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# Occurrence of *Salmonella* sp. in Slaughtered Healthy Swine and Abattoir Environment of Umuahia , Abia State Nigeria

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**Abstract:** Four hundred and fifty-seven (457) clinically healthy swine slaughtered in four abattoirs located in Umuahia area of Abia State of Nigeria were examined for the prevalence of *Salmonella* sp. in samples collected from spleen, small intestine, liver, lungs and lymph nodes. Swab samples were also collected from the surfaces of slaughtering tables, wash hand basins, butchering knives, workers hands and holden pens. Product samples collected from slaughtering house showed the presence of *Salmonella* sp. in spleen (4/40, 10.0%), small intestine (9/40, 22.5%). Liver (5/40, 12.5%), lungs (3/40, 7.5%) and lymph nodes (16/40, 40.0%). *Salmonella* sp. were recovered from slaughtering tables (3/15, 20.0%), wash hand basins (4/15, 26.7%), butchering knife (2/13, 15.4%), workers hands (4/15, 26.7%), holden pens (3/15, 20.0%). These samples were cultured for the presence of *Salmonella* sp. Simple descriptive statistics and analysis of variance were used to analyze the data collected. Although clinical Salmonellosis was not detected in the study herd, multiple serotypes of *Salmonella* were found causing endemic infection in the study herd. The most frequently detected species was *Salmonella* cholereasuis and it was resistant to all the antibiotic used in sensitivity testing except polymyxin B. This result showed the necessity for adopting more effective hygienic measures in the abattoir environment and equipment to reduce their role in the spread of *Salmonella*. This work will be relevant to abattoir and health workers, to show them the need to maintain good hygienic condition in abattoir environment.

**Key words:** *Salmonella* sp. abattoir environment, swine, pork

## INTRODUCTION

Meat and meat products play a major role in the transmission of zoonotic diseases. Pork is a major carrier of food borne salmonellosis throughout the world. A recent study of pork in retail stores found 9.6% of samples were contaminated[1]. *S. typhimurium* and *S. enteritidis* are the most common cause of food poisoning. Because many milder cases are not diagnosed or reported, the actual number of infections may be 20 or more times greater. Husbandry conditions during transport to slaughter may favour the growth or shedding of a particular pathogen, so that when the animals arrive at the slaughter house, the pathogen can be an important component in the feaces of pigs[2]. Contamination occurs during slaughtering, handling, cutting, processing and storage[3]. *Salmonella* bacteria are primarily located in the gastro-intestinal tract of the sub-clinical infected pigs and the epidemiology of *Salmonella* at the slaughter house level is first of all a question of direct or indirect faecal contamination of pigs or carcasses[4]. During transport and lairage, the proportion of sub-clinically infected pigs that excrete *Salmonella* may be increased considerably. Stress has frequently been blamed for this increase in prevalence rates. Consequently, the risk of cross-contaminating non-infected pigs is also increased[5].

Carcasses may be contaminated or cross-contaminated by manual or mechanical handling. During slaughtering, transfer of micro organisms continues from carcasses to hands of workers and equipment surface and from them to other carcasses.

Although slaughter equipment is often the immediate source of contamination, the carrier pigs plays an important role in the dissemination of *Salmonella.* As a result of the technical difficulties in detecting carrier animals before slaughter or during meat inspection, indicted carrier animals are continual sources of contamination in slaughter houses and ultimately in food products[6]. Slaughter of these animals under poor hygienic conditions leads to the contamination of carcasses by *Salmonella* sp. thus facilitating their transmission and increasing the risk of food-borne diseases in humans[7]. Transmission is thought to occur by pig to pig contact or from exposure to contaminated environment[5]. The muscle tissues of healthy living

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animals are usually free from micro-organism and their contamination during slaughtering is undesirable but cannot be avoided in the transformation of live animals into meet.

Among the various hazards that can be present in our time, micro-biological hazards, such as *Salmonella*, are by far the most important one if we consider economical and health consequences[8,9]. It is thus not surprising that during the past decade, many meat producing countries under gone national programs to control the level of *Salmonella* in finished meat products[10]. However, the characteristics of the infection caused by this in microorganism are such that efficient control measures are not easy to apply.

One of the most challenging aspect of the *Salmonella* infection in animals is that most of the infected animals won't show any clinical sign. 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 faeces for few days but will survive within lymph nodes for many weeks or months[11]. Following a stress period, such as transport to slaughter house, the positive animal will shed again the bacterium in faeces and possible contaminate other animals or meat products by direct or cross-contamination at slaughter. In poultry, once birds are infected, the bacterium will persist in caecum until birds are shipped to the abattoir[12]. Given the silent nature of the disease, the control of the contamination of abattoirs where most of the animals are positive at slaughtered is problematic. Use of serology to detect antibodies produced following the infection is the most appropriate tool to detect healthy carriers since bacteriological cultures of faeces are often found negative[13].

