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Piggery Environment as a Source of *Salmonella* Contamination for Swine

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Abstract: A study was conducted to identify the degree of salmonella sp. from March to November, 2003; there were 4 visits to each of 5 selected swine farms in Umuahia. Feaces and urine samples were collected from swine on longitudinal and cross-sectional groups on each visit to each farm. Environmental samples including feed, water, pen walls, pen floor, rodents and flies were collected. All sample were evaluated as *salmonella* positive or negative by culture. *Salmonella* sp. were detected in 84(18.4%) of the swine samples with the following distribution piglets (15/113, 13.3%), weaners (23/115,20.0%) fatteners (30/20, 25.0%) and adults (16/109,14.7%). The piggery environmental swabs showed that *Salmonella* sp. were recovered from the wall (15/97, 15.5%), feaces (25/114, 21.99) Urine (2/56,, 3.69) floor 24/100 24.0%, water trough (19/45, 20.0%), and feed trough (8/45, 17.89). The result showed age differences in the distribution of salmonella spp. in Swine that the necessity of adopting more efficient hygienic measures in the areas where swine are raised in order to reduce the role of piggery environment in the spread of *salmonella.*

Key words: Piggery environment, age differences, salmonella sp., swine

INTRODUCTION

The social and economic impact of Food borne diseases is considerable, and *Salmonellosis* flays a significant role in this phenomenon. The cost associated with Food borne-related illness in humans is estimated to be between $ 7.7 and $ 8.4 billion annually. For the Period 1987, *SalmonellaI* accounted for 42% of the outbreaks and 51% of the cases[1]. The presence of *Salmonella* species in meat animals has resulted in *Salmonellosis* becoming the most important zoonosis in developed countries[2].

The results of previous epidemiologic investigation suggest that reservoir of *Salmonella* are widespread on swine farms as well as persistent over time[3-4-5-6].Although, there is a paucity of information on pattern of transmission of *Salmonella* on swine farms. Transmission is thought to occur from introduction of carrier animals into the herd, through contaminated feed, or by exposure to infected rodents or farm personnel [7]Among the most recognized source of introduction or risk factors associated with *Salmonella,* ineffective washing and disinfections protocols, multiple sources of incoming animals and lack in biosecurity are often observed[8] .

Since the farm is the initial source of infection for swine, prevention of infection at this stage of food processing continuum could greatly enhance the safety of pork as food. Research is needed to identify the factors associated with *Salmonella* sp. occurrence in farm and the intervention practices that with keep level low[9-10]. *Salmonella* sp. are also commonly found in the environment. They can survive for several weeks to months in wet, cool environments[11]. *Salmonella* had been defected in slurry, barn, dust, and even on pen surfaces after cleaning[12]. A recent study demonstrated that *Salmonella* can infects market age pigs exposed to a contaminated environment in a period of time as short as 2 hours[13].

Pigs and other livestock that carry *Salmonella* sp. are often healthy and show no visible signs of infection. Many animals will be infected at younger ages while the gut flora is not well established. Following the infection, in swine, the bacterium will be present in feaces for few days but will survive within the lymph nodes for many weeks or months[14]. Many swine units where *Salmonella* sp. have been detected may never experience disease due to the bacteria. However, certain conditions such as stress, other diseases, poor nutrition or hygiene can trigger clinical disease, called *Salmonellosis.* Information on *Salmonella* infection, contamination and infection cycle in swine production is important for effective control measures of *Salmonella* at the farm level. Nevertheless, evaluating farm-level control options requires knowledge of basic data such as prevalence and distribution of pathogens in animals and production unit.

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Table 1: Frequency and distribution of *Salmonella* Isolates among different age groups of pig from five different piggery farms

Source	Piglets +ve/tested %	Weaners +ve/tested %	Fatteners +ve/tested %	Adult +ve/tested %	Total +ve/tested %
Amawom	^{5}/33(15.21)	^{8}/33(18.6)	^{10}/33(30.3)	^{5}/32(15.6)	26/131(19.8)
Nnono	^{2}/24(8.3)	^{4}/25(16.0)	^{3}/25(12.0)	^{2}/24(8.3)	^{11}/98(11.2)
Ndume	6/27(22.2)	^{6}/23(26.1)	^{9}/27(33.3)	^{5}/25(20.0)	^{26}/102(25.5)
Amakama	^{2}/16(12.5)	^{6}/16(25.0)	^{5}/16(31.1)	^{2}/15(13.3)	^{13}/63(20.6)
Apumiri	^{0}/13(0.0)	^{3}/18(16.7)	^{3}/19(15.8)	^{2}/13(15.4)	^{8}/63(12.7)
Total	^{15}/113(13.3)	^{23}/115(20.0)	^{30}/20(25.0)	^{16}/109(14.7)	^{4}/457(18.4)
Mean	6.3±3.3	4.6±1.3	3 ± 2.4	3.2 ±1.6	

