Salmonella* Gallinarum

The sequenced *Salmonella* Gallinarum isolate also lacked some of the T3SS effectors that are present in the S. Enteritidis genome, and sopA had an early stop codon that likely prevents the expression of a functional SopA protein (Thomson et al., 2008). In addition, there was a mutation in bscG of S. Gallinarum, whose gene product is predicted to be important for cellulose

biosynthesis, which likely contributed to the observed deficiency in its ability to form biofilms (Thomson et al., 2008). Taken together, these mutations may negatively impact the ability of S. Gallinarum to colonize mammalian hosts, thereby limiting its ability to cause illnesses in a broad range of host species. In addition to mutations in sopA, several studies have demonstrated the influence of other T3SS effectors on the ability of *Salmonella* serovars to colonize different hosts (Eswarappa et al 2008., Eswarappa et al., 2009; Araya et al., 2010). The T3SSs are multiprotein complexes composed of structural and regulatory elements that transfer functional effectors from the bacterium into host cells, thereby facilitating invasion of and survival within host cells (Galan and Wolf-Watz, 2006; Marlovits et al., 2004). The T3SSs play vital roles in the interaction of *Salmonella* with the host (Schklker et al., 2012). Variations in the gene sequences encoding T3SS factors for both the SPI-1 and SPI-2 systems have been associated with differences in the abilities to invade different hosts. Some of the main variability associated with host specificity is with the secreted effectors and the SipD T3SS tip protein rather than other structural components of the respective T3SSs (Eswarappa et al., 2008; Eswarappa et al., 2009). The SPI-1 T3SS tip protein SipD is important for cell invasion; studies showed that antibodies directed at SipD block the ability of S. Enteritidis to invade intestinal epithelial cells (Desinet al., 2010). Effectors secreted by the SPI-I T3SS may also play a role in host specificity/range; sequence variability in the genes encoding the effector proteins SipA, SopA, and SopE and the chaperones SicP and InvB showed close evolutionary similarity in S. Enteritidis and S. Gallinarum compared to other serovars not as closely associated with avian sources (Eswarappa et al., 2008). Thus, these SPI-1-associated factors may play a role in specificity in the initial invasion of the intestinal epithelium or immune cells in birds. The inflammatory responses to infections by S. Enteritidis

and S. Gal-linarum are different, with S. Enteritidis eliciting a stronger inflammatory response in chickens, which may prevent a more systemic spread, while S. Gallinarum elicits a weaker initial immune response and typically leads to systemic fowl typhoid (Kaiser, 2010).

On the SPI-2 side, at least 13 SPI-2-associated genes in S. Enteritidis and S. Gallinarum show close evolutionary similarity to each other compared to other serovars (Eswarappa et al., 2008). It was hypothesized that the sequences may have coevolved for survival in the avian host, since survival in the reticuloendothelial system has been shown to be important for host specificity in chickens (Barrow, 1994). The SPI-2 T3SS plays an important role in survival in the SCVs of macrophages (Knodler et al., 2010). Genetic variability in SPI-1 factors, such as SopE and/or SodC, along with SPI-2 factors, such as SseC, SseD, and/or SseF, may lead to differences in the abilities to survive in different host cells (Eswarappa et al., 2009). The factors SseG and, possibly, SseF appear to impact the migration of the SCVs to the Golgi apparatus in the host cell, which may serve as a potential source of nutrition for *Salmonella* (Salcedo and Holden, 2005). Hence, differences in the effector proteins may impact the ability to survive in different host cells and to be transported to sites of systemic infection. In addition to changes in the gene sequences themselves, posttranslational modifications of T3SS effectors can impact the targeting of *Salmonella* or the SCVs to different parts of the host cells.

2.5. Non -Typhoidal *Salmonella*e in Poultry Meat and egg

Non-typhoidal *Salmonella* is a Gram-negative bacterium belonging to the family Enterobacteria-ceae. These bacteria are said to be localized in the intestinal tract of several distinct groups of animals such as domestic fowls like chickens, ducks, geese, turkeys; farm animals like goats, cows,

sheep, pigs; pets such as dogs, cats, horses, and other reptiles like turtles, lizards, snakes. They are also found in frogs, toads, rodents and other birds like parakeets, parrrots and wild birds. These reservoirs of *Salmonella* can cause the disease to humans termed as non-typhoidal salmonellosis (NTS) (Hoelzer et al., 2011; Bangera et al, 2018). Human beings obtain this infection through the ingestion of raw or undercooked contaminated food from animal origin, mainly from poultry (eggs and meat), pigs (meat) and by the consumption of unpasteurized cow milk. Non-typhoidal Salmonellosis refers to the infection produced by all serotypes of *Salmonella* except for typhoidal and paratyphoidal group. The symptoms include diarrhea, vomiting and abdominal cramps which develop 12 to 72 hours after infection. Non-typhoidal Salmonellosis have a discrete adaptation to certain animals such as *Salmonella* Choleraesuis to pigs, *Salmonella* Dublin to cattle, *Salmonella* Abor- tusovis to sheep and *Salmonella* Gallinarum to poultry (Bangera et al., 2018; Bhaisare et al., 2014).

Poultry and poultry meat often get contaminated with likely pathogenic microorganisms including *Salmonella*, Campylobacter, E. coli, Listeria and S. aureus (Bhaisare et al., 2014). In the poultry industry *Salmonella* and Campylobacter are the major foodborne pathogens. The chicken meat surface can acquire *Salmonella* from intestinal contents, fecal material or from cross-contamination during slaughtering processes (Rouger et al., 2017). Chicken meat is said to be a nutritious, healthy food which is low in cholesterol and the finest source of protein in comparison with other meat. Since the chicken meat has a high moisture content, rich in nitrogenous compounds like essential amino acids, proteins, good source of minerals, vitamins and other growth factor, it serves as an ideal medium for bacterial growth as the organisms tend to remain on the surface or just under it. Both poultry muscle and skin are excellent substrates for

supporting the growth of a wide variety of microorganisms. NTS being isolated from poultry sources is well documented and data are available from many parts of the world (Rouger et al., 2017; Bhaisare et al., 2014).

Non-typhoidal *Salmonella* serovars have the ability to cause blood stream infections when they have an assemblage of virulence genes in their *Salmonella* pathogenicity islands (SPIs). Some of the virulence chromosomal genes of NTS are invA, spvC, sefA, sopB and stn. The invasion gene invA, is essential for the entry of the bacterium from the gut lumen into the epithelial cells. It is possibly responsible for the virulence of the bacteria, facilitating their entry into the bloodstream causing bacteraemia (Bangera et al., 2018). The unwarranted use of antimicrobials in largescale poultry production as veterinary medicine and also as growth promoters is a widening problem causing an increase in antimicrobial resistance in NTS and all other bacteria. The irrepressible use of antimicrobials can lead to the selection for bacterial resistance posing a threat to public health by spreading of the resistance from farm animals to the human population (Center for Science and environment, 2017, Center for Science and Environment, 2014). It is noteworthy that Non-typhoidal *Salmonella* is no doubt, a major zoonotic pathogen that plays a significant role in foodborne human salmonellosis worldwide through the consumption of contaminated foods, particularly those of animal origin. Foods of animal origin, including meat, eggs, milk and other products, play a significant role in the daily diets of Nigerians. Poultry populations, in particular chicken and turkey, are frequently colonized with *Salmonella* without detectable symptoms (sub-clinical infections/healthy carriers) by horizontal and vertical transmission at primary production level [Herrington et.al. 1988, Nataro et.al, 1998]. The presence of *Salmonella* in healthy poultry animals is suggested as the main risk factor, by allowing bacteria to easily transmit in table eggs

and poultry meat to humans [Watson et.al, 2008]. In fact, in Europe it is assumed that the observed reduction in salmonellosis cases 32% between 2008 and 2012) is mainly due to successful *Salmonella* control measures (involving surveillance, biosecurity and vaccination) implemented in poultry/egg production and focused on particular serotypes (e.g., S. Enteritidis and S. Typhimurium) that are considered of public health significance [Herrington et.al. 1988, Bhavar et.al, 2007, Watson et.al, 2008, Tang et.al, 2012]. These measures led to the achievement of reduction targets for poultry populations in most EU countries and lower non-compliance regarding *Salmonella* in poultry products [Herrington et.al. 1988, Bhavar et.al, 2007]. Moreover, decreasing contamination rates in raw poultry products are in agreement with those recently observed in industrialized countries from other geographical regions with pathogen reduction programmes, such as the USA [Tang et.al, 2012, Sharma et.al, 2004, Andersen et.al, 2005]. Since the 1980s, worldwide outbreaks of human salmonellosis have been caused by S. Enteritidis present in eggs and contaminated broiler meal. In this case, breeders and laying hens were often thought to be infected by rodent feces and urine and transmitted the bacterium vertically, resulting in infected progeny. In addition, during the same period, outbreaks of human salmonellosis, resulting from ingestion of pork and beef meat contaminated with *Salmonella* serovars, have been reported in many countries (CDC, 1981; Spitalny et al., 1984; Delpech et al., 1998), with S. Typhimurium being the main serotype involved. It should be noted that by the 2000s a high incidence of *Salmonella* in poultry products was reported in the EU, with rates >50% for several countries [Lapaque et.al 2006]. In the 2013 zoonosis EFSA/ECDC report involving data from European countries, as in previous years, *Salmonella* was most frequently reported, although at low levels, in fresh turkey (5.4%) and fresh broiler meat (3.5%), in comparison with

eggs (0.1%) or fresh pig meat (0.7%) [Bhavsar et.al, 2007]. Despite the highest incidence being detected in poultry meat, eggs still remain the most important source of food-borne *Salmonella* outbreaks [Bhavsar et.al, 2007]. In fact, using quantitative source attribution models the higher number of human salmonellosis cases in Europe was attributable to eggs (65% in 2011 and 17% in 2012) and pigs (28% in 2011 and 56.8% in 2012) compared with broilers (2.4% in 2011 and 10.6% in 2012) and turkey meat (2.6% in 2011 and 4.5% in 2012) [Scallan et.al, 2011, Andrews et.al, 2010]. However, diverse surveys targeted to detect *Salmonella* in poultry products in developing countries, some with expansion of the poultry industry, still detected high percentages of positive samples, ranging from ~13% to 39% in South America [Hoffmann et.al, 2012, Batz et.al, 2011], ~35% in Africa [Batz et.al, 2005, CDC, 1997] and ~35% to 50% in Asia [Batz et.al, 2012, Brenner et.al, 1998, Su et.al, 2007]. Those differences possibly reflect diverse poultry production husbandry practices and absence of control measures along the food chain, highlighting the importance for *Salmonella* spread of the extensive international trade in animals and their products [Herrington et.al, 1988]. Worldwide data about *Salmonella* serotype prevalence in humans and in the diverse range of foodstuffs have contributed to establish an epidemiological link between salmonellosis and poultry products, with diverse serotypes overlapping between humans and poultry meat (chicken and turkey). In the EU, recent changes in the frequency of *Salmonella* serotypes causing human infections were reported, which in some cases were in line with those occurring in poultry. Nevertheless, interpretation of these data should be cautious, owing to limitations in the number of poultry isolates serotyped each year. Of particular relevance is the decrease in S. Enteritidis human cases (19% reduction between 2011 and 2013 in the EU), a serotype typically associated with poultry meat and egg

consumption. In the USA, S. Heidelberg in particular has been identified as one of the top human and poultry serotypes, with several clones implicated in diverse large multistate outbreaks resulting from the consumption of contaminated chicken or turkey products [Tang et.al, 2012, Chiu et.al, 2004]. The spread and the global persistence of serotype S. Kentucky reflect other particular situations related to the increased globalization of travel and the food/animal trade in different geographical regions. This serotype has been associated with a worldwide (Europe, Africa and Asia) spread of a particular epidemic clone (S. Kentucky ST198X1), recovered from several livestock reservoirs, particularly poultry farms, with chicken and turkey implicated as the potential major human infection vehicles [Darwin et.al, 1999, Van Asten et.al, 2005, Stevens et.al, 2009, Carnell et.al, 2007, Morgan et.al, 2004]. These and other examples of multi-country/multi-state outbreaks or clonal expansion of *Salmonella* infections linked to poultry meat serve as a reminder of the importance of acting upon any *Salmonella* contamination in the food chain and monitoring to detect the emergence of any serotype or new clone. *Salmonella* serotypes and clones associated with human infections and with an enhanced ability to colonize several food animals, able to persist along the food chain (e.g. primary production on-farm, slaughter operations, equipment and meat handlers, retail meat) with efficient transmission and rapid spread are of public health relevance [Hoebe et.al, 2004, Vugia et.al, 2004, Morgan et.al, 2004, Dejong et.al, 1965, Fedorka et.al, 1995]. Although understanding the exact mechanisms of their persistence and spread in poultry production are still largely unknown, recent studies focusing on emergent poultry-associated *Salmonella* strains unveiled specific features that could provide a significant advantage both in the environment and in the host (poultry/ human) [Tang et.al, 2012]. For example, in Israel, an S. Infantis emergent clone possessed a mega-plasmid, which

increased its tolerance to stress factors (e.g., mercury and oxidative stress) and its virulence/pathogenicity (e.g., enhanced biofilm formation, adhesion and invasion into avian and mammalian host cells) [Winnen et.al, 2008]. Also, a genomic study of several predominant *Salmonella* serotypes from Canadian broiler chickens showed the presence of multiple features related with pathogenicity (e.g., genes encoding adhesins, flagellar proteins, iron acquisition systems, type III secretion system) and stress tolerance (e.g., metal and antiseptic tolerance genes; better acid-stress response) [Chiu et.al, 2002]. S. Heidelberg, including ground turkey outbreak isolates, carried phages and plasmids with diverse virulence factors (e.g., P2like phage-sopE1 gene, IncX-type IV secretion system), which could play a role in their virulence (a serotype highly related to invasive infections), colonization and persistence (a poultry-associated serotype) [Tang et.al,2012, Chiu et.al, 2004]. In S. Kentucky, the acquisition of an E. coli ColV virulence plasmid was also associated with enhanced colonization ability in chicken, particularly in a dominant avian clonal type [Tang et.al,2012].

2.6. Non-Typhoid Salmonella (NTS) in Africa

An estimated global burden of NTS morbidity and mortality showed that enteric NTS cause 93.8 million illnesses with 155,000 deaths annually, while invasive NTS were estimated to cause 3.4 million cases with 681,316 deaths annually [Herrington et.al, 1988, Nataro et.al, 1998]. African countries have a relatively low level of reported NTS gastroenteritis, but a much higher level of invasive non-enteric NTS infections, estimated at 227 cases per 100,000 persons per year compared to the global average of 49 cases per 100,000 persons per year [Herrington et.al, 1988]. These figures of salmonellosis make Africa the leading continent with invasive non-enteric NTS

cases accounting for more than half of the global cases [Herrington et.al, 1988]. In humans, many of the gastroenteric infections caused by NTS are self-limiting, and thus, many sporadic cases go unnoticed and/or unreported. However, a serious aspect of this situation is that some of the gastroenteric infections may develop into bacteraemia, which is an emerging opportunistic infection in individuals infected with HIV, the elderly and in children [Stephen et.al, 2001, Bhavsar et.al, 2007]. The reservoirs of food-borne NTS are often located in the primary food animal production. Many of these zoonotic NTS are able to colonize the intestinal tract of a variety of animal species, and in most of these cases, the animals are healthy and asymptomatic. Faecally contaminated foodstuffs like meat, eggs, dairy products and sometimes vegetables are the main sources of salmonellosis in humans [Tsolis et.al,2008, Guerrant et.al, 2005, Cossart et.al, 2004, Watson et.al, 2008]. The dissemination of NTS is also a growing concern due to increasing cases of drug resistant isolates and their frequent carriage of transmissible antimicrobial resistance genes. Even more worrying is the rising occurrence of multidrug-resistant NTS, including cases reported in some African countries [Nataro et.al, 1998, Hoebe et.al, 2004]. Because of multidrug-resistance, treatment with first line drugs is often no longer an alternative, and this puts pressure on the use of second- or third-line drugs. Some limited studies in Africa on antimicrobial resistance in NTS isolates from animal sources have been undertaken, but with varying results [Hoebe et.al, 2004, Tang et.al, 2012, Nguyen et.al,2004]. Many prevalence and risk factor studies of NTS in layer and broiler populations have been conducted in the USA and Europe [Sharma et.al, 2004, Andersen et.al, 2005, Lapaque et.al, 2006, Scallan et.al, et.al 2011]. A systematic review of risk factors associated with laying hen farms identified multiple risk factors related to biosecurity measures, management factors and the environment [Andrews et.al, 2010]. In

addition, developed countries have put in place monitoring and surveillance systems for antimicrobial resistance targeting important zoonotic pathogens like NTS. Unfortunately, such systematic surveillance neither exist for NTS nor other food-borne pathogens in most developing countries like Nigeria probably due to inadequate literature. Since most human NTS infections originate from animal sources, prevention and control at pre-harvest level in the primary production units is an effective way to minimize NTS dissemination and transmission to humans through the food chains [Batz et.al, 2011, Batz et.al, 2005, CDC, 1997, CDC, 2024].

2.7. Non-Typhoid Salmonella and the Nigerian Poultry Industry

The poultry industry in Nigeria has been rapidly expanding in past years despite facing many problems such as avian influenza, the global financial crisis and inadequate credit [Tsolis et.al, 2008]. The Nigerian poultry industry increased from 150,700 million chickens in 2005 to 192,313 million in 2010 [Guerrant et.al, 2005]. Across the different regions of the country, the poultry sector is characterized by a low level of production and specialization and a general weak level of biosecurity [Tsolis et.al, 2008]. In 2011, Nigerian hen egg production totaled 636,000 metric tonnes and was valued at $527.49 million, ranking 19 in world hen egg production and the top producer in Africa [Guerrant et.al, 2005]. Both large and small egg farms are scattered all over the country, although they are generally concentrated around the major urban centres [Tsolis et.al, 2008]. Poultry meat and eggs are the major sources of animal protein in Nigeria, as in many developing countries, because of their affordability and acceptability [Herrington et.al, 1988, Nataro et.al, 1998]. This source is, however, being threatened by diseases such as salmonellosis and avian influenza [Donnenberg et.al, 2000]. In food-producing animals and especially in

poultry, *Salmonella* is one of the leading causes of infection, and this has a direct impact on the global marketing of the respective food-producing animals and animal-derived food products [Cossart et.al, 2004]. Poultry salmonellosis related to host adapted serovars remains a major constraint on poultry production in all parts of Nigeria [Watson et.al, 2008, Hoebe et.al, 2004, Tang et.al, 2012]. Farmers still experience great losses (due to mortality, morbidity and drop in egg production) caused by host adapted *Salmonella* serovars despite huge amounts spent on vaccination and medication. In early life, S. Pullorum causes extremely high mortality of both broilers and commercial laying birds. Similarly, older birds succumb heavily to other serovars of *Salmonella*, and it is assumed that *Salmonella* infections of this category of birds are mainly due to S. Gallinarum (Nguyen et al., 2004). In addition to these host adapted serovars causing systemic disease, poultry harbor in their gastrointestinal tracts zoonotic serovars with no apparent signs of illness. Hence, these *Salmonella* serovars can be present in feaces excreted by healthy animals and may be transferred to raw foods of animal origin through contamination during slaughtering and processing (Sharma and Qadri, 2004). Generally, *Salmonella* in food producing animals, including poultry, manifests as long periods of latent carriage with occasional faecal shedding, which is the leading source of contamination of feed, water and environment (Cossart and Sansonetti, 2004). Relatively few African countries report surveillance data on *Salmonella* and as such, very limited information is available on *Salmonella* isolation for this continent (Anderson et al., 2005). This is also the case in Nigeria, where the few studies conducted so far show different drawbacks, such as the limited number of samples considered, the lack of representativeness of the samples selected, lack of access to serotyping facilities, or the restricted geographical coverage. Raufu et al. (2013), who collected samples from three

poultry slaughterhouses and five intensively managed poultry farms in a circumscribed area of Nigeria, reported a *Salmonella* prevalence ranging from 2 to 16%. Moreover, a study conducted in three commercial hatcheries in the Jos area reported a prevalence of 9%, with S. Kentucky and S. Hadar as the most frequent serovars [16]. Idowu et al; (2017), reported that a total of 228 of the 523 farms sampled were positive for *Salmonella* with a farm prevalence of 43.6% (CI 95 [39.7–48.3%]). A farm was considered to be infected and/or contaminated when at least one matrix tested positive. Looking at the prevalence of *Salmonella* per state in the study, the highest prevalence was recorded in Ogun state (65.4%), which was also one of the states with the largest number of farms sampled (110), whereas Edo state, which is among the states with the lowest number of farms sampled (18), registered the lowest prevalence (11.1%). For each farm, one sample each of litter, faeces, dust, water and feed were collected and 370 out of the 2615 samples collected tested positive for *Salmonella* (14.1%, CI 95 [12.8; 15.5%]). Considering the number of positive farms and the number of positive samples, Idowu et al;(2017) observed that for each positive farm, more than one matrix was generally positive for *Salmonella*.

CHAPTER THREE

3.0 ECONOMIC BURDENS OF PERSISTENT INFECTION OF NON-TYPHOIDAL *SALMONELLA* IN POULTRY FARMS, NORTH CENTRAL NIGERIA

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Abstract

The non-typhoidal salmonellosis (NTS) is a pathogenic bacterial zoonosis with substantial but often under-appreciated public health impacts. The NTS is prevalent in poultry and humans in Nigeria, yet its economic and social burden have not been determined through any empirical study. To bridge the gap, we evaluated the impact of NTS in social and economic terms. Relevant population, economic and epidemiological data were retrieved from peer-reviewed publications, open sources and relevant authorities. Additional data were obtained through experts' opinions and field surveys. Using a customized and validated Microsoft Excel® tool, economic analysis was conducted. Using the year 2020 reference point, the burden of NTS was 325,731 cases and a total of 1,043 human deaths, at a disability-adjusted life year (DALYs) of 37,321. The cost associated with infection in humans was US$ 473,982,068. A total loss of US$ 456,905,311 was estimated in poultry including the direct value of animal loss, US$ 224,236,769, loss from salvage slaughter and culling, US$ 220,386,556, and value of foregone production, US$ 12,281,987. The outcomes of this important work provide empirical evidence to support informed decisions and investments in the control and eradication of human and poultry salmonellosis (NTS) in Nigeria.

Keywords: Economic burden; Social burden; non-typhoidal *Salmonella*; Disability-adjusted life years; Years of life lost; Years lost due to disability.

3.1 Introduction

Salmonellosis is a pathogenic bacterial zoonosis with substantial public health impacts [1,2]. With over 2600 different serovars identified to date, *Salmonella* spp. is broadly divided into typhoidal and non-typhoidal *Salmonella* (NTS) serovars [3,4]. The NTS is one of the widespread causes of food-borne diarrhoeal diseases, while the invasive NTS (iNTS) is responsible for major bloodstream infections universally [1,3,5]. Humans are infected with NTS through contamination from poultry products (egg fragments, hatching eggs, chick boxes, fluff and faeces), partially cooked meat and raw eggs [2,3]. The global estimates of burden of NTS varied widely, including an estimate of over 27 million human cases and 200,000 deaths per annum [6,7]; approximately 79 million human cases and over 59,000 deaths annually [2]; and 93.8 million human infections and 155,000 fatalities annually [8]. Furthermore, in a recent ranked study in the USA, *Salmonella* spp. was the first-ranked foodborne pathogens, with the most significant cost of illness and the quality-adjusted life-year (QALY) losses [9].

The iNTS was estimated to cause 177 – 388 cases per 100,000 children under 5 years in Africa but may reach up to 2000 – 7500 cases per 100,000 humans in immunocompromised HIV-infected adults, and a case fatality ranging between 20 – 25% [10]. In Nigeria, the poultry farm level prevalence of NTS range from 41.6 - 47.9% and the risk factors for NTS infection of poultry farms in Nigeria have been fully explored [4,11,12]. Based on a recent meta-analytic study, Nigeria has a burden of prevalence (in humans) of 1.9% (2,732/143,756) *Salmonella* bacteremia and 16.3% (1,967/12,081) *Salmonella*-associated gastroenteritis [13]. In addition, a total of 53 *Salmonella* serotypes have been identified in humans in the country including 39 associated with *Salmonella*-bacteremia and 31 associated with *Salmonella*-gastroenteritis [13].

The country has an estimated human population of approximately 219 million as of 2022 and has the largest market in sub-Saharan Africa, with a GDP PPP in excess of US$ 1 trillion for the year 2020 [11,14,15]. The agriculture sector contributes 24.1% of the country's GDP in the year 2020, with the poultry sector contributing approximately 25% of the agriculture GDP, and 6 – 8% to real GDP annually [16,17]. The 2020 poultry population in Nigeria was approximately 224 million [18] and is a major source of readily available and affordable animal protein (11). In 2019, the consumption of poultry products was approximately US$ 2 billion while the industry was worth US$ 4.2 billion [19].

Previous workers have made efforts to estimate the cost of animal health challenges globally and in Nigeria, including for multiple pathogens [2,20,21], *Salmonella* [1,8], avian influenza [22], and African swine fever [23], among others. Animal diseases cause significant, often undervalued economic losses through morbi-mortality, treatment and intervention cost, effects on production and productivity, and human health components (livelihoods, psychosocial and zoonotic impacts). It is therefore important to continue to estimate the burden of animal disease and relative microbiological hazards that may originate through animal-sourced food system to prioritise interventions aimed at mitigating these impacts. The aim of this work was to determine the economic and social costs and consequences of NTS in human and poultry in Nigeria, using the year 2020 as a reference point. The outcome should provide empirical information to guide informed decision, investment, and adequate planning for human and animal health interventions against poultry salmonellosis (NTS) in Nigeria.

3.2 Materials and Method

This work is a follow-up on the previous one where *Salmonella* isolates were obtained and characterized from samples collected from poultry farms in North central Nigeria where *Salmonella enterica*, *S. arizonae*, *S. paratyphi* and *S typhi* were recovered at prevalences of 41.6% (95%CI: 38.58 to 44.68), 0.2% (95%CI: <0.01 to 0.8), 1.9% (95%CI: 1.2 to 3.0) and 2.3% (95%CI: 1.5 to 3.5) respectively [4].

3.2.1. Data sources and Evaluation tool

We used the semi-automated Microsoft Excel® costing tool developed as part of the disease estimation process under the Food and Agriculture Organization of the United Nations' (FAO) Africa Sustainable Livestock 2050 [21, Supplementary materials 3.1 – 3.7]. Extensive economic, population and poultry sector data were obtained from various sources including: 1). The United Nations' Department of Economic and Social Affairs, Population Division, 2). The World Bank, 3). The Nigerian Federal Ministry of Agriculture and Rural Development (FMARD), 4). The Federal Ministry of Finance, Nigeria, 5). The National Agricultural Extension and Research Liaison Services (NAERLS), 6). Peer reviewed literatures, 7). Field surveys, 8). Experts' opinions and calculations made from these various sources (Table 3.1; Supplementary Tables 3.1 – 3.7).

3.2.2. Additional data and Expert elicitation protocol for assembling information on zoonoses and AMR

Currently, in Nigeria, the information system may not always provide the government with sufficient and robust information on the incidence, prevalence and impact of zoonoses on society.

It is therefore challenging to have a single source of comprehensive dataset for measuring economic evaluations, and return on investment aimed at prevention, management and control of animal diseases and zoonoses. We utilised the Google form (https://docs.google.com/forms/d/e/1FAIpQLSefH1i8YASvewU1y1x-OS0sgyuvWJnOuaECXKH9ReLV4YaYZw/viewform?vc=0&c=0&w=1&flr=0) to collect data briefs on humans and poultry from key informant, experienced stakeholders and value chain actors in the poultry industry. We also utilised the Africa Sustainable Livestock 2050 (ASL2050) expert elicitation protocol to assemble information on selected zoonoses and antimicrobial resistance using consensus of judgements of carefully selected experts [21]. It should be noted that experts were drawn using the snowballing sampling approach through which 30 animal health and 11 human health experts were obtained [21. These tools provided additional sources of data needed to measure the impact of zoonoses on society in monetary terms especially where industry, population and economic data were insufficient, unreliable or physically impossible to gather such data from current datasets. Data obtained through Google forms were evaluated for measure of central tendencies (absolute counts, minimum, median, average, maximum and mode) and those from experts' opinions were triangulated with field surveys, literature search and official statistics [2,21,24].

3.2.3. Estimation of burdens of non-typhoidal salmonellosis

We estimated the losses in humans (social cost) and poultry (economic cost) using the input data described above and the excel spreadsheet developed by the Africa Sustainable Livestock project [21].

To estimate the social cost of the disease, the Disability-Adjusted Life Years (DALYs) method was used as proposed by the World Health Organization (WHO) in quantifying the burden of disease from mortality and morbidity [2,25]. One DALY represents a one year of healthy life lost (a health gap measure that combines both time lost due to premature mortality and the time spent in illness). Following the methodology of Herrera et al. [26], the "cost" of one DALY has been defined as the willingness to pay for a DALY, which was determined based on the Value of Statistical Life

(VSL). The VSL available for the United States was discounted to a yearly value and transferred into the Nigerian context using the benefit transfer methodology described in Hammit and Robinson [27].

The loss of production was calculated by estimating the value of animals lost and the value of forgone production (including losses from decrease in egg production, culling and salvage slaughter) as presented in the detailed study of FAO [21]. Input data and sources are detailed in Table 3.1.

Table 3.1. Input data for the computation of economic and social costs of non-typhoidal salmonellosis for the year 2020 in Nigeria.

Poultry	**Intensive (large-scale)**	**Intensive (small and medium-scale)**	**Free-range/Semi-intensive (indigenous)**	**Total**		**Source**
Year 2020 poultry population	33,968,841	118,377,487	72,168,465	224,514,793		[18].
Price per carcass yield (Naira)	2,240	2,002	2,000			Carcass weight (1.4 – 1.6 kg) [28,29].
Price of poultry meat (kg) (Naira)	1,400	1,400	850			[29].
Price of eggs (Naira)	37	37	30			[29]
No of eggs laid per hen/year	250	180	40			Experts' opinions, [21].
Price of culled animal (or % decrease in price due to culling) (Naira)	1340	741	739			Experts' opinions, [21,30].
Price reduction for culled bird (Naira)	40%	63%	63%			Experts' opinions, [21].
Number of cases	6,793,768	26,043,047	10,825,270	43,662,085		Experts' opinions, field survey, [18].
Number of deaths	2,717,507	10,417,219	2,706,317	15,841,044		Experts' opinions, field survey, [18].
Number of salvage slaughter	2,038,130	10,417,219	8,118,952	20,574,302		Experts' opinions, field survey, [18].
Number of culls	1,698,442	3,906,457	108,253	5,713,152		Experts' opinions, field survey, [18].
Number (eggs lost in survivor hen per year)	38	27	3			Experts' opinions, field survey, [18].
Humans	**Livestock keepers**				**Consumers**	
Number of humans involved in the poultry value chain	9,627,904	33,552,135	20,454,954	63,634,993	27,407,441	[21].
Number of cases	1,155	40,263	147,276	188,694	137,037	FMoH, Experts' opinions.
Number of deaths	4	129	471	604	439	FMoH, Experts' opinions.
Duration of disease in days	6	6	3		3	FMoH, Experts' opinions.
Average age of infection	20	16	16		25	FMoH, Experts' opinions.
Year 2020 human population	208,327,405					[14].
Exchange rate (Naira to US$) (2020)	380.26					[31].
Exchange rate (US$ PPP) (2020)	144					[32].
DALYs weight (*Salmonella*)	0.21					
Average life expectancy	55 years					[33].
VSLY (US$)	11,353					[26].

GDP, US$ PPP 2020 (Naira)	N406,878,200,000,000	(US$ 1.07 trillion) [34].
Percentage Livestock VA (2020)	1.39%	[16,19].
Livestock VA, US$ PPP (Naira) (2020)	N5,655,606,980,000	US$14,873,000,000) [35],
Animal production losses (2020)	N128,886,753,387	[21].
GDP in 2020 (Naira)	N164,382,595,400,000	(US$432,290,000,000), [34].
AG GDP %	24.1%	[17].
Loss as a % of GDP	0.08%	Calculation using [17, 19, 21, 26, 32 – 34].
Loss as a % of AG GDP	0.33%	Calculation using [17, 19, 21, 26, 32 – 34].

Federal Ministry of Health = FMoH; GDP = Gross domestic product; PPP = Purchasing power parity; DALYs = Disability-adjusted life years; VSLY = Value of statistical life year; VA = Value added; N = Naira, AG GDP = Agriculture Value Added GDP. Additional data inputs are available in the Supplementary Tables 3.1 – 3.7.

3.3. Results

The results are presented in three sections as described below.

3.3.1. Losses in humans (Social costs)

Overall, the total economic losses associated with NTS in Nigeria for the year 2020 was US$ 930,887,379 with approximate losses in humans (social costs) and animals (poultry sector) being 50.9% (US$ 473,982,068) and 49.1% (US$ 456,905,311) respectively (Table 3.2). The losses in the human population were further disaggregated into losses in workers in the poultry value chain (livestock keepers, 64.1%; US$303,911,990), and the general populace (consumers, 35.9%; US$170,070,078) (Table 3.2). Among the livestock keepers, a significant percentage of the social costs (77.9%) was borne by the value chain stakeholders in the free-range and semi-intensive (indigenous) poultry stock (Sector 4). Approximately 21.6% and 0.6% of the social costs were borne by the value chain stakeholders (humans) in the commercial intensive (small and medium scale) (Sector 3), and intensive large scale and commercial operations (Sectors 1 and 2) (Table 3.3). In total, 188,694 and 137,037 cases were estimated among poultry keepers and consumers respectively; with 1,043 deaths and 324,689 survivors predicted to be directly associated with

NTS in the year 2020 in Nigeria. The estimated DALYs was 13,391, which translated to the social cost above (US$ 473,982,068.00; Table 3.3).

3.3.2. Losses in poultry (economic costs)

Approximately 61% (US$278,732,259) of the total losses in poultry (chickens) originated from the intensive small and medium scale farms, while 23.95% (US$109,412,575) was from the free-range and semi-intensive (indigenous) poultry (Table 3.4). The direct values of poultry lost were 61.4% (US$42,187,375), 51.9% (US$144,537,197) and 34.3% (US$37,512,197) of the total losses in each evaluated sector (Intensive large-scale; Intensive small and medium scale; and free-range and semi-intensive (indigenous) poultry) (Table 3.4). Specifically, the total value of animals lost, the value of loss from salvage slaughter and culling, and the total value of forgone production were US$224,236,769 (49.1%), US$220,386,556 (48.2%) and US$ 12,281,987 (2.7%) respectively (Table 3.4).

3.3.3. Pattern of antimicrobial use in the different sectors of the poultry industry

Based on the consensus of experts' opinions, the intensive small and medium-scale poultry farms as well as the intensive large poultry farms significantly access and used antimicrobials (92.5%), with only 62% of getting antimicrobials through recognised means (formal sources from veterinary drug stores or from veterinarians [36 – 38]) compared with the free-range and semi-intensive indigenous farms' access and use (49.2%), and access through the recognised means (13%) respectively (Figure 3.1a). Similarly, 90.9% of the intensive small and medium-scale poultry farms as well as the intensive large farms have observed significant increase in the use of antimicrobials in the last decade compared with just 20% in the free-range and semi-intensive indigenous poultry farms (Figure 3.1b). While 92% of the stakeholders in the intensive small and

medium-scale poultry farms and the intensive large farms were seriously concerned with the observed trends in antimicrobial usage in poultry farms, it was shown that only 60% of the free-range and semi-intensive indigenous poultry farms stakeholders were seriously concerned (Figure 3.1c). Among the human experts, 100% of them confirmed to have observed a significant increase in antimicrobial use in humans in the last decade. Only 20% and 80% were moderately and highly concerned about the trend respectively.

Table 3.2. Overall economic and social costs of non-typhoidal salmonellosis in poultry and humans for the year 2020 in Nigeria.

Poultry	Intensive (large-scale)	Intensive (small & medium scale)	Free-range/Semi-intensive (indigenous)	Total		
Value of animals lost (US$)	42,187,375	144,537,197	37,512,197	224,236,769		
Value of forgone production (US$)	26,573,102	134,195,062	71,900,378	232,668,542		
Total loss in animals (US$)	68,760,477	278,732,259	109,412,575	456,905,311		
Loss as a % of livestock GDP	0.00%	0.00%	0.00%	0.01%		
Loss as a % of national GDP	0.00%	0.00%	0.00%	0.00%		
Loss per case (US$)	10	11	10	10		
Loss per case, as percentage of healthy animal	65%	77%	73%			
Human (social)	Losses in Livestock keepers			Total for livestock keepers	Total for consumers	Total human loss
Value of mortality (US$)	1,643,368	63,814,550	233,426,044	298,883,961	167,075,760	
Value of morbidity (US$)	50,490	1,759,506	3,218,033	5,028,029	2,994,318	
Total social cost (US$)	1,693,857	65,574,056	236,644,077	303,911,990	170,070,078	473,982,068
Social cost as % of GDP	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Overall losses in poultry and humans (US$)						930,887,379

All calculations were conducted at an exchange rate of Naira 380.26 to US$ 1.00.

Table 3.3. Economic and social costs of non-typhoidal salmonellosis in humans for the year 2020 in Nigeria.

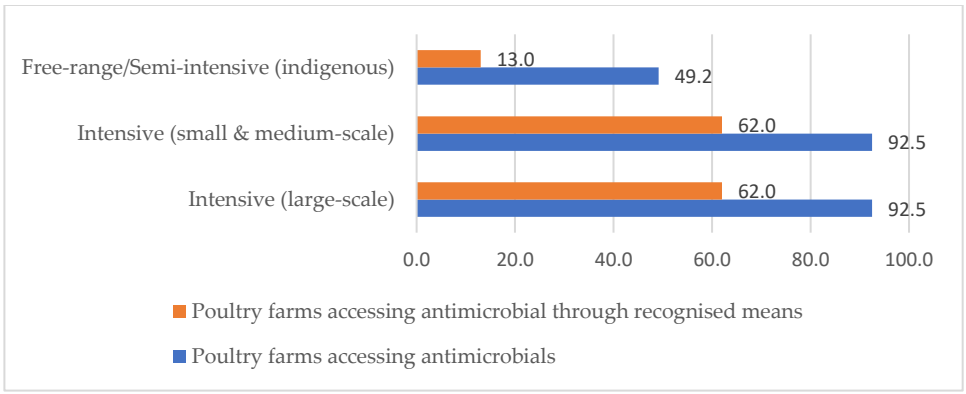
Salmonellosis parameters	Poultry keepers			Total Poultry keepers	Total Poultry consumers
	Intensive (large-scale)	Intensive (small & medium scale)	Free-range/Semi-intensive (indigenous)		
Ref. year	2020	2020	2020	2020	2020
DALY Weight	0.21	0.21	0.21	0.21	0.21
Number of cases in humans	1,155	40,263	147,276	188,694	137,037
Number of deaths in humans	4	129	471	604	439
Number of survivors	1,152	40,134	146,804	188,090	136,599
Disease duration in years	0.02	0.02	0.01		0.01
Average age of infection	20	16	16		25
YLL	129	5,025	18,380	23,534	13,156
YLD	4	139	253	396	236
DALY=YLL+YLD	133	5,163	18,633	23,930	13,391
Social cost(DALY*VSLY) (US$)	1,693,857	65,574,056	236,644,077	303,911,990	170,070,078
Total social costs in humans (US$)					473,982,068

DALYs = Disability-adjusted life years; YLL = years of life lost; YLD = years lost due to disability; VSLY = Value of statistical life year. All calculations were conducted at an exchange rate of Naira 380.26 to US$ 1.00.

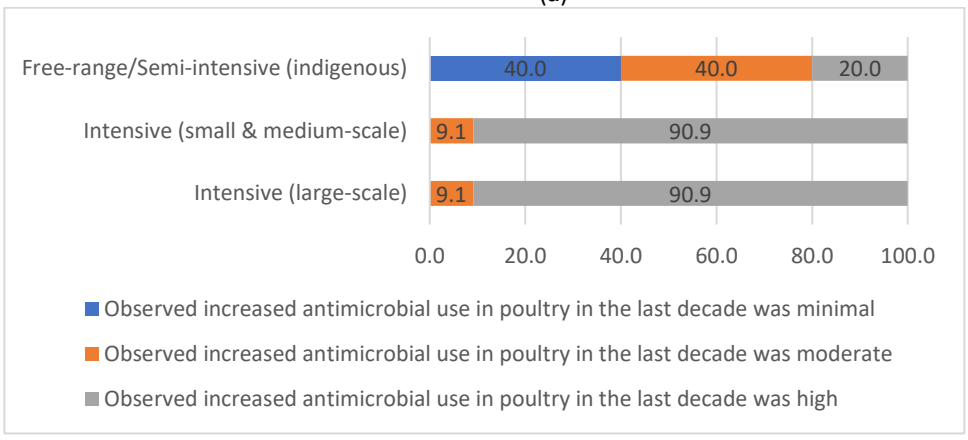
Table 3.4. Economic costs of non-typhoidal salmonellosis in poultry for the year 2020 in Nigeria.

Salmonellosis parameters	Intensive (large-scale)	Intensive (small & medium scale)	Free-range/Semi-intensive (indigenous)	Total
Number of cases in poultry	6,793,768	26,043,047	10,825,270	43,662,085
I. Value of animals Lost (US$)				
Number of deaths	2,717,507	10,417,219	2,706,317	15,841,044
Value of animals lost (US$)	42,187,375	144,537,197	37,512,197	224,236,769
II. Loss from salvage slaughter and culling				
Number of salvage slaughter*	2,038,130	10,417,219	8,118,952	20,574,302
Number of culls*	1,698,442	3,906,457	108,253	5,713,152
**this number may exceed the number of cases if the whole flock is culled / slaughtered*				
Value of loss from salvage slaughter and culling (US$)	23,306,641	125,179,537	71,900,378	220,386,556
II. Value of foregone production				
Number of survivors	339,688	1,302,152	-	1,641,841
Number of eggs lost per year in survivors	38	27	3	
Total value of forgone production (US$)	3,266,461	9,015,526	-	12,281,987
Total losses in poultry (US$)				456,905,311

All calculations were conducted at an exchange rate of Naira 380.26 to US$ 1.00.