Research is needed to identify the factor associated with *Salmonella* sp. occurrence in farms and the intervention practices, that will keep the levels low[14,15]. This study reports on the distribution of *Salmonella* sp. in healthy swine slaughtered in Umuahia swine abattoir and in slaughter house environments. We emphasize here the need to maintain good hygiene conditions in the abattoir environment. This work will be relevant to abattoir and health workers where there is need to maintain good hygienic condition in the abattoir environment.

## MATERIALS AND METHODS

**Collection of samples:** A total of 273 samples were randomly collected form healthy pigs slaughtered between January to September 2003 in 4 abattoirs in Umuahia Local Government Areia. Product samples were collected by making a small incision in spleen, small intestine, liver, lungs, lymph nodes. Samples from environment were taken from slaughtering tables, washing basins, butchering knife, workers hands and holden pens. Samples were collected from the environment using sterile swab sticks (EVEPON), were wetted in sterile physiological saline and were uniformly stroked across the surface, of the sites. A sampling round begins with the collection of sample from slaughter houses environment followed by the product samples. The slaughtering farms obtained pigs from local pigs farm and seldom were the animals shipped too long distances.

The pigs for slaughter usually remained for 1 to 3 days in holding pens before slaughter. The slaughter facilities were small and only a few animals were sampled each day.

**Isolation and biochemical identification of organism :** The surfaces of small intestine, spleen, liver, lungs and lymph nodes were sterilized with 70% ethyl alcohol. Thereafter, small parts of the above specimen were excised and grounded with a mortar and pestle. Some selenite F-broth was added during grinding to homogenize the contents of the mortar. One gramme aliquot of each macerated specimen and 1.0 mL aliquot of each swab and 1.0 mL aliquot of each swab suspension from the environment (both types of suspension prepared in sterile saline, 10 mL/swab) were transferred for pre-enrichment into 1.0%. Buffered peptone water (BPW, Merck 7228). p.H. 7.5 was incubated for 18 h at 37°C. After 18 h of incubation at 37°C, 1mL of pre-enrichment culture (BPW) was transferred for enrichment into 9 mL of selenite F-broth (BIOTECH) and incubated at 37°C for 18 hrs. Then one loopful (10 μL) of selective enrichment selenite-broth cultures was streaked into salmonella-shigella agar (SSA, LAB-M) Plates were incubated at 37°C overnight. Suspected lactose-negative colonies were sub-cultured into slants of triple sugar iron (TSL media, LAB.M) Biochemical characterization of isolates showing typical *salmonella* characteristics were performed according to[16]. The disc diffusion method of[17] was used to determine the antibiograms of isolates.

**Antigenic characterization:** All presumptive *Salmonella* isolates were characterized anti-genically by using the rapid serum agglutination technique with polyvalent and monovalent somatic and Flagella antisera (poly AL-V., Difco)[18]. Specific serovar identification was carried out at the diagnostic unit of National Veterinary Research Institute, Vom, Nigeria.

Table 1: Frequency and distribution of *Salmonella* sp. from spleen, small intestine, lungs and lymph nodes from four different pig slaughter sikes

| Source | Spleen (%) | S/Intestine (%) | Liver (%) | Lungs (%) | L/Node (%) | Total (%) |
|---|---|---|---|---|---|---|
| Umudike | 20.0 | 30.0 | 20.0 | 0.0 | 50.0 | 24.0 |
| Ibeku | 10.0 | 20.0 | 10.0 | 10.0 | 40.0 | 18.0 |
| Amakama | 0.0 | 10.0 | 0.0 | 20.0 | 30.0 | 20.0 |
| Ihie | 10.0 | 30.0 | 20.0 | 0.0 | 40.0 | 20.0 |
| Total | 10.0 | 22.5 | 12.5 | 7.5 | 40.0 | 18.5 |
| Mean±SD | 1.0±0.8 | 2.2±0.4 | 1.3±0.4 | 0.2±0.4 | 4.0±0.8 | |

p>0.05

NB. Percentage of occurrence = $\frac{\text{Actual number of Isolates x 100}}{\text{Total number tested}}$