N.B. Percentage of occurrence = Actual number Isolated x 100 / Total number of samples tested

Table 2: Percentage occurrence of *Salmonella sp.* in five different piggery farms

Source	Wall %	Feaeces %	Urine %	Floor %	Water trough %	Feed trough %	Total
Amawom	17.2	28.1	11.8	24.0	7.1	14.3	19.8
Nnono	5.0	15.4	0.0	20.0	10.0	10.0	11.2
Ndume	15.0	24.0	8.3	40.0	20.0	20.0	25.5
Amakama	15.4	26.7	12.5	26.7	16.7	16.7	20.6
Apumiri	6.7	18.8	0.0	13.3	20.0	20.0	12.7
Total	12.4	22.8	6.3	26.0	17.8	15.6	18.3
Mean ± SD	2.4±1.2	5.2±2.3	0.6±1.3	5.2±2.3	1.6±1.2	1.4±1.2	

$p<0.05$

NB: Percentage of occurrence = Actual number of Isolate x 100 / Total number tested

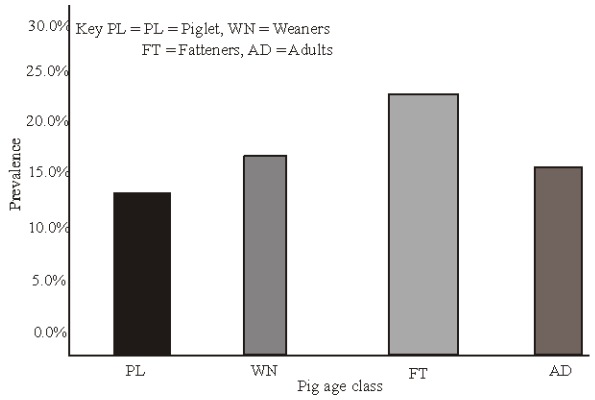

Fig. 1: Profile of age class distribution of *Salmonella* isolates

Fig. 2: Prevalence of *Salmonella* Isolates in piggery environment

This study was conducted to evaluate the degree of contamination of piggery environment with *Salmonella* and to evaluate the distribution of this pathogen among different age groups. The study will alert pig farmers on the danger of contaminated piggery environment in the transfer and maintainance of *Salmonella* species at Farm level.

MATERIALS AND METHODS

Sample collection. A total of 457 clinically healthy swine from four different age groups of pigs comprising 113(24.8%) Piglets, 115(25.1%) weaners, 120(26.3%) fatteners, 109(23.8%) adults were sampled. They were sampled from five different swine farms located in Umuahia of Abia State. Samples were obtained during a period of nine months from March to November, 2005. Rectal swab samples and swab sample of urine were taken from each animal. The piggery environment was sampled throughout this period and included feed, water, pen floor, urine, feaces and pen walls.

Isolation Procedure: 1.0 mL aliquot of each swab and 1.0ml aliquot of each environmental swab suspension (both types of suspension prepared in stercle saline, 10ml/swab) were transferred for pre-enrichment into 1.0ml Buffered peptone water (B.P.W, Merck 7228), P.H 7.5 and incubated for 18 h at 37°C. After 18 h of incubation at 37°C, 1ml of pre-enrichment culture (B.P.W) was transferred for enrichment into 9ml of selenite f-broth (BIOTECH) and incubated at 37°C for 18 h. Then one loopful (10 μL) of selective enrichment selentite F-broth culture was streaked into *Salmonella*-shigella agar

(SSA, LAB M) plates and incubated at 37°C overnight and suspected lactose-negative colonies were sub-cultured into slants of triple sugar Iron (T.S.I.) media (LAB-M). All bacterial isolates obtained were examined macroscopically for colony characteristics, Gram stained, purified and maintained in slants of nutrient agar. Further characterization was based on the result of various biochemical tests performed according to[15], and[16]. Agglutination test was carried out for confirmation.

Antigenic characterization: All presumptive *Salmonella* isolates were characterized antigenically by using the rapid serum agglutination techniques with polyvalent and monovalent somatic and flagella antisera (Poly. Al-V., Difco);[17]. All *Salmonella* isolates were serotyped at the diagnostic unit of National Veterinary Research Institute Vom, Nigeria. The disc diffusion method was used to determine the anti- biograms of Isolates.

RESULTS

Eighty four (84.4%) of the 457 pigs tested were found to be carriers of Salmonella species which represeat four pig age groups i.e Piglets, weaners, fatteners and adult (Table 1).

The prevalence and the carrier rate of different pig age group was determined (Fig.1). The bar chart showed that the fatteners had the highest carrier rate of 25.0% followed by the weaners (20.0%), while the least was piglets (13.3%).