(a)



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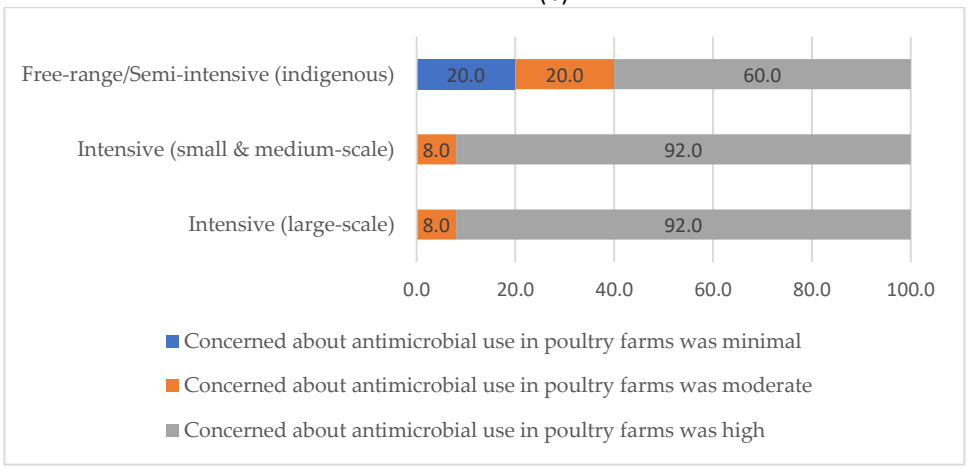

(c)

Figure 3.1. Experts' opinions elicitation on a. Antimicrobial use practice in poultry; b. Degree of increased use of antimicrobial in poultry; c. Degree of concern on increased use of antimicrobial in poultry.

Note that 100% of the human health experts observed increased antimicrobial use in humans in the last decade, and only 20% and 80% were moderately and highly concerned about the trend respectively. It should be noted that the most common antimicrobials used in poultry are: Tetracycline, Streptomycin, Penicillin, Nalidixic acid, Metronidazole, Gentamycin, Furazolidone, Furaltadone, Erythromycin, Enrofloxacin, Chloramphenicol and Ampicillin [38].

3.4 Discussion

We have estimated the overall economic burden of NTS in Nigeria for the year 2020, which came to US$ 930,887,379 (3.19% of the national budget). This represented a significant proportion in a country, which revised national budget for 2020 stood at 10.51 trillion naira ($29.19 billion) (https://www.reuters.com/article/nigeria-budget-idUSL8N2DA6Q9). Considering that the poultry sector contributed between 6 – 8% to real GDP for the year 2020, and approximately 25% of the agriculture GDP [16,17], the significance of these losses becomes more glaring. There is however lack of documentary evidence that the national government has taken account of this point, in planning and intensifying efforts to mitigate the impacts of NTS in humans and poultry. The only earlier estimates made in the past was conducted as part of the Africa Sustainable Livestock 2050 project in Nigeria [21]. The NTS typically presents as an acute onset of fever, abdominal pain, diarrhoea, nausea and sometimes vomiting, while the illness may last for 2–7 days [2]. In response to such acute self-limiting gastrointestinal illnesses, the households, particularly the poor in the rural and peri-urban households, and where healthcare services are hard to reach, primarily resort to habitual use of antibiotics and herbal medication, with high levels of self-prescription compared to antibiotic prescriptions that originate through the pharmacists [39]. In particular, metronidazole, tetracycline, amoxicillin, ampicillin or the Amplicox (a combination of ampicillin and cloxacillin) have been reported to be regularly abused [39]. In view of these observation, cases of NTS at the healthcare facilities may have been grossly underreported as only more serious cases may get to the hospital.

Comparing the case of NTS to other zoonotic diseases, which have occurred in Nigeria in recent times: 1). The highly pathogenic avian influenza subtype H5N1 (HPAI H5N1), which is a rapidly

fatal disease in poultry and humans that occurred in 2006 – 2008, and has continued to date, and somewhat become endemic in Nigeria. This virus may present in different subtypes. HPAI H5N1, may cause 70,000 - 145,000 household members to fall into poverty through loss of livelihoods associated with poultry, whereas NTS is more insidious but would affect a lot more people through morbidity (≤ 325,731 humans) but much lower mortality (≤ 1,043) [40]. In comparison, COVID-19, a largely public health issue, would produce much larger loss (up to 34.1 %) loss in the country's GDP, amounting to US$ 16 billion, primarily from the services sector and within a short period [41]. The burden of NTS is also lower than the associated burden in malaria, an endemic human disease, which burden of illness in Nigeria may be in excess of 25% of the GDP [42].

We estimated a disability-adjusted life years (DALYs) associated with NTS in 2020 to be 37,321; and more specifically, a total of 325,731 cases and a total of 1,043 human deaths was linked to NTS, with 64.1% of the social costs associated with the poultry keepers than the general populace which accounted for only 35.9%. This observation calls for systematic surveillance for NTS in humans, particularly the poultry keepers, and the need to intensify eradication of poultry salmonellosis in farmed poultry stock [13]. Two particular results were interesting. First, a significant percentage of the social costs (77.9%) originated from the free-range and semi-intensive (indigenous) poultry stock (Sectors 3 and 4). It should be understood that most of these categories of farmers reside in the rural and peri-urban often unplanned areas, and public health facilities may be inadequate or hard to access, there are imbalanced ratio of health workers to patients at such facilities, and the direct and indirect costs to patients may be relatively higher [43]. These may be directly linked to findings in the study of Adeyemi et al [39] where households regularly self-medicate using antibiotics and herbal medication, based on options of patients and

household to adopt alternative cheaper healthcare measures due to impoverishment, with consequent contribution to underreporting and underestimation of cases of NTS in Nigeria [13,43]. Secondly, although NTS is perceived as a disease of livestock, especially poultry, in view of the different serotypes of *Salmonella enterica* subsp. Enterica present in poultry [11,12], the larger proportion of the total losses (50.9%) directly related to losses in humans (social costs), pointing to the fact that human costs of salmonellosis may have been underestimated highly in the past.

It is unsurprising that the significant proportion of the losses in poultry (chickens) originated from the intensive small and medium scale farms and the free-range and semi-intensive (indigenous) poultry. These poultry sectors (3 and 4) contribute approximately 85% of the total poultry population in Nigeria, mainly in the small town, peri-urban shanties and unplanned rural areas, operate with low biosecurity and sources of day-old chicks that feed the system which may not always be standardised [30]. The aforementioned issues are significant risk factors for infection of poultry farms with *Salmonella* organism in Nigeria [4,12,30]. The total value of forgone production accounted for only 2.7% of the total losses in the poultry sector, an indication that poultry farmers hardly destroy and clean out the farms completely post infection, which is the standard recommended practice. The aforementioned factor can be a precursor to maintenance of infection in poultry farms and re-infection of new stock as emphasised in earlier study by [4].

It is noteworthy to indicate that despite the efforts made by the national and subnational government in Nigeria, the experts still observed significant usage of antimicrobials in both human and animal health, especially in the intensive small and medium-scale poultry farms, the intensive large poultry farms, and the human health facilities. There is a need for more stringent

measure to control dispensing and access to antimicrobials if efforts put in to date would yield any measurable progress.

3.4.1. Limitations of the study

Our work is subject to a number of limitations. First, while we have made effort to evaluate the comprehensive economic and social costs of Non-Typhoidal *Salmonella* infections in Nigeria, we are aware that it is generally impossible to measure everything necessary for a comprehensive analysis. Even when measurements are available, they may not adequately represent values appropriate for the analysis at hand [44]. Secondly, we assumed linear costs for lost units without considering the cost corresponding to discounted values, the costs of treatment and prevention. Hence, our evaluation may therefore be partially attributable to the overall costs. In a data-scarce environment like Nigeria, wherein comprehensive costs of prevention and control are not well detailed, especially in the sectors 1 – 3 of the poultry industry, this may serve a major limitation to conduct a comprehensive cost evaluation. Furthermore, our estimate does not take into account the cost of reducing the loss and an incompressible limit of loss inherent in the socio-economic and behavioural context from Nigeria. It was difficult to estimate these items during the evaluation.

Fourthly, we use computational model to estimate some costs and disease simulation rather than a direct clinical trial with control group, using data from the line ministry, the stakeholders and experts. We are aware that this may be subject to a degree of bias of experts submitting the data, and all outputs/outcomes are as meaningful as the input values [44,45]. While we advocate for a disease-specific collation of economic dataset informing future analysis, it becomes difficult to conduct a nationwide clinical trial for an insidious but impactful disease like NTS due to

regulations and time, and using data from other country may introduce geographical context and bias. Finally, the time factor may be a limitation to this type of economic evaluation carried out in this work as the industry is very dynamic in growth, disease contexts change, and many variables of interventions (prevention, management, treatment and controls) may demand regular remodelling using datasets available for the industry.

3.5. Conclusions

Our work has highlighted the burden of non-typhoidal salmonellosis in Nigeria, and the level of under-appreciation of its impact in the human population. Importantly, we have demonstrated that in developing countries like Nigeria, where there are constraints in public and animal health infrastructures, the overall ramifications of NTS have consequences that are detrimental to patients (human and animal), the economy and the country as a whole. It is believed that findings from this study should stimulate discussions on the effort at control and eradication of poultry salmonellosis, and by extension the reduction in the burden of NTS and iNTS in humans in Nigeria.

Supplementary Materials: The following supporting information are available as supplementary materials: Table 3.1. Number by cases data; Table 3.2. Basic Check on data; Table 3.3. Total Loss; Table 3.4. Loss (Humans); Table 3.5. Salmonellosis Loss (Poultry); Table 3.6. Poultry population (2020 estimates); and Table 3.7. Human populations estimate for the year 2006, 2020 and 2022.

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CHAPTER FOUR

4.0 COST EFFECTIVENESS ANALYSIS OF INTERVENTION AGAINST NON-TYPHOIDAL SALMONELLA IN NIGERIA

This manuscript has been published in the journal, *One Health* under the title: Sanni et al. (2024). '**Cost-effectiveness of One Health intervention to reduce risk of human exposure and infection with Non-Typhoidal Salmonellosis (NTS) in Nigeria**.' *One Health*, 18, 100703. https://doi.org/10.1016/j.onehlt.2024.100703.

Abstract

Non-typhoidal *Salmonella* infection (NTS) is an important foodborne zoonosis with underappreciated health and economic burdens, and low case fatality. It has global prevalence, with more burdens in under-resourced countries with poor health infrastructures. Using a cohort study, we determined the cost-effectiveness of NTS in humans in Nigeria for the year 2020. Using a customized Excel-based cost-effectiveness analysis tool, structured (One Health) and unstructured (episodic intervention against NTS) in Nigeria were evaluated. Input data on the disease burdens, costs surveillance, response and control of NTS were obtained from validated sources and the public health system. The non-complicated and complicated cases were 309,444 (95%) and 16,287 (5%) respectively, and the overall programme cost was US$ 31,375,434.38. The current non-systematic episodic intervention costed US$ 14,913,480.36, indicating an additional US$ 16,461,954 to introduce the proposed intervention. The intervention would avert 4,036.98 NTS DALYs in a single year. The non-complicated NTS case was US$ 60/person with significant rise in complicated cases. The cumulative costs of NTS with and without complications far outweighed the program cost for One Health intervention with an incremental cost-effectiveness ratio (ICER) of -US$ 221.30). Utilising structured One Health intervention is cost-effective against NTS in Nigeria, it carries additional mitigative benefits for other diseases and is less costly and more effective, indicative of a superior health system approach. Identified limitations must be improved to optimize benefits associated and facilitate policy discussions and resource allocation.

Key words: Non-typhoidal *Salmonella*; cost-effectiveness analysis; human; disease outbreak; One Health; Nigeria.

4.1 Introduction

Non-typhoidal Salmonellosis (NTS) is an important foodborne zoonosis globally with significant but underappreciated health and economic burdens. In low-and-middle income countries (LMICs), especially in under-resourced, unplanned and underserved areas, humans and animal live in close proximity and often share the environmental resources, hence, NTS infection and transmission may be acquired through the environment [1]. A critical evaluation and profiling of the food systems in Nigeria, and in particular, the animal sourced food (poultry), revealed an additional risk of NTS in Nigeria [2,3]. Specifically, the country's poultry meat production is approximately 0.3 million tons per annum, but poultry meat demand is in excess of 1.5 million tons [3,4]. In addition, the country imposed an import ban on live poultry (except for day-old chicks) and frozen poultry products since 2003 [5,6]. To meet the shortfalls of approximately 1.2 million tons annually, poultry meat and poultry products are being smuggled into Nigeria almost on daily basis, especially from the neighbouring Benin Republic [4,6]. These unscrupulously smuggled poultry and its products are non-assessed, unregulated and non-standardized and end up in the human food chain in Nigeria [5,7], with high likelihood of risk of salmonellosis.

In addition, workers in the poultry value chain and consumers of poultry and products that evade pre-slaughter and post-slaughter inspection and hygienic processing procedures, are considered as high-risk groups [1,8]. In Nigeria, the NTS is prevalent, and is an often-underdiagnosed persistent disease in both humans (≤ 16.3%) and animals (≤ 48.3%), particularly in poultry [7,9,10]. Human and poultry cases of NTS are complicated by the phenomenon of antimicrobial resistance, which is linked to underdiagnoses, ease of access to antimicrobials, antibiotic misuse, abuse and overuse in order to treat infections [7,11,12].

The disease spectrum and economic burden of non-typhoidal *Salmonella* infections is broad and often underestimated [8,13,14]. In a recent estimate of social and economic burdens of NTS in Nigeria, approximately 325,731 cases with 1,043 deaths and 324,689 survivors, as well as an estimated DALYs of 13,391 were directly associated with NTS at a human cost of approximately US$ 473,982,068.00, apart from similar livestock related costs [8]. Worse still, the World Health Organization in its Global Health Estimates listed diarrhoeal disease in the top four causes of disability-adjusted life year (DALY) in Nigeria for the year 2019, and the country ranked in the top slot globally for DALYs, years of life lost from mortality (YLL) and years of healthy life lost due to disability (YLD) for diarrhoeal diseases [15]. In another modeled economic evaluation, which considered full, partial and no deployment in cases of mild and severe (complicated) invasive NTS, decision to use point of care diagnostic- tests fast-track identification and differentiation between the resistant and non-resistant strains, and shorted time to treatment and patient outcomes [1]. Previous workers have also confirmed that *Salmonella*-associated gastroenteritis had a high incidence, medium to high mortality, high population burden, low individual burden but a difficult to estimate disease specific incidence in the European Union [16]. Furthermore, the age specific population burden of gastro-intestinal salmonellosis was higher in adult > 65 years, but the disease is reported more in children under 15 years and ranked as medium to high both in terms of notification rate and DALYs per 100,000 individuals in the world [16].

Furthermore, in low- and middle-income countries (LMICs), empirical data to support decision to guide evidence-based local action in public health is scarce. Hence, making cases for increased investment by governments and resource partners in the areas of intervention and surveillance systems difficult [17]. In this situation, modeling techniques are needed to bridge such statistical,

economic and data gaps. One such tool is the cost effectiveness analysis (CEA) tool [18,19]. The cost effectiveness analysis is an objective measure that compares intervention costs against common outcome(s) of interest, for instance DALYs, or number of lives saved [18,19]. It assisted in the selection of the most cost-effective intervention for this outcome while evaluating the programme costs. The CEA is particularly useful when health benefits are difficult to calculate or convert to monetary terms [18,19]. The objective of the current work is to use the customised cost-effectiveness analysis model to demonstrate the benefit of structured but systematic One Health approach to disease surveillance and control against non-typhoidal salmonellosis (NTS) versus allowing the current episodic non-systematic intervention based on the previously estimated burden of NTS in Nigeria (≤ 325,731 cases and 1,043 human deaths in a fixed year, 2020 and assuming that the utilization of One Health makes health system 50% more effective) [8]. The outcome should contribute to and supports empirical decisions on investment in national One Health approaches in tackling food-borne zoonoses like salmonellosis specifically, but also the agrifood system and other One Health challenges in Nigeria.

4.2 Materials and Methods

4.2.1. Definition of One Health Intervention against non-typhoidal salmonellosis, data collection, data management and input parameters

Based on previously validated and published data [1-3,8], we defined One Health intervention against non-typhoidal salmonellosis (NTS) as all interventions carried out by the public and animal health sectors towards mitigating the risk of NTS in humans and animals in Nigeria for the year 2020. This was inclusive of investigations, responses (epidemio-surveillance and laboratory) and

control activities aimed at NTS [8]. It was estimated that these activities were aimed at 325,731 human cases of NTS, which was estimated to occur in the year 2020 and a human mortality of 1,043 with a disability-adjusted life year (DALYs) of 37,321 [8]. In addition, a total of 43,662,085 poultry (chickens) were involved in the 2020 outbreaks from January - December 2020 with 15,841,044 deaths, 20,574,302 salvage slaughters, 5,713,152 culls and 1,533,587 chickens whose destinations were difficult to trace [8]. The total cost of these outbreaks in humans and poultry was a cumulative of US$ 930,887,379. Input parameters were collected from various sources including peer-reviewed literature, experts' opinions and field surveys. These were summarized in Table 4.1. Additional parameterisation and assumptions were detailed in Supplementary Table 4.1 and Supplementary material 4.2.

4.2.2 Study design

A decision tree analysis model was developed in Microsoft Excel v2016 (Microsoft Corporation, Redmond, USA) to evaluate the cost-effectiveness of investments in structured multisectoral One Health interventions against NTS in humans in Nigeria from a health systems perspective (Supplementary material 4.2). The model followed a cohort of 325,731 individual cases of NTS from a Nigerian human population of 208,327,405 for the year 2020. These values were representative of all individuals infected with non-typhoidal *Salmonella* organisms in the year 2020, with hospitalization or no hospitalization including 16,287 (5%) that proceeded to severe/complicated illnesses and 1,043 (0.32%) whose death were associated with NTS in the year. We estimated intervention and treatment pathways, costs and health gains. Typical symptoms of NTS are self-limiting acute gastroenteritis with the sudden onset (6 – 72 hours) of

headache, fever, nausea, vomiting, abdominal cramps, dehydration and infectious diarrhoea, usually for up to 5 – 7 days [20,21]. The 5% of individuals who have severe/complicated illnesses are expected to develop symptoms associated with bacteremia or focal invasive infection (e.g., osteomyelitis, meningitis, endovascular infection, septic arthritis) [22,23]. The inclusive criteria for the economic data used in this analysis included: 1) Relevance to research objectives, 2) Accuracy and reliability of the data or associated verification system with the national or subnational health system, 3) Completeness and consistency of the data, 4) Timeliness of the data, 5) Accessibility and availability, where possible, data were accessed directly from the health authorities, 6) Granularity and details – we utilized published peer-reviewed documents and grey literature to verify our data, and 7) Consistency with theoretical frameworks fitting into our current economic analysis.

We excluded poor quality data and those with questionable integrity, extremely large or incomparably small data (Outliers and anomalies), redundant dataset, those that did not contribute directly to the objectives of the study, those subject to bias, and those that were deemed not representative or cannot be cross verified.

4.2.3 Model structure

Two different strategies were compared including the systematic and intentional One Health approach to disease control measures against NTS (Strategy 1) and the current episodic non-systematic interventions in Nigeria (Strategy 2) (Figure 4.1; Supplementary material 4.2). Strategy 1 is defined as an enhanced investment in the investigation, management and control of NTS with the aim to make it intentional and effective, empirical administration of antimicrobials and laboratory activities (Supplementary Figure 4.3). Strategy 2 is defined as the current level of

episodic investment in NTS investigation, management and control. Both national and subnational coordination was considered with the Nigeria Center for Disease Prevention and Control (NCDC) leading the surveillance, the National Primary Health Care Development Agency (NPHCDA), and the primary, secondary, and tertiary level healthcare facilities among others contributing to the surveillance system for humans and the Federal and States' Ministries of Health (F/SMoH) coordinating the related matters. Human-level data were also cross-validated, where necessary with the Surveillance Outbreak Response Management and Analysis System (SORMAS), a tool being used by the Surveillance unit of the FMOH (Supplementary Figure 4.4) [24].

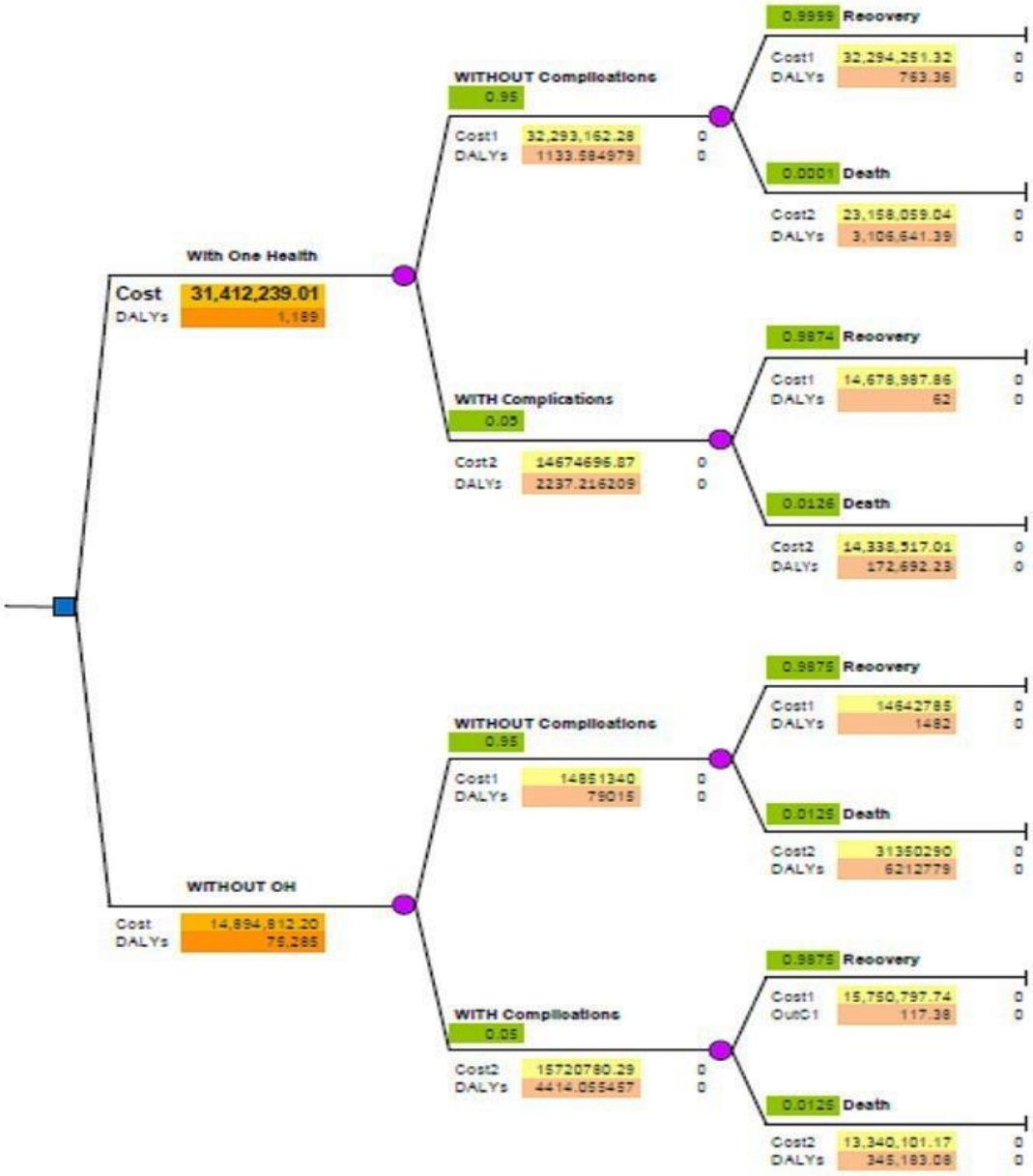

Figure 4.1. Framework of schematic decision tree to assess cost-effectiveness of One Health approach versus episodic non-systematic interventions against non-typhoidal salmonellosis in Nigeria, 2020

4.2.4 Measurement of effectiveness and cost-effectiveness

The model's primary outcome measure is the cost per disability adjusted life years (DALYs) averted using One Health intervention in structured One Health interventions against NTS in humans in Nigeria from a health systems perspective. DALYs were calculated as the sum of years of life lost (YLL) and years of life with disability (YLD). We used standard methods to compute DALYs [15]. DALYs for a disease or health condition are calculated as the sum of Years lost due to premature death (YLL) in the population and Years lived with disability (YLD) for incident cases of the health condition. Years lost due to premature death (YLL) is calculated from the number of deaths at each age multiplied by a global standard life expectancy for the age at which death occurs (Table 4.1). To estimate YLD for a particular cause for a particular time period, the number of incident cases in that period is multiplied by the average duration of the disease and a weight factor that reflects the severity of the disease on a scale from 0 (perfect health) to 1 (dead). The DALYs were calculated using a discount rate of 3%, age weighting, Nigeria's life expectancy of 53 years [25], and assumed an average duration of illness of 5 days. The applied disability weight for mild, moderate and severe diarrhoea were: Mild 0·074 (0·049–0·104); Moderate 0·188 (0·125–0·264) and Severe 0·247 (0·164–0·348), as obtained from the Global Disease Burden Study 2013 [26].

Table 4.1. Input Parameters for the Cost-Effectiveness Analysis for Non-typhoidal salmonellosis in Nigeria, 2020.

Variable	Value (CI95%)	Notes and Source*
Epidemiological, surveillance and laboratory test variables		
Prevalence of NTS in humans	0.1563%	Calculations, See Supplementary Table 4.1
Prevalence of NTS in humans (with 50% case reduction with One Health inputs)	0.07815%	
Accuracy of test kit (rapid stool antigen test)	82.92% (74.4, 89.2)	
Accuracy of test kit (Widal's antigen test)	43.00% (33.7, 52.8)	
Mortality rate of NTS (among human cases only)	0.320202867%	
Cost of laboratory testing	US$9.73	
Proportion of NTS cases hospitalized	5% (1.9, 11.5)	
Proportion of NTS death hospitalized	60% (50.2, 69.1)	

Duration of mild illness (NTS)	5 days	
Duration of severe (complicated) illness (NTS)	15 days	
Proportion of cases that proceed to severe illnesses	0.00781777	
Costs and budgeting		
Annual national budget for health, 2019	US$980,126,753.51	See Supplementary Table 4.1
Total programme cost for diarrhoeal diseases (1.3% of annual budget)	US$12,741,647.80	
Mean Health Expenditure (National)	0.516241	
Mean Health Expenditure (Sub-national)	0.483759	
Multisectoral Coordination Mechanisms (MCM) at the National Level		
Annual programme cost	US$6,577,238.59	See Supplementary Table 4.1. The costs for national and subnational were based on partial attribution of diarrhoeal diseases and annual health budget (see the footnotes).
Personnel salaries	US$5,492,678.96	
Overhead (training, administrative, secretarial and communication)	US$77,146.29	
Laboratory supplies, consumables and medications	US$1,007,413.34	
Subnational (State and Local Government) One Health Units and clinics		
Annual programme cost	US$6,164,409.20	See Supplementary Table 4.1.
Personnel salaries	US$5,147,923.44	
Overhead	US$72,304.10	
Laboratory supplies, consumables and medications	US$944,181.67	
Treatment cost/patient	US$60	See Supplementary Table 4.1.
Vaccination cost	0	Humans are not vaccinated against NTS in Nigeria
NTS cost to death	US$50,000	See Supplementary Table 4.1.
Socio-demographic data		
GDP per capital, Nigeria, 2020	US$2,074.61	See Supplementary Table 4.1.
Human population, 2020	208,327,405	
Life expectancy, 2020	53 years	
Birth rate in Nigeria, 2020	37/1000	
Death rate in Nigeria, 2020	13/1000	
Minimum wage in Nigeria, 2020	US$78.89	
Annual wage increment	12%	
Epidemiological models		
NTS DALYs	37,321	See Supplementary Table 4.1.
YLD	632	
DALY weight	0.21	
YLL	36,690	
Mean infection age	19 years	
Number of survivors	324,688	
Associated mortality (humans)	1,043	
Number of NTS cases (humans)	325,731	
Value of life lost	446,749.49	
Mean number of cases/ day (human)	892.41 cases/day	
Mean number of deaths/ day (human)	2.858 deaths/day	
Human deaths avoided/day due to One Health intervention	1.429 deaths/day	

**Details of references and sources of the values are available in Supplementary Table 4.1. Note that overhead costs are inclusive of training, indirect administrative costs and communication costs and miscellaneous costs. Attributable budgets of 51.62% and 48.38% for the national and subnational systems is based on the details from the Federal Ministry of Health (See Supplementary Table 4.1). Only 1.3% of the annual budget is spent on diarrhoeal diseases.*

4.2.5 Calculation of Disability Adjusted Life Years (DALYs) and Incremental Cost-Effectiveness Ratio

Using the formula:

DALY = Years of life lost to premature death (YLLs) + Years lived with disability (YLD)

Where, for a single individual: YLL = life expectancy – age at death, and in a population: YLL_x = number of deaths at age_x, X standard years of life lost was put at age 20, and YLD = Incidence of cases x average duration x disability weight.

The number of deaths and incident cases were obtained from the line previous findings [8], and population estimates were obtained from the *United Nations, Department of Economic and Social Affairs, Population Division* estimates [27]. The average duration of illness for NTS is 5 (mild) – 15 (severe/complicated) days (Table 4.1), hence, these values were annualized by dividing the values by 365 to get them on a year scale. These calculations were made with reference to the Microsoft Excel template developed by World Health Organization was used for computation of YLL, YLD and DALYs respectively.[15]

The incremental cost-effectiveness ratio (ICER) was the measure of cost-effectiveness calculated as the net change in total costs and DALYs averted between providing One Health interventions compared with maintaining the current intervention and management system against NTS. The ICER was calculated as:

$ICER = (CNTS_{OH} – CNTS_{nOH}) / (DALYs_{OH} – DALYs_{nOH})$,

where the $CNTS_{OH}$ is the total cost of One Health interventions against NTS for mild and severe (complicated) cases and $CNTS_{nOH}$ is the total of cost of non-One Health interventions against NTS for mild and severe (complicated) cases. The ICER was compared with the opportunity cost based

on the Nigeria cost-effectiveness threshold (US$1,037.31) (50% of the per capita GDP (US$2,074.61)) for the year 2020 [28].

Table 4.2. Outputs from the decision tree pathways, with termination in recovery or death

Cost variables*	**US$**
Programme costs with OH	31,375,434.38
Programme costs WITHOUT OH	14,913,480.36
Additional costs spent on implementing OH programme	16,461,954.02
DALYs of NTS with One Health	1,229.70
DALYs of NTS WITHOUT One Health	75,618.47
Incremental DALYs	74,388.77
ICER ($/DALY)	-221.30
Treatment costs of NTS per patient (hospitalization)	60
Vaccination cost against NTS	-
Cumulative Cost of NTS to deaths	50,000
Cost of NTS with complications that progress to Recovery (if OH is implemented)	14,678,987.86
Cost of NTS with complications that progress to Recovery (if OH is not implemented)	15,750,797.74
Cost of NTS WITHOUT complications that progress to Recovery (if OH is implemented)	32,294,251.32
Cost of NTS WITHOUT complications that progress to Recovery (if OH is not implemented)	14,642,785.00
Cost of NTS with complications that progress to Death (if OH is implemented)	14,338,517.01
Cost of NTS with complications that progress to Deaths (if OH is not implemented)	13,340,101.17
Cost of NTS WITHOUT complications that progress to Deaths (if OH is implemented)	23,158,059.04
Cost of NTS WITHOUT complications that progress to Deaths (if OH is not implemented)	31,350,290.00
DALYs	
DALYs of NTS with complications that progress to Recovery (if OH is implemented)	46
DALYs of NTS with complications that progress to Recovery (if OH is not implemented)	91
DALYs of NTS WITHOUT complications that progress to Recovery (if OH is implemented)	890
DALYs of NTS WITHOUT complications that progress to Recovery (if OH is not implemented)	1,780
DALYs of NTS with complications that progress to Death (if OH is implemented)	283,429
DALYs of NTS with complications that progress to Deaths (if OH is not implemented)	566,857
DALYs of NTS WITHOUT complications that progress to Deaths (if OH is implemented)	5,108,829
DALYs of NTS WITHOUT complications that progress to Deaths (if OH is not implemented)	10,217,658
Incremental cost-effectiveness ratio (ICER)	-US$,221.30

**This table is supported by details from Figure 4.1 and Supplementary material 4.2. The baseline DALYs (WITHOUT One Health) was 75,512. With One Health, an additional 74,389 DALYs was averted.* NTS = non-typhoidal salmonellosis; DALYs = Disability-adjusted life years; OH = One Health; ICER = incremental cost-effectiveness ratio.

4.2.6 Sensitivity analysis and dealing with uncertainty.

Sensitivity analysis was assessed using a one-way sensitivity analysis (deterministic) and a probabilistic sensitivity analysis (PSA). The one-way sensitivity analysis was conducted across selected parameters to assess the effect of selected changes on the ICER (Table 4.3; supplementary material 4.2). Using the increase or decrease of parameters without confidence bounds, the output values in the determination analysis model were compared with deterministic and probabilistic values at the lower and upper range of each output (Table 4.3). However, where possible, ranges for sensitivity analysis were based on upper and lower confidence intervals or IQR found within the systematic literature review (supplementary material 4.2 – Sensitivity analysis). The PSA (Monte Carlo simulation) was performed to explore the effect of uncertainty across our model parameters using 1,000 iterations. The key parameters included the per day costs for severe (complicated) and critical patients, DALYs, length of stay, and the transition probabilities with defined distributions prevalence of NTS without One Health, prevalence of NTS with One Health, screening accuracy of NTS test Kit (Pen side test) [29], mortality rate associated with NTS in Nigeria, probability of NTS with and without complications that progress to Recovery (if OH is implemented or not implemented), probability of NTS with and without complications that progress to Death (if OH is implemented or not implemented) (online supplementary material 4.2). The analysis randomly sampled each parameter in our model simultaneously from their probability distribution and repeated this 1000 times to generate CIs around our estimates of cost per DALY averted. The confidence intervals (CIs) or variation of parameters and the effect

on the cost-effectiveness were also evaluated. Finally, the PSA was run, and estimates were presented in Table 4.3 with details in supplementary material 4.2.

4.3 Results

The results of the costs, DALYs and the ICER associated with the two options are shown in Table 4.2. The overall programme cost using a structured and comprehensive One Health intervention was US$ 31,375,434.38, whereas the continuation of the current non-systematic episodic intervention was US$ 14,913,480.36, an indication that an additional US$ 16,461,954.02 would be needed to implement the structured systematic One Health surveillance system (including diagnosis) in combating the burden of NTS in Nigeria (Table 4.2). The One Health intervention may avert 74,221 NTS DALYs in 2020. Approximately US$ 60 is needed to treat a case of non-complicated NTS, but this cost may rise significantly with complications. Ordinarily, the Cost of NTS WITHOUT complications that progress to recovery (if OH is implemented) (US$ 32,294,251) outweighed the program cost for One Health for the year 2020 (US$ 31,375,434.38) (Table 4.2). In addition, the cost of NTS WITHOUT complications that progress to Deaths (if OH is implemented) amounted to US$ 23,158,059 in the single year. Other costs are detailed in Table 4.2.

The Incremental cost-effectiveness ratio (ICER) (-US$ 221.30) was lower than the cost-effectiveness threshold for Nigeria (US$ 1,037.31), confirming that it is cost-effective (Table 4.2; Supplementary material 4.2). Basically, non-complicated and complicated cases were 309,444 (95%) and 16,287 (5%) respectively, making a cumulative total of 325,731 human cases. Of the total recoveries, 309,290 were from the non-complicated cases and only 15,397 presented with

complications. An estimated 155 non-complicated cases proceeded to death whereas 889 cases proceeded to death from the complicated cases (Supplementary material 4.2).

4.3.1 Cost-Effectiveness of Baseline and Additional Costs Spent

Whereas the baseline (without OH approach) cost was US$ 14,913,480.36 came with the associated disability adjusted life years (DALYs) (≈ 75,618), an additional spending on One Health to the tune of US$ 16,461,954.02 would avert ≈ 74,389 DALYs. Cumulatively, the years of life lost (YLL) with and without One Health was 17,209.50 and 34,419.00 respectively (Supplementary material 4.2).

4.3.2 Sensitivity analysis

The probability evaluations in sensitivity analysis produced variations in costs and DALY outcomes as outlined in Table 4.3. Based on the cost-effectiveness plane, incremental costs ranging from over US$10 million to US$30 million would produce incremental DALYs of approximately 40,000 to over 90,000 by implementing One Health intervention against non-typhoidal *Salmonella* in Nigeria (Figure 4.2a). As willingness to pay thresholds for One Health intervention against non-typhoidal salmonellosis increases, the probability for cost-effectiveness increases correspondingly, peaking at a willingness to pay threshold of US$1,600 at a probability cost-effectiveness of 0.96 (Figure 4.2b).

In summary, with the average probabilistic runs and at 95% confidence limit, the total DALYs with One Health was 1,230 and without One Health was 75,618 at total costs of US$ 31,375,434 and US$ 14,913,480 respectively (Supplementary material 4.2). Comparing the baseline results with the average probabilistic runs, whereas the original incremental DALYs shifted from 74,389 to

65,311 (95% confidence interval: CI95%: 64,538 – 66,085), the incremental costs shifted from US$ 16,461,954 to US$ 20,007,081 (CI95%: 19,698,379 – 20,315,783) and ICER shifting from 221 to 322 (CI95%: 195.90 – 448.50) (Supplementary material 4.2).

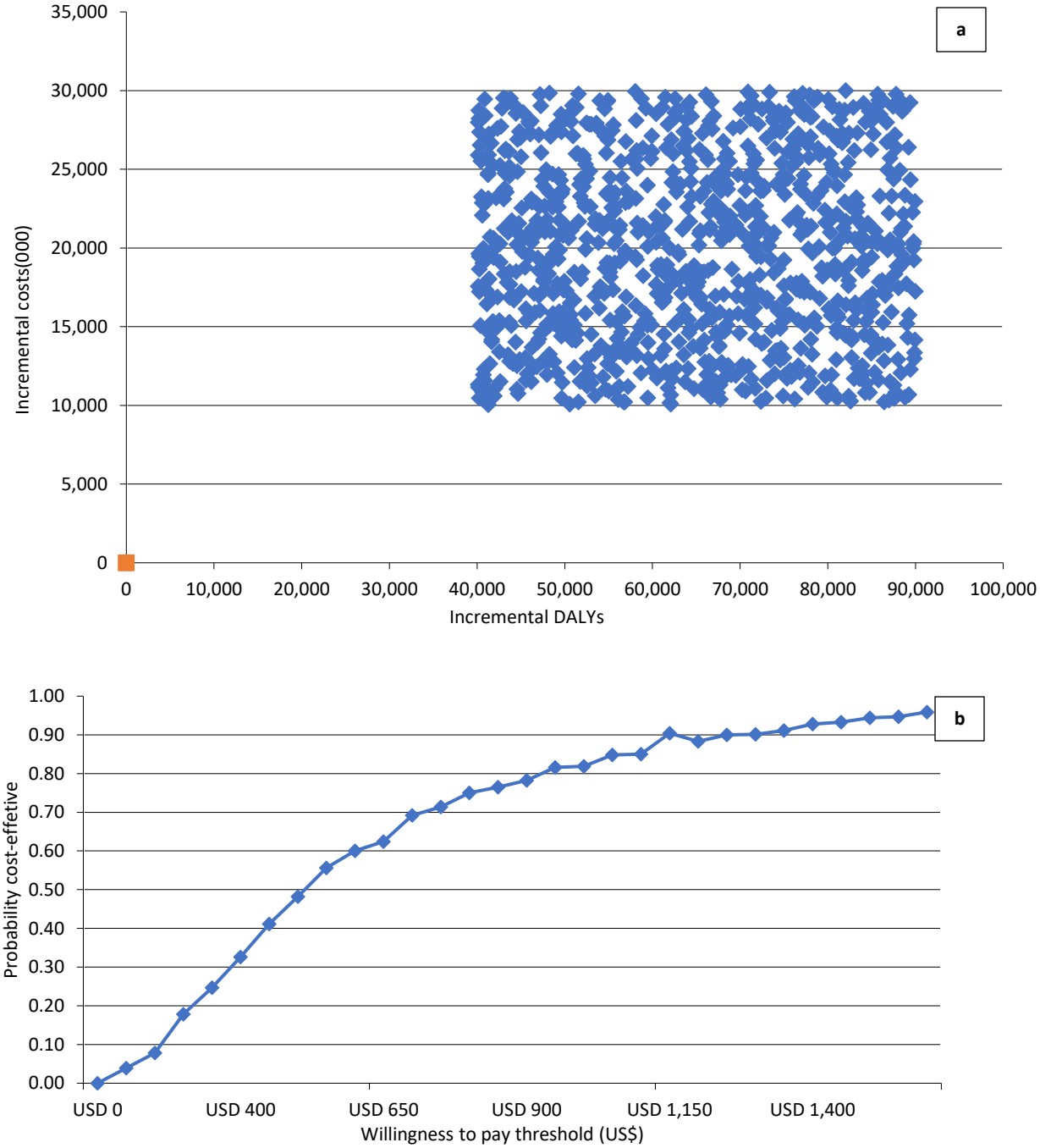

Figure 4.2a. The cost-effectiveness plane based on sensitivity analysis; 4.2b. Willingness to pay threshold for cost-effectiveness analysis for non-typhoidal salmonellosis in Nigeria, 2020.

Table 4.3. One way sensitivity analysis for cost-effectiveness analysis for non-typhoidal salmonellosis in Nigeria, 2020.

