

Prevalence and Antibiograms of *Salmonella* in Commercially Produced Crocodile meat in Zimbabwe

A.Z.Nhidza¹, C. Gufe², J.Marumure^{3*}, Z.Makuvara³, T.Chisango¹, G.T.Hanyire⁴, G.Jongi², P.V.Makaya², and T.S.Marambe²

¹Chinhoyi University of Technology, P Bag 7724, Chinhoyi, Zimbabwe

²Department of Veterinary Technical Services, Central Veterinary Laboratories, Box CY55, 18A Borrowdale Road, Harare, Zimbabwe

³School of Natural Sciences, Great Zimbabwe University, P. Bag 1235, Masvingo, Zimbabwe

⁴Department of Veterinary Technical Services, Wildlife unit, Box CY55, 18A Borrowdale Road, Harare, Zimbabwe

*Corresponding Author: Email: jmarumure@gzu.ac.zw

Article timeline

Submitted: 23-04-2021, Revised: 16-07-2021, Accepted: 03-08-2021, Published: 28-08-2021

Tanzania Veterinary Journal Vol. 36 (1) 2021

<https://dx.doi.org/10.4314/tvj.v36i1.1>

Manuscript submission, annual subscriptions and other information: <https://tvj.sua.ac.tz>

Single article purchases: <https://www.ajol.info/index.php/tvj/index>

Prevalence and Antibiograms of *Salmonella* in Commercially Produced Crocodile meat in Zimbabwe

A.Z.Nhidza¹, C. Gufe², J.Marumure^{3*}, Z.Makuvara³, T.Chisango¹, G.T.Hanyire⁴, G.Jongi², P.V.Makaya², and T.S.Marambe²

¹Chinhoyi University of Technology, P Bag 7724, Chinhoyi, Zimbabwe

²Department of Veterinary Technical Services, Central Veterinary Laboratories, Box CY55, 18A Borrowdale Road, Harare, Zimbabwe

³School of Natural Sciences, Great Zimbabwe University, P. Bag 1235, Masvingo, Zimbabwe

⁴Department of Veterinary Technical Services, Wildlife unit, Box CY55, 18A Borrowdale Road, Harare, Zimbabwe

*Corresponding Author: Email: jmarumure@gzu.ac.zw

SUMMARY

The presence of *Salmonella* in food products and emergence of antibiotic resistance are the major challenges facing public health policies. A total of 2749 crocodile meat samples obtained from the Central Veterinary Laboratories in Zimbabwe were screened for *Salmonella* species. The crocodile meat samples were collected from three Zimbabwean commercial farms between the year 2012 and 2019 for a retrospective observational study to determine the prevalence and magnitude of antibiotics resistant *Salmonella* species in crocodile meat. The isolation of *Salmonella* was in accordance with the ISO 6579:2002 and the antibiotic susceptibility testing was carried out based on Clinical and Laboratory Standard Institute's recommendations by means of the Kirby-Bauer disk diffusion method. SILAB Database was used to determine the prevalence of *Salmonella* species. Prevalence was stratified by year and farms. Twenty *Salmonella* isolates were identified using biochemical tests, and 15 were confirmed by polymerase chain reaction (PCR). Antimicrobial susceptibility profiles of the confirmed *Salmonella* isolates were examined using 14 antibiotics. The overall prevalence of *Salmonella* species in crocodile meat samples was 0.5%. The prevalence of *Salmonella* species ranged from 0.04% to 0.44% in the crocodile meat samples and annual prevalence ranged from 0.01% to 1%. The highest prevalence of *Salmonella* (4.4%) was recorded in the year 2012. *Salmonella* isolates from one of the three tested farms were resistant to Erythromycin (73.33%), Ampicillin (80%), and Penicillin G (100%). Generally, *Salmonella* isolates displayed lower resistance to Cefepime, Ceftriaxone, Amikacin, Tetracycline, Ertapenem, Florfenicol, and Erythromycin (0-53.33%) whereas all *Salmonella* isolates showed susceptibility to Cefepime, Ceftriaxone, Ertapenem, and Florfenicol. Although the study indicates low prevalence of *Salmonella* species in crocodile meat, there is a need for strict implementation of Hazard Analysis Critical Control Point (HACCP) to reduce contamination rates in meat and its products.

Keywords: Antimicrobial Susceptibility testing, *Salmonella*, Prevalence, Crocodile meat salmonellosis, typhoid

INTRODUCTION

Salmonella is a globally important cause of foodborne diseases in humans with increased morbidity, mortality, and economic loss (Wu *et al.*, 2019, Taddese *et al.*, 2019, Raseta *et al.*, 2017, Pal *et al.*, 2015). According to research, *Salmonella* has resulted in 93.8 million illnesses and 155,000 deaths per year globally (Raseta *et al.*, 2017). Reports from the World Health Organization indicate that salmonellosis cases have increased significantly over the past few years (Chen *et al.*, 2020).

WHO estimates billions of dollars worth of economic burden resulting from salmonellosis, with tens of millions of people affected every year (WHO, 2015). The increase in salmonellosis is correlated to the antimicrobial resistance of *Salmonella* (Taddese *et al.*, 2019). Foodborne pathogenic microorganisms such as *Salmonella* spp. are the leading causes of human infection and diseases in developing countries, leading to remarkably high medical expenses (Raseta *et al.*, 2017).

Salmonella species in food products have become an animal and public health challenge worldwide (Kaur *et al.*, 2018). *Salmonella* spp. are generally carried in the intestinal tracts of reptiles as part of their normal gut flora (Wikström *et al.*, 2014) and are intermittently shed when animals are predisposed to stressful conditions such as overcrowding. (Whiley *et al.*, 2017). Farmed crocodiles can be infected with *Salmonellae* in the contaminated feed from local meat sources (Huchzermeyer, 1991). Hence, the chances of transmission of drug-resistant *Salmonella* from crocodiles to humans can occur both through direct contact and along the farm to fork chain.