Environmental samples yielded *Salmonella* isolates in 82 (18-3%) of the 447 examined sampler from the piggery house environment. The greatest percentage of Isolates was from floor (26.0%), followed by feaces (22.8%) and water trough (17.8%) Table 2. The farm environment yielded positive isolates throughout the study. Fig 2 shows the percentage of Salmonella isolates from piggery environments. The highest *Salmonella* isolates was from floor (26.0%, while the least was from urine (6.3%).

Distinct Farm-to-Farm differences were detected in the overall prevalence of *Salmonella* swine water trough (Fig. 3). One Farm, designated with code ND had an overall *Salmonella* prevalence of 40.0% in water trough. The range on the other four (4) operations in the study was 7.1% to 20.0% prevalence of *Salmonella* in floor samples Fig. 4. than did the other four (4) Farms.

Table 3: Seasonal Variations of the occurrence of *Salmonella*

	Rainy	Dry	Total
Number of samples (environment)	45	350	800
Number of Isolates	100	60	160
Percentage of all Salmonella Positive sample obtained In a given season	63	37	
Percentage of samples of a season Which were positive	22	16	
Number of serovars	30	20	50
Percentage of serovars	60	40	100

Table 3. shows the number of samples and number of *Salmonella* isolates distributed through out the seasons. A total of 160 samples were positive for *Salmonella,* which meant that 20.0% of the 800 samples had *Salmonella.* The larger number of *Salmonella* isolates was obtained during the rainy season, and the smaller number was obtained during the dry season. Table 3 further illustrates percentages of the seasonal isolates of the total isolates and percentages of the samples which were positive. It also shows the number of different serovars isolated in the different seasons. The larger number of serovars was 28 isolated during rainy season while 17 was isolated during dry season.

Distinct farm-to-Farm differences were detected in the overall prevalence of *Salmonella* serotypes isolated from swine Fig. 4. One Farm designated with code ND had the highest *Salmonella* serotype isolates of 39.35%. The range in the other Farms in the study was 10.7% to 21.4%. The seven different serovars that were identified had the highest number from the faeces and floor Table 5.

The antibiotic sensitivity patterns of the Isolates are shown in Table 6. This result showed that *Salmonella* cholereasuis was resistant to all antibiotic used except for polymyxin B where there was slight sensitivity. It was found that all the isolates except *salmonella* cholereasuis were sensitive to nitrofurantoin.

DISCUSSION

This study revealed that a significant proportion of healthy pigs (18.4%) are carriers of *Salmonella* sp. The prevalence of *Salmonella* species in fatteners reported in this study was comparable to the prevalence reported in other studies[18-19].

Table 4 : Serouars by environmental sample

Sample types	Serovars
Faeces	agona, typhimurium, enteritides cholereasuis, Infantis.
Floor	agama, typhimurium, agona, cholereasuis
Feed (trough)	derby, typhimurium, infants

Table 5:Sensitivity patterns of the Isolates Using Agar diffusion method.

	S. typhi	S. Chol	S.enterides	S.Infantis
Nitrofurantion 200ng	X	R	X	X
Sulfafurazone 100ng	R	R	R	R
Streptomycin 10ng	R	R	+	X
Penicillin 1.5 Units	X	R	R	X
Engthromyxin 10ng	R	R	R	R
Tetracycline 10ng	R	R	R	R
Polymyxin B	+	+	R	X

X = very sensitive + = Slightly sensitive R = Resistant.

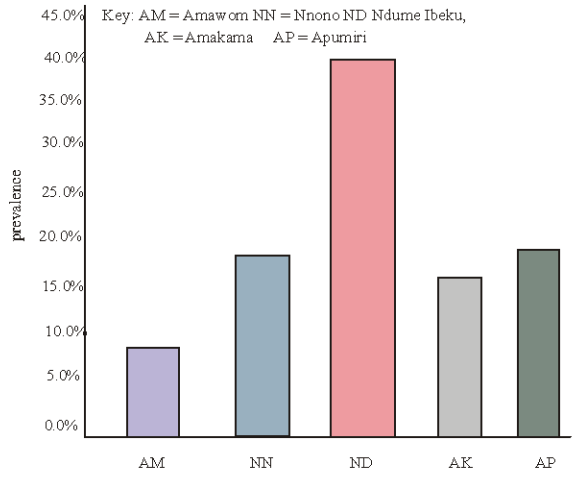

Fig. 3: Prevalence of *Salmonella* in water troughs according to pig Farms in Umuahia