Variables	Value in the model	Deterministic value	Probabilistic Value	Lower	Upper	SE
Probabilities						
Prevalence of NTS without One Health	0.0016	0.0016	0.0015	0.0015	0.0016	0.0000
Prevalence of NTS with One Health	0.0008	0.0008	0.0007	0.0007	0.0008	0.0000
Screening accuracy of NTS test Kit (Pen side test)	0.8292	0.8292	0.8565	0.7877	0.8707	0.0007
Mortality rate associated with NTS in Nigeria	0.0370	0.0370	0.0353	0.0352	0.0389	0.0000
Probability of NTS with complications that progress to Recovery (if OH is implemented)	0.9454	0.9454	0.9875	0.8981	0.9926	0.0007
Probability of NTS with complications that progress to Recovery (if OH is not implemented)	0.9454	0.9454	0.9873	0.8981	0.9926	0.0007
Probability of NTS WITHOUT complications that progress to Recovery (if OH is implemented)	0.9995	0.9995	0.9999	0.9495	0.9999	0.0004
Probability of NTS WITHOUT complications that progress to Recovery (if OH is not implemented)	0.9454	0.9454	0.9875	0.8981	0.9926	0.0007
Probability of NTS with complications that progress to Death (if OH is implemented)	0.0546	0.0546	0.0522	0.0519	0.0574	0.0000
Probability of NTS with complications that progress to Deaths (if OH is not implemented)	0.0005	0.0005	0.0005	0.0005	0.0005	0.0000
Probability of NTS WITHOUT complications that progress to Deaths (if OH is implemented)	0.0005	0.0005	0.0005	0.0005	0.0005	0.0000
Probability of NTS WITHOUT complications that progress to Deaths (if OH is not implemented)	0.0005	0.0005	0.0005	0.0005	0.0005	0.0000
Costs (US$)	**Value in the model (US$)**	**Deterministic value (US$)**	**Probabilistic value (US$)**	**Lower (US$)**	**Upper (US$)**	
Cost of NTS with complications that progress to Recovery (if OH is implemented)	14,716,351.41	14,716,351.412	14,727,785.36	11,773,081.13	17,659,621.69	
Cost of NTS with complications that progress to Recovery (if OH is not implemented)	15,666,871.41	15,666,871.412	15,651,174.69	12,533,497.13	18,800,245.69	
Cost of NTS WITHOUT complications that progress to Recovery (if OH is implemented)	32,327,851.41	32,327,851.412	32,252,834.64	25,862,281.13	38,793,421.69	
Cost of NTS WITHOUT complications that progress to Recovery (if OH is not implemented)	14,642,687.80	14,642,687.796	14,668,199.93	11,714,150.24	17,571,225.35	
Cost of NTS with complications that progress to Death (if OH is implemented)	14,331,111.41	14,331,111.412	14,294,956.26	11,464,889.13	17,197,333.69	
Cost of NTS with complications that progress to Deaths (if OH is not implemented)	13,306,927.80	13,306,927.796	13,291,166.50	10,645,542.24	15,968,313.35	
Cost of NTS WITHOUT complications that progress to Deaths (if OH is implemented)	23,103,801.41	23,103,801.412	23,134,527.01	18,483,041.13	27,724,561.69	
Cost of NTS WITHOUT complications that progress to Deaths (if OH is not implemented)	31,367,587.80	31,367,587.796	31,244,429.14	25,094,070.24	37,641,105.35	
Outcomes	**Value in the model**	**Deterministic value**	**Probabilistic Value**	**Lower**	**Upper**	
DALYs of NTS with complications that progress to Recovery (if OH is implemented)	45.57	45.57	62	28	63	
DALYs of NTS with complications that progress to Recovery (if OH is not implemented)	91.15	91.15	118	55	127	
DALYs of NTS WITHOUT complications that progress to Recovery (if OH is implemented)	890	889.96	863	541	1,239	
DALYs of NTS WITHOUT complications that progress to Recovery (if OH is not implemented)	1,779.92	1,779.92	1,409	1,082	2,478	
DALYs of NTS with complications that progress to Death (if OH is implemented)	283,428.71	283,428.71	172,766	172,325	394,533	
DALYs of NTS with complications that progress to Deaths (if OH is not implemented)	566,857.41	566,857.41	345,189	344,649	789,066	
DALYs of NTS WITHOUT complications that progress to Deaths (if OH is implemented)	5,108,828.81	5,108,828.81	3,106,691	3,106,168	7,111,490	
DALYs of NTS WITHOUT complications that progress to Deaths (if OH is not implemented)	10,217,657.63	10,217,657.63	6,212,817	6,212,336	14,222,979	

NTS = non-typhoidal salmonellosis; DALYs = Disability-adjusted life years; OH = One Health; SE = Standard Error.

4.4 Discussion

Currently, it costs an estimated US$ 60 to treat non-complicated NTS in this study but estimate for the complicated cases was difficult to obtain due to different treatment pathways and health outcomes [30]. This treatment cost may differ based on political geography, health systems' pricing and the country's GDP; for instance, such cost range between US$ 8.96 and 39.11 in Ethiopia [14], and in mild to complicated cases of NTS, it may vary between US$ 399 and US$ 760 ($CI_{95\%}$: 201 – 1285) (Hong Kong) [31], or more than US$ 3,375 (Spain), and up to US$ 7,400 (USA) [13]. We established that the cost of NTS WITHOUT complications that progress to recovery (if OH is implemented) outweighed the program cost for One Health in the single year, 2020. If the additional costs associated with NTS with and without complications, and those that proceeded to deaths or recovery (if OH is implemented) are added, the investment cost is worthwhile. This should provide justification for political economy and investment in structured One Health in NTS surveillance and control with unintended mitigative benefits for other diseases.

In this analysis, the annual allocation of the initial set-up cost was included in the One Health interventions, versus the non-One Health intervention. Debate on whether it should be annualized, and the subjectivity in determining the estimated total of start-up cost, and whether such costs and capital costs should be expensed as incurred cost remained [32]. This debate should make CEA complex, but we considered it as part of capital costs, and annualized it in the analyses. We generated a negative ICER of - US$ 221.30. Such negative ICER can mean two things, either that the new intervention is more costly and less effective, in which case the comparator is superior, and the new intervention should be rejected, or that the new intervention is less costly and more effective, in which case the new intervention is superior for adoption [33,34]. In our

case, the second position subsisted because the structured One Health intervention averted the DALYs worth 74,388.77 at a top-up cost of ≈ US$ 16,461,954. Our DALYs for One Health intervention (17,687) is much lower than WITHOUT (35,356), which is a positive outcome, hence the current analysis is suggested for implementation (Supplementary material 2).

This study is subject to a number of limitations. First, it has been reported previously that some diarrhoeal diseases, for instance salmonellosis typically have a comparative high notification rate in children - due to a testing bias including more regular tests, relative reduced immunity in the young and higher chances of exposure to infectious agents, and pattern of hospital-seeking behaviour [16,35,36], however, we did not conduct an age-segregated analysis in this study. Perhaps, we have underestimate or overestimate the age specific or overall burden of NTS. Secondly, we estimated the CEA for a year and did not apply the multi-year time-discounted factor used in economic studies, however, with the understanding that program implementation in health system with future implications typically have multi-year benefits, thus additional maintenance costs and benefits may attend this analysis. Thirdly, the difference between national assembly- approved (allocated) and released (performance) budget may have significant impact on the outcome of the analysis in varied widely. In addition, we utilized the whole of capital and set up cost for the One Health interventions in the analysis, whereas we did not utilize the same for non-One Health interventions (due to non-committal spending associated with episodic non-systematic interventions), with implication on potential over-costing for inputs in One Health. Furthermore, Widal's test (for agglutinating antibodies detection against the O and H antigens) is widely used in Nigeria, similar to in other LMICs, for the diagnosis of gastrointestinal form of salmonellosis and typhoid fever, but it is not sufficiently sensitive, specific or reliable enough to

be an optimal diagnostic assay for typhoid fever and it does not aid in the diagnosis of paratyphoid (NTS) fever, as the antibodies are not cross-reactive against S. Paratyphi A, B and C antigens. Hence, a false-negative Widal's test may result from the assay being performed early in the course of illness and a false-positive Widal's test is more likely in an area of high endemicity where antibodies may represent past infection [9,30]. This observation makes NTS specific attribution of burden of illness quite difficult and may lead to test-sensitive underestimation or overestimation of cases. Finally, the health authorities in Nigeria and globally typically cluster NTS together with other gastrointestinal health challenges as part of the diarrhoeal diseases programmes; and other Enterobacteriaceae, as well as other diseases such as malaria can create further complexities with antigenic determinants that cross-react with S. Typhi; hence, where a baseline was not established previously, as is the case in most LMICs due to additional health costs, interpreting test results may be complicated. It is encouraged that more sensitive methods like ELISA should be employed or used in combination with Widal's test [30].

4.5 Conclusion

This evaluation has produced empirical evidence suggesting that structured surveillance and control intervention against NTS in humans is cost-effective despite the low prioritization of the disease in Nigeria and similar LMICs. One Health intervention attracts enormous costs initially. However, 'structured?' One Health interventions are effective in preventing costs associated with DALYs and costs associated with illnesses and deaths. The ICER was US$ 221.30./ Based on outcomes One Health intervention for NTS is less costly and more effective in the long run. It has the potential to prevent additional illnesses and deaths. The output should support discussions

with policy makers, funders and resource allocators in robust funding of surveillance and control efforts in health. The outputs also produce data for further discussion on the burden of NTS.

Disclaimer: The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the institutions mentioned in the work.

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CHAPTER FIVE

5.0 BENEFIT-COST ANALYSIS OF INTERVENTION AGAINST NON-TYPHOIDAL *SALMONELLA* IN NIGERIA

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Abstract

Non-typhoidal salmonellosis (NTS) represents a significant disease and economic burden in Nigeria. To determine whether investment in control is economically justifiable, we used the pre-structured Outbreak Costing Tool (OCT) to estimate the robust funding of public and animal health systems for epidemio-surveillance and controlling multisectoral outbreaks of NTS in Nigeria and the benefit-cost of intervention. Data were collected and gaps were filled through key informants' consultations. The multisectoral NTS burden for the year 2020 in Nigeria costed US$ 930,887,379.00. Approximately 4,835 technical officers and 3,700 non-technical staff (n = 8,535) investing over 2.2 million work hours were needed. The NTS control programme's investment cost was US$ 53,854,660.87. The non-labour-related cost was 89.21% of the total intervention costs. The overall intervention's investment was 374.15% of the estimated national and subnational systems' annual budget for diarrhoeal diseases, and the outbreak response period incurred the highest costs (53%) of the total intervention. In conclusion, intervention against NTS was beneficial (benefit–cost ratio: 17.29), hence the need to multisectoral surveillance and response against NTS in Nigeria. However, complex sectoral silos must give way to coordinated collaborations; and delays associated with over-centralization of health interventions must be removed through decentralized framework focused on sub-national empowered for rapid investigation, response, control, data collection, and analyses. It should assist anticipatory planning, and outbreak investigation and reduce critical response time to intervention. Anticipatory planning tools, when applied preemptively, can benefit budgeting, identify gaps, and assist in the delivery of cost-saving and effective measures against infectious disease.

Key words

Non-typhoidal *Salmonella*; benefit - cost analysis; infectious disease outbreak; One Health; Nigeria.

5.1 Introduction

Non-typhoidal *Salmonella* (NTS) as a bacterial zoonosis plays a significant role in foodborne human salmonellosis worldwide [1], and can be transmitted to humans particularly through the consumption of foods of animal origin, including eggs and poultry meat, as well as through direct contact with animals or their environments, especially for people working in the agriculture industry [2,3]. Specifically, humans are infected with NTS through contamination from poultry products (egg fragments, hatching eggs, chick boxes, fluff and faeces), partially cooked meat and raw eggs [4,5]. More than 2600 serovars of *Salmonella enterica* have been identified, of which many can cause human infections. However, non-typhoidal serovars, especially *Enteritidis* and *Typhimurium*, are the most commonly isolated serotypes in human infections [6]. Salmonellosis in humans is commonly characterised by diarrhoea, abdominal cramps, fever and vomiting [7]. Although most non-typhoidal *Salmonella* infections are associated with self-limiting gastroenteritis, they have the potential to cause fatal infections among infants, young children, older adults and immunocompromised individuals [8]. The majority of non-typhoidal *Salmonella* serovars are pathogenic as a result of their ability to invade, replicate and survive in human host cells [9].

The global estimates of the burden of NTS varied widely, including an estimate of over 27 million human cases and 200,000 deaths per annum [10,11]; approximately 79 million human cases and over 59,000 deaths annually [4]; and 93.8 million human infections and 155,000 fatalities annually [12]. Furthermore, in a ranked study in the USA, *Salmonella* spp. was the first-ranked foodborne pathogens, with the most significant cost of illness and the quality-adjusted life-year (QALY) losses [13]. In Nigeria, the poultry farm level prevalence of NTS range from 39.7 - 48.3% and the risk

factors for NTS infection of poultry farms in Nigeria have been fully explored [14-17]. Based on a recent meta-analytic study, Nigeria has a burden of prevalence (in humans) of 1.9% (2,732/143,756) (95%CI: 1.3 to 2.7) *Salmonella* bacteremia and 5.7 - 16.3% (1,967/12,081) *Salmonella*-associated gastroenteritis [18]. In addition, a total of 53 *Salmonella* serotypes have been identified in humans in the country including 39 associated with *Salmonella*-bacteremia and 31 associated with *Salmonella*-gastroenteritis [18].

In the year 2018, the U.S. Centers for Disease Control and Prevention (CDC) commissioned a study in collaboration with RTI International (formerly Research Triangle Institute). This led to the development of the Outbreak Costing Tool (OCT) that estimates intervention costs and useful for a range of disease outbreak scenarios [19,20]. Based on applied intervention and control costs, which can be implemented for humans and animal specific disease outbreaks, especially where multisectoral responses are required (e.g., zoonotic disease outbreaks), and with a good understanding of disease burden for such disease, a benefit - cost analysis (BCA) may be integrated. The BCA calculates the monetary ratio of all costs to implement a program or course of action and helps determine whether a course of action is worth investing in based on the assumed worth of the associated benefits. It differs from a tool like the cost effectiveness analysis (CEA), which assist in selecting the most cost-effective intervention for a defined health outcome, even when multiple methods of intervention are cost-beneficial [21]. BCA is particularly useful for decision-makers, health leaders, policymakers and resource allocators and for ranking proposals and budgets in the public and animal health sectors. Considering NTS as a One Health challenge in Nigeria, and with a knowledge of its economic and social costs, the application of a tool like the OCT could assist the Nigerian human, animal and environmental health ministries in

preemptive planning and budgeting for intervention against NTS. The outcomes may also be potentially adaptable to other countries with related burdens of NTS or similar profiles like Nigeria.

The primary aim of this investigation was to use the OCT to retrospectively generate a cost estimate for investigation, response and control associated with an all-year-round outbreak of NTS in the year 2020 in Nigeria, and to determine whether these interventions were justifiable and cost-beneficial in view of the economic and social burdens of NTS in Nigeria.

5.2 Materials and Methods

5.2.1. Spatio-temporal Coverage of Outbreaks of non-typhoidal Salmonellosis

Based on the previously validated and published data, we assumed a total of 325,731 human cases of NTS occurred in the year 2020 with a human mortality of 1,043 and a disability-adjusted life year (DALYs) of 37,321 [22, Figure 5.1a]. A total of 188,694 cases (57.9%) occurred among people involved in the poultry value chain while 137,037 (42.1%) occurred among the consumers of poultry and poultry products [22]. In addition, a total of 43,662,085 poultry (chickens) were involved in the 2020 outbreaks from January - December 2020 with 15,841,044 deaths, 20,574,302 salvage slaughters, 5,713,152 culls and 1,533,587 unaccounted-for chickens [22, Figure 5.1a]. The total cost of these outbreaks in humans and poultry was a cumulative of US$ 930,887,379.00 [22]. All cases in humans and poultry occurred between the time point January 1 to December 31, 2020 and all cases were distributed randomly in the country, particularly in the peri-urban and rural areas, and high poultry-dense locations within the country.

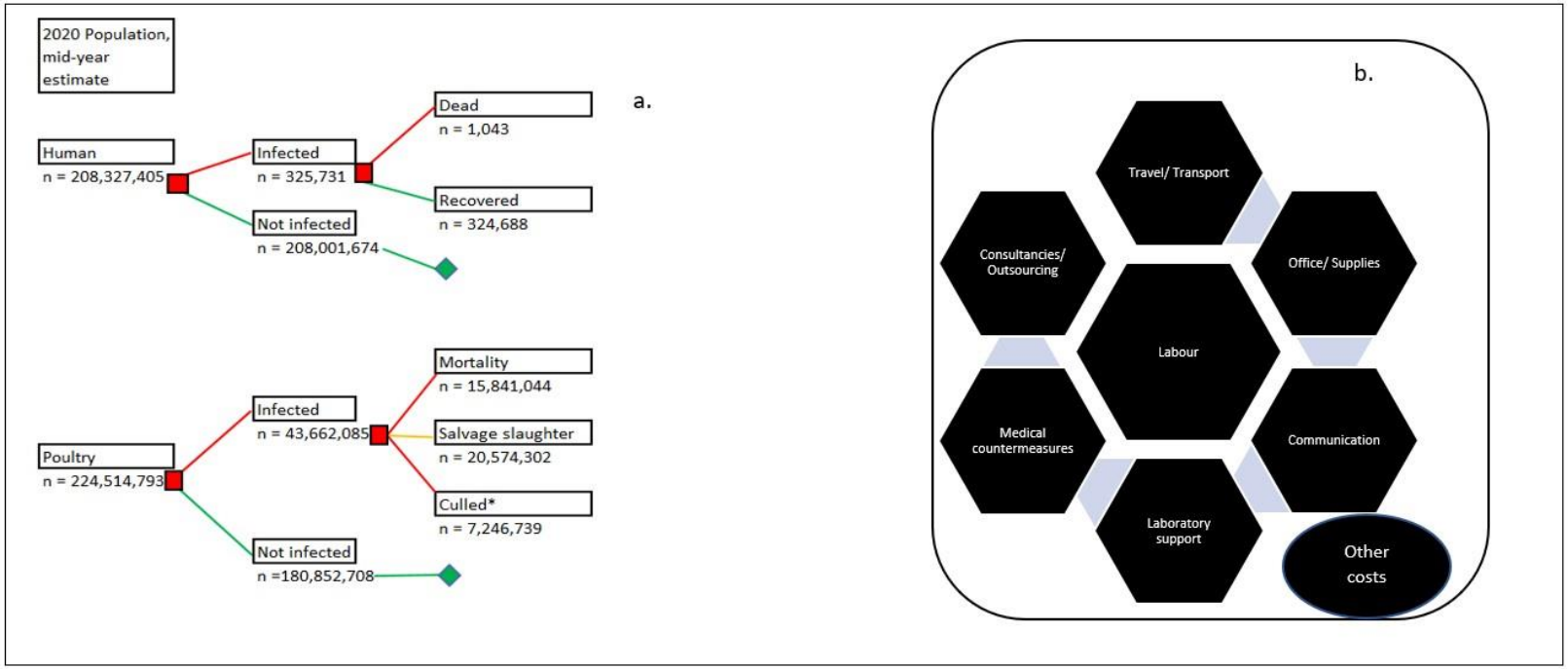


Figure 5.1. a. Modelled decision tree population dynamics for non-typhoidal Salmonella infection, Nigeria, Jan. – Dec. 2020; b. Framework for resource category in estimating the cost of surveillance and control in outbreaks of non-typhoidal Salmonella, Jan. – Dec. 2020.

*Framework for resource category was adapted from Bodenham et al., (2021) and steps in a multistate foodborne outbreak investigation from CDC (2022). These steps include: 1). Detect - detect a possible outbreak by monitoring for reported illnesses (salmonellosis) nationwide, 2). Find - define who will be included in the outbreak and look for additional sick people, 3). Generate - generate hypotheses (potential explanations) by interviewing people about what they ate before getting sick, 4). Test - test hypotheses by comparing what sick people ate to what people who are not part of the outbreak ate, 5). Solve - confirm the contaminated food using epidemiologic, laboratory, and traceback information and identify the point of contamination, 6). Control - stop the outbreak by recalling contaminated food, cleaning or closing food facilities, and providing advice to people and businesses, and 7). Decide - Decide the outbreak is over when illnesses stop and the contaminated food is no longer available. Modelled decision tree was adapted from Sanni et al., (2023). *Including 1,533,587 unaccounted infected poultry birds. These may have been consumed unscrupulously or inadvertently. The salvage slaughters also end up in the human food chain largely.*

5.2.2. Outbreak Management - Investigations, Responses and Controls

According to Ihekweazu et al. [16], salmonellosis is viewed in Nigeria as a moderate zoonosis, ranking low on severity and epidemic potentials but high to moderate on burden of diseases, ability of the health services to control, and socio-economic impacts. Furthermore, salmonellosis, combined with other diarrhoeal diseases only benefit approximately 1.3% of the total mean annual expenditure as a percentage of current health expenditure (CHE) of the Nigerian federal and states' budgets [23,24]. Hence NTS would only have partial attribution of the 1.3% funding, an indication that it does not enjoy any prioritized funding or attention as some other rapidly spreading infectious diseases. Basically, there is no budget specifically dedicated for the control of salmonellosis at the federal, state and local government levels except as part of the diarrhoeal diseases. The responsible ministries and government departments or parastatals responsible for salmonellosis management and control include the Federal and States' Ministries of Agriculture and Livestock Development or Animal Health, and the public and private veterinary clinics (for livestock), and the Federal and States' Ministries of Health (F/SMoH), Nigeria Center for Disease Prevention and Control (NCDC), the National Primary Health Care Development Agency (NPHCDA), and the primary, secondary and tertiary level healthcare facilities among others (for humans). Human-level data were also cross-validated with the Surveillance Outbreak Response Management and Analysis System (SORMAS), a tool being used by the Surveillance unit of the FMOH.

In addition, because its fatalities in human is low, the dispositions of most affected individuals with diarrhoeal diseases including NTS was to seek self-therapy at home first, including self-

administration of antibiotics, and would only seek hospitalization in case of increasing severity, which is not responsive to home treatment [25,26].

Individual human cases are treated when hospitalization is sought. In cases of aggravated or surge incidences of diarrhoeal diseases in humans at any period of the year, the government's disease surveillance and control officers at the national and sub-national level would swing into action to investigate, intervene and respond in order to implement control. Such interventions may include the following steps taken in multistate foodborne outbreak investigation [27]: 1). *Detect* - detect a possible outbreak by monitoring for reported illnesses (salmonellosis) nationwide, 2). *Find* - define who would be included in the outbreak and look for additional sick people, 3). *Generate* - generate hypotheses (potential explanations) by interviewing people about what they ate before getting sick, 4). *Test* - test hypotheses by comparing what sick people ate to what people who are not part of the outbreak ate, 5). *Solve* - confirm the contaminated food using epidemiologic, laboratory, and trace back information and identify the point of contamination, 6). *Control* - stop the outbreak by recalling contaminated food, cleaning or closing food facilities, and providing advice to people and businesses, and 7). *Decide* - decide the outbreak is over when illnesses stop and the contaminated food is no longer available. For poultry, farmers often vaccinate against fowl typhoid and fowl cholera using commercially available vaccines as preventive protocols. When there are aggravated cases of NTS in poultry, often caused by *Salmonella enterica* serovars *Enteritidis* or *Typhimurium*, farmers typically implement antimicrobial treatment protocols, and if the cases are not resolving, salvage slaughter or culling would supervene. With the above scenarios, the possibility of missing cases in humans and poultry is high.

5.2.3. Questionnaire Data Collection for Outbreak Costs

Data on costs associated with the outbreaks in humans and animals were mined from previous evaluations [22], or collected using validated tools [20, Supplementary Material 5.1]. A total of 244 field-level datasets on real and estimated costs of intervention for outbreaks of NTS were collected from the government departments and the industry identified in section 2.2 above from December 2021 to September 2023. A total of 115 datasets (47.1%) originated the public and animal health officers (medical doctors, veterinary doctors, nurses, pharmacists, consultants, project managers, epidemiologists, surveillance officers and microbiologists). One hundred and twenty (49.2%) datasets came from the laboratory officers (consultants, scientists and technicians), and nine datasets (3.7%) from administrative officers, managers and monitoring and evaluation officers. Approximately 78.7% (n = 192) of the responses were obtained through physical face-to-face questionnaire surveys and only 21.3% (n = 52) were obtained through an online survey. Officers and industry stakeholders with knowledge of costs associated with the outbreak were selected as key informants. Following informed consent, respondents completed a structured questionnaire pertaining to one or more of the seven OCT independent cost categories: labour, office materials and equipment, travel and transport, communication, laboratory support, medical countermeasures, and consultancies (Supplementary Material 5.1; Figure 5.1b). Each cost category questionnaire was designed to generate responses suitable for filling the OCT tool by cost category. When a respondent did not have enough insight or knowledge on specific aspects of a cost category, either the respondent conferred with a colleague for further information or suggested the name of a knowledgeable colleague (snowball) that could complete the remaining cost category fields, and such individual was approached for

participation [20]. Questionnaire responses were cross verified by additional government officials where possible to generate more robust cost estimates and reduce questionnaire bias [28]. This cross-verification of sub-national data was conducted by additional key-informant questionnaire administration, which occurred at national level (Supplementary Material 5.1).

5.2.4 Integration of Outbreak Costing Tool (OCT) to Determine Costs of Intervention, Scenario Analysis and Benefit - Cost Ratio

In the context of this study, 'multisectoral' is defined within the context of One Health, wherein collaborative engagement is conducted among multiple discipline and sectors with a view to ultimately integrate transdisciplinary approach in their working environment (local, regionally, and nationally), with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment. Such engagements include co-planning, co-working, co-funding and co-implementation in the field [16,20,21]. All costs for disease burdens were retrieved from a previous study [22]. Costing for the investigation, response and control against NTS in Nigeria for the year 2020 was performed using the OCT. The OCT offered a standardized, Excel-based approach to recording and summarizing outbreak costing data [20]. Multisectoral costs were integrated by direct entry of questionnaire-sourced information from multiple sectors into the OCT spreadsheet (Supplementary Material 5.2), however, we did not break down these costs per each sector based on the protocol of the OCT. All seven cost-related categories were entered comprehensively (Supplementary Material 5.2 and Supplementary Table 5.3).

In each category, the individual items, the quantity and cumulative cost per budget line, and the percentage cost for each item were entered in relation to three pre-defined categorization of timelines for outbreak investigation and management: 1) the initial response period (i.e., preparation, outbreak verification, outbreak diagnosis, case verification, case diagnosis, case definition construction, case recording, epidemiology description, hypothesis development, hypothesis evaluation and finalization, and reconciling evidence); 2) the outbreak response period (i.e., implementing infection control and prevention measures); and 3) the follow-up and reporting period (i.e., initiating or maintaining surveillance and dissemination of findings). All entries were verified independently by two of the researchers (SOA, and FOF). The results spreadsheet was shared among all the authors for internal quality control and to identify errors. Results were summarized to facilitate data interpretation, draw inferences and determine the implications of outputs. All cost estimates were calculated at the mid-year exchange rate for the year 2020 (US$1 = N380.26 (local currency) at the time of calculation) [29].

With the understanding that the political, health and financial system are dynamic, and that there are many competing interests for limited funds, we used a separate scenario analysis Excel spreadsheet (Supplementary Material 5.4), and estimated the changes in the impact of interventions and benefit-cost ratios for five scenarios targeting some of the most elaborate cost categories as follow:

Scenario 1: By increasing of labour and laboratory support costs each by 40% while decreasing travel and transport costs by 40%, the new total cost of the surveillance programme against NTS would be 100.57% of original cost, thus reducing the BCR marginally to 17.19; ***Scenario 2***: Even if labour costs are increased by 60%, laboratory support costs are increased by 40% and office

supplies costs are increased by 25%, while decreasing travel and transport costs by 40% and medical countermeasure costs by 10%, the new total cost of the surveillance programme against NTS would be 98.23% of original cost, thus increasing the BCR marginally to 17.6; ***Scenario 3***: By adding an additional 20% to the laboratory support costs of 40% to make a new cumulative of 60%, and with an additional 50% increase in the costs associated with consultancies/outsourcing, added to scenario 2, the new total cost of the surveillance programme against NTS would be 105.47% of original cost, thus reducing the BCR marginally to 16.39; ***Scenario 4***: If the travel and transport costs increase by 20%, and laboratory support costs increase by 20% while office supplies costs increase by 15% and medical countermeasure costs reduce by 15%, the new total cost of the surveillance programme against NTS would be 98.90% of original cost, thus increasing the BCR to 17.48; ***Scenario 5***: Finally, if the travel and transport costs increase by 40%, and communication costs increase by a marginal 10% while all other parameters remain the same, the new total cost of the surveillance programme against NTS would be 108.21% of original cost, thus decreasing the BCR to 15.97.

Using the total costs of the interventions, and the overall economic and social costs of the burden of NTS, we calculated the benefit-cost ratio (BCR) as follow: *Benefit – cost Ratio (BCR) of intervention against NTS = (Annual economic and social burdens of diseases ÷ Annual cost of intervention)*.

Where: Annual burden of costs of diseases = US$ 930,887,379.00 (Sanni et al., 2023), and Annual cost of intervention was calculated from the current analysis.

5.3. Results

Based on the analyses, an annual effective One Health intervention covering surveillance, management and control of NTS in poultry, and intervention and course of antibiotic treatment in humans in Nigeria would involve at the minimum, approximately 4,835 technical officers and 3,700 non-technical staff (n = 8,535), with investment of over 2.2 million work hours at a total cost of US$ 53,854,660.87 across the 774 local governments areas of Nigeria (Table 5.1, Supplementary Material 5.2). The labour-related cost was US$ 5,811,976.02 (10.79%) of the total intervention cost and the non-labour cost was US$ 48,042,684.85 (89.21%). The non-labour cost subdivided into various categories as shown in Table 5.1, with major costs going into medical countermeasures, travels and transports, and laboratory supports (Table 5.1, Figure 5.2a). Overall, the total intervention cost was 374.15% of the estimated annual budget for the national and subnational systems. Incidentally, the estimated livestock health budget contributed a paltry 11.48% compared to 88.52% contribution from the public health programme on Diarrhoeal Diseases (Table 3.1).

Table 5.1. Summary of outbreaks and intervention cost for non-typhoidal Salmonella outbreak, Nigeria, 2020

Statistics	Value	Unit
Length of outbreak (days)	365	Days
Number of regions affected	37	Number
Number of human cases	325,731*	Number
Number of human deaths	1,043*	Number
Nigerian human population (Midyear, 2020)	208,327,405*	Number
Number of animal cases	43,662,085*	Number
Number of animal deaths	15,841,044*	Number
Nigerian poultry population (Midyear, 2020)	224,326,708*	Number

Non-typhoidal disease burden and social costs	930,887,379.00*	US$
Total labor hours associated with outbreak	2,271,360	Hours
Total intervention cost	**53,854,660.87**	US$
▪ Labour	**5,811,976.02**	US$
▪ Non-labour	**48,042,684.85**	US$
✓ Office	1,524,612.05	US$
✓ Travels and transport	10,987,219.27	US$
✓ Communication	291,905.54	US$
✓ Laboratory Support	5,944,302.86	US$
✓ Medical countermeasures	28,031,667.17	US$
✓ Consultancies	1,000,000.00	US$
✓ Other costs (Miscellaneous)	262,977.96	US$
Total budget for Diarrhoeal Disease Programme	14,393,777.06[#]	US$
Total intervention cost in percentage budget for Diarrhoeal Disease programme (2020)	374.15	%

**Integrated from previous analysis on burden of NTS in Nigeria for the year 2020 (Sanni et al., 2023). [#]The Budget for Diarrhoeal Disease Programme is approximately 1.3% of the total mean annual expenditure as percentage of current health expenditure (CHE) of the Nigerian federal and states' budgets [23,24]. Approximately US$ 12,741,647.80 (88.52%) came from the Public Health Programme on Diarrhoeal Diseases and only US$ 1,652,129.26 (11.48%) came from the related Animal Health Programme. The exchange rate at the time of the analysis was Naira 380.26 = US$ 1 (Midyear value, 2020). All cost categories of total expenditure were computed in Nigerian Naira and converted to US$.*

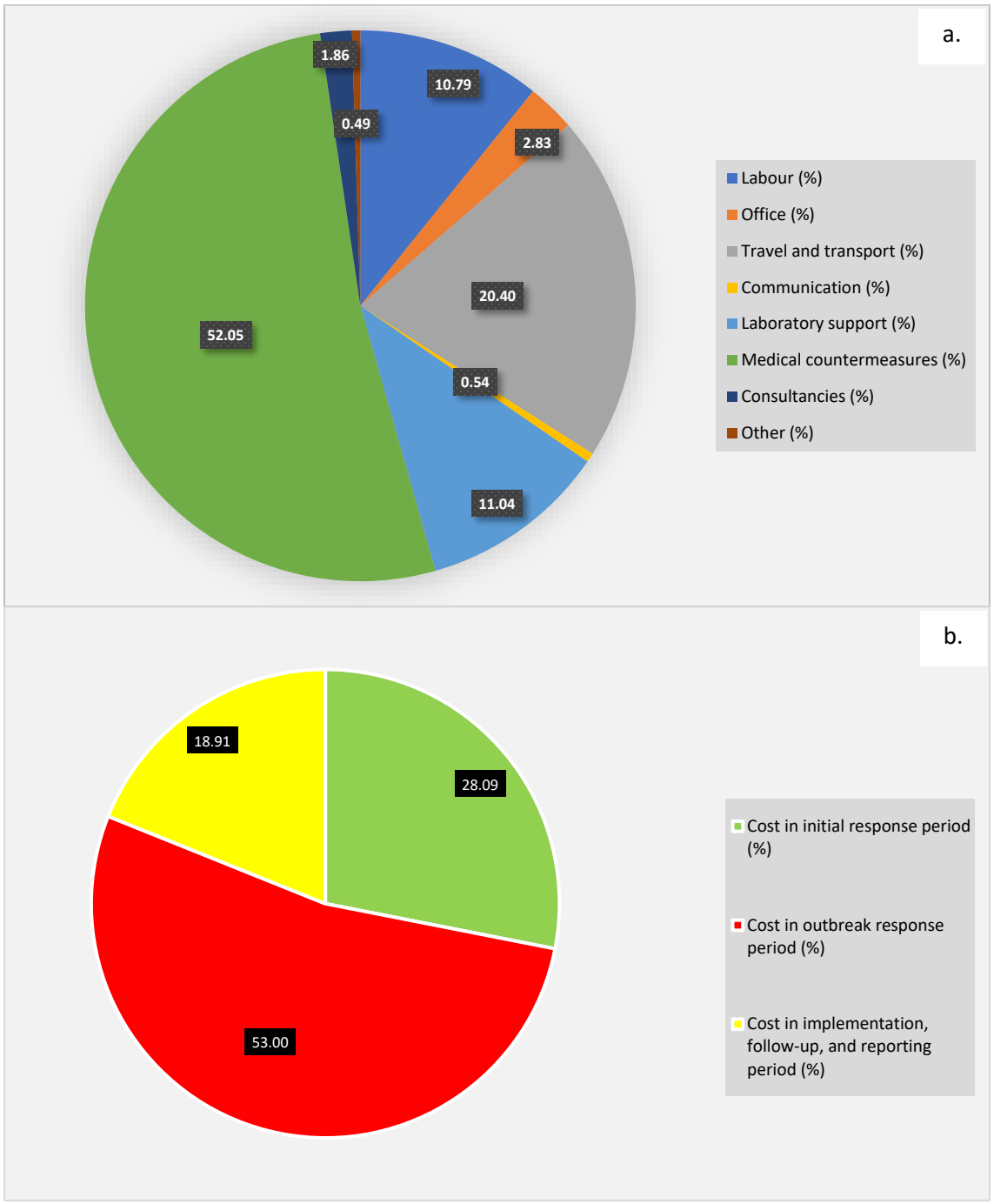


Figure 5.2. a. Percentage resource category cost of surveillance and control in outbreaks of non-typhoidal Salmonella, Jan. – Dec. 2020; b. Percentage periodic-based distribution of intervention cost for non-typhoidal salmonellosis outbreak, Nigeria, Jan. – Dec. 2020.

Comparing the clustered periods of outbreaks, the investment cost during the outbreak response period (53%) was higher than those in the preparedness and initial response period (28.09%) and those spent in the implementation, follow-up, and reporting period (18.91%) (Table 5.2). Basically, between the labour and non-labour costs for implementing treatment, control and prevention measures following outbreaks came to a total of US$ 28,541,285.02 (53.00% of the intervention cost) (Table 5.3).

Benefit – cost Ratio (BCR) of intervention = Annual burden of costs of diseases ÷ Annual cost of intervention

Where: Annual burden of costs of diseases = US$ 930,887,379.00 [22], and annual cost of intervention = US$ 53,854,660.87.

BCR = US$ (930,887,379.00 ÷ 53,854,660.87) = **17.29** (Table 5.4).

By increasing of labour and laboratory support costs each by 40% while decreasing travel and transport costs by 40%, the new total cost of the surveillance programme against NTS would be 100.57% of original cost, thus reducing the BCR marginally to 17.19; Even if labour costs are increased by 60%, laboratory support costs are increased by 40% and office supplies costs are increased by 25%, while decreasing travel and transport costs by 40% and medical countermeasure costs by 10%, the new total cost of the surveillance programme against NTS would be 98.23% of original cost, thus increasing the BCR marginally to 17.6; By adding an additional 20% to the laboratory support costs to make a new cumulative of 60%, and with an additional 50% increase in the costs associated with consultancies/outsourcing, added to scenario 2, the new total cost of the surveillance programme against NTS would be 105.47% of original cost, thus reducing the BCR marginally to 16.39 (Table 5.4).

Furthermore, if the travel and transport costs increase by 20%, and laboratory support costs increase by 20% while office supplies costs increase by 15% and medical countermeasure costs reduce by 15%, the new total cost of the surveillance programme against NTS would be 98.90% of original cost, thus increasing the BCR to 17.48. Finally, if the travel and transport costs increase by 40%, and communication costs increase by a marginal 10% while all other parameters remain the same, the new total cost of the surveillance programme against NTS would be 108.21% of original cost, thus decreasing the BCR to 15.97 (Table 5.4).

Table 5.2. Periodic-based intervention cost category for non-typhoidal salmonella outbreak, Nigeria, 2020.

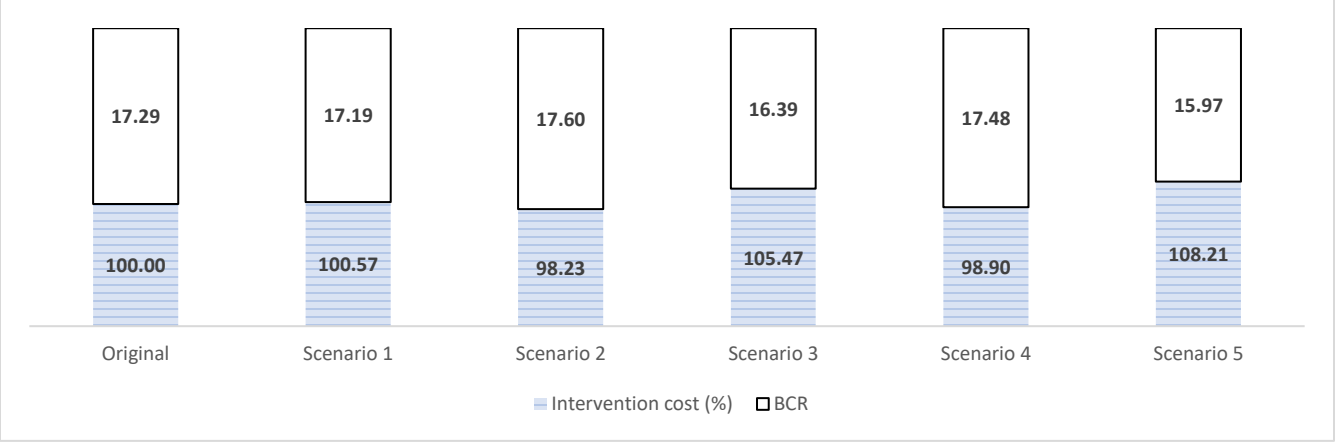
Category	Overall Cost (US$)	Cost in initial response period (US$)	Cost in outbreak response period (US$)	Cost in implementation, follow-up, and reporting period (US$)
Labor	5,811,976.02	2,982,960.61	2,272,909.59	556,105.82
Nonlabor				
Office	1,524,612.05	880,928.30	434,377.54	209,306.21
Travel and transport	10,987,219.27	1,660,337.66	7,234,628.94	2,092,252.67
Communication	291,905.54	0.00	262,714.98	29,190.55
Laboratory support	5,944,302.86	3,523,277.22	1,479,438.19	941,587.44
Medical countermeasures	28,031,667.17	5,307,424.07	16,595,524.59	6,128,718.51
Consultancies	1,000,000.00	693,000.00	156,500.00	150,500.00
Other	262,977.96	78,893.39	105,191.18	78,893.39
Total Intervention cost for NTS, 2020	**53,854,660.87**	15,126,821.26	28,541,285.02	10,186,554.59
Total intervention cost as a fraction of the budget for Diarrhoeal Disease programme (2020)	3.7415239	1.050927855	1.982890586	0.707705458

Table 5.3. Activity-based intervention cost category for non-typhoidal Salmonella outbreak, Nigeria, 2020.

Period	Activity	Cost (US$)	
		Labour	Non-labour
Initial response	Prepare for field work	445,661.55	2,724,103.79
	Establish and verify the existence of an outbreak	397,384.89	2,846,042.87
	Verify the diagnosis	447,128.23	3,432,293.38
	Construct a working case definition	217,663.13	353,076.63
	Find cases systematically and record information	243,302.00	710,951.65
	Perform descriptive epidemiology	182,253.88	839,039.01
	Develop hypothesis	239,177.04	247,521.19
	Evaluate hypothesis epidemiologically	201,986.17	294,338.16
	Reconsider, refine, and re-evaluate hypothesis	176,298.53	263,831.10
	Compare and reconcile with laboratory and/or environmental studies	432,105.19	432,662.86
Outbreak response	Implement treatment, control and prevention measures	2,272,909.59	26,268,375.43
Follow up and reporting	Initiate or maintain surveillance to determine whether the prevention and control measures are working	90,632.67	7,666,217.76
	Write an outbreak investigation report and disseminate findings appropriately	465,473.15	1,964,231.00
Total intervention cost per category		5,811,976.02	48,042,684.85
Total intervention cost as fraction of category allocated budget (%)		48.35	2,024.10

Table 5.4. Scenario Analysis of variation in Intervention costs and impacts on Benefit-Cost Ratio of Programme Intervention against NTS, 2020.

Resource Category	Original	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
Labour	5,811,976.02	8,136,766.43	9,299,161.63	9,299,161.63	5,811,976.02	5,811,976.02
Travel/ transport	10,987,219.27	6,592,331.56	6,592,331.56	6,592,331.56	13,184,663.12	15,382,106.98
Office supplies	1,524,612.05	1,524,612.05	1,905,765.06	1,905,765.06	1,753,303.86	1,524,612.05
Communications	291,905.54	291,905.54	291,905.54	291,905.54	291,905.54	321,096.09
Laboratory support	5,944,302.86	8,322,024.00	8,322,024.00	8,916,454.29	7,133,163.43	5,944,302.86
Medical countermeasures	28,031,667.17	28,031,667.17	25,228,500.45	28,031,667.17	23,826,917.09	28,031,667.17
Consultancies/ outsourcing	1,000,000.00	1,000,000.00	1,000,000.00	1,500,000.00	1,000,000.00	1,000,000.00
Others/miscellaneous	262,977.96	262,977.96	262,977.96	262,977.96	262,977.96	262,977.96
Total Program cost	**53,854,660.87**	**54,162,284.71**	**52,902,666.21**	**56,800,263.22**	**53,264,907.03**	**58,278,739.13**
Percentage of original	**100.00**	**100.57**	**98.23**	**105.47**	**98.90**	**108.21**



Scenario 1: *By increasing of labour and laboratory support costs each by 40% while decreasing travel and transport costs by 40%, the new total cost of the surveillance programme against NTS would be 100.57% of original cost, thus reducing the BCR marginally to 17.19;* ***Scenario 2:*** *Even if labour costs are increased by 60%, laboratory support costs are increased by 40% and office supplies costs are increased by 25%, while decreasing travel and transport costs by 40% and medical countermeasure costs by 10%, the new total cost of the surveillance programme against NTS would be 98.23% of original cost, thus increasing the BCR marginally to 17.6;* ***Scenario 3:*** *By adding an additional 20% to the laboratory support costs to make a new cumulative of 60%, and with an additional 50% increase in the costs associated with consultancies/outsourcing, added to scenario 2, the new total cost of the surveillance programme against NTS would be 105.47% of original cost, thus reducing the BCR marginally to 16.39;* ***Scenario 4:*** *If the travel and transport costs increase by 20%, and laboratory support costs increase by 20% while office supplies costs increase by 15% and medical countermeasure costs reduce by 15%, the new total cost of the surveillance programme against NTS would be 98.90% of original cost, thus increasing the BCR to 17.48;* ***Scenario 5:*** *Finally, if the travel and transport costs increase by 40%, and communication costs increase by a marginal 10% while all other parameters remain the same, the new total cost of the surveillance programme against NTS would be 108.21% of original cost, thus decreasing the BCR to 15.97.*

5.4. Discussion

In this analysis, first, we aimed to estimate the costs of multisectoral (human–animal) investigation and response activities associated with a year outbreak of non-typhoidal salmonellosis in Nigeria for the year 2020. In addition, we conducted a benefit – cost analysis of the intervention to determine whether it is worth investing in the epidemio-surveillance, prevention and control of NTS in Nigeria and evaluated different scenarios considering the multisectoral competing interests for limited available funds, other health priorities and unplanned but emergent needs of the country. The OCT estimated the comprehensive costs of interventions against NTS in humans and poultry in Nigeria for the year 2020 (US$ 53,854,660.87) and categorized the cost into various subheads and stages of outbreak investigation and response periods. Such division becomes necessary in order to prioritize anticipatory planning, budgeting and identify funding gaps while providing effective responses against infectious diseases [20,30]. As previously suggested by Bodenham and colleagues (2021), the OCT is a utility tool that can be used at multiple tiers and levels – for example, at different government ministries, departments and parastatals, and can be coordinated with other tools like the *Multisectoral Coordination Mechanism Operational Tool* for annual coordination and costing for all anticipated One Health activities in the country [31].