Salmonellae are primarily enteric bacteria and widespread in the environment, mainly because of animals and human excreta (Regalado-pineda *et al.*, 2020, OIE TERRESTRIAL MANUAL, 2012). The leading cause of *Salmonella*-related illnesses is *Salmonella enterica*, and this pathogen can be transmitted via a myriad of routes from animal and environmental sources to human hosts (Raseta *et al.*, 2017). Foodborne diseases emanate from a wide

range of contaminated foods that include beef, chicken, fish, and eggs. Research has shown that some countries have recorded higher *Salmonella* prevalence rates in crocodile flesh for human consumption than beef rates (Manolis *et al.*, 1991). Foods, including crocodile meat, can get contaminated during slaughtering, transportation, processing, storage, and distribution to shops (Taddese *et al.*, 2019). Salmonellosis can result in nontyphoidal or typhoid fever in humans, and the incubation period of *Salmonella* ranges from 12 to 72 hours (Chai *et al.*, 2019). Generally, salmonellosis in humans manifests itself as diarrhea, fever, and abdominal pain.

Of late, human health threat has been embedded in pathogenic microorganisms' evolution, including multidrug-resistant strains. The development of antibiotic-resistant *Salmonella* spp. is connected to the extensive and intensive use of antibiotics for prophylactic and treatment purposes in veterinary medicine (Wu *et al.*, 2019, Ecker *et al.*, 2011, Reda *et al.*, 2011, Yang *et al.*, 2010). *Salmonella* species possess several mobile elements such as plasmids, genomic islands, and transposons that are sources of antimicrobial resistance (Wu *et al.*, 2019, V T Nair *et al.*, 2018, Xia *et al.*, 2009). *Salmonella* spp. antimicrobial resistance may be due to mutations in genes responsible for antimicrobial resistance (Raseta *et al.*, 2017).

Increased antimicrobial resistance levels observed in zoonotic pathogens such as *Salmonella* have become a global public health concern, as this contributes to salmonellosis re-emergence (Francesca *et al.*, 2015). The resistance towards traditional first-line antibiotics such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole define multidrug resistance (MDR) in *Salmonella enterica* (Kaur *et al.*, 2018, Crump and Mintz, 2010).

In line with antibiotics resistance and reduction of MDR *Salmonella* transfer risk to humans, there is an urgent need to monitor the indiscriminate and injudicious use of antibiotics in food animals worldwide (Chen *et al.*, 2020, Zhao *et al.*, 2003).

Salmonella is now defined in the standard operating procedure for the microbiological monitoring of imported meat as a biological agent associated with severe illness or death (Edel, 1994). In this study, we hypothesized that all *Salmonella* isolates in crocodile meat from three selected crocodile farms in Zimbabwe during the period 2012 to 2019 are susceptible to antibiotics, and the percentage prevalence is the same per year per farm. In Zimbabwe, crocodile skin and meat are traded legally on European and local markets (Makanyanga *et al.*, 2014) however; there are limited reports on the

prevalence and antimicrobial susceptibility of *Salmonella* from crocodile meat in Zimbabwe. To this end, there is also a need for continuous *Salmonella* surveillance and dissemination of their antimicrobial sensitivity data. Therefore, the current study examines the prevalence and antibiograms of *Salmonella* species in crocodile meat from three commercialized farms in Zimbabwe. The information will assist health policymakers to establish health policies on the handling and processing of crocodile meat.

MATERIALS AND METHODS

The study was approved and conducted at Central Veterinary Laboratories (CVL), Division of Veterinary Services, Harare, Zimbabwe which is among the accredited diagnostic laboratories in Zimbabwe for microbiology tests including microscopy, culture, identification and antibiotic sensitivity testing.

Study Design

A retrospective observational study design was conducted from 2012 to 2019 on *Salmonella* isolates from 2749 crocodile meat samples. Crocodile meat samples were collected from three commercialized farms in Zimbabwe. The significance of the crocodile meat sample size was estimated based on previous reports and a formula for Quantitative Veterinary Epidemiology at a 95% level of confidence and an expected prevalence of 20% with an absolute precision of 0.05.

The calculated minimum sample size was 196. SILAB laboratory management system and the conventional filing system were used to search for the samples tested during the timeline. Crocodile meat samples were stratified according to the timeline and farms. The other part of this study involves an antimicrobial susceptibility test (AST) where a Barium Sulfate turbidity, equivalent to a 0.5 McFarland standard, was used to standardize the inoculum.

Sample Collection

The study was based on crocodile meat samples from three commercialized crocodile

farms in Zimbabwe that were submitted for analysis at CVL during the 2012-2019 period. The farms were named X, Y, Z for convenience and meat samples distribution was 45.4% (1247), 39.3% (1081) and 15.3% (421) respectively. Tail meat samples were randomly selected at slaughtering stage and three samples were collected from each carcass.

Media and cotton swab preparation

All media was prepared according to the manufacturer's instructions. The media was sterilized by autoclaving at 121°C for 15 minutes and poured into sterile sample tubes following aseptic techniques. Sterile cotton swabs were prepared by wrapping the ends of the swab sticks with 0.85% saline, moistened cotton wool, and these cotton swabs were autoclaved while wrapped in aluminum foil paper.

Salmonella identification

Salmonella species were identified using colony morphology on Xylose Lysine Deoxycholate Agar (XLD), Blood agar (BA), and MacConkey agar (MAC). The positive colonies from the three nutrient media (XLD, BA, and MAC) were further analyzed using gram staining, biochemical tests, serotyping, and PCR.

Salmonella detection was achieved by inoculating 25g of the sample into Buffered Peptone Water (BPW) and incubating at 37 ± 2°C for 18-24 hours. After incubation, 1ml of the incubated crocodile meat BPW broth was

transferred into a selective enrichment medium, Rappaport Vassiliadis (RV) broth, and incubated at $37 \pm 2^\circ\text{C}$ for 18-24 hours. A loopful of the incubated RV broth was then streaked on Xylose Lysine Deoxycholate agar using sterile loops and incubated at $37 \pm 2^\circ\text{C}$ for 18-24 hours.