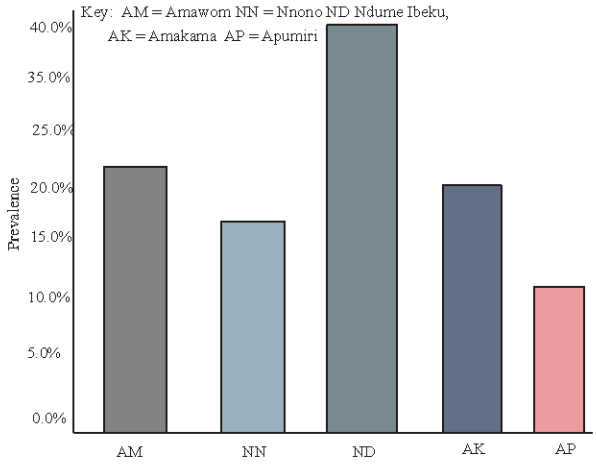

Fig. 4: Prevalence of *Salmonella* in floor Samples

The result of microbiological examination of environmental samples from the five farms showed an unexpected high percentage of materials on swine farms being contaminated by *Salmonella* species. The number of different serovars identified per farm was also unexpectedly high, which leads to the conclusion that there is a multitude of sources for the introduction of *Salmonella* sp. into swine herd.

Examination of the variation among farms in the distribution of *Salmonella* suggested one possible mechanism for transmission. The farm with the highest prevalence of infection for swine faecal samples also had the highest prevalence for floor samples that were *Salmonella* positive. This suggests that pen floors are a primary source of new infection for swine, and human tracking may be a major mode of transmission of *Salmonella* between pens. The occurrence of *Salmonella sp.* in numerous reservoir (pen floor,

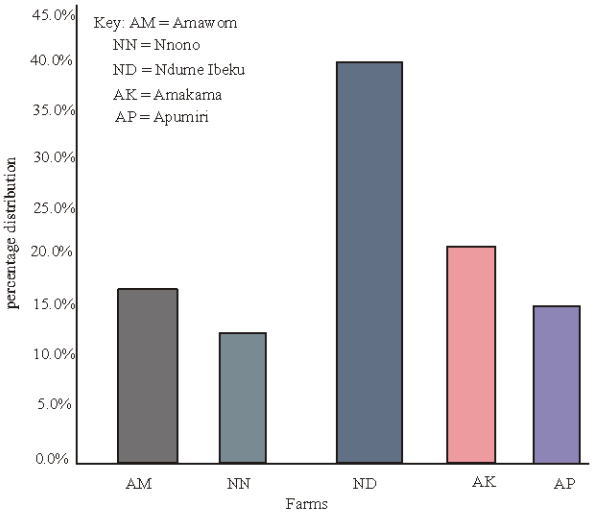

Fig. 5: *Salmonella* serovars occurring in Pig farms in Umuahia zone

water, feed, wall, swine) indicates that there are many possible sources of transmission to swine.

It has been suggested that *Salmonella* species are isolated with a higher frequency from animals under the intensive husbandry system because it is conducive to the spread of infection[20]. It is however, known that variables such as type of specimen and techniques of Isolation influenced the rate of isolation[21], while management practices within each of the systems may also affect spread of *Salmonella* between animals.

Except for *S.derby, S. typhimurium and S. infantis* the serovars identified in the present study occur only sporadically in men[22]. The same serovars have been considered to be the most frequent among healthy swine [23-24-25]and are occasionally involved in pathological conditions observed in this animal species.

The use of Buffered peptone water (BPW) for pre enrichment in most cases yielded lower percentages of isolates than when enrichment alone was used. This may be explained by supposing that *Salmonella* spp. present in low numbers, were inhibited by competing microorganisms, a situation that does not occur when incubation time is reduced to 6 hours[23].

Among the *Salmonella* serovars isolated, *Salmonella* cholereasuis was the most occurring and ecovered from the five piggery farms; this is in accordance with[26]. The source of S. Cholereasuis seem to be limited to carrier pigs and facilities previously contaminated with this serotype[27]. Other serovars such as S. typhimurium and S. enterides had been shown to exist in swine without showing symptoms. This is in support of who described the carrier state of these serovars in swine. The isolation of S. agama must be due

to the presence of Agama lizard which contaminates the environment with this servers. It would appear that the organism is ubiquitous and infective for both man and animals.

The *Salmonella* isolates were resistant to sensitivity tests. All of them grew in the presence of more than 500 mcg of tetracycline, and only one of them S. agama was sensitive to chloramphenicol, the rest being resistant at a concentration of 10 mcg. The strain of *Salmonella* cholereasuis was the most resistant. Since *Salmonella* can survive within the phagocyte cells for a long periods, the bacterium can thus be protected from the anti-microbial agent activity. The use of broad spectrum antibiotics and some growth promoters were found to be linked to the presence of *Salmonella* in swine herds[28]. The emergence and the spread of *Salmonella* typhimurium DT 104, a microorganism that resist to at least five anti-microbial agents should prompt us to reconsider the use of some anti-microbial agents in swine and poultry production.

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