First, we observed significant under-resourcing and under-provisioning for the overall Diarrhoeal Disease Programme, salmonellosis and more specifically, the NTS intervention in humans and animals. For instance, based on our estimates, the budget needed to perform efficiently an annual intervention against NTS was 274.15% above the allocated budget for the year 2020. Not surprisingly, salmonellosis is not a high-prioritized foodborne zoonoses in Nigeria despite its

ranking as high to moderate on the burden of diseases, the ability of the health services to control it, and its socio-economic impacts [16]. Secondly, budget distribution among the subheads (personnel, overhead and capital) in Nigeria weighs heavily in favour of personnel. Our analysis indicated that while the personnel may utilize less than 50% of its resources, the non-labour category utilized over 2000% of its allocated resources (Table 5.3), a pointer that there may be a need to relook at the whole budgeting process to allocate more to activities and possibly rationalize the workforce where necessary. Worse still, the estimated livestock health budget contributed a paltry 11.48% of the Diarrhoeal Disease Programme for the year 2020, an indication that much less allocation would be directed at non-typhoidal salmonellosis' surveillance, management and control. In this wise, there is bound to be ineffective Veterinary Services to tackle diseases like NTS at both national and subnational levels [20,30]. It should be noted that poultry remains one of the major sources of NTS in humans, and the Nigerian poultry value chain and informal trade enables random nationwide distribution of untested poultry and its products, with risk of long-distance transmission of NTS within Nigeria. It is expected that mitigating NTS risks in poultry would significantly reduce the social and economic burdens of NTS in humans. Thus, we advocate more investment in vaccination against fowl typhoid in poultry, and in effective surveillance, monitoring and control of salmonellosis in the poultry value chain – in particular, at the hatcheries, day-old-chicks, eggs and poultry meat distribution networks to mitigate NTS impacts. Recently, the World Bank Group has shown that investment in One Health Systems based on disease prevalence would generate expected returns and prevent mild pandemics by half or entirely [32]. Such investment scenarios could be facilitated or reviewed through tabletop or limited simulation exercises to test the likely effectiveness of such investment.

The overall estimated intervention costs cover the entirety of the outbreak year from 1st January until 31st December 2020 considering the burden of infection and deaths in human and animals, however, the estimate is for planning purposes since the dynamics of disease outbreaks is absolutely unpredictable and may respond differently under many circumstances. Distilling this further, the cost associated with the period of initial response was 28.09% of the total costs, while those related to outbreak response and follow-up and reporting were 53.00% and 18.91% respectively (Figure 5.2b). This is similar to cost distribution for scenario analysis for anthrax intervention in Tanzania in 2018 - 2019 [20]. Perhaps, an investment in preparedness and initial response period (pre-outbreak periods otherwise known as peacetime and alert period) would aid early detection, limit the scale of outbreaks thus limiting disease burdens and the eventual impact and costs of managing the outbreaks as indicated in the scenario analysis [33]. The bulk of the costs invested in the annual management of NTS in Nigeria are embedded in the outbreak period's medical countermeasures, travel and transport, laboratory and labour. Hence, every effort aimed at reducing the unit costs in these categories would have overall impacts in increasing the benefit-cost, reducing the associated disease burdens and the costs of intervention.

Understanding the distribution of these estimated costs associated with different NTS outbreak and response periods and categories can assist in effective budgeting and planning for future outbreaks, and possibly has lateral positive effects in planning for other diseases. Bodenham and colleagues [20], have earlier stressed the benefit of such planning. Whereas such plans must be innovatively engaged by the technical and non-technical officers, it can also be used with the planners and policymakers for advocacy both at the national and subnational levels.

A limitation of our investigation was the use of small number of participants to obtain the cost data, as this may have influenced the cost estimates generated. It should be noted that the tool was applied for the scenarios for the year 2020, approximately 2 years from the hypothetical outbreak events, because the calculations on the burdens of the disease had been set for the year 2020; this may have subjected the study to a degree of recall bias. We however cross-validated several pieces of information obtained from key informants and institutions. Where some degree of inconsistency exists in qualitative information, we checked official record or other information sources.

Though cost analyses for infectious disease outbreaks is challenging due to data scarcity of cost data, and dearth of records or a single repository where all the data can be obtained [34], its outputs and outcomes are vital for pushing boundaries and getting supports for investment in public and animal health. The availability and use of simple, fast and adaptable tools, such as the OCT, may assist in bridging these data gaps and building capacity in this area. Overall, the proposed intervention in this study was 17.29 cost beneficial for NTS and different scenarios presented with different positive benefit-cost ratio. Hence investment in diarrhoeal disease programme and foodborne zoonoses like NTS would be at least 16 folds worthwhile with benefits for other health programmes since many labour and non-labour resources would be shared across platforms.

In view of the burden of costs associated with medical countermeasures and travels and transport and considering the many competing yet important interests for the depleting resources in many low-and-middle income countries (LMICs), a re-prioritization of budgeting and allocation of scarce resources are desirable using innovative approach. For instance, highly trained and very

competent sub-national veterinary workforce would reduce heavy dependence on national officers, and shorten the critical response time to intervention, the burden of diseases and ultimately the heavy costs associated with travel [35]. However, such trained manpower must be capacitated with resources (surveillance materials, tools, consumables and equipment) to carry out their mandate, with the consequent effective utilization of sub-national officers. It may also improve the utilization of national officers as these would have more time to focus on planning, coordination and provision of overall backup services (surge capacity) to sub-national systems where needed. Introduction and use of electronic assistance (tools, apps, artificial intelligence etc.) for reporting, coordination, response and control may improve the four-way linkages among veterinary and public health's field and laboratory workforce at both national and sub-national levels [36,37]. It may be important to consider zonal or regional logistic supplies or stores for public health and veterinary services, to eliminate long waiting time and aid easy access to logistics, supplies and consumables that supports epidemio-surveillance and monitoring. While such coordination and lead distributions may be central, utilization and unhindered access should be subnational once any significant health event occurs or at short notice [38].

Our work is subjected to certain limitations. First, in a realistic world, disease situation, financial and political dynamics could change rapidly, hence, we made a number of assumptions as outlined in the supplementary material 1 (Supplemental: Table of and basis for assumptions) and premise on the stable political economy. It is hoped that the situation remains as suggested as any significant change may affect the outputs and outcomes of the analyses. Considering this dynamic, we suggested some scenarios and presented a supplemental material that may guide scenario planning (Supplementary Material 4). In addition, the salary category for labour is

subjected to some subjectivity either because salaries are personal and individuals do not want to talk about their salaries, or the total emoluments per each intervention may be difficult to predict since the length and scope of outbreaks may differ. To adjust for this, we utilized the admin and finance-level information to benchmark personal-level information and use mean (or median) figures where applicable and we used subject matters specialists' opinions to determine lengths and potential scopes of NTS.

5.5. Conclusions

Multisectoral investigation and response against NTS in Nigeria may benefit from health re-focusing and re-prioritization. However, it may also become complex due to current sectoral silos, uneven sectoral financing, coordination challenges and delays associated with over-centralization of public and animal health interventions. A decentralized framework with sub-national focus and empowerment for rapid investigation, response and control, as well as for collecting and analyzing useful cost and epidemio-surveillance data would be useful for robust understanding of under-estimated outbreaks like NTS. It should assist anticipatory planning, early outbreak investigation and reduce critical response time to intervention. Tools like OCT, if applied preemptively, can benefit budget planning, identify gaps in current surveillance methods, and assist in proposition of cost saving but effective measures against infectious disease.

Supplementary Materials

Supplementary Material 5.1 – Participant information note, consent form and the questionnaire.

Supplementary Material 5.2 – filled Outbreak Costing Tool (OCT) Excel Spreadsheet.

Supplementary Table 5.3 – Explanatory table on the details of the Cost Categories in the OCT.

Supplementary Material 5.4 – Scenario analysis evaluation.

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CHAPTER SIX

6.0 RISK FACTORS FOR PERSISTENT INFECTION OF NON-TYPHOIDAL *SALMONELLA* IN POULTRY FARMS, NORTH CENTRAL NIGERIA

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Abstract

Salmonellosis is a bacterial zoonosis causing an array of health conditions. Non-typhoidal salmonellosis (NTS) has a discrete adaptation to certain animals; in poultry, pullorum and fowl typhoid are its primary disease manifestations. The diseases are prevalent in Nigerian poultry and have been well-studied in Nigeria, but less so in North Central Nigeria (NCN). Using field sampling, laboratory methods and a semi-structured questionnaire for 1000 poultry farms in NCN, we explored the incidence and risk factors for the persistence of NTS infection in poultry. Approximately 41.6% of the farms had experienced NTS over the last 18 months. Farm experience of NTS moderately predicted awareness of salmonellosis. Increasing stock in smallholder farms, self-mixing of concentrate on the farm, usage of stream water, pen odour, non-adherence and partial adherence of farms to recommended poultry vaccination against pullorum and fowl typhoid and lack of and non-adherence to biosecurity were identified risk factors that increased the odds of NTS infection in poultry. Antibiotic use practice may have reduced the isolation rate of NTS, yet NTS continues to challenge poultry farms in Nigeria. Identified risk practices must be mitigated intentionally and biosecurity and hygiene must be improved to reduce the burden of NTS.

Keywords: Non-typhoidal *Salmonella*; poultry; risk factor; Nigeria; fowl typhoid; pullorum disease.

6.1. Introduction

Fowl typhoid and pullorum disease are bacterial infections (salmonellosis) found in farmed poultry caused by the *Salmonella* enterica subspecies enterica serovars Gallinarum biovars Gallinarum and *Salmonella* enterica subspecies enterica serovar Gallinarum biovar Pullorum, respectively, and they are widely distributed globally [1,2]. Recent evidence has also suggested a tendency towards increasing antimicrobial resistance in strains of these organisms obtained from poultry [3–5]. Although its eradication is possible, and this has been largely achieved in many commercial poultry in developed countries in Western Europe, the United States of America (USA), Canada, Australia and Japan, its eradication in developing countries, particularly in Africa, Asia and South America, remains debatable [6–8].

Salmonellosis is a bacterial zoonoses with considerable public health impacts, and it can be caused by typhoidal and non-typhoidal *Salmonella* organisms, including those mentioned above [8,9]. According to FoodNet surveillance data, *Salmonella* causes more disease burden in humans than any other foodborne pathogen, and globally, it causes up to 20 million human cases annually [8–10]. In the USA alone, *Salmonella*-contaminated poultry is responsible for an estimated loss of USD 2.5 billion annually, or the loss of 15,000 QALYs in annual disease burden [9,10]. This considerable burden of disease is caused by food handling and preparation problems in food service and retail settings, some of which may have been associated with contaminations along the food chain [5,9,10].

Non-typhoidal *Salmonella* (NTS) refers to the infection produced by all serotypes of *Salmonella* except for the typhoidal and paratyphoidal groups. Although there have been at least 2463 serotypes of *Salmonella* found to date (over 2500 by other estimates) [11–14], the laborious

traditional phenotypic serotyping method is still popular. It is challenging because it involves more than 150 specific antisera and expert interpreters to analyse the results [12]. In recent times, proposals for genome-based *Salmonella* serotyping and microarray methods have been made [12,15]. The symptoms of NTS in humans include diarrhoea, vomiting and abdominal cramps, which develop 12 to 72 h after infection. NTS has a discrete adaptation to certain animals, such as the adaptations of *Salmonella* Choleraesuis to pigs, *Salmonella* Dublin to cattle, *Salmonella* Abortusovis to sheep and *Salmonella* Gallinarum (*Salmonella* enterica subspecies enterica serovars Gallinarum biovars Gallinarum) and *Salmonella* Pullorum (*Salmonella* enterica subspecies enterica serovar Gallinarum biovar Pullorum) to poultry [2,11,16,17].

In Nigeria, the burden of zoonotic salmonellosis is unknown in humans or poultry; however, significant research has been produced on salmonellosis in poultry [3,18–25]. However, these studies have been concentrated in the extreme north and the southern belt of the country. North Central Nigeria (NCN), which connects the southern belt of the country, where most of the commercial poultry activities occur, with the north, where most of the indigenous poultry populations predominate, has been less investigated. It is estimated NCN had a significant poultry population in excess of 44,789,854 in 2020 [26], and it is the producer of the majority of meat and eggs supplied to the Federal Capital Territory and its neighbourhood. There is therefore a need to carry out a series of empirical studies, including one on the risk factor for continuing infections of poultry farms with *Salmonella* in North Central Nigeria, to bridge the existing knowledge gaps that exist in *Salmonella* studies in Nigeria in order to inform policy aimed at reducing the burden of this bacteria zoonosis. The goals of this study were (i) to investigate the

prevalence of non-typhoidal *Salmonella* in the poultry farms in North Central Nigeria, and (ii) to explore potential risk factors in commercial and backyard poultry farms in North Central Nigeria.

6.2. Materials and Methods

6.2.1. Selection of States and Sampling Sites

The states in this geopolitical zone include: Kogi, Niger, Nasarawa, Kwara, Benue, Plateau and the FCT (Figure 1). The selection of this study site was informed by the lack of empirical data sources on non-typhoidal *Salmonella* (NTS) from North Central Nigeria (NCN), and the need to aggregate the risk factors for persistence of non-typhoidal *Salmonella* in poultry farms in NCN.

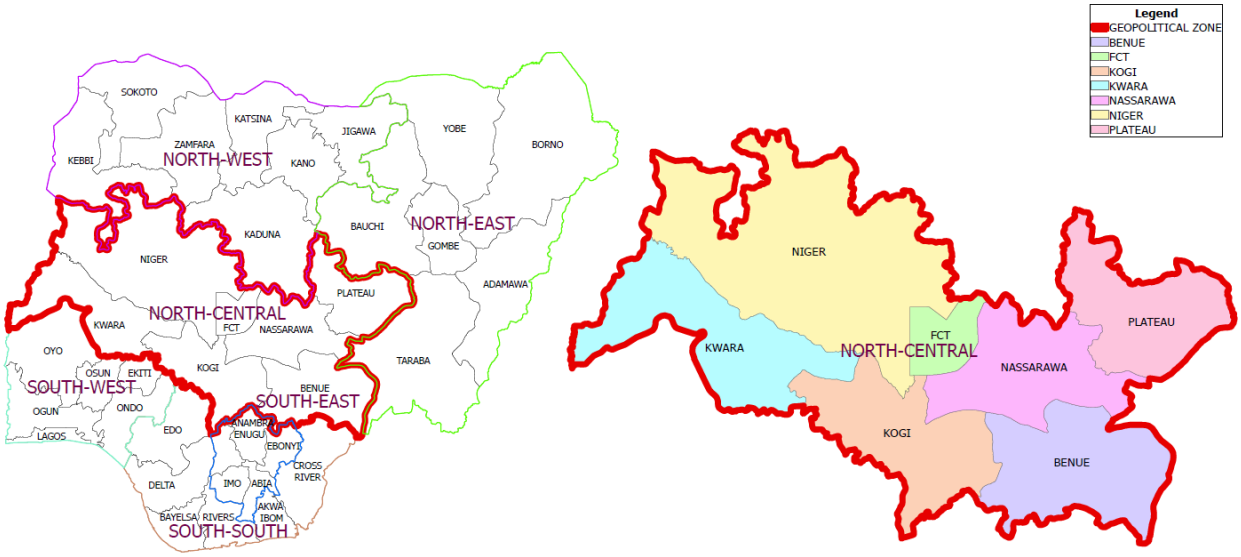

Figure 6.1. Map of Nigeria with a call-out map of the North Central zone.

6.2.2. Development of Questionnaire and Training of Data Collectors

Through a literature review and probing questions to veterinarians and animal health assistants, a list of previously identified risk factors for *Salmonella* in poultry in Nigeria was developed ([27,28]; Supplementary Material 6.2). A semi-structured questionnaire was prepared based on

this list of identified risk factors and drivers of NTS infection on farms. Although the questionnaire was prepared in English, and approximately 90% of all respondents had at least a secondary level of education, respondents were allowed to choose a convenient language for communication during the interview. All communication was in the English language or local dialects, as selected by the respondent, to enable the respondents to communicate effectively or provide detailed inputs. The questionnaire targeted data on location, demographics, years of experience, type of management and chickens kept, housing and farm environment details, awareness of *Salmonella*, case and mortality patterns and some economic variables, as well as access to professional support.

Hired research assistants (HRAs/data collectors) (n = 21) were recruited from the localities of the sampling sites in each of the states. The lead researcher (AOS) organized a training session for the HRAs on the objectives of the study, how to avoid bias during the field data collection and how to include internal quality control to enhance data validity. Five of the trained HRAs/data collectors conducted the role play exercise and served as respondents. Feedback from the role play exercise was used to improve the questionnaire. All questions were checked for consistencies, avoidance of ambiguity and misinterpretation. The pretested questions were printed in hard copies for the use of data collectors in the field.

6.2.3. Field Sampling and Laboratory Analysis

The maximum number of poultry farms was targeted for sampling per each state (n = 150 X six states = 900 samples, except for the state of Plateau, where 100 farms were visited; total = 1000). On each farm, up to five freshly voided faecal samples were pooled and collected in a sterile

sample container. Pooling of each sample per farm was considered because a farm is considered as an epidemiological unit and a single case of salmonellosis on a farm makes the farm positive in this study. While samples were collected in sterile sample containers, a lead person (typically, the farm manager, farm owner or his/her designated assistant) was interviewed using the pretested questionnaire. The farms were randomly selected and recruited once they determined to qualify for the definition of a poultry farm, without bias regarding the bird types available on the farm or the farm size. All samples were transported on ice to the laboratory, and a total of 1000 samples and 1000 questionnaires were collected. The preferred sample was the freshly voided faeces or faeces collected directly using cloacal swab/massage. In a few cases, other samples (swabs of organs and tissues) were picked from dead carcasses (n = 12) [2] and were identified using the bacterial culture methods described below at the STEP-B laboratory of the Federal University of Technology Minna, Niger, and Central Research and Diagnostic Laboratory, Ilorin.

6.2.4. Bacteriological Culture and Phenotypic and Biochemical Characterization

Collected and transported faecal swabs and organ samples were macerated in peptone water, and cultured for identification as previously described [2,29]. Briefly, approximately 25 g of each sample was weighed and added to 225 mL of 0.1% peptone water, and incubated overnight at 37 °C. The overnight-incubated suspension was transferred (0.1 mL of each to 10 mL of Rappaport-Vassiliadis Soy Peptone (RVS) Broth) (Merck, Darmstadt, Germany) and re-incubated overnight at 41.5 °C. Following the incubation, samples were cultured on Xylose Lysine Desoxycholate (XLD) agar (Merck, Germany) and incubated again overnight at 37 °C. Red colonies with a black centre

were sub-cultured in nutrient agar (NA) (Merck, Germany) to perform Gram staining and biochemical tests [29]. Colonies were Gram-stained for identification, and biochemical characterization was performed for confirmation [2,29,30].

6.2.5. DNA Extraction and Polymerase Chain Reaction

Following bacteriological culture, selected bacterial-culture-positive isolates were subjected to further molecular characterization, as described here. DNA was extracted using the protocol stated by Zhang et al. [30]. The extracted DNA was processed for PCR using the 16S rRNA gene PCR for-ward and reverse primers: (27F, 5'-AGAGTTTGATCMTGGCTCAG-3' and 1525R, 5'-AAGGAGGTGATCCAGCC-3') and 0.3 units of Taq DNA polymerase (Promega, Madison, WI, USA). PCR was carried out in a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., Foster City, CA, USA) using the predefined PCR profiles (initial denaturation at 94 °C for 5 min; followed by 30 cycles at 94 °C for 30 s, 50 °C for 60 s and 72 °C for 1 min 30 s; a final termination at 72 °C for 10 min; and chilled at 4 °C) [22,49]. The final PCR product was electrophoresed on the 1.5% agarose gel using a 100 bp molecular weight ladder as a marker.

6.2.6. Definition of Case and Control Farms

For the purpose of risk factor evaluation, a case farm was defined as a poultry farm from which a biological sample collected from a suspect-ed/unsuspected clinical case, tested in the laboratory according to the protocol mentioned above, and was consistently positive according to the test methods (culture and biochemical confirmation) in accordance with the international regulations for confirmed positive cases of poultry salmonellosis (fowl typhoid and pullorum diseases) [2].

Alternatively, poultry farms that had also experienced salmonellosis non-typhoidal *Salmonella* (NTS) within the period under consideration (≤18 months, equivalent to the maximum period for the current cycle of stocking of poultry chickens), and had been confirmed both clinico-pathologically and through laboratory confirmation, were included as case farms. For this work, a total of 416 case farms were found to have experienced NTS and tested positive for poultry salmonellosis in the last ≤18 months. A control farm was described as a farm where a sample was collected and tested as described for the case farm above but was negative according to all test protocols. Such farms must have been negative according to clinico-pathological as well as laboratory diagnostic tests. A total of 584 farms had not experienced poultry salmonellosis in the last batch of chickens present on their farms (≤15 months).

6.2.7. Statistical Analysis

Data were cleaned in Microsoft Excel 2018 and imported to Stata v 15 (Stata Corporation, College Station, 4905 Lakeway Dr., TX, USA) for analysis. Initially, we conducted descriptive statistics for all farm and collected field-level data to determine their proportions, standard errors (SEs) and 95% confidence intervals (CIs95%) for each variable, using the method of Agresti and Coull [31]. Categorical variables were also summarized as proportions. The disease prevalence was computed as the number of farms reporting to have had NTS at the time of the study or in the past, divided by the total number of study farms as a percentage. We aggregated selected risk-related variables and ran comparisons using pairwise correlation to determine whether there were significant correlations among the variables. Since the observations were not independent, a logistic regression model was used to investigate the association between the various potential

risk factors and the outcome variable (defined as a farm having experienced NTS or not, and confirmed through clinical and laboratory diagnosis). The predictor variables used in the analysis are listed in Tables 6.1–6.4. The effect of each independent variable was first run in the univariable logistic regression model. Variables associated with the outcome (non-typhoidal *Salmonella* (NTS) infection) at $p \leq 0.2$ were considered for inclusion in the multivariable logistic regression model. Independent variables were tested for pairwise associations, using a two-tailed chi-square test. The model was progressively simplified using the backward stepwise elimination method. Backward stepwise regression is a stepwise regression approach that be-gins with a full (saturated) model and at each step gradually eliminates variables from the regression model to find a reduced model that best explains the data. The stepwise approach is useful because it reduces the number of predictors, reducing the multicollinearity problem, and it is one of the ways to resolve overfitting. Variables that were found not to have strong evidence of an association, or a Wald test with a p-value (>0.05), were excluded one at a time with the least statistically significant excluded at each step. To check that the variables removed did not have a huge effect on the model, the log likelihood ratio test was calculated each time.

The Hosmer and Lemeshow test goodness of fit test was used to show how well the data fit the model. Model discrimination was assessed by using the area under the receiver operating characteristic curve (AUROC). The AUROC was used to compare the goodness of fit of logistic regression models, where values for the measurement ranged from 0.5 to 1.0. A value of 0.5 indicated that the model was no better than chance at making a pre-diction about membership in a group, and a value of 1.0 indicated that the model perfectly identified those within a group and those not. At each stage of backward stepwise elimination, the models' discrimination and

overall fit was assessed. All analyses were carried out in Stata v 15 (Stata Corporation, College Station, TX, USA). A statistical significance level was set at $p < 0.05$.

6.3. Results

This work covered the six states of the North Central zone of Nigeria (Kogi, Niger, Nasarawa, Kwara, Benue and Plateau) and the Federal Capital Territory (FCT) (Figure 1). One hundred and fifty (150) samples were collected from three local government areas (LGAs) (50 farms per LGA) in every state surveyed except in the state of Plateau, where 100 samples were collected from two LGAs (n = 1000). In the period under consideration (≤18 months, September 2020–March 2022), 416 farms (41.6%) (95%CI: 38.58 to 44.68) experienced non-typhoidal *Salmonella* (NTS)—S. enterica, as confirmed by veterinary laboratory evaluations and reports, and based on clinico-pathological evaluations of the farms. Apart from *Salmonella enterica*, *Klebsiella pneumoniae* was detected in 92.9% of the samples, *Lactobacillus bulgaris* was found in 0.9% of the samples, *Salmonella arizonae* was detected in 0.2% (95%CI: <0.01 to 0.8), *S. paratyphi* in 1.9% and S. typhi in 2.3% (95%CI: 1.5 to 3.5) of all samples (Table 6.1). A total of 392 of the 416 S. Enterica-positive samples (94.5%) exhibited mixed infections with *Klebsiella pneumoniae*, *Lactobacillus bulgarius*, *S. arizonae* and/or *S. paratyphi*.

Table 6.1. Descriptive statistics of cultured bacteria found in faecal samples collected from smallholder poultry farms, September 2020–March 2022, North Central Nigeria.

Isolates	Number	Percentage
Klebsiella pneumonia	929	92.9
Lactobacillus bulgarius	9	0.9
Salmonella enterica	416 *	41.6
S. arizonae	2	0.2
S. paratyphi	19	1.9
S. typhi	23	2.3

*A total of 392/416 (94.5%) of the samples with *S. enterica* infection had mixed infections with *Klebsiella pneumoniae*, *Lactobacillus bulgarius*, *S. arizonae* and/or *S. paratyphi*.

The percentages of farmers with ≤2 years, >2–≤4 years, >4–≤6 years and >6 years of experience were 22.4%, 31.9%, 23.9% and 21.8%, respectively. The majority of the interviewed farmers had a tertiary education (50.8%), and only 49.2% had other forms of education, up to the secondary level. Among the farms surveyed, 44.4% practiced broiler operations, 22.5% carried out layer operations, and 29.4% carried out mixed operations (layers and broilers on the farm) (Table 6.2). Details of other descriptive statistics on all farm- and field-level data are described in Table 6.2.

Table 6.2. Descriptive analysis of the respondents' variables for the incidence of non-typhoidal Salmonella in poultry farms, North Central Nigeria.

Variable * (n)	Categories	Proportion (%)	95% Confidence Interval
States (1000)	Kwara	15.00	12.78–17.22
	Nasarawa	15.00	12.78–17.22
	Kogi	15.00	12.78–17.22
	Niger	15.00	12.78–17.22
	Plateau	10.00	8.14–11.86
	Benue	15.00	12.78–17.22
	FCT	15.00	12.78–17.22
Experienced confirmed cases of salmonellosis in the last 18 months (1000)	No	58.40	55.27–61.48
	Yes	41.60	38.54–44.66
Gender (1000)	Male	56.90	53.83–59.97
	Female	43.10	40.02–46.17
Experience in years on poultry farms (1000)	≤2 years	22.40	19.81–24.99
	>2–≤4 years	31.90	29.01–34.79
	>4–≤6 years	23.90	21.25–26.55
	>6 years	21.80	19.23–24.36
Educational level of the poultry farmer (1000)	Primary	8.80	7.04–10.56
	Secondary	38.10	35.08–41.12
	Tertiary	50.80	47.70–53.90
	Others	2.30	1.37–3.23
Type of poultry (1000)	Broilers	44.40	41.31–47.48
	Layers	22.50	19.91–25.09
	Others	3.70	25.28–4.87
	Mixed	29.40	26.57–32.23
Number of chickens (1000)	≤200	34.90	31.94–37.86
	201–500	27.50	24.73–30.27
	501–1000	25.90	23.18–28.62
	≥1000	11.70	9.70–13.70
Source/type of feed (999)	Concentrate	59.46	56.41–62.51
	Mix	23.72	21.08–26.37
	Self-compounded	16.82	14.49–19.14
Source of water for chickens (999)	Borehole	46.05	42.95–49.14
	Tap borne (municipal)	20.22	17.73–22.72
	Well	29.53	26.70–32.36
	Stream	4.00	2.79–5.22

	Other	0.20	0.07–0.48
Pen type (998)	Standard block	30.06	27.21–32.91
	Dwarf block	41.98	38.92–45.05
	Zinc type	24.64	21.97–27.33
	Others	3.31	2.20–4.42
System of management (1000)	Deep litter	64.20	61.22–67.18
	Battery cage	31.80	28.91–34.69
	Others	4.00	2.78–5.22
Type of litter material used (1000)	Sawdust	42.90	38.83–45.97
	Wood shavings	30.20	27.35–35.05
	Sand	11.70	9.70–13.70
	Cement floor	14.00	11.85–16.15
	Others	1.20	0.52–1.88
Litter management (1000)	Poor	65.20	62.24–68.16
	Fair	9.50	7.68–11.32
	Good	25.30	22.60–28.00
Pen odour (1000)	No	41.60	38.54–44.66
	Yes	58.40	55.34–61.46
Stocking density (chickens per square meter of available floor space) (998)	12–14	17.43	15.08–19.79
	14–16	18.24	15.84–20.64
	16–18	22.04	19.47–24.62
	18–20	11.52	9.54–13.51
	20 and above	6.71	5.16–8.27
	Unknown	24.05	21.39–26.70
Adherence to vaccination (1000)	No	8.10	6.41–9.79
	Yes	64.40	61.43–67.37
	Partial	27.50	24.73–30.27
Practiced biosecurity (1000)	No	11.40	9.43–13.37
	Yes	55.50	52.41–58.59
	Partial	33.10	30.18–36.02
Had previously heard of salmonellosis (1000)	No	34.90	31.94–37.86
	Yes	64.90	61.94–67.86
	Do not know	0.20	0.08–0.48
Experienced confirmed cases of salmonellosis in the last 1–2 years (1000)	No	30.90	28.03–33.77
	Yes	41.60	38.54–44.66
	Do not know	27.50	24.73–30.27
When salmonellosis or mixed infection was experienced on the farm, how was it handled? Or what protocol was used? (1000)	Antibiotics	0.70	0.18–1.21
	Vaccination	36.90	33.90–39.90
	Antibiotics combined with vaccination	11.50	9.52–13.48
	Culling	27.00	24.24–29.76
	Sales	13.20	11.10–15.30
	Others	10.60	8.69–12.51
	No response	0.10	0.09–0.30
Had the knowledge (awareness) of salmonellosis as a zoonotic disease (1000)	No	38.00	34.99–41.01
	Yes	60.80	57.77–63.83
	No response	1.20	0.66–2.11
Source of knowledge (1000)	Electronic media	11.00	0.45–1.75
	Print media	35.40	32.43–38.37
	Extension agent	86.00	6.86–10.34
	Vet/AHO	9.40	7.59–11.21
	Other farmers	26.10	23.37–28.83
	Hospital	15.80	13.54–18.07
	Other sources	3.60	2.44–4.76

Had previously taken samples to veterinary service (1000)	No	36.00	33.02–38.98
	Yes	62.10	59.09–65.11
	No response	1.90	1.20–2.97
Access to professional support (1000)	No	26.70	23.95–29.44
	Yes	33.90	30.96–36.84
	Not always	37.40	34.40–40.40
	Others	2.00	1.13–2.87

All analysis was conducted using the method of Agresti and Coull [31] and reported using the binomial Wald method. * Categorization of variables based on selected industry standards and the peer-reviewed literature (Supplementary Table 6.1).

Using pairwise correlations, most of the risk- and management-related variables evaluated against the experience of *Salmonella* in farms were weakly or negatively correlated, except for the awareness of Salmonellosis (NTS) as a potential zoonosis, which was moderately correlated with the experience of *Salmonella* in poultry farms (Table 6.3). The higher the number of poultry chickens on the farm, the higher the odds of NTS on the farms. In particular, having between 500 and 1000 chickens on the farm increased the risk of infection three-fold ($p < 0.001$), and having >1000 chickens increased the risk of persistent infection by ≈4-fold ($p < 0.001$) (Table 6.4). Farmers who self-mixed concentrate on the farm had a 2-fold-increased risk of persistent NTS infection ($p < 0.001$), and the use of stream water produced the same odds ($p < 0.01$). Chickens in poultry cages had 2-fold-increased odds of persistent NTS infection ($p < 0.001$), and non-adherence of farms to recommended poultry vaccination against pullorum and fowl typhoid increased the odds of NTS infection by >7-fold ($p < 0.001$), and even partial adherence increased the risk over four-fold ($p < 0.001$) (Table 6.4). Farmers who were not implementing and applying the principles of biosecurity strictly had 2-fold-increased odds of NTS infection on their farms (Table 6.4). The laying stock was approximately two-fold as likely to be infected with persistent NTS compared with short-cycled broilers ($p = 0.002$). Finally, farms with no pen odour were 8-fold less likely to experience NTS infection compared with pens with a persistent odour ($p < 0.001$) (Table 6.4).

Table 6.3. Pairwise correlation of selected variables for incidence of non-typhoidal *Salmonella* on poultry farms, North Central Nigeria.

	Experienced *Salmonella*	Gender	Farming Experience in Years	Education Level	Type of Farms	No. of Chickens	Feed Source	Water Source	Management System	Litter Management	Pen Odour	Stocking Density	Adherence to Vaccination	Practice Biosecurity	Had Heard of *Salmonella*	Knowledge of *Salmonella*
Experienced *Salmonella*	1.000															
Gender	−0.003	1.000														
Farming experience in years	0.041	0.083 *	1.000													
Education level	0.017	0.032	0.234 *	1.000												
Type of farm	0.097 *	0.084 *	0.189 *	0.120 *	1.000											
No. of chickens	0.233 *	0.084 *	0.145 *	0.080 *	0.149 *	1.000										
Feed source	−0.156 *	−0.004	0.099	0.004	0.095 *	−0.079 *	1.000									
Water source	−0.172 *	0.009	0.090 *	−0.068 *	0.025	−0.157 *	0.257 *	1.000								
Management system	−0.125 *	−0.022	−0.014	0.008	−0.096	−0.237	0.100	0.136 *	1.000							
Litter management	−0.071 *	−0.051	−0.116 *	−0.151 *	−0.049	−0.108 *	0.177 *	0.136 *	0.044	1.000						
Pen odour	0.029	−0.005	0.003	−0.021	−0.007	0.014	0.075 *	0.232 *	0.086 *	0.152 *	1.000					
Stocking density	−0.110 *	0.011	0.063 *	−0.022	−0.063 *	−0.009	0.053	0.021	0.056	0.093 *	−0.006	1.000				
Adherence to vaccination	0.178 *	0.116 *	0.074 *	0.109 *	0.071 *	0.219 *	−0.237	−0.165 *	−0.059 *	−0.224 *	−0.017	−0.127 *	1.000			
Practiced biosecurity	0.143 *	0.046	0.141 *	0.110 *	0.050	0.084 *	−0.051	−0.180 *	0.037	−0.267 *	−0.143 *	−0.065 *	0.322 *	1.000		
Had heard of *Salmonella*	0.478 *	0.011	0.026	0.081	0.123 *	0.196 *	−0.198 *	−0.174 *	−0.054	−0.126 *	0.038	−0.046	−0.227 *	0.172 *	1.000	
Knowledge of *Salmonella*	0.343 *	−0.003	−0.066 *	−0.084 *	0.101 *	0.221 *	−0.122 *	−0.209 *	−0.057	−0.042	−0.017	−0.053	0.119 *	0.170 *	0.456 *	1.000

* Significant at $p = 0.05$. Only the 'Heard of *Salmonella*' variable was moderately correlated with 'Experienced *Salmonella*', while the 'Knowledge of *Salmonella*' was weakly predicted by the variable 'Experienced *Salmonella*'. All other variables were poorly or negatively correlated with the experience of *Salmonella*.

Table 6.4. Univariable analysis for contamination of poultry farms with Non-Typhoidal *Salmonella* (NTS) in North Central Nigeria.

Variable	Category	OR (95% CI)	Chi-Square Value	*p*-Value *
Farming Experience in Years	<2 years	1.00	2.54	Ref
	2–4 years	0.87 (0.61; 1.23)		0.43
	>4–6 years	0.99 (0.69; 1.44)		0.98
	>6 years	1.15 (0.79; 1.68)		0.47
Level of education of the poultry farmer	Primary	1.00	3.90	Ref
	Secondary	0.79 (0.49; 1.26)		0.32
	Tertiary	0.91 (0.58; 1.43)		0.68
	Other forms (skill learning, etc.)	0.42 (0.15; 1.18)		0.10
Number of chickens on the farm	<200	1.00	60.09	Ref
	201–500	1.47 (1.05; 2.06)		0.03
	501–1000	2.93 (2.10; 4.11)		<0.001
	>1000	3.79 (2.45; 5.87)		<0.001
Source of feed	Multi-sourced commercial	1.00	41.28	Ref
	Bought-in concentrate and mix	1.87 (1.38; 2.54)		<0.001
	Self-compounded	0.47 (0.32; 0.70)		<0.001
Source of water	Borehole	1.00	59.83	Ref
	Pipe-borne municipal water	1.53 (1.10; 2.13)		0.01
	Dug-up well	0.42 (0.30; 0.58)		<0.001
	Stream	2.33 (1.19; 4.58)		0.01
Pen type	Standard type house (fully built)	1.00	8.81	Ref
	Dwarf block with side nets	0.90 (0.67; 1.22)		0.51
	Zinc-sided (roofing sheet) house	0.61 (0.43; 0.86)		0.005
	Other forms of buildings	0.77 (0.37; 1.61)		0.49
Management system	Deep litter	1.00	16.10	Ref
	Battery cage	1.74 (1.33; 2.28		<0.001
	Others (semi-intensive, etc.)	1.25 (0.66; 2.40)		0.49
Litter management	Good	1.00	11.13	Ref
	Poor	1.14 (0.74; 1.75)		0.59
	Fair	0.62 (0.46; 0.84)		0.002
Litter materials used	Saw dust	1.00	4.62	Ref
	Wood shavings	1.00 (0.74; 1.35)		0.99
	Sand (non-cemented floor)	0.87 (0.57; 1.33)		0.53
	Cemented floor	1.33 (0.91; 1.95)		0.14
	Other types (straw, etc.)	2.03 (0.63; 6.51)		0.23
Pen odour	Yes	1.00	0.72	Ref
	No	0.13 (0.87; 1.46)		0.36
Stocking density (chickens per square meter of available floor space)	12–14	1.00	3.59	Ref
	15–16	0.84 (0.55; 1.27)		0.40
	17–18	0.83 (0.55; 1.23)		0.35
	19–20	0.68 (0.43; 1.10)		0.12
	>20	0.64 (0.36; 1.14)		0.13
Adherence to vaccination	Yes	1.00	46.85	Ref
	No	7.43 (3.65; 15.10)		<0.001
	Partial	4.36 (2.09; 9.10)		<0.001
Implementation and adherence to biosecurity	Yes	1.00	20.84	Ref
	No	1.99 (1.30; 3.06)		0.002
	Partial	1.14 (0.72; 1.79)		0.58
Types of chickens on the poultry farm	Broiler	1.00	14.71	Ref
	Laying stock	1.87 (1.35; 2.59)		<0.001
	Other species/stock	1.07 (0.54; 2.14)		0.85
	Mixed	1.30 (0.96; 1.76)		0.09

* *p*-values were obtained through Wald test.

According to the multivariable logistic regression model, the higher the number of poultry chickens on the farm, the higher the odds of NTS on the farm (500–1000 chickens, OR = 2.20, $p < 0.001$; >1000 chickens, OR = 2.17, $p = 0.004$), whereas dug-up wells reduced the odds of infection by half (OR = 0.57, $p = 0.01$), and use of stream water as a source of drinking water for poultry birds increased the odds of NTS infection by >3-fold ($p = 0.005$) (Table 6.5). Of note, both the partial and non-adherence of farms to the recommended poultry vaccination against pullorum and fowl typhoid increased the odds of NTS infection in the poultry farms five-fold for each (Table 6.5). The Hosmer–Lemeshow goodness of fit = $\chi 2 = 2.58$; $p = 0.96$; Akaike information criterion (AIC) = 945.52; Area under curve (receiver operating characteristics (ROC)) = 0.72 (Figure 6.2).

Table 6.5. Multivariable analysis for contamination of poultry farms with non-typhoidal *Salmonella* (NTS) in North Central Nigeria.

Variable	Category	Crude OR (95% CI)	Adjusted OR (95% CI)	*p*-Value *
Number of chickens on the farm	<200	1.00	1.00	Ref
	201–500	1.41 (0.95; 2.10)	1.42 (0.92; 2.20)	0.11
	501–1000	2.82 (1.92; 4.15)	2.20 (1.44; 3.37)	**<0.001**
	>1000	3.32 (2.03; 5.44)	2.17 (1.28; 3.71)	**0.004**
Source of feed	Multi-sourced commercial	1.00	1.00	Ref
	Bought concentrate and mix	1.55 (0.92; 1.92)	1.49 (0.99; 2.25)	0.07
	Self-compounded	0.54 (0.35; 0.84)	0.70 (0.42; 1.18)	0.18
Source of water	Borehole	1.00	1.00	Ref
	Pipe-borne municipal water	1.33 (0.92; 1.92)	1.49 (0.99; 2.25)	0.06
	Dug up well	0.43 (0.29; 0.62)	0.57 (0.37; 0. 87)	**0.01**
	Stream	2.18 (1.03; 4.60)	3.31 (1.45; 7.58)	**0.005**
Litter management	Good	1.00	1.00	Ref
	Poor	1.03 (0.65; 1.64)	1.16 (0.67; 2.01)	0.59
	Fair	0.55 (0.38; 0.80)	0.67 (0.44; 1.02)	0.06
Pen odour	No	1.00	1.00	Ref
	Yes	1.26 (0.94; 1.69)	1.56 (1.12; 2.18)	**<0.01**
Adherence to vaccination (*Fowl typhoid and fowl cholera (pullorum)*)	Yes	1.00	1.00	Ref
	No	8.33 (3.49; 19.84)	5.18 (1.96; 13.66)	**<0.001**
	Partial	5.09 (2.07; 12.51)	5.10 (1.85; 14.04)	**0.002**
Implementation and adherence to biosecurity	Yes	1.00	1.00	Ref
	No	2.08 (1.26; 3.41)	1.54 (0.87; 2.72)	0.14
	Partial	1.14 (0.67; 1.94)	0.73 (0.40; 1.33)	0.31

* *P*-values were obtained through Wald test. Bold *P*-values were significant. Akaike information criterion (AIC) = 945.52; Hosmer–Lemeshow goodness of fit = $X^2 = 2.58$; *p*-value = 0.96; area under curve (receiver operating characteristics (ROC)) = 0.72.