Pure colonies were tested for motility, catalase, oxidase, urease, indole, triple iron sugar, Lysine/Decarboxylase and Gram's stain. Colonies showing *Salmonella* growth characteristics and on biochemical tests were then sub-cultured on Blood agar (BA) and MacConkey and incubated at $37 \pm 2^\circ\text{C}$ for 18-24 hours.

A few pure colonies of BA were suspended in saline on a microscope slide, and *Salmonella* antisera were added. *Salmonella* serotyping was only done to Genus level using Polyvalent O antigens (A-S groups) and polyvalent H antigen (Phase 1 and Phase 2) antisera.

Salmonella isolates identified using biochemical tests were further confirmed with polymerase chain reaction (PCR). Colonies from pure culture plates were used for DNA extraction. DNA extraction was done according to the Zymo Research DNA extraction kit. All *Salmonella* isolates were confirmed by PCR using sets of primers specific to the *Salmonella* serotypes. Amplification was carried out using a Gene

Amp Thermal PCR System 2400 (Perkin Elmer Corp, Norwalk, CT, USA), under the following cycling parameters: initial denaturation at 94°C for 4 minutes, 30 cycles of 30 s for denaturation at 94°C , 1 minute 30 s for annealing at 56°C , and 30 s for primer extension at 72°C , followed by a terminal extension at 72°C for 7 minutes. The master mix was prepared as a multiple of the samples tested, with negative control and an equivalent of one volume master mix allowance for pipetting error.

No positive control sample was available for the test. The mixture was vortexed and aliquoted into reaction tubes. For PCR, using ST11/ST14 primers: **ST11** 5'-GCCAACCATTGCTAAATTGGCGCA,

ST14

5'GGTAGAAATTCCCAGCGGGTACTGG) (Pan *et al.*, 2002). These primers were specific for *Salmonella* species and randomly cloned chromosomal fragment of 429 bp. Amplified products were electrophoresed in 2% Ethidium Bromide-stained agarose gels with a 100-bp DNA ladder for a size marker. The gel was visualized under UV light in a gel documentation.

Antibiograms of *Salmonella* species

The Kirby-Bauer disc diffusion method was used to determine the confirmed *Salmonella* species' susceptibility patterns to antibiotics. A few colonies of *Salmonella* isolates were picked and emulsified into saline water until turbidity of 0.5 McFarland was reached.

A sterile swab was dipped in the test suspension and streaked in three directions over the entire surface of the Muller Hinton agar, and a final sweep with the swab was made against the agar around the rim of the plate. Antibiotic impregnated discs (Oxoid) were applied onto the plates using an antibiotic disk dispenser. The plates were incubated for 18-24 hours at $37 \pm 2^\circ\text{C}$.

Antimicrobial susceptibility testing was conducted using 14 antibiotics (Amoxicillin (10 μg), Amikacin (30 μg), Ampicillin (10 μg), Cefepime (30 μg), Ceftriaxone (30 μg), Erythromycin (15 μg), Ertapenem (10 μg), Florfenicol (30 μg), Kanamycin (30 μg), Neomycin (10 μg), Penicillin G (10 μg), Trimethoprim (5 μg),

Trimethoprim/Sulphamethoxazole(co-trimoxazole, 1.25/23.75 μg), Tetracycline (30 μg)). The assay was performed based on the Standard Operating Procedure (SOP) adapted from the Clinical and Laboratory Standards Institute (CLSI). Zones of inhibition were measured for each isolate for the antibiotics used. Using breakpoints adopted from CLSI, the results were then reported as sensitive, intermediate, and resistant. An isolate was defined as multidrug-resistant (MDR) if resistant to three or more of the antimicrobial classes tested. Multiple antibiotic resistance (MAR) index was also calculated.

Data Analysis

Graphpad Prism 8.01 was used to analyze the percentage prevalence of *Salmonella* in crocodile meat per farm and yearly. Prevalence of *Salmonella* species was calculated as a proportion of positives against the number of samples tested. WHONET software for antimicrobial

resistance was used to analyse the zones of inhibition and MDR was determined by identifying isolates showing resistance to more than three antimicrobial classes. Data obtained were used for calculating the MAR index as shown below

Prevalence of *Salmonella* in crocodile meat per farm per year

$$= \left(\frac{\text{number of positives}}{\text{number of samples tested}} \right)$$

$$\text{MAR index} = \frac{\text{number of resistant antibiotics to which isolates are resistant to}}{\text{number of antibiotics used}}$$

RESULTS

Crocodile meat sample distribution and biochemical testing

The submission was highest in 2013, while the lowest was recorded in 2016. Generally, farm Z submitted the least number of samples each year, not exceeding 100 samples (Figure 1).

Biochemical tests and serotyping

On XLD agar, positive colonies were red or pink with distinct black centers. The colonies on MAC were round, raised, and had a pale color. *Salmonella* spp. produced colonies that were round, raised, shiny, non-hemolytic, and grey on BA.

Gram staining results showed short gram-negative rods.

Twenty putative *Salmonella* positive isolates were obtained from 2749 crocodile meat samples submitted, and these isolates were further screened using PCR, and the results are shown in Figure 2.

Prevalence Rates of *Salmonella* spp

The overall prevalence was 0.55% (15/2749). Farm X had a prevalence of 0.07% (2/2749) and farm Y with 0.04% (1/2749). The prevalence was highest in farm Z, which recorded 0.44% (12/2749).

The Tropical Veterinarian

Using the Wilcoxon Signed Rank Test (Graphpad prism 8.01) at a significance level of 0.05, there were no significant differences ($P=0.25$) between the prevalence of *Salmonella* spp. from crocodile meat from the three farms (Table 1). Year-on-year prevalence rates of *Salmonella* spp. from crocodile meat samples were low during the period 2013 to 2019, ranging from 0.01% to 1%, and the year 2012 recorded the highest prevalence of 4.4% (Figure 3). Statistical analysis using a one-sample t-test (Graphpad prism 8.01) at a significance level of 0.05, showed no significant ($P=0.079$) differences between the prevalence of *Salmonella* spp. in crocodile meat per year.

