Occurrence of *Salmonella* on meat and products in an ostrich abattoir as determined with a DNA probe

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This study was conducted to determine the status of *Salmonella* during the processing of ostriches to ostrich meat and products. When a total of 1429 samples, collected from fillet, liver, gizzards, bloodmeal, skins, heart, faeces, large and small intestines, carcases, wash-water from feathers and from carcases, water before wash and other sources during the ostrich processing, were screened for *Salmonella* spp. using a *Salmonella* specific DNA probe, the results showed that 16.9% of the samples were positive for presence of *Salmonella*. Further analysis showed that (61/120) 50.8% of all the ostriches tested vwere positive for *Salmonella* upon arrival at the slaughter house. These results further showed that 33.3% of the carcases tested were positive for *Salmonella*. This indicated that the ostriches may have been contaminated at the rearing farm environment, during transportation or even at the abattoir environment itself. The products which were *Salmonella* positive were: gizzards (5%); the skins (8.3%); bloodmeal (4.2%); large intestines (26.2%); small intestines (16.1%) and faeces (44.2%). Products which were negative for *Salmonella* presence included: heart tissue, liver, fillet steak and meat-and-bone-meal. When the positive samples were further analysed to determine the level of bacterial concentrations in each positive sample, the results showed that the main ostrich products for export, such as ostrich meat, meat and bone-meal and ostrich fillet were negative for *Salmonella*. The only export products that showed *Salmonella* presence, were the skins with only an 8.3% positivity rate. The bacterial concentrations in the positive skin samples were so low that *Salmonella* contamination in this product is probably eliminated through further processing, such as tanning before export of this product.

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Introduction

The anticipated world-wide increase in the prevalence of human salmonellosis has generated great concern among clinicians, bacteriologists, veterinarians and epidemiologists (Turnbull, 1979). *Salmonella* spp. continue to be implicated as the principal cause of foodborne human salmonellosis in many countries of the world. The prevalence of this pathogen in the material environment and in the agricultural sectors involved in the rearing of animals for meat, constitutes a major threat to animal husbandry, meat processers and meat producers, in their efforts to supply safe foods for the consumers (Sockett, 1991). In developed countries, some foods such as milk, ice cream, whole egg mixed in bulk and canned foods are regarded as 'safe' owing to the enforced application of well controlled decontamination processes, such as treatment of food manufacturing water before use (Roberts, 1990), processing of food for safety by longitudinal integration of steps including strict hygiene care of raw materials, thorough pasteurisation and prevention of adverse post-process handling of products (Mossel & Drake, 1990; Mossel & Striujk, 1993). In the developing countries of Africa, Asia and Latin America, the enforcement of the above is not practicable in every country owing to financial constraints and lack of appropriate technology as well as lack of expert personnel.

Poultry is a major reservoir of *Salmonella*, (D'Aust *et al.* 1992), and the principal site of colonization in the intestine with *Salmonella*, is the caecum. In infected birds, the intestines carry the bacteria, but the caecum is likely to carry a greater bacterial load (Xu *et al.* 1988). In this study, poultry is defined as farmyard birds of any kind, such as hens or ducks, kept for supplying eggs and meat. Ostriches as birds which provide meat, eggs and ostrich products, are considered in this study, as poultry, even though they are wild birds, which for commercial reasons, have been domesticated for the supply of meat and skins. Infection with *Salmonella* can cause a serious problem in the exportation of ostrich meat, skins and other products.

Poultry feeds constitute a major source of contamination of poultry with *Salmonella* serotypes. These serotypes are transmitted from contaminated feeds to ostriches and other poultry stock and eventually to the people through the consumption of contaminated meat obtained from infected poultry (Nabbut 1993). The ability for *Salmonella* to spread from one ostrich carcass to another during the defeathering and dehairing operations, and to contaminate the slaughtering plant environment tends to render ineffective contemporary in-plant control measures (Oosterom 1991). Moreover, the making of feed with contaminated offal, promotes recycling of *Salmonella* into the meat production chain at the farm level (Williams 1981).

Some studies have indicated that microbial contamination of poultry carcasses may be the result of the slaughtering and dressing processes, necessary for the conversion of poultry to food for human consumption (Dickson & Anderson 1992). The ostrich industry in Zimbabwe is a fledgling one and has taken a boom of late. In this light, it was necessary to carry out a study to establish the status of *Salmonella* in ostrich meat and products. Ostriches, ostrich skins, ostrich feathers and other products are processesd for the export and local markets. The skin is exported overseas and is used to make ostrich skin products. To ensure the provision of safe products to the consumer, contamination rates, and points of contamination with *Salmonella* in the production line should be

kept in check. Some of the products obtained from the ostriches which are made into pet foods include small and large intestine, the crop and gizzards. In Zimbabwe, insufficient research has been carried out to determine the status of *Salmonella* in ostriches. This study is one of the first to be published to determine current trends of *Salmonella* infection in ostriches in Zimbabwe.