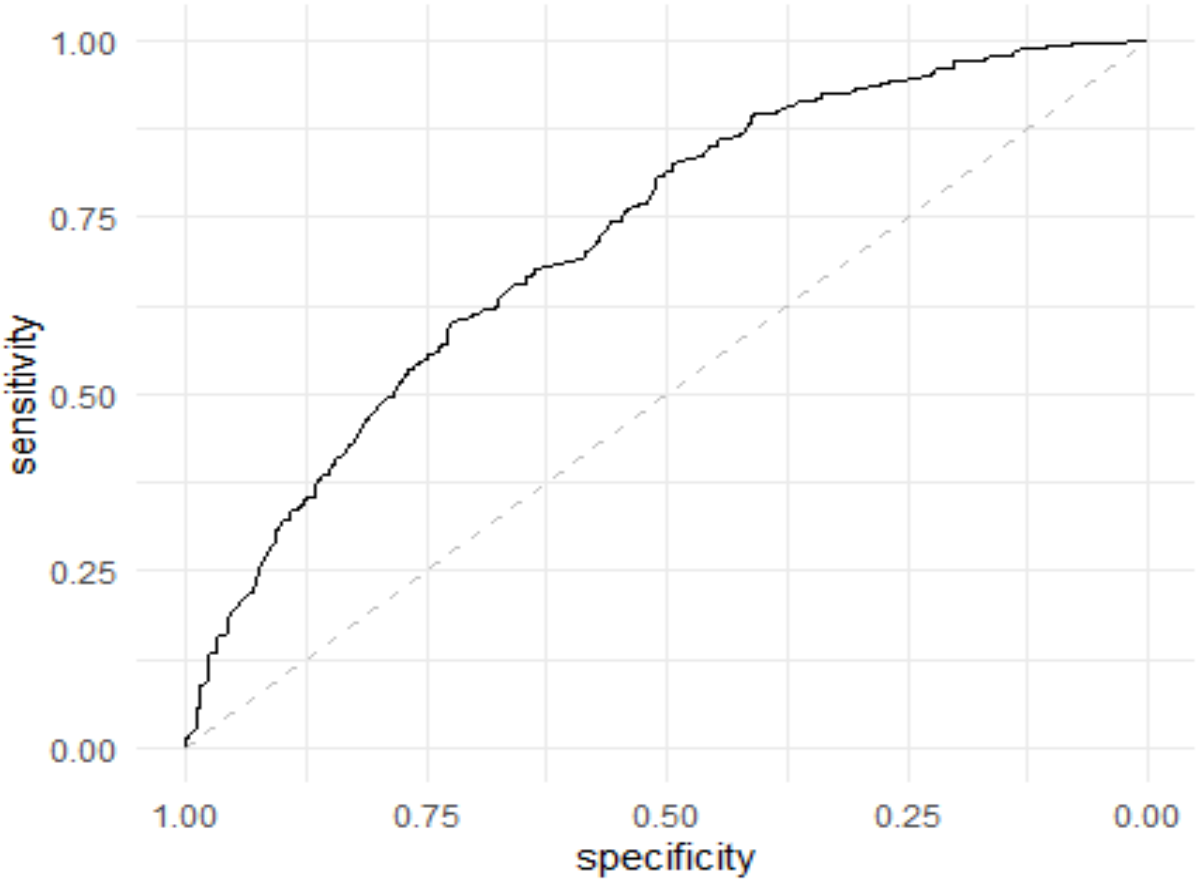

Figure 6.2. Receiver operating characteristics of risk factor model for persistent infection of non-typhoidal *Salmonella* on poultry farms, North Central Nigeria. The ROC curve (solid curve) performed better than the diagonal line (dotted line) at 0.72, a reflection that the performance of the diagnostic test that is better than chance level.

6.4. Discussion

The total burden of zoonotic salmonellosis in humans or poultry in Nigeria is unknown [3,18–25]. NCN serves the Federal Capital Territory and burgeoning neighbourhoods with food, including animal-sourced food. In this regard, this work is timely and meets the need to prevent food-borne zoonoses and related infections in the North Central belt of Nigeria (Figure 6.1; [32]). In this study, bacteria culture and phenotypic and biochemical characterization were used as the basis for identification and confirmation of non-typhoidal *Salmonella*. Culture and phenotypic and

biochemical characterization have been confirmed as very sensitive and specific for the identification of NTS, and they compare favourably with PCR and ELISA [2,33,34].

Although Klebsiella pneumoniae and other isolated organisms were incidental findings in this study, a recent report has documented the prevalence of Klebsiella pneumoniae in 41.7% of healthy poultry [35]. Klebsiella pneumoniae is an opportunistic pathogen, and a commonly isolated cause of nosocomial infections in humans, together with five other bacteria, referred to as the ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp) [36]. It is unsurprising that it was the most isolated pathogen in this study because other studies have confirmed that K. pneumoniae may cause disease in poultry and may co-habit with *Salmonella* spp. and be resistant to extended-spectrum beta-lactamase (ESBL) and carbapenemase antimicrobials, some of which may be passed onto the human food chain, causing resistant pathogens in humans [27,37–38]. In Trinidad and Tobago, 23 different *Salmonella*e have been found in broiler production with a prevalence of between 8.9 and 20.5% [5]. Similarly, in a recent survey in Great Britain involving 23 commercial broiler hatcheries, a prevalence of between 0 and 35% was obtained for the chick-handling areas, hatcher areas, macerator areas, tray wash/storage areas, external areas and other waste-handling areas, which are more contaminated in hatchery operations [40].

The prevalence of NTS in the surveyed smallholder poultry farms was 41.6% based on laboratory findings and following clinico-pathological evaluations over a period of 18 months. This prevalence was similar to previous findings from Nigeria by Jibril et al. [27] and Fagbamila et al. [21,41], who previously reported a farm-level prevalence of 47.9% and 43.6% in Nigeria. We obtained samples from broiler and layer farms but did not consider the hatcheries and

parent/grandparent farms. These latter farms need special permission to access and may have to be considered separately in a specialized study. Such a study may ascertain whether there are linkages between hatcheries and parent/grandparent farms on one hand and commercial farms on the other hand, particularly in the transmission and dispersal of NTS in the poultry food chain [42–44]. The weak correlations among the risk factors observed in the study meant that most of the factors considered cannot predict other factors and anthropogenic influence may affect how each factor plays a role. However, the awareness of *Salmonella* was moderately correlated with having experienced *Salmonella* on the farm (Table 3), an indication that previous or current experience of NTS on the farm is a positive predictor for awareness of *Salmonella* infection.

In our observation, the source of water and litter materials varied from farm to farm, and there was wide disparity in adherence to sanitary practices (Table 2). These sources, especially when they come from untreated sources, predispose farms to infection. Extension agents were confirmed as significant sources of knowledge for the farmers in this study (86%), and access to veterinary professionals and paraprofessionals was not always guaranteed (33.9%); thus, extension agents could be used as agents of change in risk communication and community engagement with regard to awareness and targeted messaging to farmers about the risk of poultry salmonellosis. For effectiveness and efficiency, the extension agents would need to be trained appropriately in relevant animal health matters, as anecdotal evidence revealed that most of the extension agents were skewed towards plant production and health.

It should be noted that the pathogen population increases with farm intensification and crowding of poultry per unit space [45]; thus, it is not surprising that the more chickens there were on the poultry farms, the higher the odds of infection with NTS were (Table 4). Similarly, the use of stream water as a source of drinking water for chickens increased the risk of infection with NTS

by 3-fold. It is highly likely that stream water is perpetually contaminated and its use without treatment would predispose poultry farms to infection. Farms are encouraged to pretreat stream water for use on their farms. While it is expected that ground water would increase the risk [46], the well water decreased the risk by half (Table 4). We are aware that most dug-up well are regularly treated with chlorine, and this may have positively influenced the reduction in the burden of risk observed in this case. We confirmed that the odds of NTS infection through feed was slightly high. Other workers [47] have recently confirmed that the incidence of NTS (S. enterica) in poultry feed and feed ingredients may range from 0 to 78%, and these may serve as a source of infection on poultry farms. Pen odour increased the risk by almost two-fold, which is more an indication of the poor hygiene practices and poor litter management on the farm rather than a risk factor itself. It is therefore important to advocate for better litter management and good farm hygiene practices to mitigate against infection with NTS.

Most importantly, the non-adherence to pullorum and fowl typhoid vaccinations (AOR = 5.2) and partial adherence to vaccinations (AOR = 5.1) both significantly increased the risk of infection with NTS infection in poultry. It is confirmed that vaccination against *Salmonella* infection in poultry is not capable of eradicating infection from flocks but only offer an extra layer of protection, increase the threshold for infection, reduce the level of shedding of the organism and reduce vertical transmission in poultry, thus preventing contamination of hatching or table eggs [2]. The advantage of such vaccinations in reducing the risk of NTS in smallholder poultry farms is obvious. However, we advocated for support with other practices as emphasized in the standard protocol for control and eradication of NTS in poultry [2]. In this work, only 64.4% of farms adhered to vaccination protocol, and only 55.5% of the farmers implemented and adhered to biosecurity practices, and only 27% of the farmers adhered to the protocol of culling of

infected flocks. However, a number of surveyed farmers continued to practice non-recommended practices against NTS eradication, including the administration of antibiotics (0.7%), vaccinations (36.9%), a combination of antibiotics and vaccination (11.5%) and the sale of infected poultry to consumers (13.2%). These practices are likely to further horizontal transmission of NTS to other farms and increase the risk for zoonosis. (Tables 6.2 and 6.4).

We are aware that this work is subject to some limitations. Firstly, complete serotyping of all classified positive cases was not performed, as this may have revealed all the serotypes of *Salmonella*e harvested over the 18 -month period. While full serotyping may be beneficial research-wise, and to inform policy, it should be noted that serotyping for *Salmonella* is a relatively expensive procedure, and smallholder poultry farms may consider this too burdensome to bear financially. Perhaps the authorities may consider covering the full cost of diagnosis for smallholder farms with cases of NTS. Secondly, several laboratories were utilized to determine the positivity for NTS, and not all farm cases were submitted for laboratory evaluation, some of which may have been salmonellosis. This potentially exposed the study to misclassification, a situation that may have increased/decreased the total prevalence determined in the study.

6.5. Conclusions

NTS continues to challenge poultry farms in North Central Nigeria, and some risk factors contributing to farm infection have been identified. Farm practices must be mitigated intentionally, and biosecurity and hygiene must be improved in order to reduce the burden of NTS. Finally, full compliance with vaccination protocols against pullorum and fowl typhoid in poultry combined with other control measures would assist in eradicating infection with NTS from poultry flocks in Nigeria.

Supplementary Materials: Supplementary Table 6.1: Categorization of variables based on selected industry standards and peer-reviewed literature; Supplementary Material 6.2: Sample questionnaire for risk factor data collection in the field.

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CHAPTER SEVEN

7.0 MOLECULAR EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE PATTERNS OF NON-TYPHOIDAL *SALMONELLA* SPP FOUND IN POULTRY FARMS, NORTH CENTRAL NIGERIA

This manuscript is being drafted with the title, '***Salmonella enterica* isolates from poultry, North-Central Nigeria reveal multi-drug resistant patterns and risk of contamination to the human food chain**' for submission to ***Frontiers in Veterinary Microbiology***.

Abstract (298 words)

Background: The non-typhoidal salmonellosis (NTS) is a significant poultry disease, manifesting as fowl cholera and fowl typhoid. It is a widely prevalent bacterial zoonosis with under-appreciated public health consequences. We undertook environmental sampling (poultry faeces and dusts in the poultry environment) to evaluate the prevalence of non-typhoidal *Salmonella* spp. in North Central Nigeria (NCN).

Methods: 600 samples were collected from 5/6 of the NCN's states and the federal capital territory, processed using standard bacterial culture and *invA*-based PCR method. Isolates obtained were tested against 11 most used antimicrobials in poultry using Kirby–Bauer disk diffusion methods. Inhibition zones were measured using WHONET version 5.6 to classify isolates as resistant, intermediate and sensitive. Statistical analysis was conducted to analyse results.

Results: An overall prevalence rate of 18.7% (95%CI: 15.8 to 22.0) (112/600) was obtained using classical bacteriology and molecular analysis. Prevalence in dusts and faeces were 20.5% (95%CI: 16.3 to 25.5) and 17.1% (95%CI: 12.1 to 23.5) respectively. Prevalence was lower in battery cages than in deep litter system, in flock > 1,000 birds compared to those less than 1,000, in older birds (> 52 weeks) versus younger birds, and in layer farms compared to in broiler farms. The odds of infection with non-typhoidal *Salmonella* spp. are at least 2 folds higher in younger birds, and in Niger state compared to other states. Isolates were most resistant to commonly used antimicrobials: tetracycline (73.8%), nalidixic acid (59.5%), sulphonamides (54.8%), and ciprofloxacin (47.6%), and most sensitive to ceftazidime (88.1%) and cefotaxime (78.6%). We observed single-resistant, multidrug-resistant, extensively drug-resistant and a pandrug-resistant isolate.

Conclusion: The research outcome indicates that the poultry industry use and abuse of antimicrobial is a key driver that increases risk of AMR. Animal health authority must implement stricter control on access to antimicrobials to mitigate AMR pathogens, likely to enter and complicate human food chain with health and economic implications.

Keywords: non-typhoidal *Salmonella* spp.; multidrug resistant; antimicrobial resistance; Nigeria; dust; poultry faeces.

7.1. Introduction

Salmonellosis is a global pathogenic bacterial zoonosis with significant public, animal and environmental health impacts (Ao et al., 2015; WHO; 2015). The *Salmonella*e serovars are broadly divided into typhoidal and non-typhoidal *Salmonella* (NTS) serovars (Brenner et al., 2000; Gal-Mor et al., 2014; Sanni et al., 2022). Both typhoidal and NTS can cause food-borne diseases, diarrhoea and associated complications (Batz et al, 2012; Gal-Mor et al., 2014; Ao et al., 2015). In Nigeria, poultry associated NTS are widespread and are spread by pathogens including *Salmonella enterica* subspecies *enterica* serovar Enteritidis and Typhimurium primarily, as well as other serovars such as *S. Newport*, *S. Abadina, S. Schwarzengrund, S. Takoradi, S. Telelkebir, S. Kentucky, S. Poona, S. Isangi, S. Heidelberg, S. Saintpaul, S. Nigeria, S. Virchow, S. Laroche* and *S. Javiana* among others (Feasey at al., 2012; Fagbamila et al., 2017; Jibril et al., 2020; WHO, 2021). The farm level prevalence of NTS in poultry may range from 39.7 - 48.3% (Fagbamila et al., 2017; Jibril et al., 2020). In various African countries, prevalence of *Salmonella* in poultry have ranged from 12.1% to 100% (Ramtahal et al., 2022). These pathogens do colonize poultry host and may carry asymptomatic infection, with consequent transmission to human through the food chain or by contamination, especially the *Salmonella enterica* serotype Enteritidis and Typhimurium (Feasey at al., 2012; WHO, 2021). For instance, the non-typhoidal *Salmonella* enterica infections has been confirmed as the pathogen with the largest burden of diarrheal disease and invasive infections globally (Kirk et al., 2015). In the USA, *Salmonella* spp. remained the top-ranked foodborne pathogens, attracting the heaviest economic and health impacts among the foodborne illnesses (Batz et al., 2011). In the European Union, the non-typhoidal *Salmonella* spp. is the third leading causes of foodborne illnesses and are responsible for the majority of foodborne-associated deaths (WHO, 2017).

In Africa, bacterial gastroenteritis has been identified as the most common disease and high burden of salmonellosis has been acknowledged (Feasey at al., 2012; Kirk et al., 2015; Smith et al., 2016). Based on a recent review, the overall prevalence of *Salmonella*-associated gastroenteritis (SAG) in humans in Nigeria is 16.3% and for *Salmonella*-bacteraemia, it is 1.9% (Akinyemi et al., 2021); and over 53 *Salmonella* serotypes have been isolated from humans in Nigeria (Akinyemi et al., 2021). It is believed that this prevalence in humans may have been underestimated as health seeking behaviour for non-rapidly fatal illnesses in formal health facilities is a last option only after self-treatment has failed (Uzochukwu and Onwujekwe, 2004; Omolase et al., 2007; Wegbom et al., 2021).

Furthermore, because the environment plays a huge role in the introduction and transmission of salmonellosis, it is important to evaluate critically *Salmonella* pathogens in poultry environments (Chinivasagam et al., 2009; Pal et al., 2022; Ramtahal et al., 2022). In this work, we collected poultry faeces and dusts from poultry environments in North Central Nigeria, and evaluate their potentials to carry NTS, including the drug resistant patterns.

7.2. Materials and methods

7.2.1. Ethical Considerations

Poultry farmers were notified of the need to collect poultry faecal samples and drag swabs of dusts from their premises, and oral consent was obtained. The protocol for the work was part of the protocol and ethical approval of the Research Ethics Committee of the Faculty of Veterinary Science, University of Pretoria, with ethical approval number REC 142-22 (July 2023). It also got additional approval from the Federal University of Technology, Minna's Ethical Review Committee with approval number: 000030, May 2022. The work was endorsed through a notification to the Veterinary Teaching Hospital (VTH) of Usmanu Danfodiyo University, Sokoto (UDUS). Sequel to the approvals, feacal and dust samples were collected from broilers and layers flocks of various ages.

7.2.2. Study area

The study was conducted in North-Central Nigeria (Figure 7.1). With a population of about twenty million (20 million), the region has a land mass of two hundred and forty-two thousand, four hundred and twenty-five square kilometer (242,425km^2). The region has an estimated indigenous

and exotic poultry population of 44,789,854 in 2020 approximately 20% of the poultry population in Nigeria (Sanni et al., 2022). To estimate variables of *Salmonella*, at farm and state level, we used cross-sectional sampling in layers and broilers from various farms in six (6) states of the North Central, Nigeria. Poultry farms in Nigeria either raise birds using deep litter, battery cage or combination of both depending on the farm size, production type and age of the birds (Adene and Oguntade, 2008).

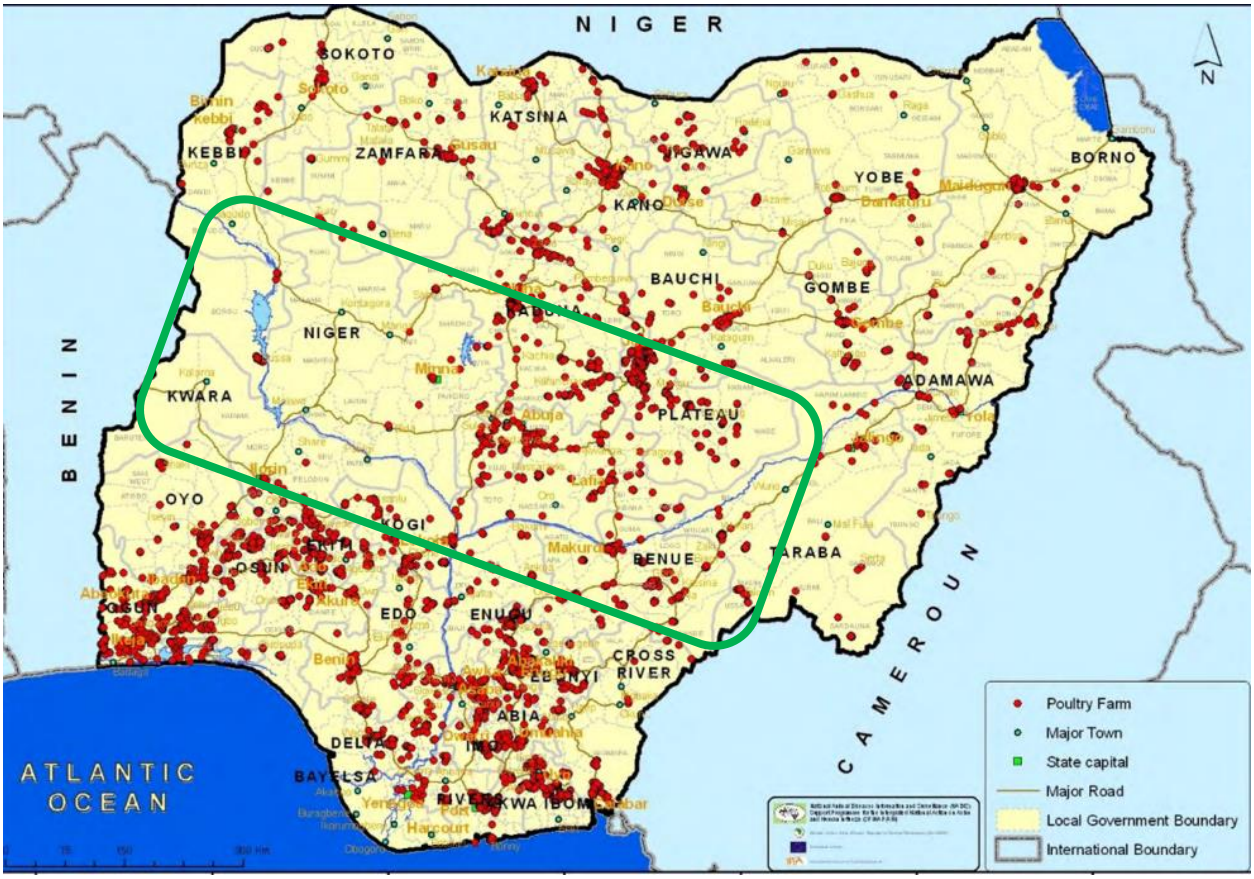


Figure 7.1. Rough spatial distribution of commercial poultry farms in Nigeria, (2022). The green square roughly represented the region where samples were collected from poultry farms.
Source: Federal Ministry of Agriculture and Rural Development, Abuja, Nigeria (2022).

7.2.3. Feacal and dust sample collection

Feacal and dust samples were secured randomly from numerous spots in poultry pens into aseptic sample container. Six hundred (600) feacal and dust samples (300 (or 299) feacal and 300 (or 297) dust samples and 4 mixed samples) representing approximately 50 feacal and 50 dust sample from each state. The sample containers were labelled accordingly and transported on ice packs to the molecular biology laboratory of the Department of Veterinary Public Health and Preventive Medicine, Usmanu Danfodiyo University Sokoto for microbiological analysis and sensitivity.

7.2.4. Salmonella isolation and identification

Cultural evaluation of samples for the presence of *Salmonella* was carried out in accordance with ISO6579-1 (International Standard horizontal method for the detection, enumeration and serotyping of Salmonella spp., 2022).

Concisely, one gram (1g) each of the feacal and dust sample was weighed and introduce into a 9 ml of buffered peptone water (BPW, Oxoid UK) for wide-reaching pre-enrichment of samples at 37°C for 18-24 hrs. Subsequently, selective pre-enrichment was done by transferring an aliquot of 0.1ml of the suspension from the overnight culture mixture into 10mls of Rappaport-Vassiliadis (RV) broth (Oxoid, UK) overnight at 41.5°C. Lastly, selective plating was done from the RV mix in a parallel striking on a Xylose Lysine Deoxycholate, (XLD) (Oxoid, UK) and subsequently onto Brilliance *Salmonella* Agar, (BSA) (Oxoid, UK) at 37°C the plates were incubated overnight. The plates were thereafter examined for the presence of colonies with black center and pinkish background typical for *Salmonella* on XLD and BSA. For every sample unit, one isolates was collected from a pure culture and spiked into selected samples for maintenance and quality control. *Salmonella* ATCC 14028 was used as quality control strain.

Using Biochemical test (commercially available media, Oxoid, UK), presumptive *Salmonella* isolates were tested. A loopful of colonies was stabbed into citrate and sulphide, indole, motility

(SIM) agar, and incubated at 37°C overnight. Isolates showing positive citrate, H2S production, and motility but a negative indole reaction were categorized as presumptive *Salmonella* and sub-cultured onto Nutrient agar (Oxoid, UK) and incubated at 37°C overnight.

7.2.5. PCR-based *Salmonella* identification

For further confirmation of *Salmonella*, isolates that were positive from biochemical test were subjected to PCR identification using the *invA*-based method (Waghamare, 2018). Briefly, one to two bacterial colonies were suspended into 100 µL of molecular grade water (Gibco, Life technologies, USA) and subjected to boiling at 100°C for 10 min. The mixture was centrifuged (Eppendorf, AG Germany) at 12,000 rpm for 2 min. PCR was mixture done with PCR Master Mix (2X) (ThermoFisher, UK) containing buffers, dNTPs, Taq DNA polymerase, reaction buffer and MgCl2. In addition to 1 µL of sample DNA and 0.5 µL of the primers (TAG Copenhagen, Denmark) (100 µM) *invA* forward (5'GTGAAATTATCGCCACGTTCGGGCA3') and *invA* reverse (5'TCATCGCACCGTCAAAGGAACC3') in 25 µl final volume reaction. Amplification was performed using T100 Thermal cycler (BIORAD, USA) with 95°C for 5 min, 94°C for 30 sec, 63°C for 30 sec and 72°C for 2 min for 35 cycles. A final cycle at 72°C for 5 min was used (Tennant SM, 2010). Amplicons were visualized in 1.5% agarose gels stained with SafeView nucleic acid stain using GelDoc Go Imaging System (BIORAD, USA). Isolates that showed a band size of 284 bp was considered *Salmonella* using 100 bp standard DNA ladder (TransGen Biotech, China). The reference strain *Salmonella* ATCC 14028 was used as positive control and water without DNA as negative control.

7.2.6. Sensitivity testing of isolates

Confirmed *Salmonella enterica* isolates by PCR was subjected to a panel of 11 commonly used antimicrobials that include ampicillin (10µg), gentamicin (10µg), ciprofloxaxin (5µg), chloramphenicol (30µg), cefotaxime (30µg), ceftazidime (10µg), kanamycin (30µg), sulphonamides (300µg), trimethoprim (5µg), tetracycline (30µg) and nalidixic acid (30µg), (Oxoid, UK). The tested antimicrobials covered seven classes including the aminoglycosides (kanamycin and gentamicin), quinolones (nalidixic acid and ciprofloxacin), cephems (cephalosporin I – IV) (ceftazidime and cefotaxime), penicillins (ampicillin), tetracyclines (tetracycline), phenicols (chloramphenicol) and the folate pathways antagonists (sulphonamides and trimethoprim). Kirby–Bauer disk diffusion methods was used in accordance with the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2020). Inhibition zone was entered into WHONET version 5.6 configured with the tested antimicrobials. Isolates was categorized as sensitive, intermediate or resistant using CLSI clinical breakpoints and CLSI guidelines for disc diffusion (Clinical and Laboratory Standards Institute 2022). Strains that showed resistant to at least one drug in at least three different antimicrobial classes was categorized as multidrug-resistant (MDR), extensively drug-resistant (XDR) or and pandrug-resistant bacteria (PDR) according to (Magiorakos et al. 2012).

7.2.7. Data analysis

Based on confirmation of *Salmonella enterica* using culture and biochemical tests, the exact measure of association was calculated using 2X2 table statistics in OpenEpi (Dean et al., 2013). The Odds-based estimates and confidence limits were calculated according to the method of Martin and Austin, (1991).

7.3. Results

7.3.1. Description of environmental samples

A total of 600 samples including 102 (17.0%) (95%CI: 14.2 to 20.2) each from Kogi and Nasarawa states, 100 (16.7%) (95%CI: 13.9 to 19.9) each from Benue and Kwara, 98 (16.3%) (95%CI: 13.6 to 19.5) each from Niger state and the federal Capital Territory, Abuja (Table 7.1). Almost half (49.8%) (95%CI: 45.9 to 53.8) of the samples originated from faeces and 49.5% (95%CI: 45.5 to 53.5) were dust samples from the poultry environments, with 0.7% as mixed/unclassified samples (Table 7.1). These samples were collected from birds housed in battery cages and high-rise buildings (36.3%) (95%CI: 32.6 to 40.3), deep litters (63.2%) (95%CI: 59.1 to 66.8) and unspecified type of houses (0.5%). Based on bird types, 358 of the samples were from broilers (59.7%) (95%CI: 55.7 to 63.5), 228 (38.0%) (95%CI: 34.2 to 42.0) from laying birds, and 10 (1.7%) from Noiler (dual-purpose breed (for both eggs and meat) of chicken developed for the Nigerian poultry sector) (Table 7.1). The remainder 4 flocks (0.7%) were not specified. Although the flock sizes (n = 15 – 50,000) and ages (1 week up to 3 years) were widely varied, the median flock size and age were 195 and 8 weeks respectively. Using classical bacteriological culture and biochemical tests, 112/600 (18.7%) (95%CI: 18.7 to 22.0) of the samples were positive including 61 from dusts and 51 from faecal samples. The positive samples were confirmed using *invA*-based PCR method (supplementary figure 7.1).

The disaggregated category level prevalence ranged from 14.7% for Kogi and Nasarawa states (95%CI: 9.0 to 23.0) to 29.6% (95%CI: 21.4 to 39.3) for Niger State (Table 7.1). The prevalence in the faeces (17.1%) (95%CI: 13.2 to 21.8) was less compared to the one from dusts (20.5%) (95%CI: 16.3 to 25.5). A higher prevalence was obtained from the deep litter system (20.9%) (95%CI: 17.1

to 25.3) compared to in the battery cages (15.1%) (95%CI: 11.0 to 20.5) (Table 7.1). Farms with ≤ 1,000 birds have higher prevalence (20.2%) (95%CI: 16.6 to 24.4) than farms with > 1,000 birds (17.1%) (95%CI: 12.1 to 23.5) and poultry stock less than or equal to one year have higher prevalence (20.2%) (95%CI: 16.9 to 23.8) than those older than one year (10.3%) (95%CI: 5.1 to 19.2) (Table 7.1).

Table 7.1. Percentage of antimicrobial resistance isolates recovered from poultry farms, North Central, Nigeria.

Samples (n)	Categories (n)	Positive (%)	Negative (%)	Odds ratio	CMLE ORs	p-value(2-tail)
Sample state of origin (600)	Abuja (FCT) (98)	16 (16.3)	82 (83.7)	1.13	0.52, 2.47	0.76
	Benue (100)	19 (19.0)	81 (81.0)	1.36	0.64, 2.90	0.42
	Kwara (100)	18 (18.0)	82 (82.0)	1.27	0.60, 2.73	0.53
	Kogi (102)	15 (14.7)	87 (85.3)	1.0	Ref.	NA
	Nasarawa (102)	15 (14.7)	87 (85.3)	1.0	0.45, 2.20	1.00
	Niger (98)	29 (29.6)	69 (70.4)	**2.42**	**1.21, 4.99**	**0.01**
Sample type (596)	Faeces (299)	51 (17.1)	248 (82.9)	1.0	Ref.	NA
	Dust (297)	61 (20.5)	236 (79.5)	1.26	0.83, 1.90	0.28
Farm management type (596)	Battery cages (218)	33 (15.1)	185 (84.9)	1.48	0.95, 2.33	0.08
	Deep litter (378)	79 (20.9)	299 (79.1)	1.0	Ref.	NA
Flock size (580)	≤ 1,000 birds (410)	83 (20.2)	327 (79.8)	1.23	0.78, 1.99	0.38
	> 1,000 birds (170)	29 (17.1)	141 (82.9)	1.0	Ref.	NA
Bird type/ purpose (596)	Broiler/Noiler* (368)	77 (20.9)	291 (79.1)	1.46	0.94, 2.28	0.09
	Layers (228)	35 (15.4)	193 (84.6)	1.0	Ref.	NA
Age of birds (594)	≤ one year (516)	104 (20.2)	412 (79.8)	**2.21**	**1.07, 5.06**	**0.03**
	> one year (78)	8 (10.3)	70 (89.7)	1.0	Ref.	NA

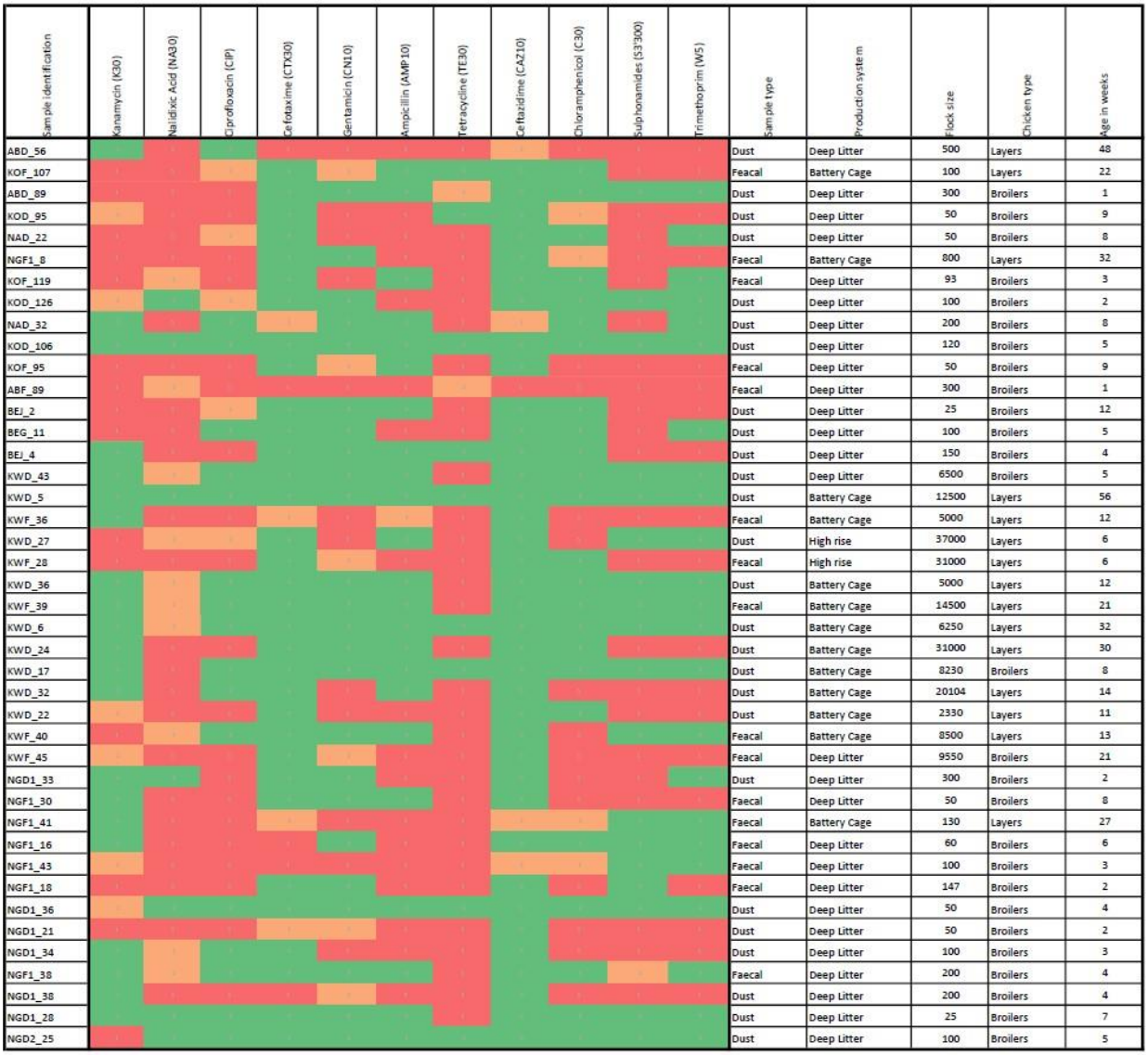
*Noiler is a dual-purpose breed (for both eggs and meat) of chicken developed by Amo Farm Sieberer Hatchery Ltd. (ASFH) in Nigeria; CMLE ORs = Conditional maximum likelihood estimates of Odds Ratio; Ref. = Reference; NA = Not applicable.

7.3.2. Association of environmental samples with odds of culture positivity

Among the states sampled, Niger state has 2.4 folds odds of non-typhoidal *Salmonella* spp. culture positivity compared to the other states, and poultry birds less than one year old have 2.2 folds odds of non-typhoidal *Salmonella* spp. culture positivity compared with birds older than one year. All other parameters were comparable to the references in the analysis (Table 7.1).

7.3.3. Antimicrobial resistance pattern

Of the 42 isolates tested by PCR and for antimicrobial sensitivity, only 2 (KWD_5 and KOD_106; 4.8%) have no resistance to any antimicrobial while 95.2% have resistance to at least one antimicrobial. The resistant organisms were from dust (24/40; 60%) or faeces (16/40; 40%) (Figure 7.2). In addition, 35% of the resistant *Salmonella* were from laying flocks while 65% were from broilers. The prevalence of AMR organisms was irrespective of farm sizes (minimum = 25; median = 200; maximum = 37,000), ages of chicken flock (minimum = 1 week; median = 7.5 weeks; maximum = 56 weeks) or states in North Central Nigeria (Figure 7.2). Furthermore, though we have four (4) single antimicrobial resistant isolates, several multidrug-resistant and extensively drug-resistant isolates were obtained as well as a pandrug-resistant isolate (ABF_89) (Figure 7.2). Based on the resistance pattern, the isolates were most resistant to tetracycline (TE30) (73.8%), nalidixic acid (NA30) (59.5%), sulphonamides (S3'300) (54.8%), ciprofloxacin (CIP) (47.6%), trimethoprim (W5) (45.2%), ampicillin (AMP10) (42.9%), kanamycin (K30) (35.7%), chloramphenicol (C30) (33.3%), gentamicin (CN10) (28.6%) , and most sensitive to ceftazidime (CAZ10) (88.1%) and cefotaxime (CTX30) (78.6%) (Figure 7.2, Table 7.2).



Sample identification	Kanamycin (K30)	Nalidixic Acid (NA30)	Ciprofloxacin (CIP)	Cefotaxime (CTX30)	Gentamicin (CN10)	Ampicillin (AMP10)	Tetracycline (TE30)	Ceftazidime (CAZ10)	Chloramphenicol (C30)	Sulphonamides (S3300)	Trimethoprim (W5)	Sample type	Production system	Flock size	Chicken type	Age in weeks
ABD_56												Dust	Deep Litter	500	Layers	48
KOF_107												Feacal	Battery Cage	100	Layers	22
ABD_89												Dust	Deep Litter	300	Broilers	1
KOD_95												Dust	Deep Litter	50	Broilers	9
NAD_22												Dust	Deep Litter	50	Broilers	8
NGF1_8												Faecal	Battery Cage	800	Layers	32
KOF_119												Feacal	Deep Litter	93	Broilers	3
KOD_126												Dust	Deep Litter	100	Broilers	2
NAD_32												Dust	Deep Litter	200	Broilers	8
KOD_106												Dust	Deep Litter	120	Broilers	5
KOF_95												Feacal	Deep Litter	50	Broilers	9
ABF_89												Feacal	Deep Litter	300	Broilers	1
BEJ_2												Dust	Deep Litter	25	Broilers	12
BEG_11												Dust	Deep Litter	100	Broilers	5
BEJ_4												Dust	Deep Litter	150	Broilers	4
KWD_43												Dust	Deep Litter	6500	Broilers	5
KWD_5												Dust	Battery Cage	12500	Layers	56
KWF_36												Feacal	Battery Cage	5000	Layers	12
KWD_27												Dust	High rise	37000	Layers	6
KWF_28												Feacal	High rise	31000	Layers	6
KWD_36												Dust	Battery Cage	5000	Layers	12
KWF_39												Feacal	Battery Cage	14500	Layers	21
KWD_6												Dust	Battery Cage	6250	Layers	32
KWD_24												Dust	Battery Cage	31000	Layers	30
KWD_17												Dust	Battery Cage	8230	Broilers	8
KWD_32												Dust	Battery Cage	20104	Layers	14
KWD_22												Dust	Battery Cage	2330	Layers	11
KWF_40												Feacal	Battery Cage	8500	Layers	13
KWF_45												Feacal	Deep Litter	9550	Broilers	21
NGD1_33												Dust	Deep Litter	300	Broilers	2
NGF1_30												Faecal	Deep Litter	50	Broilers	8
NGF1_41												Faecal	Battery Cage	130	Layers	27
NGF1_16												Faecal	Deep Litter	60	Broilers	6
NGF1_43												Faecal	Deep Litter	100	Broilers	3
NGF1_18												Faecal	Deep Litter	147	Broilers	2
NGD1_36												Dust	Deep Litter	50	Broilers	4
NGD1_21												Dust	Deep Litter	50	Broilers	2
NGD1_34												Dust	Deep Litter	100	Broilers	3
NGF1_38												Faecal	Deep Litter	200	Broilers	4
NGD1_38												Dust	Deep Litter	200	Broilers	4
NGD1_28												Dust	Deep Litter	25	Broilers	7
NGD2_25												Dust	Deep Litter	100	Broilers	5

Red colour = resistance, orange colour = intermediate and green colour = sensitive.

Figure 7.2. Heat map of antimicrobial resistance patterns of *Salmonella* isolates recovered from poultry farms, North Central, Nigeria.

Table 7.2. Percentage of antimicrobial resistance isolates recovered from poultry farms, North Central, Nigeria.

	Kanamycin (K30)	Nalidixic Acid (NA30)	Ciprofloxacin (CIP)	Cefotaxime (CTX30)	Gentamicin (CN10)	Ampicillin (AMP10)	Tetracycline (TE30)	Ceftazidime (CAZ10)	Chloramphenicol (C30)	Sulphonamides (S3'300)	Trimethoprim (W5)
Resistant (%)	35.7	59.5	47.6	11.9	28.6	42.9	73.8	2.4	33.3	54.8	45.2
Intermediate (%)	14.3	23.8	11.9	9.5	14.3	2.4	4.8	9.5	9.5	2.4	0.0
Sensitive (%)	50.0	16.7	40.5	78.6	57.1	54.8	21.4	88.1	57.1	42.9	54.8

7.4. Discussion

In this work, a total of 600 environmental samples (faeces and dusts) were collected and analyzed with an overall prevalence of 18.7% (112/600) from North Central Nigeria. The prevalence of NTS was similar across the states except for Niger state, which has a much higher prevalence compared to the others and a higher odd of isolation of NTS in poultry farms. While the immediate cause of this spatial distribution is unknown, the proliferation of unregulated marketing and hatcheries services, and the distribution therefrom may have assisted in dispersing poultry and hatchery associated NTS widely. Such observations have been made in other African countries (Ipara et al., 2019; RADARR, 2022). Jibril et al., (2020) and Samper-Cativiela et al., (2023) have earlier confirmed that relatively higher prevalence is detected in older layer flocks (>83 weeks) compared to younger flocks. This is contrary to our findings. Perhaps, our observation is linked with the higher prevalence and greater odds of *Salmonella* positivity in younger flocks compared to the older ones. It should be noted that broilers are slaughtered between 6-12 weeks while layer flocks stay much longer than a year in the farm.