Salmonella isolates show high resistance levels of over 73.33% to Erythromycin, ampicillin, and Penicillin G. However, the isolates were susceptible to Cefepime, Ceftriaxone, Amikacin, tetracycline, Ertapenem, Florfenicol, and Erythromycin (0–53.33%). Antimicrobials for which *Salmonella* isolates possess different resistance mechanisms (Ampicillin, Kanamycin, Amikacin, Tetracycline, and Trimethoprim–Sulphamethoxazole) were multiple resistance markers. Multidrug resistance was highest *Salmonella* species from farm Z, with >50% isolates (Table 2).

<https://dx.doi.org/10.4314/tvj.v36i1.1>

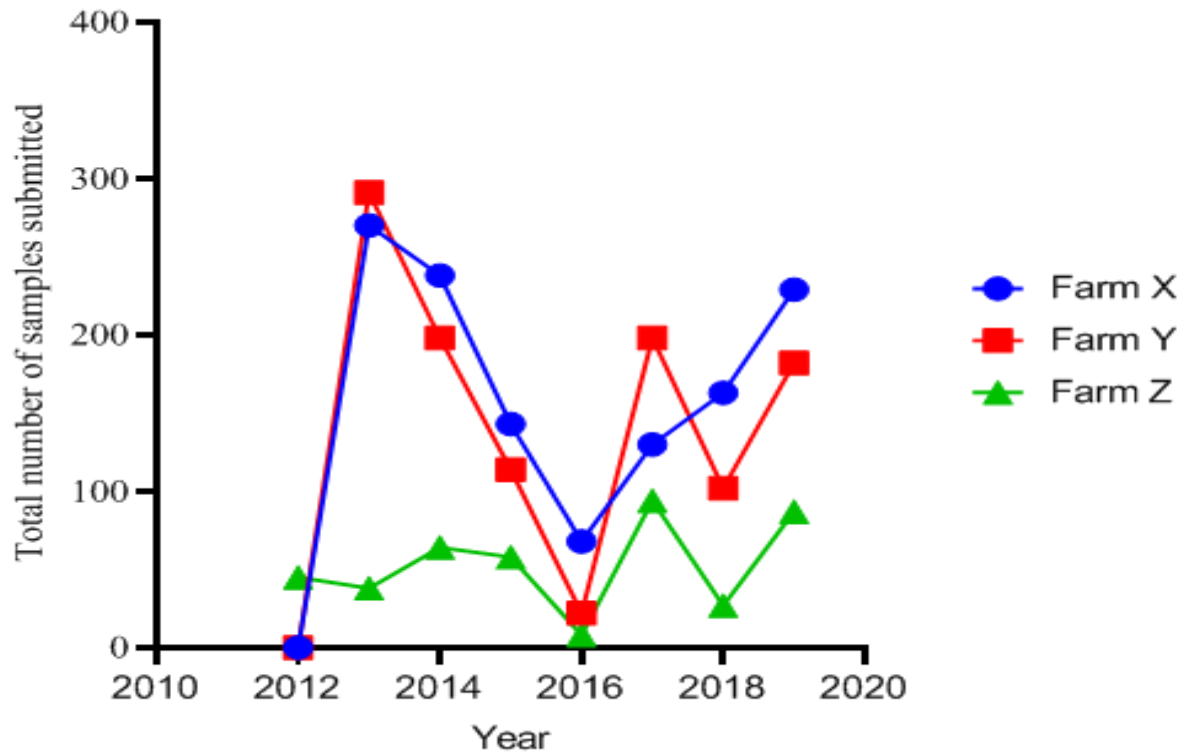


Figure 1. Distribution of samples submitted from different farms during the period 2012-2019.

Table 1: Prevalence of *Salmonella* isolated in crocodile meat from three farms in Zimbabwe, from 2012 - 2019

Farm	Crocodile meat samples (n)	Number of <i>Salmonella</i> isolates	% prevalence	Wilcoxon Signed-Rank Test
X	1247	2	0.07	P value = 0.25 95% CI 0.020- 0.400
Y	1081	1	0.04	
Z	421	12	0.44	
Total	2749	15	0.55	

X, Y, Z: Hypothetical farm names used for convenience to conceal the identity of actual farm names.