Table 2: Distribution of samples positive for *Salmonella* sp. among the samples from the environment of four (4) slaughtering houses

| Source | Slaughtering tables (%) | Washing basins (%) | Butchering knife (%) | Workers hands (%) | Hold pen (%) | Total (%) |
|---|---|---|---|---|---|---|
| Umudike | 0.0 | 25.0 | 0.0 | 50.0 | 20.0 | 25.0 |
| Ibeku | 50.0 | 25.0 | 33.3 | 33.3 | 0.0 | 29.4 |
| Amakama | 33.3 | 0.0 | 0.0 | 25.0 | 33.3 | 17.6 |
| Ihie | 0.0 | 66.7 | 25.0 | 0.0 | 25.0 | 21.1 |
| Total | 20.0 | 26.7 | 15.1 | 26.7 | 20.0 | 21.9 |
| Mean± SD | 0.8±0.4 | 1.0±0.8 | 0. 5±0.5 | 1.0±0.8 | 0.8±0.4 | |

p>0.05

NB. Percentage of occurrence = $\frac{\text{Actual number of Isolates x 100}}{\text{Total number tested}}$

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

| | Rainy | Dry | Total |
|---|---|---|---|
| Number of samples (Product samples and environment) | 400 | 330 | 130 |
| Number of Isolates | 87 | 28 | 115 |
| Percentage of all *Salmonella* positive sample obtained in a given season | 76 | 24 | |
| Percentage of samples of a season which where positive | 22 | 8 | |
| Number of serovars | 35 | 21 | 56 |
| Percentage of serovars | 62.5 | 37.5 | 100 |

## RESULTS

*salmonella* sp. were isolated from 18.5% of product samples (Table 1). The greatest percentage of isolates was from lymph nodes 40.0%, Liver 12.5%, spleen 10.0% and lungs 7.5%.

Samples from abattoir environment yielded *Salmonella* isolates from 21.9% of the 73 examined samples from slaughtering house, with a greater percentage of isolates from hand washing basins and worker's hands 26.7%, followed by hold pens and slaughtering table 20.0% and butchering knife 15.4%. Table 2.

Table 3 shows the number of samples and number of *Salmonella* isolates distributed.

Throughout the seasons, a total of 115 samples were positive for *salmonella* which meant that 15.8% of the 730 samples had *s almonella*. The larger number of *salmonella* isolates was obtained during the rainy season and the smaller number was obtained during the dry season.

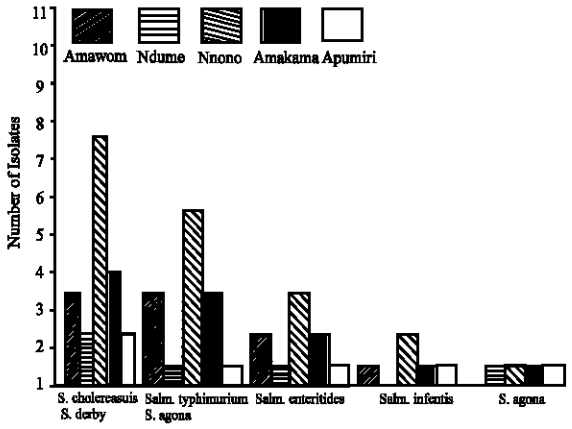


Fig. 1: Distribution of *Salmonella* sp. in Five Pig Farms in Umuahia Zone

Table 3 further shows the number of different serovars isolated in the different seasons. The larger number of serovars was 35 isolated during rainy season while 21 was isolated during dry season.

A total of seven *Salmonella* serotypes was identified (Fig. 1). The most Frequently isolated serotypes were *Salmonella cholereasuis* (33.99), *S. typhimurium* (25.0%) *S. enteritides* (16.1%) and *S. infantis* (8.9%). The antibiotic sensitivity patterns of the isolates are shown in Table 4. This result showed that *salmonella cholereasuis* was resistant to all the antibiotic used except for polymyxim B where there was slight sensitivity. It was found that all isolates were sensitive to nitrofurantoin except *Salmonella cholereasuis.*

## DISCUSSION

The result obtained from this study indicate that swine slaughtered in Umuahia Zone are frequently

Table 4: Sensitivity patterns of the isolates using agar diffusion method

| | *S. typhimurium* | *S.cholereasuis* | *S.enteritides* | S.infantis | S.agona | S. derby |
|---|---|---|---|---|---|---|
| Nitrofurantoin200 µg | X | R | X | X | X | X |
| Sulphafurazone100 µg | R | R | R | R | X | R |
| Streptomycin 10 µg | R | R | + | X | X | R |
| Penicilin I. 5units | X | R | R | X | X | X |
| Erythromycin 10 µg | R | R | R | R | R | R |
| Tetracycline 10 µg | R | R | R | R | R | R |
| Tetracycline 10 µg | R | R | R | R | R | R |
| Polymyxin B 100 µg | + | + | R | X | + | X |