Although, poultry and poultry products have been implicated as a major source of NTS for poultry farm infection and contamination of the human food chain (Wang et al., 2023), our results showed that the dust in the poultry environment may serve as a higher source of infection than poultry faeces. This is consistent with previous findings of Chinivasagam et al., (2009) and Pal et al (2021, 2022). Similarly, though insignificant, we observed slightly higher prevalence in the deep litter system compared to in battery cages. This is consistent with the findings of Jibril et al., (2020). However, a number of studies have also confirmed that battery-caged raised poultry may have higher odds of shedding of *Salmonella* organisms (Van Hoorebeke et al., 2011). Farms with

≤ 1,000 birds have higher prevalence (20.2%) than farms with > 1,000 birds (17.1%) and poultry stock less than or equal to one year have higher prevalence (20.2% than those older than one year (10.3%) (Table 7,1).

The pattern of resistance observed in this study aligns closely with previous reports on the use of antimicrobials in livestock farming in Nigeria, which have confirmed that the most used and abused antimicrobials in the poultry sector in Nigeria include tetracycline, tylosin, chloramphenicol, metronidazole, ciprofloxacin, gentamycin, and colistin (Adesokan et al., 2015; Oloso et al., 2018, 2020; Adebowale et al., 2020, 2022; Odey et al., 2024). Similar patterns of antimicrobial use and abuse prevail in other parts of Africa (Johnson et al. 2017; Gemeda et al., 2020; Gebeyehu et al., 2021; Mdegela et al., 2021; Paintsil et al., 2021). Only two of the 42 isolates were sensitive to all antimicrobial classes tested with four being resistant to one antimicrobial and the remainder 36 (85.7%) having multiple resistance across antimicrobial classes. This situation of multi drug resistance would continue to predominate in the poultry and other livestock sectors leading to many difficult to treat infections in the livestock industry with implications on higher rates of morbidity and mortality, reduced productivity, complications and long-term health impacts, treatment challenges and increasing health costs among others. Furthermore, since these are production animals, they may likely pass resistant pathogens through the human food chain. The result of this work calls for a relook at the policies and guidelines guiding antimicrobial use and surveillance in poultry and general livestock production, and a need for intensified effort to stringently control access to these antimicrobials.

7.5. Conclusion

This work revealed some important highlight useful for the poultry sector in Nigeria. One, Salmonellosis, especially the NTS is still prevalent in Nigerian poultry farms. While the national mean prevalence rate is ≈ 25%, the prevalences in the North Central Nigeria vary between 14.7% and 29.6%, irrespective of the type of housing. Secondly, both poultry and the poultry environment have the potential to contaminate other farms as well as the human food chain, hence the need to be more circumspect when dealing with poultry, poultry litter and its byproducts. It would appear that the use and abuse of antimicrobial is driving the increasing risk of antimicrobial resistance as the resistance pattern generated in this work closely match the most used antimicrobials used prevalently in the field, hence the need for the authority to implement stricter control on access to antimicrobials; otherwise, we risk the introduction of AMR pathogens into the human food chain with health and economic implications. Finally, this work provides empirical evidence for policy makers and implementers in the control of NTS in Nigeria.

7.6 Supplementary materials

Supplementary figure 7.1. Amplicons of *Salmonella* spp. (284 bp) with 100 bp standard DNA ladder visualized on stained 1.5% agarose gels. The reference strain *Salmonella* used as positive control was the ATCC 14028 and sterile water without DNA was used as negative control.

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CHAPTER EIGHT

8.0 SPATIAL DISTRIBUTION AND PREDICTIVE RISK OF PERPETUATION OF NON-TYPHOIDAL SALMONELLOSIS IN POULTRY FARMS AND HUMAN COMMUNITIES, NIGERIA

This manuscript is submited to ***Geospatial Health*** and undergoing review.

Abstract

Background: Salmonellosis in poultry and non-typhoidal salmonellosis (NTS) in humans are pathogenic bacterial zoonosis, which is widely prevalent in Nigeria. To understand the historical perspective, determine the prevalence and mitigate continued risk of salmonellosis in poultry and NTS in humans, the spatial and temporal distribution of prevalence and hotspots for risks of *Salmonella*, particularly the poultry-associated ones were determined.

Methods: Peer reviewed data, hospital record, laboratory data and District Health Information Software (DHIS) – 2 data were harmonized and filtered. The data were entered into meta-analytic excel tool and analysed to determine national and subnational prevalence of salmonellosis in poultry. Spatial and correlation analyses of NTS in humans and poultry were done using prevalence and diarrhoea data.

Results: The overall prevalence of salmonellosis in poultry was 31.6% with state-level prevalence highest in Ogun (70.2%), Lagos (61.8%), Zamfara (58.2%) and Bauchi (57.1%). Regionally, the North-West, South-West and South-South regions of Nigeria have the highest regional level prevalence of 38.5%, 36.9% and 33.6% respectively. Thirteen (13) states have higher than the national average prevalence (31.6%). Spatially, the prevalent pattern was similar to what was

determined statistically. The correlation analyses indicated that prevalence of NTS in humans negatively predicted salmonellosis in poultry, but prevalence of diarrhoea in humans positively predicted salmonellosis in poultry. Furthermore, reported prevalence of NTS in humans negatively predicted diarrhoea in humans, while prevalence of NTS in poultry was positively predicted by poultry populations.

Conclusion: The correlation patterns pointed to health data gaps. The humans NTS – poultry salmonellosis correlation was counterfactual to logic and plausibility as high poultry density and contamination in poultry is expected to predict human infection. Outcome points out underreporting linked to self-treatment, under-testing in the laboratory, and lack of uniform primary healthcare services in underserved areas of Nigeria. This work highlighted the continued burden of non-typhoidal salmonellosis in humans and poultry Nigeria and clear data gaps.

Keywords: Non-typhoidal Salmonella; prevalence; spatial distribution; Nigeria; foodborne zoonoses.

8. 1. Introduction

Salmonellosis is a pathogenic bacterial zoonosis and continues to impact the public and animal health substantially (Ao et al., 2015; WHO; 2015). The Salmonellae family has large number of identified serovars (> 2600) and its species are broadly divided into typhoidal and non-typhoidal Salmonella (NTS) (Gal-Mor et al., 2014; Sanni et al., 2022). Both typhoidal and NTS cause widespread food-borne diarrhoeal diseases, and in complicated situation, invasive NTS (iNTS) leads to major bloodstream infections universally (Batz et al, 2012; Gal-Mor et al., 2014; Ao et al., 2015). Though, it is a ubiquitous global health problem, a recent attempt at prioritizing zoonoses in Nigeria indicated that salmonellosis was viewed as a moderate zoonosis ranking low on severity and epidemic potentials but high to moderate on burden of diseases, ability of the health services to control, and socio-economic impacts, scoring 0.50 on a maximum scale of 1.00 (Ihekweazu et al., 2021). In Nigeria, human prevalence of salmonellosis and associated gastroenteritis may range from 5.7 - 16.3% (Akinyemi et al., 2021, Ihekweazu et al., 2021), with a Salmonella bacteremia of approximately 1.9% (Akinyemi et al., 2021). In poultry, farm level prevalence of NTS range from 39.7 - 48.3% (Fagbamila et al., 2017; Jibril et al., 2020; Ihekweazu et al., 2021) but individual poultry bird level prevalence may be less. Akinyemi and colleagues (2021) have earlier reported that a total of 53 Salmonella serotypes have been identified in humans in Nigeria including 39 associated with Salmonella-bacteremia and 31 associated with Salmonella-gastroenteritis. For instance, an estimate for the year 2020 in Nigeria indicated that NTS is grossly under-appreciated because whereas the industry experts perceived that its burden is not significant, and not rapidly fatal, it can potentially cause 325,731 cases and 1,043 human deaths in a single year, with a disability-adjusted life year (DALYs) of 37,321 (Sanni et al., 2023). Its economic burdens in humans

were US$ 473,982,068 and in poultry, it was US$ 456,905,311 for the year 2020 alone (Sanni et al., 2023). Similarly, in the USA, *Salmonella* spp. was the first-ranked foodborne pathogens, and it has the most significant cost of illness in billions of dollars and heavy QALY losses (Batz et al., 2011; Ihekweazu et al., 2021).

Nigeria is heavily populated with human and livestock resources, with a mid-2020 human population of 208,327,405 and poultry population of 224,326,708 (FMARD, 2020; UN-DESA, 2022; Sanni et al., 2023). The human and poultry population dynamics in Nigeria and elsewhere come with the increasing need for enormous animal-sourced foods, especially in the large cities. This situation has led to the multiplication of rural, peri-urban and urban farming, especially for the white meat, primarily poultry and pigs (Omodele and Okere, 2014; FMARD, 2022). Although there are statutory guidelines and Acts that regulate the industry, and which most large scale commercial operations may adhere to, many backyard poultry, semi-commercial farms, informal hatcheries, opaque operators in the poultry value chain, and vendors of chicken carcasses in outlets and informal markets may not comply fully with hygiene and biosecurity protocols, hence they may portend significant but inadvertent source of risk for the horizontal and vertical transmission of salmonella pathogens in the course of their operations (Awojulugbe, 2019; FAO, EU and CIRAD, 2022; Oloso et al., 2020; Mokgophi et al., 2021). Apart from salmonellosis from animal-sourced foods, NTS in humans may originate from fruits, seeded vegetables and other Produce (IFSAC, 2022).

To mitigate the scenario above, at least in the animal food value chain, the knowledge and understanding of the spatial and temporal distribution of hotspots for risk of Salmonella, particularly the poultry-associated ones, can assist in pre-emptive planning, and predictive

disease intelligence to control the disease and reduce the burdens associated with salmonellosis in Nigeria. Such information may also be useful for scenario planning elsewhere, particularly, those with similar poultry industry profile like Nigeria. In this work, historical and peer-reviewed information and grey literature from multiple data sources were utilized to map the current situation of salmonellosis in poultry and predict the risk of poultry-associated salmonellosis in the Nigerian poultry, and with possible zoonotic transmission to humans. Such outcome may assist the health authorities to focus informed decision and provide tools for control and reduce the burden of salmonellosis in poultry and humans.

8.2. Materials and methods

8.2.1. Data sources

Extensive data and peer-reviewed document search on poultry and human salmonellosis in Nigeria for the year 2000 - 2020 were conducted using available tool and following the Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) guidelines (Moher et al., 2015). Specifically, based on the search using the search term 'Salmonella', 'poultry', and 'Nigeria', published within the year January 2000 until December 2020 in *Publish or Perish* software (https://harzing.com/), relevant papers were retrieved from the following websites: 1. Crossref (1,000 papers), Google Scholar (200 papers), OpenAlex (689 papers), Pubmed (34 papers), Semantic Scholar (1,004 papers). Additional search was conducted in scholarly websites (Scopus and Web of Science), and using the artificial intelligence-linked applications 1) *Dimensions* research database (https://app.dimensions.ai/discover/publication) and 2) Connected Papers research database (https://www.connectedpapers.com/) to include additional

papers that may be missing using the original search. These publications were screened using the PRISMA-P principles (Figure 1 to retain 77 publications. All duplicates and non qualifying publications were removed henceforth. In total, 158,222 samples were retained yielding 8,279 positive isolates originating from hiumans and poultry, from 77 publications. For animal-level specific data, a total of 3,693 poultry-related *Salmonella* spp. isolates from 14,402 samples were retained (Supplementary material 8.1).

8.2.2. Inclusion and exclusion criteria

All full text of the search results from the above-mentioned databases including the abstracts were reviewed independently screened for inclusion or exclusion. Any article that mentioned *Salmonella* in humans and animals in poultry from Nigeria were recruited as candidates for full review. Prevalence data for inclusion were based on bacteria culture on human (blood or stool) or poultry (faeces, dust, environmental and pathological samples) to obtain *Salmonella* isolates in Nigeria, or other validated tests. All direct human typhoidal isolates were excluded in the analysis since only poultry-related *Salmonella* were of interest. Articles that did not mention Nigeria specifically, or simply comparing findings from other territories to previous studies from Nigeria were also excluded. All relevant articles were retrieved and thoroughly reviewed using inclusion and exclusion criteria set for this study.

8.2.3. Meta-analysis of cases in human and poultry

All data were individually checked and validated and entered into a spreadsheet. (Supplementary material 8.1). Using the previously validated and peer-reviewed meta-analytic Excel spreadsheet

(Neyeloff et al., 2012), all data from the reviewed studies were cumulated and plugged into the tool. The final state-level prevalence of salmonellosis in poultry were obtained and a national prevalence was also derived. Based on the result, a forest plot was produced for the national level data on poultry. All calculations are reflected in Supplementary material 8.2 (Figure 8.1, Screenshot below). Outcome (effect size, es) was calculated using the formula: the number of events divided by the number of subjects (D = B/C) in Excel. Standard Error (SE) was derived using the formula: E = D/SQRT(D*C) (Neyeloff et al., 2012).

Furthermore, using the data from poultry and humans, spatial distribution of salmonellosis in humans and poultry were created. To account for data gaps in humans, which may have arisen due to a number of reasons: 1) poor healthcare delivery in the underserved areas of the country, lack of laboratory services, abuse of antimicrobials before cause of diarrhoea is determined, and seeking hospitalization only when diarrhoea refused to resolve following home-level care (Uzochukwu and Onwujekwe, 2004; Adeyemi et al., 2021, Sanni et al., 2024), the proxy map for all diarrhoea cases was also drawn. Finally, correlation analysis was drawn to compare prevalences of salmonellosis (and diarrhoea) in human versus in poultry.

8.3. Results

8.3.1. Prevalence of poultry salmonellosis

The overall prevalence of salmonellosis in poultry was 31.6% (95%CI: 9.2 to 64.2) with differing state-level prevalence. While Ekiti, Nasarawa and Bayelsa have 8.0% (95%CI: 7.8 to 23.8), 9.6% and 9.7% respectively, Ogun, Lagos, Zamfara and Bauchi has the highest state-level prevalence of 70.2% (95%CI: 55.9 to 84.6), 61.8%, 58.2% and 57.1% respectively (Table 8.1). Regionally, the

North-West, South-West and South-South regions of Nigeria have the highest regional level prevalence of 38.5% (95%CI: 35.5 to 41.6), 36.9% (95%CI: 34.0 to 40.0) and 33.6% (95%CI: 30.7 to 36.6) respectively (Table 8.2). Based on the forest plot, 13 states, three regions and the multi-state evaluation were higher than the national average of 31.6% (95%CI: 9.2 to 64.2) (Figure 8.2).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
2	Prevalence of Salmonellosis in poultry, Nigeria	Events	Sample Size	Outcome (es)	SE	Var	w	w*es	w*(es²)	w²		w_v	w_v*es	w_v*(es²)	w_v^2
3	Abia	58	240	0.242	0.03	0.00	993.10	240	58	986254.46		58.39	14.11	3.41	3409.14
4	Anambra	102	220	0.464	0.05	0.00	474.51	220	102	225159.55		54.86	25.44	11.79	3009.91
5	Eboinyi	110	259	0.425	0.04	0.00	609.83	259	110	371889.30		56.31	23.91	10.16	3170.50
6	Enugu	84	458	0.183	0.02	0.00	2497.19	458	84	6235960.27		60.53	11.10	2.04	3664.05
7	Imo	57	412	0.138	0.02	0.00	2977.96	412	57	8868275.02		60.77	8.41	1.16	3692.89
8	Akwa-Ibom	48	366	0.131	0.02	0.00	2790.75	366	48	7788285.56		60.69	7.96	1.04	3682.80
9	Bayelsa	30	310	0.097	0.02	0.00	3203.33	310	30	10261344.44		60.86	5.89	0.57	3703.52
10	Cross-river	206	374	0.551	0.04	0.00	679.01	374	206	461054.18		56.84	31.31	17.24	3231.01
11	Edo	352	786	0.448	0.02	0.00	1755.10	786	352	3080383.99		59.92	26.83	12.02	3590.08
12	Delta	43	150	0.287	0.04	0.00	523.26	150	43	273796.65		55.46	15.90	4.56	3075.81
13	Rivers	11	22	0.500	0.15	0.02	44.00	22	11	1936.00		25.74	12.87	6.44	662.65
14	Ekiti	4	50	0.080	0.04	0.00	625.00	50	4	390625.00		56.43	4.51	0.36	3184.76
15	Ogun	92	131	0.702	0.07	0.01	186.53	131	92	34794.41		46.55	32.69	22.96	2167.18
16	Ondo	114	384	0.297	0.03	0.00	1293.47	384	114	1673074.17		59.20	17.57	5.22	3504.17
17	Osun	114	384	0.297	0.03	0.00	1293.47	384	114	1673074.17		59.20	17.57	5.22	3504.17
18	Oyo	81	366	0.221	0.02	0.00	1653.78	366	81	2734980.94		59.79	13.23	2.93	3575.11
19	Lagos	21	34	0.618	0.13	0.02	55.05	34	21	3030.24		29.17	18.01	11.13	850.68
20	Kogi	15	102	0.147	0.04	0.00	693.60	102	15	481080.96		56.94	8.37	1.23	3242.42
21	Niger	29	98	0.296	0.05	0.00	331.17	98	29	109675.17		52.25	15.46	4.58	2729.85
22	Nasarawa	305	3170	0.096	0.01	0.00	32947.21	3170	305	1085518852.03		61.92	5.96	0.57	3833.90
23	Kwara	18	100	0.180	0.04	0.00	555.56	100	18	308641.98		55.80	10.04	1.81	3114.07
24	Benue	117	688	0.170	0.02	0.00	4045.68	688	117	16367487.93		61.10	10.39	1.77	3732.99
25	Plateau	314	854	0.368	0.02	0.00	2322.66	854	314	5394760.72		60.42	22.22	8.17	3650.74
26	Taraba	96	500	0.192	0.02	0.00	2604.17	500	96	6781684.03		60.59	11.63	2.23	3671.36
27	Borno	130	525	0.248	0.02	0.00	2120.19	525	130	4495215.42		60.27	14.92	3.70	3632.67
28	Adamawa	39	196	0.199	0.03	0.00	985.03	196	39	970275.51		58.36	11.61	2.31	3405.86
29	Bauchi	12	21	0.571	0.16	0.03	36.75	21	12	1350.56		23.08	13.19	7.54	532.61
30	Gombe	3	11	0.273	0.16	0.02	40.33	11	3	1626.78		24.44	6.67	1.82	597.41
31	Yobe	114	384	0.297	0.03	0.00	1293.47	384	114	1673074.17		59.20	17.57	5.22	3504.17
32	F.C.T, Abuja	16	98	0.163	0.04	0.00	600.25	98	16	360300.06		56.22	9.18	1.50	3161.18
33	Jigawa	114	384	0.297	0.03	0.00	1293.47	384	114	1673074.17		59.20	17.57	5.22	3504.17
34	Kaduna	162	809	0.200	0.02	0.00	4040.01	809	162	16321649.88		61.10	12.23	2.45	3732.84
35	Kano	21	45	0.467	0.10	0.01	96.43	45	21	9298.47		37.75	17.62	8.22	1425.04
36	Katsina	12	39	0.308	0.09	0.01	126.75	39	12	16065.56		41.65	12.82	3.94	1734.74
37	Kebbi	17	48	0.354	0.09	0.01	135.53	48	17	18368.22		42.56	15.07	5.34	1811.02
38	Sokoto	30	62	0.484	0.09	0.01	128.13	62	30	16418.15		41.80	20.23	9.79	1747.12
39	Zamfara	32	55	0.582	0.10	0.01	94.53	55	32	8936.16		37.46	21.79	12.68	1402.91
40	Multistate-NG	570	1267	0.450	0.02	0.00	2816.30	1267	570	7931525.93		60.70	27.31	12.28	3684.26
41															
42	k	38				Sums:	78962.57	14402	3693	1193523280.23		1993.50	589.19	220.59	109529.74
43	df	37													
44												v	0.016119909		
45	Q	1066.216			Q_v	46.453									
46	I^2	96.530			I^2_v	20.349									
47															
48	es (fixed)	0.182			es (random)	0.296									
49	SEes (fixed)	0.004			SEes (random)	0.022									
50	CI (fixed)	0.175	0.189		CI (random)	0.252	0.339								

Figure 8.1. Screenshot of the calculations done to arrive at the state and national-level prevalence for Salmonellosis in poultry.
Data were meta-analysed using the previously validated Excel spreadsheet (Neyeloff et al., 2012). The spreadsheet is available as Supplementary material 8.2.

Table 8.1. Mean prevalence of Salmonellosis in poultry, per state, 2000 – 2020, Nigeria.

State	No.	Events	Sample Size	Outcome	SE	CI lower	CI upper	Rate	CI lower	CI upper
Abia	1	58	240	0.242	0.032	0.179	0.304	24.167	6.220	54.553
Anambra	2	102	220	0.464	0.046	0.374	0.554	46.364	8.998	101.725
Ebonyi	3	110	259	0.425	0.040	0.345	0.504	42.471	7.937	92.879
Enugu	4	84	458	0.183	0.020	0.144	0.223	18.341	3.922	40.603
Imo	5	57	412	0.138	0.018	0.102	0.174	13.835	3.592	31.262
Akwa-Ibom	6	48	366	0.131	0.019	0.094	0.168	13.115	3.710	29.940
Bayelsa	7	30	310	0.097	0.018	0.062	0.131	9.677	3.463	22.818
Cross-river	8	206	374	0.551	0.038	0.476	0.626	55.080	7.522	117.682
Edo	9	352	786	0.448	0.024	0.401	0.495	44.784	4.678	94.246
Delta	10	43	150	0.287	0.044	0.201	0.372	28.667	8.568	65.902
Rivers	11	11	22	0.500	0.151	0.205	0.795	50.000	29.548	129.548
Ekiti	12	4	50	0.080	0.040	0.002	0.158	8.000	7.840	23.840
Ogun	13	92	131	0.702	0.073	0.559	0.846	70.229	14.351	154.809
Ondo	14	114	384	0.297	0.028	0.242	0.351	29.688	5.450	64.825
Osun	15	114	384	0.297	0.028	0.242	0.351	29.688	5.450	64.825
Oyo	16	81	366	0.221	0.025	0.173	0.270	22.131	4.820	49.082
Lagos	17	21	34	0.618	0.135	0.353	0.882	61.765	26.417	149.947
Kogi	18	15	102	0.147	0.038	0.073	0.221	14.706	7.442	36.854
Niger	19	29	98	0.296	0.055	0.188	0.404	29.592	10.770	69.954
Nasarawa	20	305	3170	0.096	0.006	0.085	0.107	9.621	1.080	20.323
Kwara	21	18	100	0.180	0.042	0.097	0.263	18.000	8.316	44.316
Benue	22	117	688	0.170	0.016	0.139	0.201	17.006	3.081	37.093
Plateau	23	314	854	0.368	0.021	0.327	0.408	36.768	4.067	77.603
Taraba	24	96	500	0.192	0.020	0.154	0.230	19.200	3.841	42.241
Borno	25	130	525	0.248	0.022	0.205	0.290	24.762	4.257	53.780
Adamawa	26	39	196	0.199	0.032	0.137	0.261	19.898	6.245	46.041
Bauchi	27	12	21	0.571	0.165	0.248	0.895	57.143	32.332	146.617
Gombe	28	3	11	0.273	0.157	-0.036	0.581	27.273	30.862	85.407
Yobe	29	114	384	0.297	0.028	0.242	0.351	29.688	5.450	64.825
F.C.T, Abuja	30	16	98	0.163	0.041	0.083	0.243	16.327	8.000	40.653
Jigawa	31	114	384	0.297	0.028	0.242	0.351	29.688	5.450	64.825
Kaduna	32	162	809	0.200	0.016	0.169	0.231	20.025	3.084	43.133
Kano	33	21	45	0.467	0.102	0.267	0.666	46.667	19.960	113.293

Katsina	34	12	39	0.308	0.089	0.134	0.482	30.769	17.409	78.948
Kebbi	35	17	48	0.354	0.086	0.186	0.523	35.417	16.836	87.669
Sokoto	36	30	62	0.484	0.088	0.311	0.657	48.387	17.315	114.089
Zamfara	37	32	55	0.582	0.103	0.380	0.783	58.182	20.159	136.523
Multistate-NG	38	570	1267	0.450	0.019	0.413	0.487	44.988	3.693	93.670
Summary				**0.316**	**0.005**	**0.307**	**0.326**	**31.634**	**0.921**	**64.190**

Note that for this analysis, there was no literature available for prevalence of Salmonellosis in poultry for Ondo, Osun, Yobe and Jigawa, hence, the national average was used for those four states.

Table 8.2. Summary Table of mean prevalence of Salmonellosis in poultry, per region and poultry population dynamics, 2000 – 2020, Nigeria.

Geopolitical Zones (Region), Nigeria	Regional Prevalence	Poultry Population Dynamics, 2020*		
		Indigenous	Exotic	Estimated total
South-East	29.04	8,682,064	22,439,738	30,933,716
South-South	33.55	2,466,606	11,757,575	14,224,181
South-West	36.92	3,347,106	23,388,375	26,735,481
North-Central + Abuja (FCT)	20.29	9,691,867	22,726,618	32,418,485
North-East	29.66	10,130,102	11,044,134	21,174,236
North-West	38.45	20,484,256	28,697,419	49,181,675

*Poultry population data were from the Federal Ministry of Agriculture and Rural Development (FMARD, 2020)

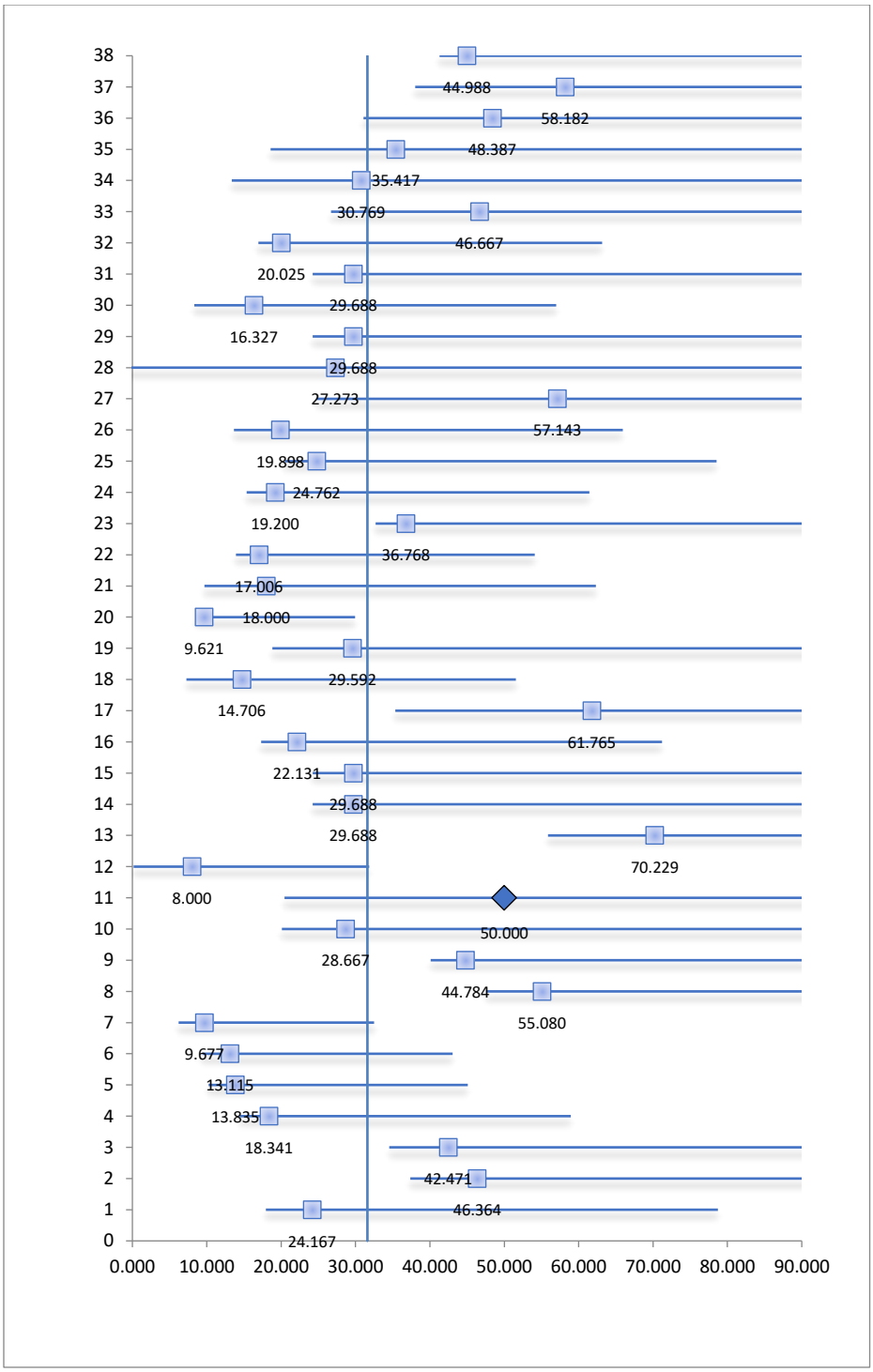

Figure 8.2. Forest plot of mean prevalence of non-typhoidal salmonellosis in poultry in the different state, Nigeria, 2000 – 2020. *Note that 1 = Abia, 2 = Anambra, 3 = Ebonyi, 4 = Enugu, 5 = Imo, 6 = Akwa-Ibom, 7 = Bayelsa, 8 = Cross-River, 9 = Edo, 10 = Delta, 11 = Rivers, 12 = Ekiti, 13 = Ogun, 14 = Ondo, 15 = Osun, 16 = Oyo, 17 = Lagos, 18 = Kogi, 19 = Niger, 20 = Nasarawa, 21 = Kwara, 22 = Benue, 23 = Plateau, 24 = Taraba, 25 = Borno, 26 = Adamawa, 27 = Bauchi, 28 = Gombe, 29 = Yobe, 30 = F.C.T, Abuja, 31 = Jigawa, 32 = Kaduna, 33 = Kano, 34 = Katsina, 35 = Kebbi, 36 = Sokoto, 37 = Zamfara, 38 = Multistate. Note that for this analysis, there was no literature available for prevalence of Salmonellosis in poultry for Ondo, Osun, Yobe and Jigawa, hence, the national average was used for those four states.*

8.3.2. Spatial spread of salmonellosis in poultry and humans

Based on the spatial map of prevalence of NTS and diarrhoea in humans and salmonellosis in poultry, some interesting patterns were observed. Only 21 of the 37 subnationals (states and the FCT) have NTS data for humans but 33 of the 37 subnationals have data for poultry (Fig. 8.3 a and b). The prevalent pattern was widely different between states, with the South-West, North-West and South-South displaying more endemicity than the three other regions, a pattern that is consistent with the outcome of the statistical analysis (Fig. 8.3 a – c). The correlation analyses indicated that prevalence of NTS in humans negatively predicted salmonellosis in poultry, but prevalence of diarrhoea in humans positively predicted salmonellosis in poultry. Furthermore, reported prevalence of NTS in humans negatively predicted diarrhoea in humans, while prevalence of NTS in poultry was positively predicted by poultry populations (Fig. 8.3 d – g).

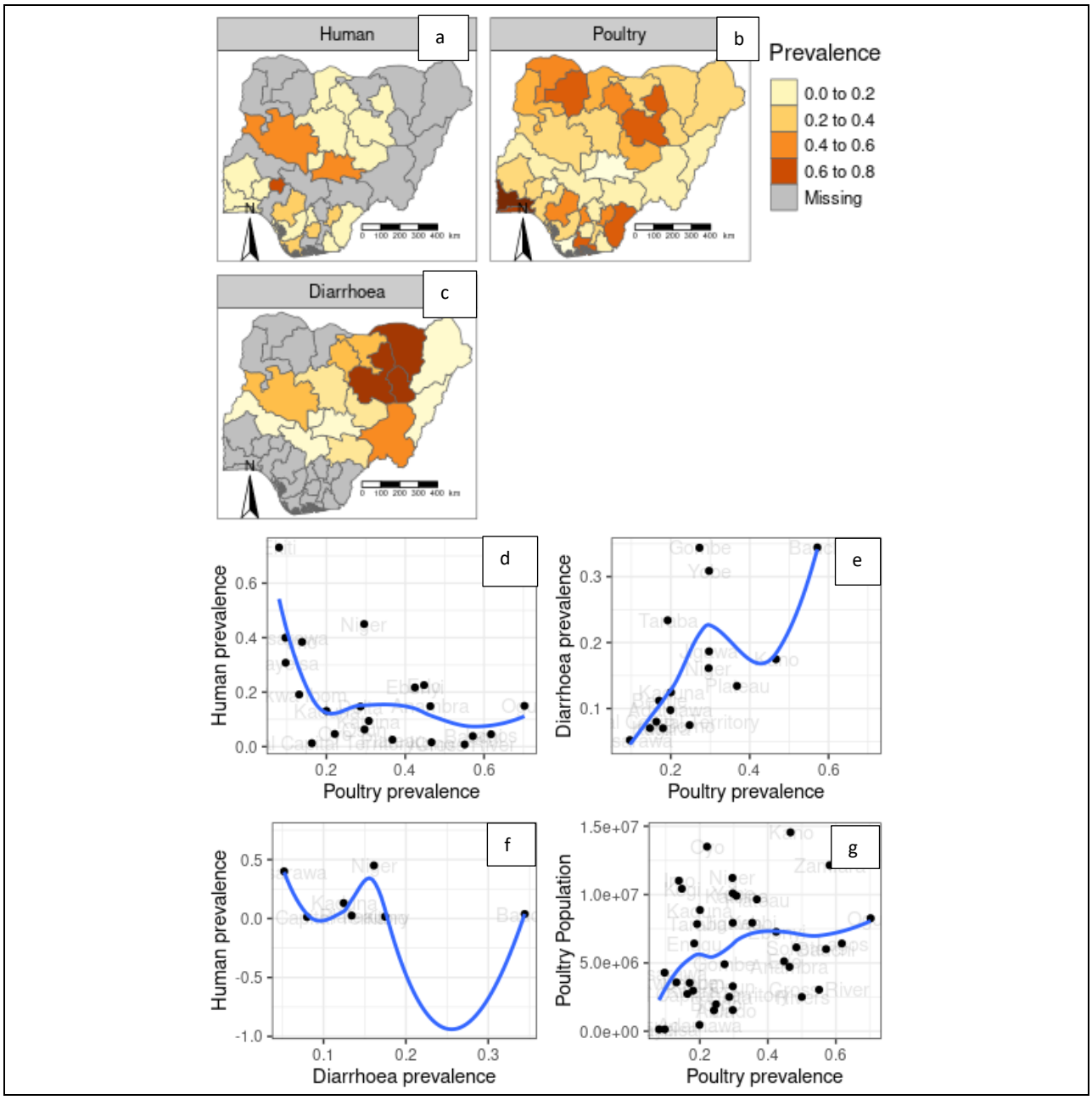

Figure 8.3. Mean prevalence of poultry salmonellosis and non-typhoidal salmonellosis in humans per state based on historical and peer-reviewed data, 2000 – 2020. (a). Prevalence of NTS in humans based on hospital records and peer reviewed publications. (b). Prevalence of salmonellosis in poultry based on peer reviewed publications and laboratory records. (c). Prevalence of diarrhoea in humans based on the District Health Information Software (DHIS) – 2 data. (d). Correlation analysis of poultry versus human salmonellosis prevalence. (e). Correlation analysis of poultry salmonellosis versus human diarrhoea prevalence. (f). Correlation analysis of reported human salmonellosis versus human diarrhoea prevalence. (g). Correlation analysis of poultry population versus poultry salmonellosis prevalence.

Salmonellosis in poultry manifest primarily as fowl typhoid and pullorum disease (WOAH, 2023a, b). where there were missing data in humans, the DHIS-2 data for diarrhoea were used as proxy. For poultry-level data, only 4 states (Ondo, Osun, Gombe and Jigawa) (10.8%) lacked data, hence, the national average of poultry salmonellosis was used for those states.

8.4. Discussion

In this work, the re-evaluation of prevalence of salmonellosis in poultry from 2000 until 2020, and mapped spatio-temporally prevalence of salmonellosis This work revealed that salmonellosis in poultry is still a major challenge for the poultry industry at 31.6% national prevalence and differing prevalences among states. However, the health authorities are yet to give the disease in humans and poultry the attention it deserved. For example, in the West African subregion, salmonellosis ranked 20 out of the 30 prioritized zoonoses (Goryoka et al., 2021), and in Nigeria, experts viewed it as a moderate zoonosis, and ranked it low on severity and epidemic potentials but high to moderate on burden of diseases, ability of the health services to control, and socio-economic impacts with a score of 0.50 out of 1.00 (Ihekweazu et al., 2021). It would appear that states and regions with dense human populations, many peri-urban poultry and day-old-chick hatchery and distribution services tend to have higher prevalence compared to relatively sparse areas of the country. In this analysis, Ogun, Lagos, Zamfara and Bauchi has the highest state-level prevalence, and the North-West, South-West and South-South regions have higher prevalence.

The patterns of correlation analyses are of a concern. The humans NTS negatively predicted salmonellosis in poultry; it is known that NTS is a foodborne zoonosis, and the disease has been linked with contaminated poultry getting into the human food chain among others (Batz et al., 2012; Ao et al., 2015). It is expected that as more poultry cases are detected per state, more human cases are expected to be reported in the hospital. This negative correlation contradicted this view. It is highly likely that many cases of acute self-limiting gastrointestinal illnesses, possibly caused by nontyphoidal Salmonella never get reported to the health authorities, or never get tested in the laboratory, hence the low record (Baba et al., 2013; Enabulele and Awunor, 2016;

Rockers and McConnell, 2017). This is further reinforced because only 21 states have peer-reviewed records out of 37 distinct subnational systems (Figure 8.3a). It is unlikely that there has been no case of NTS in humans in those non-reporting states. In addition, the primary healthcare services may not adequately reach the underserved areas of the country or may be delivering poor services (Makinde et al., 2018; Adedibu et al., 2022). In addition, abuse of antimicrobials to treat acute diarrhoea is prevalent nationwide. Anecdotal evidence reveals that in homesteads, diarrhoea is initially treated with metronidazole (flagyl) or tetracycline, or some mix of complementary or home remedies, and only in unresolved cases, there may be hospital follow-ups. In resolved cases, many such patient do not complete the course of medication. In this situation, such cases go unreported and undiagnosed (Uzochukwu and Onwujekwe, 2004; Omolase et al., 2007; Adeyemi et al., 2021; Wegbom et al., 2021)). This may have been responsible for the observed negative correlation between NTS prevalence and diarrhoea in humans, while he same diarrhoea in humans was a positive predictor for salmonellosis in poultry as found in this study (Figure 8.3e).

It is unsurprising that areas with high poultry populations in our study also have high prevalence of poultry salmonellosis. Previous studies have confirmed that anthropogenic risk factors including age of the birds, flock size, feed, hygienic condition of the farm, environmental determinants, among others may drive farm-level infection and transmission of *Salmonella* Gallinarum and *S*. Enteritides in poultry farms (Sirdar et al., 2012; Neogi et al., 2020)

This work presents with certain limitations. The lack of health data, both in humans and poultry (livestock) and especially for mildly symptomatic illnesses like diarrhoea must be addressed by the relevant health authorities. First, the cause of such lack must be established and addressed

without delay. Adequate risk communication and community engagement to ensure robust data collection must become systemized. Even, where data exist, the quality and consistency of the data are questionable. It would be important to set a standard template to collect all diarrhoeic-related data, and back it up with laboratory confirmation to make future works based on quantitative epidemiology more robust.

8.5. Conclusion

This work has highlighted the continued burden of non-typhoidal salmonellosis in humans and poultry Nigeria. It shows clear data gaps based on hospital, clinical and laboratory records, and surveillance data. Real effort at disease reduction, control, and eradication in poultry and human would benefit from robust and comprehensive data to inform these efforts in Nigeria.

Ethical Approval

The protocol for the work was part of the protocol approval from the Federal University of Technology, Minna's Ethical Review Committee approval number: 000030, May 2022.

Supplementary materials

Supplementary materials 8.1 and 8.2 are found in the section for supplementary materials.

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CHAPTER NINE

9.0 GENERAL CONCLUSION

This work was conducted with a view to conduct microbiological evaluation of non-typhoidal salmonellosis in North Central zone of Nigeria, re-analyse the risk of introduction of non-typhoidal Salmonella into poultry farms in Nigeria and contribute to the determination of the epidemiology of foodborne Salmonella among poultry farmers and consumers. It was also aimed at determining the economic burden of food borne salmonellosis in humans and poultry, demonstrating the benefit of disease control measures against salmonellosis using the modified benefit-cost model to evaluate the cost of comprehensive control and of not taking any action against salmonellosis in poultry, and map spatial heterogeneity of habitat suitability for salmonellosis in poultry farms. Overall, the economic burden of NTS in Nigeria for the year 2020 was US$ 930,887,379 (3.19% of the national budget), a significant proportion of a country's budget. It was doubtful if the national authorities have taken account of this magnitude of losses in planning and intensifying efforts to mitigate the impacts of NTS in humans and poultry. The NTS burden is significant, although it does not kill at the scale of rapidly spreading infectious diseases. It can cause a disability-adjusted life years (DALYs) of 37,321 per annum, leading to 325,731 cases and over a thousand deaths in humans. Although, the estimated treatment cost for a human case of acute gastro-intestinal salmonellosis was US$ 60 in non-complicated situation, this cost can significantly increase in complicated cases due to different treatment pathways and health outcomes. In addition, setting a benchmark for One Health and the whole plan inclusive in such lan is a major challenge,

however, this work justified the need for economic investment in NTS surveillance and control rather than to wait until outbreaks occur.

The benefit-cost analyses conducted in this work is important to prioritise anticipatory planning, budget allocations and identify funding gaps while providing effective responses against infectious diseases. For instance, the analuses identified the under-resourcing of the animal health component of salmonellosis control in Nigeria, and this may be linked with the ineffective Veterinary Services to tackle diseases like NTS at both national and subnational levels. Poultry remains a major source of livelihoods, hence, mitigating NTS risks in poultry would significantly reduce the social and economic burdens of NTS in humans. An investment in preparedness and response would limit the scale of outbreaks and the associated disease burdens, the eventual impact and costs of managing outbreaks. The availability and use of simple, fast and adaptable tools to assist in national and subnational planning is apt and should be encouraged. However, such planning must be followed up by trained manpower capacitated with resources (surveillance materials, tools, consumables and equipment) to carry out their mandate. Coordination and some degree of decentralization would also be beneficial to the overall surveillance system.