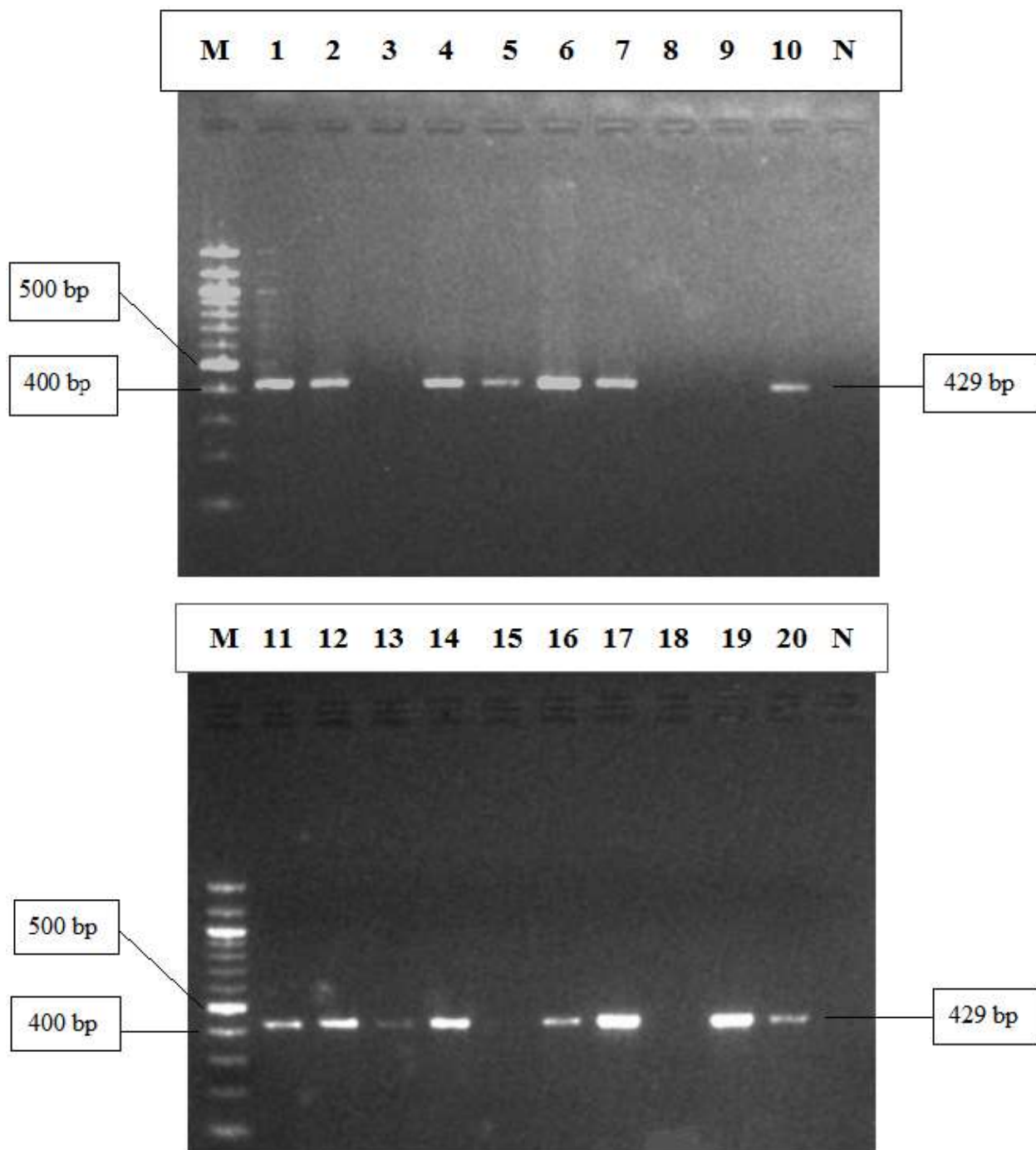


Figure 2. PCR results for *Salmonella* isolates from crocodile meat samples. M represents the 100 bp ladder, While lane N indicates the negative control. Lane 1, 2, 4, 5, 6, 7, 10, 11, 12, 13, 14, 16, 17, 19 and 20 show presence of *Salmonella* spp (429 bp). Out of the 20 isolates presumed positive for *Salmonella*, 15 isolates produced bands of approximately 429 bp.

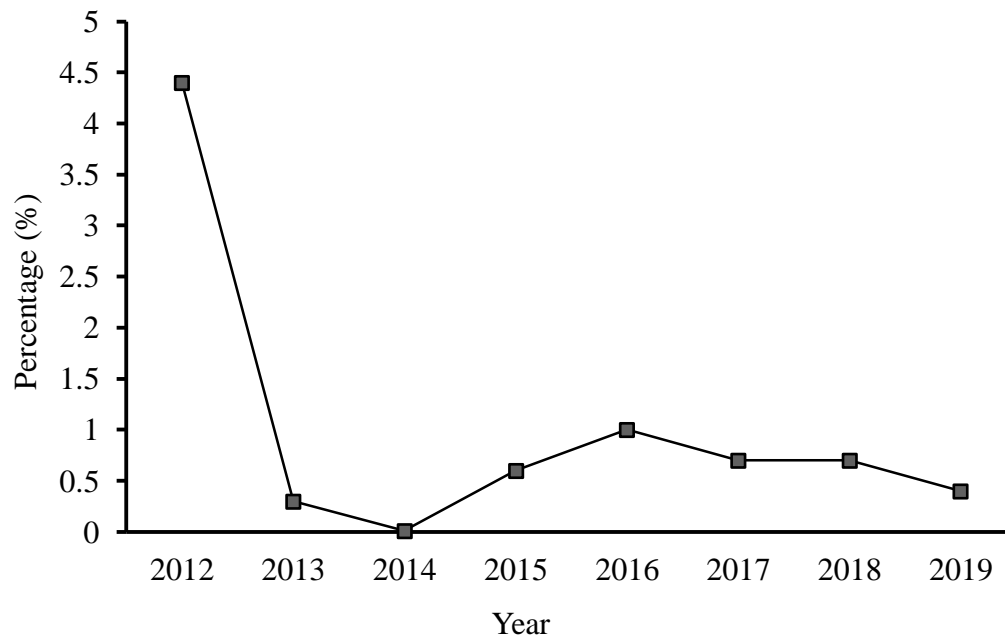


Figure 3. Yearly prevalence rates of *Salmonella* spp. over the period 2012-2019

Table 2: Antibiotic susceptibility profiles of *Salmonella* isolates from crocodile meat.

Antibiotic name	Code	Break points	NO. of Isolates	Resistance (R) % (n/N)	Intermediate (I) % (n/N)	Susceptible (S) % (n/N)	MAR Index & MDR
Amikacin	AN	<15-16>	15	0 (0/15)	13.33 (2/15)	86.67 (13/15)	
Amoxicillin	AMX	<14-17>	15	53.33 (8/15)	20 (3/15)	26.67 (4/15)	
Ampicillin	AM	<14-16>	15	80 (12/15)	0(0/15)	20(3/15)	MAR Index.
Cefepime	FEP	<19-24>	15	0 (0/15)	0 (0/15)	100 (15/15)	0.7
Ceftriaxone	CRO	<20-22>	15	0(0/15)	0(0/15)	100(15/15)	
Ertapenem	ETP	<19-21>	15	0(0/15)	0(0/15)	100(15/15)	
Erythromycin	E	<14-22>	15	73.33 (11/15)	6.67(1/15)	20 (3/15)	
Florfenicol	FLR	<15-18>	15	0(0/15)	0(0/15)	100(15/15)	MDR
Kanamycin	K	<14-17>	15	20(3/15)	20(3/15)	60(9/15)	5/15(33%)
Neomycin	NEO	<13-14>	15	26.67	46.67	26.67	
Penicillin G	P	S _≥ 29	15	100(15/15)	0(0/15)	0(0/15)	
Tetracycline	TE	<12-14>	15	20(3/15)	26.67(4/15)	53.33 (8/15)	
Trimethoprim	TMP	<11-15>	15	33.33(5/15)	6.67(1/15)	60(9/15)	
Sulfamethoxazole	SXT	<11-15>	15	33.33(5/15)	6.67(1/15)	60(9/15)	