X = very sensitive+slightly Sensitive R = Resistant

infected with *Salmonella* sp. It was shown that rates of *Salmonella* positive swine vary widely, probably due to pre-slaughter practices and hygiene standards, as well as the bacteriological methodology employed in the isolation of this micro-organism and the size of sample examined. The presence of *Salmonella* sp. in lymph nodes and other tissues could represent either a past or a recent infection, or one occurring during processing. The lymph nodes could be considered as important sources of contamination of *Salmonella* sp. because they are often cut while the carcass is being butchered thus contaminating cutting utensils and consequently the meat[4]. The percentage of positive lymph nodes (40.0%) in this study agrees with this report.

A high number of strains were isolated in the pigs slaughtered at Umudike slaughtering house few organisms wore isolated from Amakama (home slaughtered) animals. This is probably due to the small number of pigs slaughtered per slaughtered day (1 to 2 animals) in this situation and also to the fact that animals were slaughtered immediately after arrival. It was observed that lymph nodes show a lower frequency of *salmonella* sp. when collected at the site where the animals are raised. The researcher therefore, postulated that the transmission of *Salmonella* in swine tends to occur during transportation from the farms to the slaughter houses where the animals are usually crowded and under the influence of stressful condition. These factors result in a reduced resistance of the animal to infection and favour the feacal-oral cycle of *Salmonella* transmission on the farm.

The abattoir environment was positive for *salmonella* in all the study area. This suggests that the slaughter house environment contributes greatly to contamination of pork in the killing room. A wide variation exists in the extent of slaughter house contamination[7] in Canada evaluated the degree of contaminations of the slaughter house environment by *Salmonella* and reported a considerable *Salmonella* contamination (25% of Floor abattoir swabs and 12.5% of cold room swabs). The differences in results are likely due to the structural characteristics of slaughter houses, the species of the slaughtering practices, the sanitation practices and the sampling procedure.

Results from this study also show that slaughter house holder pens are frequently contaminated with a variety of *Salmonella* serovars[19] also found high levels of contamination with *Salmonella* in holder pen from two slaughter house in Europe. It is possible that pigs harbour *Salmonella* while on the farm, but they do not shed the organism into faces. The stress of pre-slaughter events may then induce these non-shedding infected pigs to start shedding. However, it is also possible that pigs become infected during transportation and pre-slaughter holding, through cross infection and expose to a contaminated environment.

The detection of *Salmonella* sp. in the holden pen and on the surfaces of the slaughtering tables agree with [20], who report that swine is the principal source of *Salmonella* contamination in abattoir and that the slaughtering process promotes dissemination. Of the contamination. These findings are also reinforced by the fact that the bacteria were found in the butchering knife used to cut the carcasses before refrigeration and sell in the market. The isolation of *Salmonella* sp. from wash hand basins and workers hands further proved the cross-contamination of carcass by abattoir workers.

The level of contamination in the slaughter house at Ibeku and Umudike could be as a result of such factors as high number of swine from different ranches slaughtered per day. This is further evidenced by the high numbers of carriers as shown in the lymph nodes and small intestine samples and also the length of time spent in the slaughter house holden pen (72 hrs). In addition the crowding (less than 1.70$m^2$/animal) and poor sanitation practices associated with the environment also contribute to the high level of abattoir contamination. Other contributing factors include the inadequate design of the pen (Unpaved floor with no slopes or drainage). All these could have contributed to the spread of bacteria from one animal to another and then to the slaughter house environment and the equipment utilized in the slaughtering process.

The high distribution of *Salmonella cholereasuis* is associated with carrier pigs and facilities, previously contaminated with this serotype[2] her serovars such as *S. typhimurium* and *S. enteritids* had been shown to

exist in swine without showing symptoms. This agrees with the reports of[21] Wilcock and[21] who described the carrier state of these serovars in swine.

The *Salmonella* isolates were resistant to the most of the antibiotics used in the sensitivity tests. All of them grew in the presence of more than 50mcg of tetracycline and only one of them *S. agama* was sensitive to chloramphenicol, the rest were resistant to antibiotic concentration of 10mcg. The strains of *Salmonella cholereasuis* was the most resistant. The resistance may be associated with antibiotic (particularly the tetracycline) used as additives in food, given to animal, as concentrates or supplements to grass.

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