In this work, certain risk factors have also been identified includen pathogen population increases with farm intensification and crowding of poultry per unit space, the use of stream water as a source of drinking water for chickens, and the non-adherence to pullorum and fowl typhoid vaccination protocols. Behavioural change communication, and the empowerment of subnational officers, veterinarians, and paraveterinarians, as well as extension agentsand use them as agents of change in risk communication and community engagement would benefit infection control.

Based on the microbiological analysis, poultry and poultry products, including environmental samples from the poultry remain major sources of NTS for poultry farm infection and contamination of the human food chain. The pattern of resistance confirmed the pattern of antimicrobials use in the poultry sector in Nigeria include tetracycline, tylosin, chloramphenicol, metronidazole, ciprofloxacin, gentamycin, and colistin, a similar pattern to what exist elsewhere in Africa and many low- and middle-income countries. We identified many multi drug resistance organisms, a condition that may predicate many difficult to treat infections in the livestock and in humans. Many states (subnational system) are challenged with the burden of NTS in poultry although health data gaps exist for both poultry and human. It would appear that country with high dense poultry population, and human population predicts outbreaks in poultry, hence, comprehensive data on prevalence of NTS would inform better analysis in the future. Towards this end, healthcare services should be ensured to reach underserved areas of the country and behavioural change communication to seek hospitalization and report incidence of NTS should be promoted in humans.

This work has some limitations, including the lack of data in many respects and the need to dig up data from various sources, some of which may be subjective or biased by personal expert's view and expertise. Secondly, linear costs without considering the discounted values in a multi-year study is challenging. In addition, the computational model to estimate some costs and disease simulation did not consider the dynamics of changes that the industry is subjected to. The disease, NTS in humans, as some other diarrhoeal diseases, typically have a comparative high notification rate in children compared to in adult, hence, the hospital-level record may be subjected to reporting and testing biases. Furthermore, Widal's test (for agglutinating antibodies

detection against the O and H antigens) is widely used for testing NTS and typhoidal salmonellosis in Nigeria, but it is not sufficiently sensitive, specific or reliable enough to be an optimal diagnostic assay for typhoid fever and it does not aid in the diagnosis of paratyphoid (NTS) fever, as the antibodies are not cross-reactive against S. Paratyphi A, B and C antigens, hence, false-negative/false positive results may have supervene. The current sectoral silos, uneven sectoral financing, coordination challenges and delays associated with over-centralization of public and animal health interventions are still limiting the full implementation of One Health approach in full scale, hence, our theoretical approach may face some practical challenge. In some situation, and for some data, this work used small number of participants, because cost data are difficult to obtain, and this may be subjected to a degree of recall and courtesy biases.

In this work, complete serotyping of all classified positive cases was not performed, a limitation that future work should consider. While full serotyping may be beneficial research-wise, and to inform policy, it should be noted that serotyping for *Salmonella* is a relatively expensive procedure, and smallholder poultry farms may consider this too burdensome to bear financially. It is also possible that some of our isolates are subject to misclassification, a situation that may have increased/decreased the total prevalence determined in the study. The lack of health data, both in humans and poultry (livestock) and especially for mildly symptomatic illnesses like diarrhoea remains a limitation that must be addressed by the relevant health authorities. In addition, the quality and consistency of health data are often questionable and need standardisation.

It is recommended that the national health and veterinary authorities consider the following:

1. The integration of structured surveillance and control intervention against NTS in humans and poultry. Such integration has potential benefits in preventing additional illnesses and deaths.
2. Outcomes of this work should promote evidence-based advocacy with the policy makers, funders and resource allocators, in robust funding of surveillance and control efforts in health.
3. The outputs from this work should trigger interests in supporting intentional data gathering against NTS and other diseases in humans and animals.
4. More investment in targeted vaccination against fowl typhoid in poultry, and in effective surveillance, monitoring and control of salmonellosis in the poultry value chain is necessary and should be promoted.
5. Because the NTS continues to challenge poultry farms in North Central Nigeria, good farm practices must be implimented intentionally, and biosecurity and hygiene protocols must be improved in order to reduce the burden of NTS. In addition, the full compliance with vaccination protocols against pullorum and fowl typhoid in poultry combined with other control measures would assist in eradicating infection with NTS from poultry flocks in Nigeria.
6. There is a need to relook at the policies and guidelines guiding antimicrobial use and surveillance in poultry and general livestock production, and a need for intensified effort to stringently control access to these antimicrobials.

Overall, this work has highlighted the continued burden of non-typhoidal salmonellosis in humans and poultry Nigeria. Real effort at disease reduction, control, and eradication in poultry and human would benefit from robust and comprehensive data availability in Nigeria.

CHAPTER TEN

10.0 GENERAL REFERENCES

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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Research Ethics Committee

11 April 2023

CONDITIONALLY APPROVAL

Ethics Reference No	**REC142-22**
Protocol Title	**Eco-epidemiology and microbiological evaluation of poultry salmonellosis in North Central Nigeria, and its socio-economics and public health impacts**
Principal Investigator	**Dr AO Sanni**
Supervisors	**Prof FO Fasina**

Dear Dr AO Sanni,

We are pleased to inform you that your submission has been conditionally approved by the Faculty of Veterinary Sciences Research Ethics committee, subject to other relevant approvals.

Please note the following about your ethics approval:

1. Please use your reference number (REC142-22) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application for post graduate studies (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

Conditionally approved pending obtaining ALL other relevant approvals.

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

100 YEARS OF VETERINARY EDUCATION

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Conditional Ethical Approval document

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

3 July 2023

Approval Certificate
New Application

AEC Reference No.: REC142-22
Title: Eco-epidemiology and microbiological evaluation of poultry salmonellosis in North Central Nigeria, and its socio-economics and public health impacts
Researcher: Dr AO Sanni
Student's Supervisor: Prof FO Fasina

Dear Dr AO Sanni,

The **New Application** as supported by documents received between 2022-12-05 and 2023-06-26 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2023-06-26.

Please note the following about your ethics approval: Please note that Humanities must also review and approve the application (email refers)

1. The use of species is approved:

Samples	Number
Poultry- faeces, organs from post mortem samples (Stored- Historic/Retrospective) Nigeria	1000

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2024-07-03.
3. Please remember to use your protocol number (REC142-22) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All Incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

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Fakulteit Veeartsenykunde
Lefapha la Disaense tša Diphoofadiruiwa

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Final Ethical Approval document

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA
DIRECTORATE FOR RESEARCH, INNOVATION AND DEVELOPMENT (DRID)
(OFFICE OF THE VICE-CHANCELLOR)

9th May, 2022

Dear Sir/Ma,

Notice of Full Approval after Full Committee Review

Re: Protocol titled: **Eco-Epidemiology and Microbiological Evaluation of Poultry Salmonellosis in North Central Nigeria, and its Socio-economics and Public Health Impacts**

Assign Number: 000030

Principal Investigator: Sanni Abdullahi Ozomata

Address **of Principal Investigator:** Department of Animal Production, Federal University of Technology, Minna, P.M.B 65, Niger State, Nigeria
Date of receipt of application: 3rd May, 2022
Date of Approval of application: 9th May, 2022

The ethical committee of the Federal University of Technology, Minna has given the approval for the implementation of the research after several reviewed.
You are required to submit periodically, a review of the study to this committee and on completion of the study, a final full review must be submitted to this committee. Any adverse effect in the course of the studies must be brought to the notice of the committee within seven (7) days. This committee must be informed before your research findings are published.

Yours faithfully,

Professor Moses Aderemi Olutoye
Director, DRID
For Chairman, Research Ethics Committee

1

Local Protocol and Ethical Approval document, Nigeria

List of Supplementary Materials accompanying the Thesis

Chapter 3: Economic Burdens of Persistent Infection of Non-Typhoidal *Salmonella* in Poultry Farms, North Central Nigeria.

Supplementary Table 3.1. Number by cases data

Serial No.	Data	Data source
1.	Total value of animal loss, PPP	Calculation
2.	Total value of production decrease, PPP	Calculation
3.	Sum	Calculation
4.	*2020 Population (poultry)*	Federal Ministry of Agriculture and Rural Development (FMARD) (2020)
5.	*Pop. Weight*	Calculation (from FMARD source)
6.	*Number of cases*	Calculation from Salmonellosis_loss_Animal, Peer-reviewed literature, experts' opinions.
7.	*Farm-gate price of a healthy animal (local currency)*	Field survey
8.	*Farm-gate price of a healthy animal (USD PPP)*	Calculation from row 8
9.	Value of animals lost per case (USD PPP)	Calculation
10.	Value of production lost per case (USD PPP)	Calculation
11.	TOTAL loss per case (USD PPP)	Calculation
12.	Loss per case as a percentage of the farm-gate price of a healthy animal	Calculation
13.	Total loss from livestock keepers, PPP	Calculation
14.	Total loss from consumers, PPP	Calculation
15.	Total loss caused by \NTS (salmonellosis)	Calculation
16.	PPP conversion factor (GDP, World Bank) 2020	World Bank, https://data.worldbank.org/indicator/PA.NUS.PPP?locations=NG
17.	Agriculture, value added (% of GDP), 2010 – 2021	World Bank, https://data.worldbank.org/indicator/NV.AGR.TOTL.ZS?locations=NG
18.	GDP (current US$), 2010 – 2021	World Bank, https://data.worldbank.org/indicator/NY.GDP.MKTP.CD?locations=NG&start=2010
19.	GDP, PPP (constant 2011 international $), 2010 – 2021	World Bank, https://data.worldbank.org/indicator/NY.GDP.MKTP.PP.CD?end=2021&locations=NG&start=2009
20.	GDP, PPP (constant 2017 international $), 2010 – 2021	World Bank, https://data.worldbank.org/indicator/NY.GNP.MKTP.PP.KD?end=2021&locations=NG&start=2009
21.	Animal losses as percentage of Agric. GDP	Calculation
22.	Animal losses as percentage of Poultry GDP	Calculation
23.	Animal losses as percentage of Agriculture Budget Expenditure	Calculation
24.	Human losses as percentage of GDP	Calculation
25.	Human losses as percentage of Health Budget Expenditure	Calculation
26.	Budget allocation (2016/2017)	Federal Ministry of Finance (2020)
27.	Budget allocation (2017/2018)	Federal Ministry of Finance (2020)
28.	Budget allocation (2018/2019)	Federal Ministry of Finance (2020)
29.	Budget allocation (2019/2020)	Federal Ministry of Finance (2020)
30.	Budget expenditure (2016/2017)	Federal Ministry of Finance (2020)
31.	Budget expenditure (2017/2018)	Federal Ministry of Finance (2020)
32.	Budget expenditure (2018/2019)	Federal Ministry of Finance (2020)
33.	Budget expenditure (2019/2020)	Federal Ministry of Finance (2020)
34.	Animal loss, PPP	Calculation
35.	Production Decrease, PPP	Calculation
36.	Social cost - Livestock keepers, PPP	Calculation
37.	Social cost - Consumers, PPP	Calculation
38.	Total costs	Calculation

Data from database: World Development Indicators, Last Updated: 26/08/2022 (https://databank.worldbank.org/source/world-development-indicators).

Supplementary Table 3.2. Basic Check on data

Serial No.	Salmonellosis	Poultry	Poultry Keeper	Consumers	Sources
1.	Prevalence	Calculation	Calculation	Calculation	Field data, PS brief, Literature
2.	Fatality (over total case = case fatality)	Calculation	Calculation	Calculation	Field data, PS brief, Literature

	Salmonellosis	Poultry			Livestock keepers			Consumers
		Intensive (large-scale)	Intensive (small & medium-scale)	Free-range/Semi-intensive (indigenous)	Intensive (large-scale)	Intensive (small & medium-scale)	Free-range/Semi-intensive (indigenous)	
3	Case/ population	Calculation	Calculation	Calculation	Calculation	Calculation	Calculation	Calculation
4	Fatality (over total case = case fatality)	Calculation	Calculation	Calculation	Calculation	Calculation	Calculation	Calculation

Data from Federal Ministry of Agriculture and Rural Development (2020).

Supplementary Table 3.3. Total Loss

Serial No.	Data	Data source
1.	Total value of animal loss, PPP	Calculation
2.	Total value of production decrease, PPP	Calculation
3.	Total loss from livestock keepers, PPP	Calculation
4.	Total loss from consumers, PPP	Calculation
5.	Total loss caused by *Salmonella*	Calculation
6.	Animal losses as percentage of Agric. GDP	Calculation
7.	Animal losses as percentage of Poultry GDP	Calculation
8.	Animal losses as percentage of Agriculture Budget Expenditure	Calculation
9.	Human losses as percentage of GDP	Calculation
10.	Human losses as percentage of Health Budget Expenditure	Calculation
11.	PPP conversion factor (GDP, World Bank) 2020	World Bank, https://data.worldbank.org/indicator/PA.NUS.PPP?locations=NG
12.	Agriculture, value added (% of GDP), 2010 – 2021	World Bank, https://data.worldbank.org/indicator/NV.AGR.TOTL.ZS?locations=NG
13.	GDP (current US$), 2010 – 2021	World Bank, https://data.worldbank.org/indicator/NY.GDP.MKTP.CD?locations=NG&start=2010
14.	GDP, PPP (constant 2011 international $), 2010 – 2021	World Bank, https://data.worldbank.org/indicator/NY.GDP.MKTP.PP.CD?end=2021&locations=NG&start=2009
15.	GDP, PPP (constant 2017 international $), 2010 - 2021	World Bank, https://data.worldbank.org/indicator/NY.GNP.MKTP.PP.KD?end=2021&locations=NG&start=2009

Data from database: World Development Indicators, Last Updated: 26/08/2022 (https://databank.worldbank.org/source/world-development-indicators).

Supplementary Table 3.4. Loss (Humans)

Serial No.	Population group	Intensive (large-scale)	Intensive (small & medium-scale)	Free-range/Semi-intensive (indigenous)	Total Poultry keepers	Total Poultry consumers	Source
1.	Diseases	Salmonellosis	Salmonellosis	Salmonellosis	Salmonellosis	Salmonellosis	Predetermined for the work
2.	Ref. year(s)	2020	2020	2020	2020	2020	Predetermined for the work
3.	Daly Weight	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
4.	Human Population Total Nigeria (2020)	Determined (Mid-year estimate)	Determined (Mid-year estimate)	Determined (Mid-year estimate)	Determined (Mid-year estimate)	Determined (Mid-year estimate)	United Nations, Department of Economic and Social Affairs, Population Division (2022). World Population Prospects 2022, Online Edition.
5.	Population of group (**)	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
6.	Number of cases (**)	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
7.	Number of deaths (**)	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
8.	Number of survivors	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
9.	Duration in years(*)	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
10	Average Life expectancy at birth	Determined	Determined	Determined	Determined	Determined	World Bank, https://data.worldbank.org/indicator/SP.DYN.LE00.IN?locations=NG
11	Average age of infection (***)	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
12	YLL	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
13	YLD	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
14	DALY=YLL+YLD	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
15	WTP for a DALY(++)	Calculation	Calculation	Calculation	Calculation	Calculation	Input data
16	DALYs in monetary terms (+++)	Calculation	Calculation	Calculation	Calculation	Calculation	Input data

Supplementary Table 3.5. Salmonellosis Loss (Poultry)

Serial No.	Data	Intensive (large-scale)	Intensive (small & medium-scale)	Free-range/Semi-intensive (indigenous)	Total	Source
1.	Poultry population	Calculation	Calculation	Calculation	Calculation	Input data
2.	Number of cases	Calculation	Calculation	Calculation	Calculation	Input data
	I. Value of animals Lost	Calculation	Calculation	Calculation	Calculation	Input data
3.	Number of deaths	Calculation	Calculation	Calculation	Calculation	Input data
4.	Price per animal	Calculation	Calculation	Calculation	Calculation	Input data
5.	Value of animals lost	Calculation	Calculation	Calculation	Calculation	Input data
6.	II. Loss from salvage slaughter and culling	Calculation	Calculation	Calculation	Calculation	Input data
7.	Number of salvage slaughter*	Calculation	Calculation	Calculation	Calculation	Input data
8.	Number of culls*	Calculation	Calculation	Calculation	Calculation	Input data
9.	Price of salvage slaughtered/culled animal	Calculation	Calculation	Calculation	Calculation	Input data
10	*Number of salvage slaughter and deaths is higher than number of cases as often the whole flock is slaughtered/culled	Calculation	Calculation	Calculation	Calculation	Input data
11	Value of loss from salvage slaughter and culling	Calculation	Calculation	Calculation	Calculation	Input data
12	III. Value of foregone production	Calculation	Calculation	Calculation	Calculation	Input data
13	Percentage of cases in salvage slaughter	Calculation	Calculation	Calculation	Calculation	Input data
14	Number of survivors	Calculation	Calculation	Calculation	Calculation	Input data
15	Percentage of eggs lost per year in survivors	Calculation	Calculation	Calculation	Calculation	Input data
16	Number of eggs per hen per year	Calculation	Calculation	Calculation	Calculation	Input data
17	Price of eggs	Calculation	Calculation	Calculation	Calculation	Input data
18	Value of eggs lost	Calculation	Calculation	Calculation	Calculation	Input data
19	Total value of forgone production	Calculation	Calculation	Calculation	Calculation	Input data

Supplementary Table 3.6. Poultry population (2020 estimates)

Nigerian States	Population (Number)				Proportion of production system (absolute numbers)				
					Indigenous chicken		Commercial exotic breeds		
	Indigenous chicken	Improved or exotic breeds	TOTAL (2020 Estimation)		Free-range (extensive)	Backyard (improved free range/semi intensive)	Small scale	Medium scale	Large scale
Abia	305,083	1,220,332	1,525,415		122,033	183,050	610,166	366,100	244,066
Anambra	1,034,471	3,855,755	4,702,140		362,065	672,406	2,313,453	771,151	771,151
Ebonyi	1,817,232	5,451,697	7,268,929		454,308	1,362,924	2,180,679	1,908,094	1,362,924
Enugu	1,669,065	4,750,416	6,419,481		500,720	1,168,346	1,662,646	1,662,646	1,425,125
Imo	3,856,213	7,161,538	11,017,751		578,432	3,277,781	4,296,923	1,790,385	1,074,231
Akwa-Ibom	356,985	3,212,861	3,569,845		160,643	196,341	1,124,501	1,606,430	481,929
Bayelsa	NA	NA	NA		NA	NA	NA	NA	NA
Cross-river	454,998	2,578,325	3,033,323		81,900	373,099	1,675,911	644,581	257,832
Edo	1,278,678	3,836,033	5,114,710		191,802	1,086,876	1,726,215	1,342,611	767,207
Delta	375,945	2,130,358	2,506,303		37,595	338,351	894,750	809,536	426,072
Rivers	200,504	2,305,799	2,506,303		26,066	174,439	922,320	830,088	553,392
Ekiti	40,360	94,174	134,534		20,180	20,180	58,388	23,543	12,243
Ogun	826,583	7,439,243	8,265,825		289,304	537,279	1,859,811	3,719,621	1,859,811
Ondo	231,695	1,312,937	1,544,632		69,508	162,186	590,822	459,528	262,587
Osun	492,788	2,792,465	3,285,253		123,197	369,591	1,256,609	837,740	698,116
Oyo	1,755,681	11,749,556	13,505,237		438,920	1,316,761	1,762,433	5,287,300	4,699,822
Lagos	320,912	6,097,330	6,418,242		25,673	295,239	1,219,466	2,743,798	2,134,065
Kogi	3,127,220	7,296,847	10,424,067		1,407,249	1,719,971	3,648,423	2,189,054	1,459,369
Niger	4,486,550	6,729,825	11,216,375		1,570,293	2,916,258	4,037,895	1,682,456	1,009,474
Nasarawa	856,702	3,426,810	4,283,512		214,176	642,527	1,370,724	1,199,383	856,702
Kwara	444,434	2,518,460	2,962,894		186,662	257,772	1,259,230	856,276	402,954
Benue	776,960	2,754,677	3,531,637		388,480	388,480	1,239,605	826,403	688,669
Plateau	3,183,027	6,462,508	9,645,535		732,096	2,450,930	1,874,127	3,231,254	1,357,127
Taraba	3,058,112	4,783,202	7,841,314		1,070,339	1,987,773	2,152,441	2,152,441	478,320
Borno	1,279,846	689,148	1,968,994		255,969	1,023,877	379,031	254,985	55,132
Adamawa	329,483	128,132	457,615		82,371	247,112	76,879	44,846	6,407
Bauchi	2,522,631	3,483,633	6,006,264		605,431	1,917,199	1,567,635	1,393,453	522,545
Gombe	2,940,029	1,960,020	4,900,049		940,809	1,999,220	862,409	784,008	313,603
Yobe	5,546,533	4,538,072	10,084,605		831,980	4,714,553	1,951,371	2,223,655	363,046
F.C.T, Abuja	681,459	2,044,376	2,725,834		68,146	613,313	1,226,625	449,763	367,988
Jigawa	3,567,244	4,359,964	7,927,208		1,605,260	1,961,984	1,743,986	1,525,988	1,089,991
Kaduna	3,546,760	5,320,140	8,866,900		851,222	2,695,538	2,394,063	1,596,042	1,330,035
Kano	4,947,357	9,603,693	14,551,050		1,583,154	3,364,203	2,881,108	3,841,477	2,881,108
Katsina	4,458,271	5,448,998	9,907,269		1,783,308	2,674,963	1,907,149	2,724,499	817,350
Kebbi	3,964,624	3,964,624	7,929,248		1,585,850	2,378,774	991,156	2,061,604	911,864
Sokoto	2,575,968	3,557,289	6,133,257		901,589	1,674,379	1,387,343	1,600,780	569,166
Zamfara	4,858,063	7,287,095	12,145,158		1,943,225	2,914,838	4,007,902	1,821,774	1,457,419
Total	**72,168,465**	**152,346,328**	**224,326,708**		**22,089,954**	**50,078,511**	**61,114,194**	**57,263,294**	**33,968,841**
Percentage	32.17	67.91	100		9.85	22.32	27.24	25.53	15.14

Source: Federal Ministry of Agriculture and Rural Development (2020)

Supplementary Table 3.7. Human populations estimate for the year 2006 and 2022

STATE	Geopolitical Zones	No of LGAs	Population 2006	Growth Rate	Males2006	Females2006	Population 2022
Abia	SEZ	17	2,833,999	2.7	1,434,193	1,399,806	4,340,370
Adamawa	NEZ	21	3,168,101	2.9	1,606,123	1,561,978	5,005,472
Akwa Ibom	SSZ	31	3,920,208	3.4	2,044,510	1,875,698	6,693,261
Anambra	SEZ	21	4,182,032	2.8	2,174,641	2,007,391	6,505,448
Bauchi	NEZ	20	4,676,465	3.4	2,426,215	2,250,250	7,984,474
Bayelsa	SSZ	8	1,703,358	2.9	902,648	800,710	2,691,237
Benue	NCZ	23	4,219,244	3.0	2,164,058	2,055,186	6,770,648
Borno	NEZ	27	4,151,193	3.4	2,161,157	1,990,036	7,087,638
Cross River	SSZ	18	2,888,966	2.9	1,492,465	1,396,501	4,564,450
Delta	SSZ	25	4,098,391	3.2	2,074,306	2,024,085	6,784,042
Ebonyi	SEZ	13	2,173,501	2.8	1,040,984	1,132,517	3,381,035
Edo	SSZ	18	3,218,332	2.7	1,640,461	1,577,871	4,928,990
Ekiti	SWZ	16	2,384,212	3.1	1,212,609	1,171,603	3,885,827
Enugu	SEZ	17	3,257,298	3.0	1,624,202	1,633,096	5,227,007
FCT	NCZ	6	1,405,201	9.3	740,489	664,712	5,829,899
Gombe	NEZ	11	2,353,879	3.2	1,230,722	1,123,157	3,896,362
Imo	SEZ	27	3,934,899	3.2	2,032,286	1,902,613	6,513,415
Jigawa	NWZ	27	4,348,649	2.9	2,215,907	2,132,742	6,870,690
Kaduna	NWZ	23	6,066,562	3.0	3,112,028	2,954,534	9,735,051
Kano	NWZ	44	9,383,682	3.3	4,844,128	4,539,554	15,775,329
Katsina	NWZ	34	5,792,578	3.0	2,978,682	2,813,896	9,295,387
Kebbi	NWZ	21	3,238,628	3.1	1,617,498	1,621,130	5,278,369
Kogi	NCZ	21	3,278,487	3.0	1,691,737	1,586,750	5,261,009
Kwara	NCZ	16	2,371,089	3.0	1,220,581	1,150,508	3,804,902
Lagos	SWZ	20	9,013,534	3.2	4,678,020	4,335,514	14,920,049
Nasarawa	NCZ	13	1,863,275	3.0	945,556	917,719	2,990,009
Niger	NCZ	25	3,950,249	3.4	2,032,725	1,917,524	6,744,552
Ogun	SWZ	20	3,728,098	3.3	1,847,243	1,880,855	6,267,473
Ondo	SWZ	18	3,441,024	3.0	1,761,263	1,679,761	5,521,833
Osun	SWZ	30	3,423,535	3.2	1,740,619	1,682,916	5,666,957
Oyo	SWZ	33	5,591,589	3.4	2,809,840	2,781,749	9,546,933
Plateau	NCZ	17	3,178,712	2.7	1,593,033	1,585,679	4,868,311
Rivers	SSZ	23	5,185,400	3.4	2,710,665	2,474,735	8,853,416
Sokoto	NWZ	23	3,696,999	3.0	1,872,069	1,824,930	5,932,598
Taraba	NEZ	16	2,280,483	2.9	1,189,463	1,091,020	3,603,071
Yobe	NEZ	17	2,321,591	3.5	1,206,003	1,115,588	4,025,606
Zamfara	NWZ	14	3,259,846	3.2	1,630,344	1,629,502	5,396,004
Grand Total		**774**	**139,983,289**	**3.2**	**71,699,473**	**68,283,816**	**232,447,125**

The total mid-year estimates for **2020 human population in Nigeria was 208,327,405**. There was no disaggregation by the subnational system (States). Source: United Nations, Department of Economic and Social Affairs, Population Division (2022). World Population Prospects 2022, Online Edition, https://population.un.org/wpp/Download/Files/1_Indicators%20(Standard)/EXCEL_FILES/1_General/WPP2022_GEN_F01_DEMOGRAPHIC_INDICATORS_REV1.xlsx.

Online link to Google form for data collection: https://docs.google.com/forms/d/e/1FAIpQLSefH1i8YASvewU1y1x-OS0sgyuvWJnOuaECXKH9ReLV4YaYZw/viewform?vc=0&c=0&w=1&flr=0

Data brief in Google_19.08.2022.pd

Salmonellosis-2020_ Nigeria_Expert Elicitat

Evaluation sheet

Chapter 4: Cost Effectiveness Analysis of Intervention Against Non-Typhoidal *Salmonella* in Nigeria

Supplementary Table 4.1. Assumptions and parameters

S/no	Input parameters	Value	Source/ Assumption	Comments (if any)
1.	Proportion of the population with non-typhoidal salmonellosis (NTS) (n = 325,731)	0.1563%	Sanni et al., 2023	Calculated using 325,731 NTS cases.
2.	Prevalence of NTS in humans (with 50% case reduction with One Health inputs)	0.07815%		
3.	Proportion of infected population with NTS who recovered	99.68% of 0.1563%	Sanni et al., 2023	Calculated from 325,731 - 1,043 = 324,688
4.	Proportion of the population with NTS who died	0.3202% of 0.1563%	Sanni et al., 2023	Calculated using 1,043 NTS deaths
5.	Infected but non-complicated cases (NTS)	95%	Feasey et al., 2012; Plumb, 2023; Sanni et al., 2023	Calculated using 1,043 NTS deaths/total cases
6.	Infected and complicated cases (NTS)	5%		
7.	Proportion of deaths among the infected but non-complicated cases (NTS)	0.50%		
8.	Infected but non-complicated cases (NTS)	5.46%		
9.	Proportion of NTS deaths who get hospitalized	60%	Uzochukwu and Onwujekwe, 2004; Feasey et al., 2012; Plumb et al., 2023; Assumed that 3/5 of cases presented at hospital died due to delayed case presentation. Based on record of hospitalization, only severe (complicated) cases get to hospital.	
10	Average age of onset of NTS (hospitalized)	19 years	Sanni et al., 2023. This median value does not preclude NTS in younger individuals.	
11	Average age of NTS death (hospitalized)	20 years	Median value assumed from the epi-model	
12	Accuracy of test kit (rapid stool antigen test).	82.92%	Geteneh et al., 2023	Safari et al., 2015 for the calculation.
13	Accuracy of test kit (Widal's antigen test).	43.00%	Enabulele and Awunor, 2016	Safari et al., 2015 for the calculation.
14	Mean cost of treatment per case of NTS	Naira 22,815.60 (US$ 60)	Akinyemi et al., 2007; Broughton et al., 2010; Orszagh et al., 2020 ; van Wagenberg et al., 2022; Subject matter specialist' opinions. This includes doctor's visits, stool/blood culture, outpatient costs, medications and associated travel costs. This cost may vary widely depending on geography and medical costs.	
15	Cost of laboratory testing (Widal's test) per patient	US$9.73	https://www.surjen.com/lab-test	
16	Vaccination cost	0	Humans are not vaccinated against NTS in Nigeria	
17	Cumulative Cost of NTS to deaths	US$50,000	US Value: US$ 1,764,112 - 17,641,121 (USDA-ERS, 2020)	
18	Mid-year human population, Nigeria, 2020	208,327,405	UN DESA, 2022	World Population Prospects, (UN DESA, 2022)
19	Human deaths per day without One Health intervention	2.858/day	Sanni et al., 2023	(325,731 - 324,688)/365 derived from Epi model
20	Human deaths avoided per day with One Health intervention	1.429/day	Sanni et al., 2023	Calculation above reduced by 50%
21	Minimum wage (Nigeria, 2020)	(US$78.89)	Trading Economics, 2023	Naira 30,000 converted to US$
22	Annual wage increment	12%	Channels TV, 2023	Calculated from the 5-year projected increase.
23	Crude death rate (Nigeria, 2020))	13/1000	World Bank, 2023a	
24	Crude birth rate (Nigeria, 2020)	37/1000	World Bank, 2023b	

25	GDP per capital in Nigeria (2020)	$2074.61	world bank 2023c	
26	Standard Life expectancy (for Nigeria) (2020)	53 years	World Bank, 2023d	52.46 years (men) & 53.32 years (women)
27	Number of NTS recoveries amongst those hospitalized	324,688	Sanni et al., 2023; calculations	
28	NTS DALYs (Disability-adjusted life years)	37,321		
29	YLD (years of healthy life lost due to disability)	632		
30	DALY weight	0.21		
31	YLL (years of life lost due to premature mortality)	36,690		
32	Value of life lost	446,749.49		
33	Mean number of cases/ day (human)	892.41 cases/day		
34	Mean number of deaths/ day (human)	2.858 deaths/day		
35	Human deaths avoided/day due to One Health intervention	1.429 deaths/day		
36	Duration of mild NTS illness	5 days (≤5)	Taliha et al., 2022; Sanni et al., 2023	
37	Duration of severe(complicated) NTS illness	15 days (10 – 15 days)	Taliha et al., 2022; Sanni et al., 2023	
38	Annual Health budget (National + Subnational), 2020	$980,126,753.51	Fasanmi et al., 2018; Orszagh et al., 2020 ; FMOH, 2017; Chaitkin, 2022.	Calculated from the Nigeria Health Financing Policy and Strategy, 2017 (FMOH, 2017). 51.62% of annual health cost comes from the national government and 48.38% comes from the subnational system. 1.3% of the annual budget is dedicated to diarrhoeal diseases at national and subnational levels.
39	Annual Health Budget (National only, 2020)	$505,941,430.16		
40	Mean Health Expenditure (National)	0.516241		
41	Mean Health Expenditure (Sub-national)	0.483759		
42	Total Program costs - diarrhoeal diseases (National)	US$6,577,238.59		
43	Personnel (National)	US$5,492,678.96		
44	Overheads (secretariat, office etc.)	US$77,146.29		
45	Laboratory supplies, consumables and transport (National)	US$1,007,413.34		
46	Annual Health Budget (Subnational, 2020)	$474,185,323.35	Fasanmi et al., 2018; Orszagh et al., 2020 ; FMOH, 2017; Chaitkin, 2022.	Calculated from the Nigeria Health Financing Policy and Strategy, 2017 (FMOH, 2017). 51.62% of annual health cost comes from the national government and 48.38% comes from the subnational system.
47	Total Program costs - diarrhoeal diseases (Subntional)	US$6,164,409.20		
48	Personnel salaries	US$5,147,923.44		
49	Overhead	US$72,304.10		
50	Laboratory supplies, consumables and medications	US$944,181.67		
51	Out of pocket expenses for health financing (2020)	71%	EGH, 2023.	
52	Total health expenditure per capita (2020)	US$83	EGH, 2023.	
53	Total health expenditure as percentage of GDP (2020)	4.2% (range: 2.2 – 17.8)	FMOH, 2017; Ojiugo et al., 2019.	
54	Mean annual expenditure on diarrhoeal diseases as percentage of Current health expenditure (CHE)	1.3%	FMOH, 2017; Chaitkin, 2022.	

Note that Personnel, Overheads and Capital budgets at national and subnational levels are partially attributable (1.3%) of total cost for the subheads) from the overall budget FMOH, 2017; Chaitkin, 2022. US$1 = N380.26 at the time of calculation; Trading Economics: https://tradingeconomics.com/nigeria/minimum-wages; Channels TV: https://www.channelstv.com/2023/03/29/tinubus-govt-should-review-minimum-wage-ngige/; EGH = Exemplars in Global Health; NCDC spent US$24,193,972.55 from 2020 to mid-2023 (https://www.icirnigeria.org/exclusive-nigerian-government-spends-over-n16-billion-on-disease-control-since-2020/).

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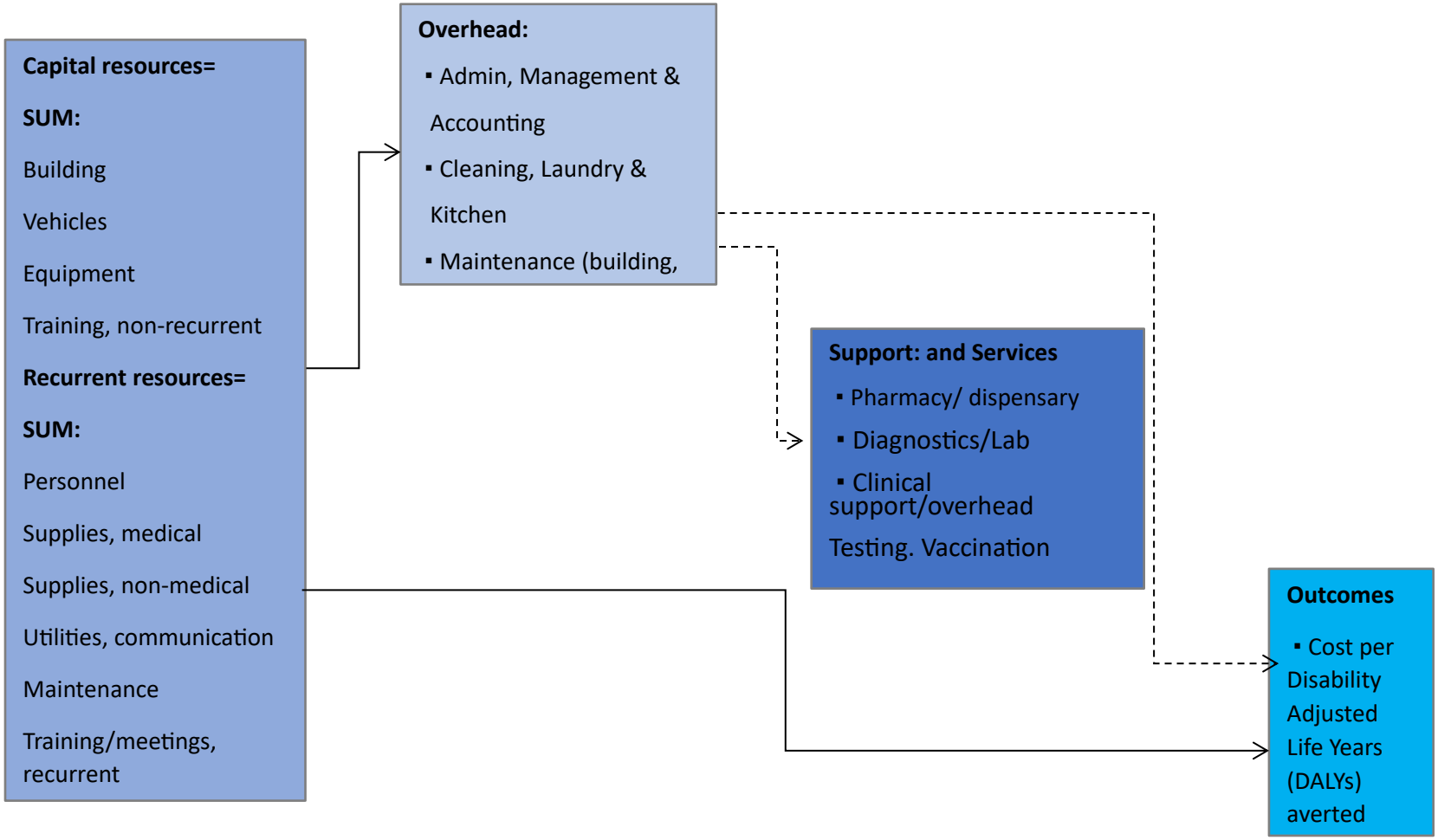
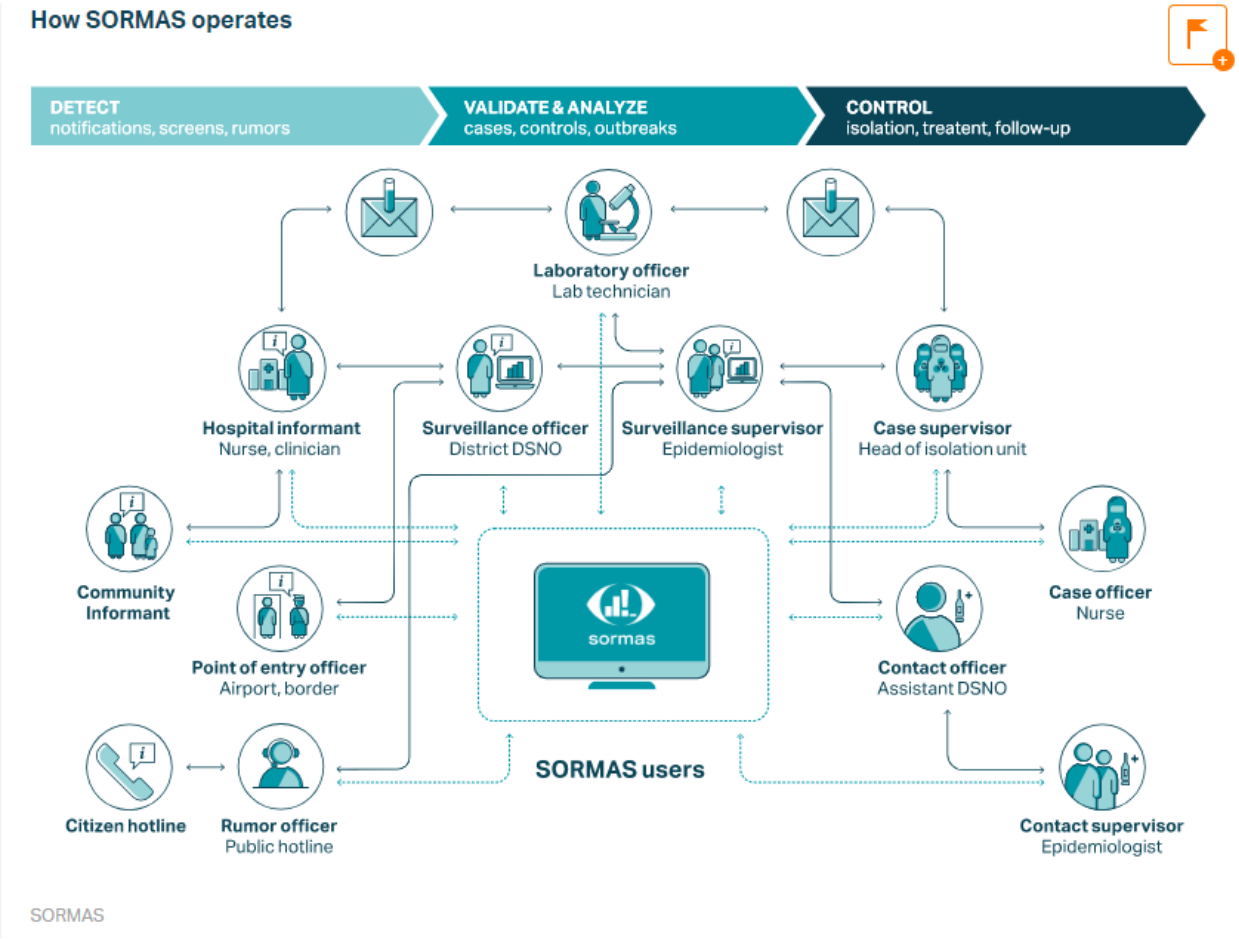
Supplementary material 4.3. *A conceptual Framework guiding the Budgeting and Costs of One Health Intervention in Non-typhoidal Salmonellosis.*





***Supplementary Figure 4.4**. Schematic Diagram of how SORMAS operate.*

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Chapter 5: Benefit-Cost Analysis of Intervention Against Non-Typhoidal *Salmonella* in Nigeria

Baseline information as framework for the current study

Non-typhoidal *Salmonella* play a significant role in foodborne human salmonellosis worldwide [1] and can be transmitted to humans particularly through the consumption of foods of animal origin, including eggs and poultry meat, as well as through direct contact with animals or their environments, especially for people working in the agriculture industry [2,3]. More than 2500 serovars of *Salmonella enterica* have been identified, of which many can cause human infections. However, non-typhoidal serovars, especially *Enteritidis* and *Typhimurium*, are the most commonly isolated serotypes in human infections [4]. Salmonellosis in humans is commonly characterised by diarrhoea, abdominal cramps, fever and vomiting [5]. Although most non-typhoidal *Salmonella* infections are associated with self-limiting gastroenteritis, they have the potential to cause fatal infections among infants, young children, older adults and immunocompromised individuals [6]. The majority of non-typhoidal *Salmonella* serovars are pathogenic as a result of their ability to invade, replicate and survive in human host cells [7].