Key: % percentages, n= number of isolates R/I/S for each antibiotic, N= total number of isolates tested for each antibiotic; < resistant, > sensitive and – intermediate, MDR: Multidrug Resistant, MAR: Multiple antibiotic resistance

DISCUSSION

The number of samples submitted during the period 2012-2019 was uneven (Fig 1). This could be correlated with variations in the market over the period under study. The uneven distribution of samples may be

linked to changes in the European market during the 2012 to 2019 period. The uneven distribution of crocodile meat samples submitted can also be attributed to economic challenges, which have reduced the samples,

as farmers could not afford to send crocodile meat to Europe. Farm priority in crocodile farming can also be a contributing fact since some farmers might not find it necessary to have their meat tested since they concentrate mainly on crocodile skin production.

Twenty *Salmonella* isolates were obtained from 2749 crocodile meat samples submitted, and only 15 *Salmonella* isolates were confirmed using PCR. The primers used in this study checked for *Salmonella* species' presence that could infect the crocodile population under study. PCR primers used managed to amplify only 15 of the 20 presumed positives, and this could be due to the existence of bacteria with the same antigenic properties as *Salmonella*.

In this current study, the low overall *Salmonella* spp. with the prevalence rate of 0.55% (Table 1) is in line with reports by Taddese *et al* (2019). The overall prevalence rate was low in crocodile meat from the three farms due to little crocodiles stock per farm and strict HACCP. Farms X and Y recorded less than 0.1% prevalence rate of *Salmonella* species in meat, which corroborates the studies done by other researchers (Makaya *et al.*,2012). In Poland's research, significantly lower prevalence (0.4-0.7%) of *Salmonella* spp. in meat preparations were reported (Raseta *et al.*, 2017). However, a higher prevalence of *Salmonella* spp. in meat was reported (Asfaw *et al.*,2020, Nghiem *et al.*, 2019, Magwedere *et al.*, 2015, Makaya *et al.*, 2012).

Although the differences in the prevalence of *Salmonella* spp. in crocodile meat were not statistically significant (Table 1), the three farms' prevalence rates were different. The differences in the prevalence of *Salmonella* sp. in crocodile meat from the three farms can be attributed to geographical and seasonal variation, animal management practices, and hygienic conditions. Prevalence of *Salmonella* spp. in crocodile meat farm Z is ten times higher than farms X and Y (Table 1), and this is linked to the tight HACCP at farms X and Y. Farms X and Y are involved in skin and meat production for export. Therefore rigorous quality controls are implemented. More so, farms X and Y are using meat for human

consumption, and the procedures for crocodile slaughter and meat processing minimize the risks of *Salmonella* contamination.

On the contrary, farm Z is solely for skin production; hence the quality controls might not be so tight. *Salmonella* spp. are generally carried in crocodiles' intestinal tracts and released when crocodiles are stressed and could contribute to the high prevalence at farm Z. Moreover, intestinal *Salmonella* spp. in asymptomatic crocodile and crocodile pond water may act as possible sources of contamination for crocodile meat (Makanyanga *et al.*,2014).

Furthermore, the other contributing factors to a high prevalence of *Salmonella* spp. in crocodile meat from farm Z could be feeding off the crocodiles with *Salmonella* contaminated meat and poor hygienic practices slaughter and handling. Variation in prevalence rates of *Salmonella* can be linked to environmental contamination, breed, sample size, and method of isolation.

Although there were no significant differences between the yearly prevalence, the highest annual prevalence (4%) of *Salmonella* spp. was recorded in 2012 (Figure 3). The highest prevalence of 4% was similar to the results reported for ready-to-eat meat samples in Bangladesh (5%) and Egypt (4.4%) (Dhary, 2019).

Salmonella prevalence in our study from 2013 to 2019 was less than 1%, which was in line with what was reported on one farm in Zimbabwe (0.05%) (Makanyanga *et al.*,2014). The low prevalence of *Salmonella* isolates during the period 2013 to 2019 (0.01% to 1%) is in contrast with the higher prevalence reported for ready to eat meat in Korea (22.4%), Australia (38.8%), and China (54%) (Dhary, 2019). Variation in *Salmonella* isolates' yearly prevalence (Figure 3) may be linked to seasonality, sanitation practices, and microbial detection methods. The observed low isolates of *Salmonella* spp. from 2013 to 2019 contrasted with previous findings where the prevalence of 15–30.9% was reported (Gelaw *et al.*, 2018, Makanyanga *et al.*,2014). In one study conducted by Magwedere *et al* (2015), the highest

prevalence of *Salmonella* of 5.7% was recorded in 2013, and this was almost similar to our study findings. Generally, *Salmonella* yearly prevalence in the current study was low, which could be linked to the slow recovery of *Salmonella* spp. after disinfection by chlorination and pasteurization of carcasses.

In the current study, *Salmonella* isolates were highly susceptible to second and third-generation antibiotics like Cefepime (100%), Ceftriaxone (100%), Ertapenem (100%), and Amikacin (100%). However, significant antimicrobial resistance was recorded with traditional first-line antibiotics Ampicillin (80%), Erythromycin (73.33%), and Penicillin G (100%). Our results were similar to Italy's reports during 2005-2006, Poland during 2008-2012, and Thailand (Raseta *et al.*, 2017). Moreover, antimicrobial resistance to antibiotics such as ampicillin (80%) and susceptibility to antibiotics such as Ceftriaxone (100%) concurs with findings by Taddese *et al* (2019).

In other researches, resistance to Cefepime and Ceftriaxone was reported (Zhang *et al.*, 2014). In a comparative study, crocodile isolates' resistance rates were lower than isolates from domesticated animals and humans (Reda *et al.*, 2011), which indicates that crocodiles are not customarily treated with antibiotics in these farms. This study's findings were in line with other studies that reported high resistance rates towards Ampicillin and Tetracycline (Mthembu *et al.*, 2019).

Of the 15 isolates tested, 10 showed resistance to at least a single antibiotic. Multidrug resistance was indicated in 33% (5/15) of the isolates tested, and this contrasts studies that have shown high MDR percentages above 50 (Mutai *et al.*, 2018,

Zhang *et al.*, 2014). According to Taddese *et al* (2019) and Jajere (2019), the resistance of *Salmonella* spp can be linked to inappropriate medication and imprudent use of antibiotics. The overall multidrug resistance percentage (33) recorded in this study is almost similar to the findings of Nghiem *et al* (2019) (multi antimicrobial resistance= 36%). A high MAR index (0.71) was observed in our study, which indicates the high use/misuse of antibiotics in three farms (X, Y, Z).

The observed MAR index was higher compared to the findings of other studies (Beshiru *et al.*, 2019). The bacterial resistance development is linked to the selective pressure of antibiotics, which have resulted in the emergence of new strains that are adapted to a wide range of antimicrobials. Notably, MAR index >0.2 indicates a high-risk contamination source in cases where antibiotics are often used. The MAR of 0.71 shows that there might be a high risk of antibiotics contamination from the environment and excreta from humans and animals.

The low prevalence rates of *Salmonella* spp. from crocodile meat recorded can be due to HACCP implemented by the crocodile farmers. However, the isolation of *Salmonella* spp. from some of the crocodile meat samples is a cause for concern from a public health point of view. Thus, stricter HACCP and implementation of measures to avoid contamination of the meat after pasteurization need to be carefully considered. Further work should focus on multiplex PCR targeting antimicrobial resistance genes, stratification of *Salmonella* isolates resistance patterns according to species and continuous *Salmonella* surveillance and dissemination of their antimicrobial sensitivity data.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Pious Vengesayi Makaya, Managing Director of Central Veterinary Laboratories, to provide reagents and access the equipment used in

this study. Special thanks go to the crocodile farmers for supplying meat for bacterial isolation

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

- Asfaw Ali D, Tadesse B, Ebabu, A. Prevalence and Antibiotic Resistance Pattern of *Salmonella* Isolated from Caecal contents of Exotic Chicken in Debre Zeit and Modjo, Ethiopia. *Int.J. Microbiol*, <https://doi.org/10.1155/2020/1910630>, 2020.
- Beshiru A, Igbinsosa I H, Igbinsosa, E O. Prevalence of antimicrobial resistance and virulence gene elements of *Salmonella* serovars from ready-to-eat (RTE) shrimps. *Front. microbiol*, 10, 1–11. <https://doi.org/10.3389/fmicb.2019.0161>, 2019.
- Chai SJ, Gu W, O'Connor KA, Richardson LC, Tauxe RV. Incubation periods of enteric illnesses in foodborne outbreaks, United States, 1998-2013. *Epidemiology and infection*, 147, e285. <https://doi.org/10.1017/S0950268819001651>, 2019.
- Chen Z, Bai J, Wang S, Zhang X, Zhan Z, Shen H, Zhang H, Wen J, Gao Y, Liao M, Zhang J. Prevalence, Antimicrobial Resistance, Virulence Genes and Genetic Diversity of *Salmonella* Isolated from Retail Duck Meat in Southern China. 1–12, *Microorganisms*, 2020.
- Crump J A, MintzE. D. Global trends in typhoid and paratyphoid fever. *Emerg Infect Dis*, 50: 241-246, 2010.
- Dhary A. Occurrence and antimicrobial susceptibility of *Salmonella* isolates from grilled chicken meat sold at retail outlets in Erbil City, Kurdistan Region, Iraq. *Ital. J. Food Saf*, volume 8:8233, 2019.
- Ecker L, Olarte L, Vilchez G, Ochoa T J, Amemiya I. Physicians' responsibility for antibiotic use in infants from periurban Lima, Peru. *Rev Panam Salud Publica* 30: 574-579, 2011.
- Edel W. *Salmonella* Enteritidis eradication program in poultry breeder flocks in The Netherlands, *Int. J. Food Microbiol*, 21: 171–178, 1994.
- Prestinaci F, Pezzotti P, Pantosti A. Review: Antimicrobial resistance: a global, multifaceted phenomenon, *Pathog Glob Health*, 109(7): 309–318. doi: 10.1179/2047773215Y.0000000030, 2015.
- Gelaw A K, Nthaba P, Matle I. Detection of *Salmonella* from animal sources in South Africa between 2007 and 2014. *J S AFR VET ASSOC*, 89, 1–10. <https://doi.org/10.4102/jsava.v89i0.1643>, 2018.
- Huchzermeyer KDA. Treatment and control of an outbreak of salmonellosis in hatchling Nile crocodiles (*Crocodylus niloticus*), *J S Afr Vet Assoc*, 62(1):23-25, 1991.
- Jajere S M. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity, and antimicrobial resistance, including multidrug resistance, *Vet World*, 12, 504–521, 2019.
- Kaur A, Kapil A, ElangovanR, Jha S, Kalyanasundaram D. Highly-sensitive detection of *Salmonella typhi* in clinical blood samples by magnetic nanoparticle-based enrichment and in-situ measurement of isothermal amplification of nucleic acids, *PLoS ONE*, 1–14, 2018.
- Magwedere K, Rauff D, De Klerk G, Keddy K H, Dziva F. Incidence of nontyphoidal *Salmonella* in food-producing animals, animal feed, and the associated environment in South Africa, 2012-