Annual counts of number of human cases	325,731	[8]
Annual counts of number of outbreak-associated human deaths	1,043	[8]
Annual counts of number of animal cases	43,662,085	[8]
Annual counts of number of outbreak-associated animal deaths	15,841,044	[8]

Salmonellosis is a pathogenic bacterial zoonosis with substantial public health impacts [13,14]. With over 2600 different serovars identified to date, *Salmonella* spp. are broadly divided into

typhoidal and non-typhoidal *Salmonella* (NTS) serovars [15,16]. The NTS is one of the widespread causes of food-borne diarrhoeal diseases, while the invasive NTS (iNTS) is responsible for major bloodstream infections universally [13,15,17]. Humans are infected with NTS through contamination from poultry products (egg fragments, hatching eggs, chick boxes, fluff and faeces), partially cooked meat and raw eggs [14,15]. The global estimates of burden of NTS varied widely, including an estimate of over 27 million human cases and 200,000 deaths per annum [18,19]; approximately 79 million human cases and over 59,000 deaths annually [14]; and 93.8 million human infections and 155,000 fatalities annually [20]. Furthermore, in a recent ranked study in the USA, *Salmonella* spp. was the first-ranked foodborne pathogens, with the most significant cost of illness and the quality-adjusted life-year (QALY) losses [21].

The iNTS was estimated to cause 177 – 388 cases per 100,000 children under 5 years in Africa but may reach up to 2000 – 7500 cases per 100,000 humans in immunocompromised HIV-infected adults, and a case fatality ranging between 20 – 25% [22]. In Nigeria, the poultry farm level prevalence of NTS range from 41.6 - 47.9% and the risk factors for NTS infection of poultry farms in Nigeria have been fully explored [10,11,16]. Based on a recent meta-analytic study, Nigeria has a burden of prevalence (in humans) of 1.9% (2,732/143,756) *Salmonella* bacteremia and 16.3% (1,967/12,081) *Salmonella*-associated gastroenteritis [12]. In addition, a total of 53 *Salmonella* serotypes have been identified in humans in the country including 39 associated with *Salmonella*-bacteremia and 31 associated with *Salmonella*-gastroenteritis [12].

Supplementary Materials

Supplementary Material 5.1 – Participant information note, consent form and the questionnaire.

Supplementary Material 5.2 – filled Outbreak Costing Tool (OCT) Excel Spreadsheet.

Supplementary Table 5.3 – Explanatory table on the details of the Cost Categories in the OCT.

Supplementary Material 5.4 – Scenario analysis evaluation.

Supplementary Material 5.1

APPENDIX 1: CONSENT FORM

PARTICIPANT INFORMATION SHEET

_____/_____ 202

Title: Eco-Epidemiology and Microbiological Evaluation of Poultry Salmonellosis in North Central Nigeria, and its Socio-economics and Public Health Impacts

Lead Researcher/Student Name: Sanni Abdullahi OZOMATA

Student Number: 22959590

University: University Of Pretoria, South Africa

Faculty: Veterinary Science

Department: Veterinary Tropical Diseases

Programme: PhD (Veterinary Tropical Diseases)

Candidate Physical address: House 57, Aviation Housing Estate, F.C.T Abuja, Nigeria.

Email address: drsao.epidem@gmail.com

Phone number: +234 803 608 0269

Under the supervision of the following persons:

Supervisor Prof. Folorunso O. FASINA (daydupe2003@yahoo.co.uk)

Co-Supervisor Dr. Annelize JONKER (annelize.jonker@up.ac.za)

Dear Prospective Participant

My name is Abdullahi Ozomata SANNI, I am doing research under the supervision of Prof. Folorunso O. FASINA, an Extraordinary Professor in the Department of Vet. Tropical Diseases at the University of Pretoria. My study will lead to the award of PhD (Vet Sc.) Degree from the University of Pretoria. We are inviting you to participate in a study under the broad title "**Eco-Epidemiology and Microbiological Evaluation of Poultry Salmonellosis in North Central Nigeria, and its Socio-economics and Public Health Impacts**." This specific study is aimed at understanding the **Economic and Social Burdens of Non-Typhoidal *Salmonella* Infections**.

WHAT IS THE PURPOSE OF THE STUDY?

The aim of this study is understanding the Economic and Social Burdens of Non-Typhoidal *Salmonella* Infections.

WHY AM I BEING INVITED TO PARTICIPATE?

You have been selected as a stakeholder in the industry through direct identification, recommendation or nomination from your area of expertise or contributions.

The totality of the study has been discussed with the authorities of the Federal Ministry of Agriculture and Rural Development, Abuja Nigeria. Permission has been obtained and the total number of participants in this study will be dependent on when the saturation point is reached because we are using industry and publicly available data and participants are recruited through snowballing method.

WHAT IS THE NATURE OF MY PARTICIPATION IN THIS STUDY?

The study involves the use of questionnaires, which will be administered using face to face method, or through Google Forms

(https://docs.google.com/forms/d/e/1FAIpQLSefH1i8YASvewU1y1x-OS0sgyuvWJnOuaECXKH9ReLV4YaYZw/viewform?vc=0&c=0&w=1&flr=0). The expected duration of participation and the time needed to collect data is approximately 30 – 60 minutes per participant, depending on areas that concern each participant.

CAN I WITHDRAW FROM THIS STUDY EVEN AFTER HAVING AGREED TO PARTICIPATE?

Participation in this research is entirely voluntary. It is participant's choice whether to participate or not. Participant may change their mind later and stop participating even if they agreed earlier.

WHAT ARE THE POTENTIAL BENEFITS OF TAKING PART IN THIS STUDY?

This study should make empirical data available, which should assist implementation research, decision science, inform future government policy and benefit the poultry sector of the agricultural industry.

ARE THEIR ANY NEGATIVE CONSEQUENCES FOR ME IF I PARTICIPATE IN THE RESEARCH PROJECT?

There are no foreseeable risks of harm or side effects to you by participating in this study. The only inconvenience to you will be your valuable time that you will sacrifice answering the questions in the questionnaire.

WILL THE INFORMATION THAT I CONVEY TO THE RESEARCHER AND MY IDENTITY BE KEPT CONFIDENTIAL?

All the answers from the participants to be used will be viewed as strictly confidential, and only members of the research team will have access to the information. No data published in

dissertations and journals will contain any information about name, address and picture. Your anonymity is therefore ensured.

HOW WILL THE RESEARCHER(S) PROTECT THE SECURITY OF DATA?

Questionnaires will be kept under lock and key until the capturing has been completed. Only the researcher will have access to the questionnaires. The raw data will be captured in Microsoft Excel Spread Sheet and stored with on the researcher's computer with a protective password and an external drive as a backup. After the study had been completed the data will be kept for a period of 3 years, but will not be used in any further studies.

WILL I RECEIVE PAYMENT OR ANY INCENTIVES FOR PARTICIPATING IN THIS STUDY?

Participating in this study is voluntary and participants are not entitled to any payment.

HAS THE STUDY RECEIVED ETHICS APPROVAL?

This study has received the necessary ethical approval from the Research Ethics Review Committee of the Federal University of Technology Minna, Nigeria and the Faculty of Veterinary Science, University of Pretoria, South Africa.

HOW WILL I BE INFORMED OF THE FINDINGS/RESULTS OF THE RESEARCH?

If you would like to be informed of the final research findings, please contact Dr Abdullahi Sanni with the email and phone numbers displayed on the first page of this document. The findings are accessible from the time of publication in the journal that accept the manuscript for peer-review publication, and also permanently in the associated PhD Thesis of Abdullahi Ozomata SANNI at the University of Pretoria.

Should you have concerns about the way in which the research has been conducted, you may contact Prof. Folorunso O. FASINA, e-mail: folorunso.fasina@fao.org.

Thank you for taking time to read this information sheet and for participating in this study.

If you agree with the above content, you will sign or thumbprint the following, or use digital signature (for online Google Form) and we will now proceed with the interview.

Participant name:	
Participant signature and date	

Regards
Dr. A. O. Sanni
Department of Veterinary Tropical Diseases, University of Pretoria
E-mail: drsao.epidem@gmail.com

Phone number: +234 803 608 0269

Supplementary Material 5.1. Questionnaires to target specific cost head for the Outbreak Costing Tool

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Specific Labour Costs

Respondent details

Name:

Position:

Email:

Phone:

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Section 1 of 7 - Specific Labour Costs

*Please complete any monetary questions in **Nigerian Naira***

If any section below is not applicable to the current outbreak, please write N/A

The following 4 questions are to be answered for each of the listed job titles below (where applicable) and for any additional job titles absent from the list:

1 - Average monthly salary (including benefits)

2 - Typical work hours in a month (in an average month)

3 - Average number of hours spent in outbreak investigation & response activities (over duration of the outbreak)

4 - Number of staff in this particular role that worked on outbreak investigation & response activities.

Job title 1: **Epidemiologist**

1 –

2 –

3 –

4 –

Job title 2: **Public Health Specialist**

1 –

2 –

3 –

4 –

Job title 3: **Medical Specialist**

1 –

2 –

3 –

4 –

Job title 4: **Nurse**

1 –

2 –

3 –

4 –

Job title 5: **Pharmacist**

1 –

2 –

3 –

4 –

Job title 6: **Lab Technician**

1 –

2 –

3 –

4 –

Job title 7: **Data analyst**

1 –

2 –

3 –

4 –

Job title 8: **Community engagement specialist**

1 –

2 –

3 –

4 –

Job title 9: **Project Manager**

1 –

2 –

3 –

4 –

Job title 10: **Director of Outbreak Response**

1 –

2 –

3 –

4 –

Job title 11: **Veterinarian**

1 –

2 –

3 –

4 –

Job title 12: **Other(specify) _____**

1 –

2 –

3 –

4 –

Job title 13: **Other(specify) _____**

1 –

2 –

3 –

4

NB - There is a more complex addition to this section, covering the percentage of value hours and how they were distributed for each individual job title across each stage of the outbreak investigation. A percentage of total expenditure form for this section is to be completed after this initial section is complete.

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Specific labour costs questionnaire: Percentage of total expenditure form

For your job role, please indicated below what percentage of your total value hours was incurred during each period of the outbreak (each applicable row should total 100%):

JOB TITLE	**INITIAL RESPONSE PERIOD:** ***% of value hours reported*** Including the following activities: • Prepare • Verify outbreak • Verify diagnosis • Construct case definition • Record cases • Perform descriptive epidemiology • Develop hypothesis • Evaluate hypothesis • Refine hypothesis • Reconcile evidence	**OUTBREAK RESPONSE PERIOD:** ***% of value hours reported*** Including: • Implement control & prevention measures	**IMPLEMENTATION FOLLOW UP AND REPORTING PERIOD:** ***% of value hours reported*** Including: • Initiate or maintain surveillance • Disseminate findings	**TOTAL (%)** **(each individual row should total 100%)**

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Office Materials & Equipment Costs

Respondent details

Name:

Position:

Email:

Phone:

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Section 2 of 7 – Office Material & Equipment Costs

*Please complete any monetary questions in **Nigerian Naira***

If any section below is not applicable to the current outbreak, please write N/A

The following 2 questions are to be answered for each of the listed office consumables below (where applicable), and for any additional office-related items absent from the list:

1 - Quantity used

2 - Total expenditure on item to support outbreak investigation and response activities

Office supplies 1: **Stationeries**

1 –

2 –

2 –

Office supplies 2: **Printing/copies**

1 –

2 –

Building & Office Equip Rental 1:

Rented building space

1 – N/A

2 –

Building & Office Equip Rental 2:

Rented equipment

1 – N/A

2 –

Building & Office Equip Rental 3:

Rented furniture

1 – N/A

2 –

Telecommunications/Electronics 1:

Internet/Wifi

1 – N/A

2 –

Telecommunications/Electronics 2:

Mobile phone data

1 – N/A

2 –

Telecommunications/Electronics 3:

Specialty software

1 – N/A

2 –

Telecommunications/Electronics 4:

Mobile phones

1 –

2 –

Telecommunications/Electronics 5:

Solar panels to charge phones & computers

1 –

2 –

Telecommunications/Electronics 6:

GPS

1 –

2 –

Telecommunications/Electronics 7:

Mobile Hotspots

1 –

2 –

Other 1 (specify)**:** _____

1 –

2 –

Other 2 (specify): _____

1 –

2 –

Please identify any office staff positions directly related to these outbreak investigation and response activities:

1 Job title:

1 Number of individuals:

2 Job title:

2 Number of individuals:

3 Job title:

3 Number of individuals:

4 Job title:

4 Number of individual

NB - There is a more complex addition to this section, covering the percentage of total expenditure for each item reported and how it is distributed across each individual stage of the outbreak investigation. A percentage of total expenditure form for this section is to be completed after this initial section is complete.

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Travel & Transport Costs

Respondent details

Name:

Position:

Email:

Phone:

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Section 3 of 7 – Travel & Transport Costs

Please complete any monetary questions in ***Nigerian Naira***

If any section below is not applicable to the current outbreak, please write N/A

The following 2 questions are to be answered for each of the listed travel and transport items below (where applicable), and for any additional travel and transport-related items absent from the list:

1 - Quantity used

2 - Total expenditure on item to support outbreak investigation and response activities

Vehicle related costs 1: **Fuel costs**

1 – N/A

2 –

Vehicle related costs 2: **Rented or hired vehicles**

1 –

2 –

Vehicle related costs 3: **Parking (quantity = days)**

1 –

2 –

Vehicle related costs 4: **Purchased vehicles**

1 –

2 –

Vehicle related costs 5: **Maintenance & repair costs**

1 – N/A

2 –

Travel & Lodging 1: **Lodging (quantity = nights)**

1 –

2 –

Travel & Lodging 2: **Per diem expenses (food etc.) (quantity = days)**

1 –

2 –

Travel & Lodging 3: **Airfare for deployed personnel**

1 – N/A

2 –

Travel & Lodging 4: **Taxi & Bus fares**

1 – N/A

2 –

Other 1 (specify)**:** _____

1 –

2 –

Other 2 (specify): _____

1 –

2 –

Please identify any travel and transport staff positions directly related to these outbreak investigation and response activities:

1 Job title:

1 Number of individuals:

2 Job title:

2 Number of individuals:

3 Job title:

3 Number of individuals:

4 Job title:

4 Number of individuals:

NB - There is a more complex addition to this section, covering the percentage of total expenditure for each item reported and how it is distributed across each individual stage of the outbreak investigation. A percentage of total expenditure form for this section is to be completed after this initial section is complete.

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Communication Costs

Respondent details

Name:

Position:

Email:

Phone:

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Section 4 of 7 – Communication Costs

*Please complete any monetary questions in **Nigerian Naira***

If any section below is not applicable to the current outbreak, please write N/A

The following 2 questions are to be answered for each of the listed communication items below (where applicable), and for any additional communication-related items absent from the list:

1 - Quantity used

2 - Total expenditure on item to support outbreak investigation and response activities

Outreach/Awareness 1: **Airtime for national radio broadcasts to communicate/warn about outbreak**

1 – N/A

2 –

Outreach/Awareness 2: **Airtime for national television broadcasts to communicate/warn about outbreak**

1 – N/A

2 –

Outreach/Awareness 3: **Outbreak ads in national newspapers**

1 –

2 –

Outreach/Awareness 4: **Airtime for local radio broadcast to communicate/warn about outbreak**

1 – N/A

2 –

Outreach/Awareness 5: **Airtime for local television broadcasts communicate/warn about outbreak**

1 – N/A

2 –

Outreach/Awareness 6: **Ads in local newspapers to communicate/warn about outbreak**

1 –

2 –

Outreach/Awareness 7: **Wall Posters to communicate/warn about outbreak**

1 –

2 –

Outreach/Awareness 8: **T-shirts to raise awareness for outbreaks**

1 –

2 –

Other 1 (specify)**:**

_____

1 –

2 –

Other 2 (specify):

_____

1 –

2 –

Please identify any communications staff positions directly related to these outbreak investigation and response activities:

1 Job title:

1 Number of individuals:

2 Job title:

2 Number of individuals:

3 Job title:

3 Number of individuals:

4 Job title:

4 Number of individuals:

NB - There is a more complex addition to this section, covering the percentage of total expenditure for each item reported and how it is distributed across each individual stage of the outbreak investigation. A percentage of total expenditure form for this section is to be completed after this initial section is complete.

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Laboratory Support Costs

Respondent details

Name:

Position:

Email:

Phone:

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Section 5 of 7 – Laboratory Support Costs

*Please complete any monetary questions in **Nigerian Naira***

If any section below is not applicable to the current outbreak, please write N/A

The following question is to be answered for the laboratory items listed below (where applicable), and for any additional laboratory-related items absent from the list:

1 - Total expenditure on item to support outbreak investigation and response activities

Specimen testing 1: **Identification of pathogens** 1 –

1 –

Specimen testing 2: **Data management**

1 –

Other 1 (specify)**:**

_____

1 –

Specimen testing 3: **Data analysis and results**

1 –

Other 2 (specify):

_____

1 –

Specimen testing 4: **Waste management**

Please identify any laboratory staff positions directly related to these outbreak investigation and response activities:

1 Job title:

1 Number of individuals:

3 Job title:

3 Number of individuals:

2 Job title:

2 Number of individuals:

4 Job title:

4 Number of individuals:

NB - There is a more complex addition to this section, covering the percentage of total expenditure for each item reported and how it is distributed across each individual stage of the outbreak investigation. A percentage of total expenditure form for this section is to be completed after this initial section is complete.

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Medical Countermeasures Costs

Respondent details

Name:

Position:

Email:

Phone:

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Section 6 of 7 – Medical Countermeasures (Non-labour) Costs

*Please complete any monetary questions in **Nigerian Naira***

If any section below is not applicable to the current outbreak, please write N/A

The following 2 questions are to be answered for each of the listed medical countermeasures items below (where applicable), and for any additional medical countermeasures-related items absent from the list:

1 - Quantity used

2 - Total expenditure on item to support outbreak investigation and response activities

Drugs 1: **Drugs for prevention: Vaccines**

1 –

2 –

Drugs 2: **Antibiotic prophylaxis**

1 –

2 –

Drugs 3: **Additional drugs (specify):** _____

1 –

2 –

Drugs 4: **Additional drugs (specify):** _____

1 –

2 –

Drugs 5: **Additional drugs (specify):** _____

1 –

2 –

Control measures 1: **Quarantine**

1 – N/A

2 –

Control measures 2: **Closing food premises**

1 – N/A

2 –

Control measures 3: **Animal culls**

1 – N/A

2 –

2 –

Other 1 (specify)**:**

_____

Control measures 4: **Disposal or decontamination of contaminated items**

1 –

1 – N/A

2 –

2 –

Other 2 (specify):

_____

Prevention measures 1: **Water chlorination**

1 –

1 – N/A

2 –

2 –

Prevention measures 2:
Impregnated bed nets

1 –

Please identify any medical staff positions directly related to these outbreak investigation and response activities:

1 Job title:

2 Number of individuals:

1 Number of individuals:

3 Job title:

2 Job title:

3 Number of individuals:

4 Job title:

4 Number of individuals:

5 Job title:

5 Number of individuals:

6 Job title:

6 Number of individuals:

NB - There is a more complex addition to this section, covering the percentage of total expenditure for each item reported and how it is distributed across each individual stage of the outbreak investigation. A percentage of total expenditure form for this section is to be completed after this initial section is complete.

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Consultancies Costs

Respondent details

Name:

Position:

Email:

Phone:

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Section 7 of 7 – Consultancies Costs

Please complete any monetary questions in ***Nigerian Naira***

If any section below is not applicable to the current outbreak, please write N/A

The following question is to be answered for each of the listed consultancy areas below (where applicable), and for any additional consultancy areas absent from the list:

1 - Total expenditure on consultancy used to support outbreak response activities

1 Consultancy for database development	
1 –	2 Consultancy for database management

1 –

3 Consultancy for data collection

1 –

4 Consultancy for data analysis

1 –

5 Consultancy for field epidemiology

1 –

6 Consultancy for biology/entomology

1 –

7 Consultancy for training

1 –

8 Consultancy for risk communications and media trainings

1 –

9 Consultancy on development of case management guidelines for safety hazards (zoonotic, food safety etc.)

1 –

Other 1 (specify)**:**
_____

1 –

Other 2 (specify):
_____

1 –

Please identify any consultancy staff positions directly related to these outbreak investigation and response activities:

1 Job title:

1 Number of individuals:

2 Job title:

2 Number of individuals:

3 Job title:

3 Number of individuals:

4 Job title:

4 Number of individuals:

NB - There is a more complex addition to this section, covering the percentage of total expenditure for each item reported and how it is distributed across each individual stage of the outbreak investigation. A percentage of total expenditure form for this section is to be completed after this initial section is complete.

Costing for hypothetical non-typhoidal *Salmonella* outbreak, 2020, Nigeria

Non-labour costs questionnaire: Percentage of total expenditure form

Respondent details

Name: _____

Institute & Position: _____

Email: _____

Phone: _____

Date: _____/_____/_____

Instructions

Please complete the box below.

*Each of the items listed by yourself in the previous **Office Materials Questionnaire** are listed below. For each item, please indicate below what **percentage** of total expenditure was incurred during each period of the outbreak. Periods of outbreak include:*

- ***Initial response period***
- ***Outbreak response period***

- ***Implementation, follow up & reporting period***

  *Each box should contain a **single percentage**.*

  *Each item row should total **100%***

ITEM	**INITIAL RESPONSE PERIOD: *% of total expenditures*** Including the following activities: • Prepare • Verify outbreak • Verify diagnosis • Construct case definition • Record cases • Perform descriptive epidemiology • Develop hypothesis • Evaluate hypothesis • Refine hypothesis • Reconcile evidence	**OUTBREAK RESPONSE PERIOD: *% of total expenditures*** Including: • Implement control & prevention measures	**IMPLEMENTATION, FOLLOW UP AND REPORTING PERIOD: *% of total expenditures*** Including: • Initiate or maintain surveillance • Disseminate findings	**TOTAL (%)** (each individual row should total 100% - this is like the final questions)

Supplementary Material 5.2

Supplemental: Table of and basis for assumptions

S/No.	Assumptions and sources of Costs	Reference
1.	Budget allocation – Labour (83.51%) versus non-labour (16.49%) Total attributable budget (Diarrhoeal Diseases Programme – Salmonellosis (1.3%): Health (Fed and States MoH) = N4,845,138,991; Agric (Fed and States MoA&RD) = N628,238,672.96	Federal Ministry of Health (2017). Nigeria Health Financing Policy and Strategy, 2017. Available at: https://nesgroup.org/download_policy_drafts/Nigeria-Health-Financing-Policy-Strategy_2017-21032019_1661875118.pdf. Accessed 03 June 2023. Chaitkin M. 2022. Intergovernmental Rivalry and Fragmentation: How Federalism Shapes Public Financial Management and Health Financing in Nigeria. Case Study Series on Devolution, Health Financing, and Public Financial Management. Washington, DC: ThinkWell. Available at: https://thinkwell.global/wp-content/uploads/2022/04/Nigeria-Case-Study-April-2022.pdf. Accessed 03 June 2023. BudGIT, 2022. Appropriation Amendment: Federal Ministry of Agriculture And Rural Development, and National Veterinary Research Institute budgets. Available at: https://budgit.org/wp-content/uploads/2023/01/2022-Appropritation-Bill.pdf. Accessed 03 October 2023. Vanguard Newspaper, 2019. 2019: Buhari, 33 governors budget N15.737 trillion, available: https://www.vanguardngr.com/2019/01/2019-buhari-33-governors-budget-n15-737-trillion/. Accessed 3 October 2023.
2.	Mean monthly salaries and time contributed to diarrhoeal disease programme	***Field Survey***: Med lab: new intake N140,000, seniors 320,000; Pharmacists: new intake N130,000, seniors N300,000; Medical officers: new intake N240,000, seniors N620,000; Public Health Specialist N620,000; Epidemiologist Junior N240,000, Senior N620,000; Technicians N75,000; Veterinarian: new intake N240,000, seniors N325,000; Veterinary epidemiologist: new intake N240,000, seniors N480,000; Zonal Veterinary officers: state N500,000, Federal 490,000; Veterinary technician N85,000; Veterinary extension officer N240,000; Veterinary laboratory scientist N170,000; Project managers N780,000; Others: 60,000 – 150,000. Minimum wage for not technical officers: N30,000.
3.	Office costs	Field survey
4.	Purchase and distribution of resources	Resources (human and material) are purchased or distributed at different levels based on different considerations: National (n = 1), States (n = 36 plus FCT = 37), Regional (Zonal) (n = 109) and Local Government Authority (n = 774)
5.	Vehicle price (N25 million) (Range: N6.93m – N25m)	Available at: https://nigerianprice.com/prices-of-toyota-hilux-in-nigeria/
6.	Daily allowances to investigate an outbreak (limited) (N350,000 per outbreak)	Field survey
7.	Communication costs	Based on co-contribution from the Diarrhoeal Programme
8.	PPE Cost (US$ 13.04)	Bolas, T., Werner, K., Alkenbrack, S., Uribe, M. V., Wang, M., & Risko, N. (2023). The economic value of personal protective equipment for healthcare workers. PLOS global public health, 3(6), e0002043. https://doi.org/10.1371/journal.pgph.0002043
9.	100 pieces syringes with needles (N14,999.00)	Available at: https://www.jumia.com.ng/mlp-syringes/
10.	Pipette tips (N14,091 per 1000 pieces)	Available at: https://www.jumia.com.ng/generic-10ul-200ul-1000ul-5ml-pipette-micropipette-tip-for-123627222.html
11.	Laboratory support costs	Field survey
12.	Poultry Fowl typhoid vaccines (NVRI) (N1000 per 100 doses)	Available at: https://www.nvri.gov.ng/products Personal Communication: Dr. David Lazarus
13.	Antibiotic therapy (Assuming that 50% of all human cases implement self-treatment at a cost of N13,309.10)	Field survey, Uzochukwu and Onwujekwe, 2004.
14.	Cost of Widal's test	Available at: https://www.surjen.com/lab-test
15.	Hospitalization and Treatment costs (N22,815.60)	Field survey
16.	Miscellaneous medical countermeasures costs (10% of cumulative)	Subject matters experts' opinions
17.	Consultancy costs (Equivalent of mean GHS support cost per country = US$1,000,000)	Subject matters experts' opinions
18.	Contingencies and Miscellaneous expenses (N100,000,000)	Subject matters experts' opinions

Please note that a number of the non-labour costs are contributory services where many activities budget contribute to the Health budget pool, hence partial attribution in cost contribution was given to Non-Typhoidal Salmonellosis.

Supplementary material 2_Outbreak_c

Outbreak Costing Tool.

Supplementary Table 5.3. Outbreak Costing Tool individual cost items for each non-labor cost category

Office materials and equipment	Travel and transport	Communication	Laboratory support	Medical countermeasures	Consultancies
Stationeries	Fuel costs	Airtime for national radio broadcasts	Personal protective equipment	Drugs for prevention: Vaccines	Database development
Printing/copies	Rented or hired vehicles	Airtime for national television broadcasts	Syringes	Antibiotic prophylaxis	Database management
Rented building space	Parking	Advertisements in national newspapers	Pipettes	Quarantine	Data collection
Rented equipment	Purchased vehicles	Airtime for local radio broadcasts	Reagents	Closing of food premises	Data analyses
Rented furniture	Maintenance and repair costs	Airtime for local television broadcasts	Shipment of materials	Animal culls	Field epidemiology
Internet	Lodging	Advertisements in local newspapers	Specimen collection	Disposal or decontamination of contaminated items	Biology/ entomology
Cellular data	Per diem expenses (e.g., food)	Wall poster advertisements	Specimen transport	Water chlorination	Training
Speciality software	Airfare for deployed personnel	T-shirts to raise outbreak awareness	Specimen processing	Impregnated bed nets	Risk communication and media trainings
Mobile phones	Taxi and bus fares		Identification of pathogens		Development of case management guidelines for safety hazards (e.g., zoonotic, food safety)
Solar panels to charge phones and computers			Data management		
Global Positioning System devices			Data analysis and results		
Mobile hotspots			Waste management		

Supplementary Material 5.4

Supplementary material 4_Scenario ai

Scenario Analysis.

Chapter 6: Risk Factors for Persistent Infection of Non-Typhoidal *Salmonella* in Poultry Farms, North Central Nigeria

Supplementary Table 6.1. Categorization of Variables based on selected industry standards and peer reviewed literature.

Question	Category	Reference	Reclassification	Notes	Reference
State	State by state	No		-	-
Serial number	-	No		-	-
L .G. A	L. G. A by L. G. A	No		-	-
Gender	0 vs 1	Y	M=1 F=0	There 57% of women and 51% of men with a gender gap of 7.2% are involved in poultry.	The World Bank Nigeria development report (2021) (https://www.worldbank.org/en/country/nigeria/publication/nigeria-development-update-ndu).
Age of respondents	0 vs 1	Y	41 and above = 1 1-40 = 0	The mean age of poultry farmers is 40 years	Gender participation in commercial poultry production (http://www.lrrd.org/lrrd22/9/okoh22160)
Length in poultry farming	0 vs 1	Y	6 and above =1 1-5 = 0	65% of farmers have mean farming experience of 6 years	Differentials in technical efficiency among broiler farmers in Imo state Nigeria (https://www.ajol.infor/index.php/naj/article/view/196166/185183)
Education level	0 vs 1	Y	Primary &secondary =0 Tertiary & others higher qualifications =1	40% of poultry farm owners hold secondary school cert, 35% a university degree and 8.3% a primary school certificate.	Socio-economic factors as determinants of farm management skills (www.resarchgate.net/publication/321650666)
Farm location				Skipped, not analyzed	
Name of farm				Skipped, not analyzed	
Type of poultry	0 vs 1	Y	Broiler = 0 Layer & others =1		Short cycle and long cycle

Number of chickens in the farm	0 vs 1	Y	500 and above = 0 1-499 = 1		-
Source of feed	0 vs 1	Y	Self-compounded = 1 Commercial = 0	Poultry farmers prefer to use self-compounded feeds than commercial feeds	Poultry farmers preference and use of commercial and self-formulated feeds (https://www.researchgate.net/publication/223151006)
Source of water for birds	0 vs 1	Y	Borehole/tap borne = 0 Stream/well/ others = 1	27% of poultry farmers depend on borehole, tap water combined and 3% on depended solely well, stream or river.	quality of different water sources used in poultry and piggery farms in southeastern Nigeria (https://www.researchgate.net/publication/349180707)
Pen type	0 vs 1	Y	Standard block= 0 Others = 1	In commercial and semi commercial setting in developing countries, chickens are normally housed in naturally ventilated pen with additional lightning provided in form of electricity	Poultry development review (https://wwwfao.org/3/i3531e/i3531e.pdf)
System of management	0 vs 1	Y	Deep litter = 1 Battery cage = 0	There are three primary intensive control: deep litter, battery case and wire floor system.	Types of poultry management systems (https://fabioclass.com/poultry-management-systems/)
Litter material	0 vs 1	Y	Saw dust/wood shavings/sand =0 Cement floor= 1		Beddings and no beddings
Litter management	0 vs 1	Y	Good=0 Poor/fair=1	Daily grading of litter should be done.it is advisable to also use dry lime in order to keep litre dry.	Poultry litter management for better performance and production (https://www.pashudhanpraharee.com/poultry-

					litter-management-for-better-performance-and-production/)
Pen odour	0 vs 1	Y	Yes =1 No =0	Ammonia is the cause of pen odour and the most environmentally significant aerial pollution associated with poultry production	Poultry development review (https://wwwfao.org/3/i3531e/i3531e.pdf)
Stocking density	0 vs 1	Y	1-16 =0 17 & above = 1		Code of practice 2012, broiler production South Africa http://www.sapoultry.co.za/pdf-docs/code-practice-broilers.pdf
Adherence to Vaccination	0 vs 1	Y	Yes=1 No/partial=0	87% of poultry farmers vaccinate their chickens	An appraisal of the use of vaccination for disease prevention in poultry in Ibadan, Nigeria. (www.ajol.info/index.php/bahpa/article/view/76526)
Practice biosecurity	0 vs 1	Y	Yes = 1 No/partial = 0	Practice of biosecurity in the study area was high	Adoption of biosecurity for disease prevention and control by poultry farmers in Imo state, Nigeria (www.ajol.info/index.php/jafs/article/view/204206)
Ever administered fowl typhoid/ cholera vaccine	0 vs 1	Y	Yes = 1 No = 0	4% of farmers vaccinate chickens against fowl typhoid and fowl cholera	An appraisal of the use of vaccination for disease prevention in poultry in Ibadan, Nigeria. (www.ajol.info/index.php/bahpa/article/view/76526)
Ever heard of *Salmonella* infection in poultry	0 vs 1	Y	Yes = 1 No = 0	-	-
Ever experienced Salmonellosis	0 vs 1	Y	Yes=1 No=0	Large scale farms had experienced more *Salmonella* prevalence at 33% prevalence rate	Prevalence of *Salmonella* in chicken , farm attendants and beddings (www.researchgat.net/figure/prevalence-of-*Salmonella*-in-chickens-

					farm-attendants-and-bediing -in-hawassa-and-bonga-_tbi2_317032593)
If *Salmonella* ever encountered how was it managed/controlled	0 vs 1	Y	Antibiotic/Vaccination = 1 Others = 0	-	Survey
Knowledge of *Salmonella* as a zoonotic agent	0 vs 1	Y	No knowledge = 0 Knowledge = 1	Majority of respondent have good knowledge about poultry diseases but not poultry zoonotic diseases	Understanding attitude, practices and knowledge of zoonotic infectious disease risks among poultry farmers in Ghana (https://onlinelibrary.wiley.com/doi/10.1002/vms3.257)
Source of knowledge of *Salmonella* as major zoonotic agent	0 vs 1	Y	Media = 1 Others = 0	Farmers with higher education level and longer experience have improved knowledge of zoonotic poultry diseases	Understanding attitude, practices and knowledge of zoonotic infectious disease risks among poultry farmers in Ghana (https://onlinelibrary.wiley.com/doi/10.1002/vms3.257)
Ever encounter mortality of chickens	0 vs 1	Y	No = 0 Yes = 1	A mortality rate of 1.5% or less is normal however zero mortality is the aim.	Mortality in poultry (https://agreenerworld.org/wp-content/uploads/2018/05/TAFS-8-Mortality-in-poultry-v3.pdf)
Type of mortality	0 vs 1	Y	High & moderate = 1 Low/normal = 0		Survey
Ever taken sample to a veterinarian/ animal health lab.	0 vs 1	Y	No = 0 Yes = 1	-	-
Type of Sample	-	No			Survey
What was the result?	0 vs 1	Y	*Salmonella* / *Salmonella* + others=1	-	-

			No *Salmonella* = 0		
What did you do after the result?	0 vs 1	Y	Sell = 1 Others = 0	-	Survey
What is the cost of treatment?	0 vs 1	Y	High = 1 Others = 1	-	Survey
Estimated cost of mortality from *Salmonella*	0 vs 1	Y	High= 1 Others= 0	-	Survey
Did *Salmonella* affect your production?	-	No		-	Survey
Nature of effect on production	-	No		-	Survey
Profit after sales	-	No		-	Survey
Access to professional support	0 vs 1	Y	Yes= 1 Others= 0	Shortage of professional affects availability of support.	Challenges and prospect of poultry industry (https://www.grin.com/document/296347)

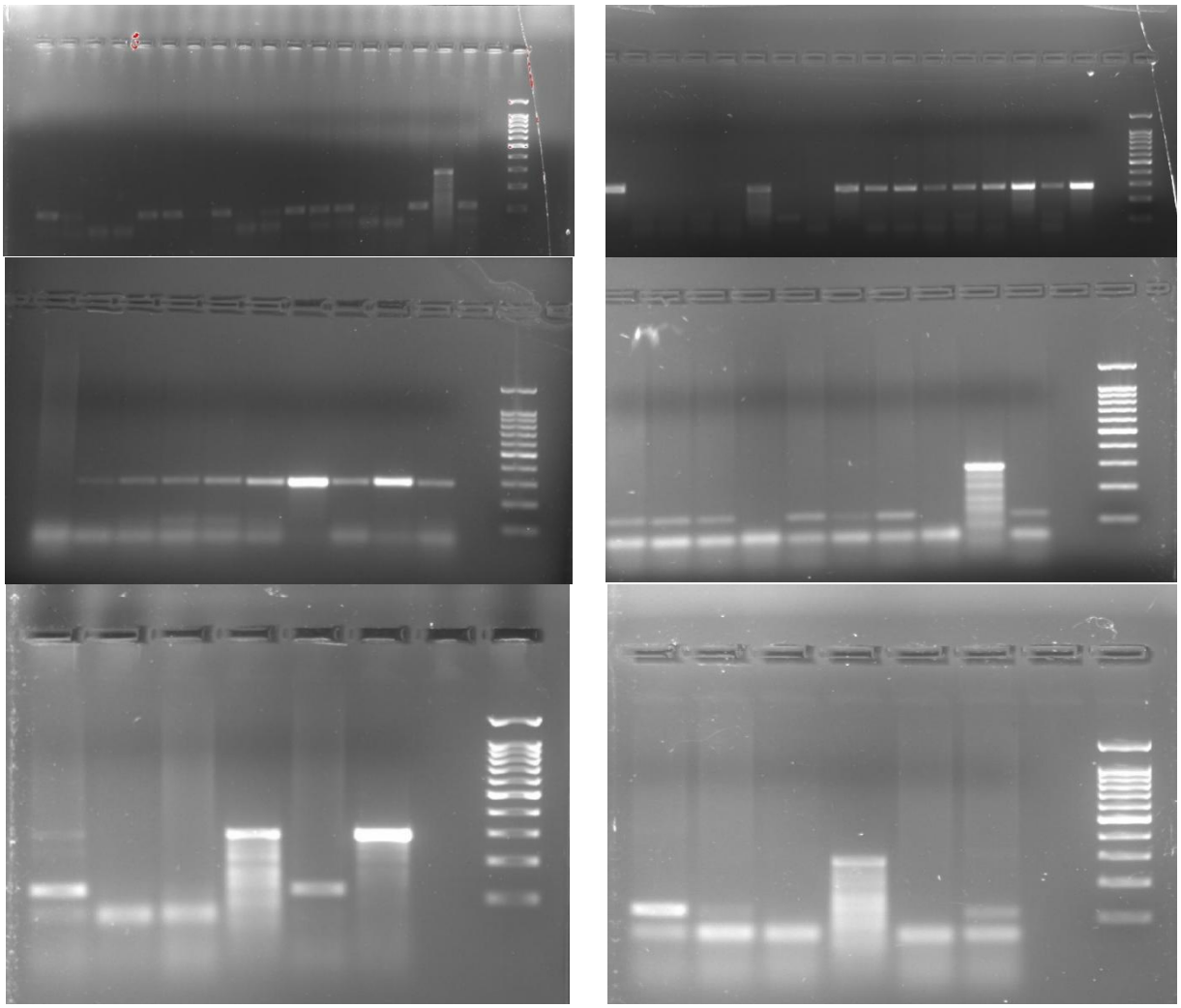
LGA = Local Government Authority; Y = Yes.

Supplementary material 6.2. Sample Questionnaire for risk factor data collection in the field

1. State -
2. Serial Number -
3. LGA -
4. Gender - A. Male, B. Female
5. Age of respondents – A. >60, B. 41-60, C. 21-40, D.<20
6. Length in Poultry Farming - <2years, B. 2-4years, C. 4-6years, D. >6years
7. Educational level – A. primary B. Secondary C. Tertiary D. others
8. Farm location -
9. Name of farm -
10. Type of poultry (commercial or backyard) – A. broilers B. Layers C. mixed D. others
11. Number of chickens in the farm – A. <200 B. 201-500 C. 501-1000 D. >1000
12. Source of feed- A. commercial feed B. concentrate mix C. self-compounded
13. Source of water for birds – A. borehole B. tap borne C. Well D. Stream E. others (describe)
14. Pen type – A. standard block B. dwarf block C. zinc type D. others
15. System of management – A. deep litter B. battery cage C. others
16. Litter material – A. Sawdust B. wood shavings C. Sand D. cement floor E. others
17. Litter management – A. Good B. Poor C. Fair
18. Pen odour – A. Yes B. No
19. Stocking density – A. 12-14/M^2 B. 14-16/M^2 C. 16-18/M^2 D. 18-20/M^2 E. >20/M^2 F. not known
20. Adherence to vaccination - A. Yes B. No C. Partial
21. Practice biosecurity - A. Yes B. No C. Partial
22. Ever administered fowl typhoid/ cholera vaccine - A. Yes B. No
23. Ever heard of *Salmonella* infections in poultry - A. Yes B. No
24. Ever experienced *Salmonella* infection on farm - A. Yes B. No C. Don't Know
25. If *Salmonella* ever encountered how was it managed/controlled – A. antibiotics B. Vaccination C. antibiotics and vaccination D. culling and sale E. others
26. Knowledge of *Salmonella* as a zoonotic agent - A. Yes B. No
27. Source of knowledge of *Salmonella* as a zoonotic agent A. electronic media B. print media C. extension agent D. vet/animal health officer E. other farmers F. hospital
28. Ever encountered mortality of chickens - A. Yes B. No
29. Type of mortality A. high B. Moderate C. Low D. normal occurrence

30. Ever taken samples to a veterinarian/animal health lab - A. Yes B. No
31. What type of samples – A. feces B. Egg C. whole bird D. all E. others
32. What was the result – A. *Salmonella* B. *Salmonella* and another infection C. other
33. What did you do after the result? A. Treat B. Sell C. others
34. What is the cost of treatment – A. high B. Moderate C. Low D. others
35. Estimated cost of mortality from *Salmonella* – A. high B. Moderate C. Low D. others
36. Did *Salmonella* affect your production - A. Yes B. No
37. Nature of effect on production: A. high B. Moderate C. Low D. others
38. Profit after sales - A. Yes B. No
39. Access to professional support - A. Yes B. No C. Not always D. others

Chapter 7: Molecular Epidemiology and Antimicrobial Resistance Patterns of Non-Typhoidal *Salmonella* Spp. Found in Poultry Farms, North Central Nigeria



Amplicons of *Salmonella* spp. visualized on stained 1.5% agarose gels. The amplicon size was 284 bp measured against a 100 bp standard DNA ladder. The reference *Salmonella* strain (ATCC 14028) was used as positive control and sterile water without DNA was used as negative control.

Supplementary figure 7.1. Amplicons of Salmonella spp. visualized on stained 1.5% agarose gels.

*Note that the 42 *Salmonella* isolates identified in this work will be sequenced and published at a later date outside the scope of the PhD.

Chapter 8: Spatial Distribution and Predictive Risk of Perpetuation of Non-Typhoidal Salmonellosis in Poultry Farms and Human Communities, Nigeria

Supplementary material 8.1. Prevalence of poultry salmonellosis and non-typhoidal salmonellosis in Nigeria

Supplementary material 1_prev of poi

Supplementary material 8.2. Meta-analyses and forest plot of poultry salmonellosis, 2000 – 2020, Nigeria

Supplementary material 2-Meta-analy