2014. Clin. Infec. Dis, 61(Suppl 4), S283–S289.
<https://doi.org/10.1093/cid/civ663>, 2015.
- Makanyanga T B, Mutema G, Mukarati N L, Chikerema S M, Makaya P V, Musari S, Matope G. International Journal of Food Microbiology Microbial quality of frozen Nile crocodile (*Crocodylus niloticus*) meat samples from three selected farms in Zimbabwe. Int. J. Food Microbiol, 170, 44–47.
<https://doi.org/10.1016/j.ijfoodmicro.2013.10.020>, 2014.
- Makaya P V, Matope G, Pfukenyi D. M. Distribution of *Salmonella* serovars and antimicrobial susceptibility of *Salmonella* Enteritidis from poultry Zimbabwe. Avian Pathol, 41(2), 221226, 2012.
- Manolis S C, Webb G J W, Pinch D, Melville L, Hollis G. *Salmonella* in captive crocodiles. Aust. Vet. J, 68: 102–105, 1991.
- Mthembu T P, Zishiri O T, El Zowalaty M E. Molecular detection of multidrug-resistant *Salmonella* isolated from livestock production systems in South Africa. INFECT DRUG RESIST, 12, 3537–3548.
<https://doi.org/10.2147/IDR.S211618>, 2019.
- Mutai W C, Muigai AWT, Waiyaki P, Kariuki S. Multidrug-resistant *Salmonella* enterica serovar *Typhi* isolates with reduced susceptibility to ciprofloxacin in Kenya. BMC Microbiol, 18(1), 4–8.
<https://doi.org/10.1186/s12866-018-1332-3>, 2018.
- Nghiem M N, Nguyen VT, Jeung EB, Vo TTB. Alternate antimicrobial resistance genes in multidrug-resistant *Salmonella* spp. isolated from retail meats in Vietnam using RNA-sequencing analysis. J.Food Saf, 39(6), 1–8.
<https://doi.org/10.1111/jfs.12707>, 2019.
- Obwolo MJ, Zwart P. Prevalence of *Salmonella* in the intestinal tracts of farm-reared crocodiles (*Crocodylus niloticus*) in Zimbabwe. J. Zoo Wildl. Med. 24. 175-176, 1993.
- OIE terrestrial manual. Chapter 2.9.9 – Salmonellosis, 2012.
- Pal M, Merera O, Abera F, Rahman MT, Hazarika RA. Salmonellosis: A major foodborne disease of Global significance, Beverage & Food World, 42: 12, 2015.
- Pan TM, liu YJ. Identification of *Salmonella* Enteritidis isolate by polymerase chain reaction and multiplex polymerase chain reaction, J. Microbiol. Immuno. Infect., 35: 147-151, 2002.
- Rašeta M, Mrdović B , Janković V, Bečkei Z, Lakićević B, Vidanović D, Polaček V. Prevalence and antibiotic resistance of *Salmonella* spp . in meat products, meat preparations, and minced meat, IOP Conf. Ser. Earth Environ. Sci, 2017.
- Reda AA, Seyoum B, Yimam J, Andualem G, Fiseha S, Vandeweerd JM. Antibiotic susceptibility patterns of *Salmonella* and *Shigella* isolates in Harar, Eastern, Ethiopia. J. Infect. Dis. Immun. 3: 134-139, 2011.
- Regalado-pineda ID, Rodarte-medina R, Resendiz-nava CN. Three-Year Longitudinal Study: Prevalence of *Salmonella* Enterica in Chicken Meat is Higher in Supermarkets than Wet Markets from Mexico, Foods , 2020.
- Taddese D, Tolosa T, Deresa B, Olani A, Shumi E. Antibigrams and risk factors of *Salmonella* isolates from laying hens and eggs in Jimma. BMC Res Notes, 1–7.
<https://doi.org/10.1186/s13104-019-4516-5>, 2019.
- V T Nair D, Venkitanarayanan K, Kollanoor Johny A. Antibiotic-Resistant *Salmonella* in the Food Supply and the Potential Role of Antibiotic Alternatives for Control. *Foods (Basel, Switzerland)*, 7(10), 167.
<https://doi.org/10.3390/foods7100167>, 2018.

- Wikström VO, Fernström LL, Melin L, Boqvist S. *Salmonella* isolated from individual reptiles and environmental samples from terraria in private households in Sweden. *Acta Vet Scand.* doi: 10.1186/1751-0147-56-7. PMID: 24461167; PMCID: PMC3922756, 2014.
- Whiley H, Gardner MG, Ross K. A Review of *Salmonella* and Squamates (Lizards, Snakes and Amphisbians): Implications for Public Health. *Pathogens.* doi: 10.3390/pathogens6030038. PMID: 28829352; PMCID: PMC5617995, 2017.
- WHO. Estimates of the global burden of foodborne diseases: Foodborne Diseases burden epidemiology reference group 2007-2015, 2015.
- Wu X, Suksawat F, Richards AL, Phommachanh S, Phongaran D, Angkititrakul S. Evaluation of the Containment of Antimicrobial-Resistant *Salmonella* Species from a Hazard Analysis and Critical Control Point (HACCP) and a Non-HACCP Pig Slaughterhouses in Northeast Thailand. *Pathogens,* 24;9(1):20. doi: 10.3390/pathogens9010020. PMID: 31878260; PMCID: PMC7168580, 2019.
- Xia S, Hendriksen RS, Xie Z, Huang L, Zhang J. Molecular characterization and antimicrobial susceptibility of *Salmonella* isolates from infections in humans in Henan Province, China. *J Clin Microbiol,* 47: 401-409, 2009.
- Yang B, Qu D, Zhang X, Shen J, Cui S, Shi Y, Xi M, Sheng M, Zhi S, Meng J. Prevalence and characterization of *Salmonella* serovars in retail meats of the marketplace in Shaanxi, China. *Int. J. Food Microbiol,* 141(1-2): 63-72. doi: 10.1016/j.ijfoodmicro.2010.04.015, 2010.
- Zhang L, Fu Y, Xiong Z, Ma Y, Wei Y, Qu X, Zhang H, Zhang J, Liao M. Highly Prevalent Multidrug-Resistant *Salmonella* From Chicken and Pork Meat at Retail Markets in Guangdong, China. *Front Microbiol.* 10;9:2104. doi: 10.3389/fmicb.2018.02104. PMID: 30258419; PMCID: PMC6143800, 2018.
- Zhao S, Datta AR, Ayers S, Friedman S, Walker RD, White DG. Antimicrobial-resistant *Salmonella* serovars isolated from imported foods. *Int. J. Food Microbiol,* 84(1): 87- 92. doi: 10.1016/S0168-1605(02)00402-6, 2003.