A *Salmonella*-specific DNA probe that has been extensively tested for specificity, sensitivity of detection, reliability of results and cost-effectiveness (Gopo *et al.*, 1991), was employed in this study. A comparison between the *Salmonella*-specific DNA probe method and the conventional microbiological method, showed that the results obtained using the probe method, were more specific and reliable as they did not show false negatives.

Materials and methods

Materials

A *Salmonella*-specific DNA probe (1.8 Kb), was used in this study (Gopo *et al.*, 1988). Restriction endonuclease Hpal and radio-isotopes (^{32}p-d-ATP and ^{32}p-d-CTP) were obtained from Amersham, UK and used according to manufacturers' conditions.

Sample collection

A total of 1429 samples were collected from gizzards, liver, blood meal, heart, faeces, small intestines, large intestines, raw water and wash waters from carcasses and feathers. Five grams (5g) of each of ostrich fillet, liver, gizzards, large intestines, small intestines and heart tissue, were collected at various stages of the slaughter process (Figure 1) and were transported to the laboratory in 10 ml of selenite broth (5.0 g tryptone, 4.0 g lactose, 10.0 g disodium phosphate, 4.0 g

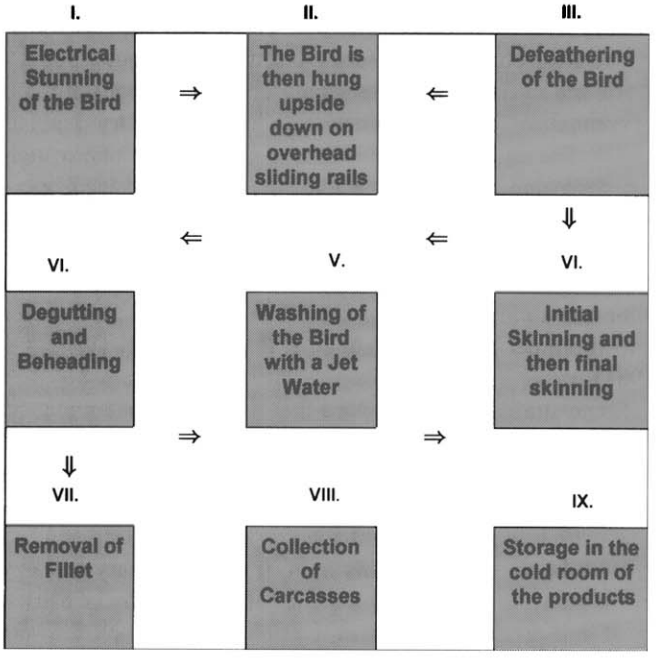

Figure 1 This figure shows the stages in the slaughter process at the ostrich abattoir, from the electrical stunning of the birds to the storage of the products. Different types of samples were collected at the various stages during the slaughter process.

sodium selenite, 0.01g cystine, 1000 ml distilled water — pH 7.0). Ten millilitres (10 ml) of blood tissue were taken from each ostrich and inoculated into 40 ml of selenite broth. The raw and wash-water samples were collected in 10 ml volumes and pipetted into 40 ml of selenite broth. All the samples were incubated at 37°C for 6 h before processing.

Sample processing

The tissue samples were homogenized in 10 ml of selenite broth and tissue debris was removed by low-speed centrifugation at 300 rpm in a Beckman GPR bench-top centrifuge, using a GH 37 rotor, followed by filtration through two layers of cheesecloth. The supernatants of these tissue samples, as well as the wash-water samples were each centrifuged at 6 000 rpm in a Beckman bench centrifuge for 15 min, to pellet the bacteria. The supernatants were carefully discarded and the bacterial pellets were resuspended in 2 ml volumes of distilled water, and transferred to microfuge tubes and centrifuged at 12 000 rpm for 15 min. The supernatants were carefully discarded and the bacterial pellets resuspended in 20 µl of distilled water. Each whole 20 µl bacterial suspension was dot-spotted onto a nitrocellulose filter (High Bond C, Amersham), to form dot-blots, for filter colonization. The colonized filters were transferred, colony side up, onto Whatman 3 MM filter papers, presoaked in lysing buffer [0.5 M NaOH, 1.5 M NaCl, 0.1% sodium dodecyl sulfate (SDS)] and incubated at room temperature for 15 min, to lyse the colonised bacteria and denature their chromosomal DNAs. After the lysing step, the colonized filters were neutralised by transferring them, colony side up onto Whatman 3 MM filter papers, presoaked in 1.0 M Tris-HCl, pH 7.4 and incubated, at room temperature for 10 min. In order to complete the neutralization process the filters were transferred to 3 MM filter papers, presoaked in 0.5 M NaCl, 1.0 M/Tris-HCl, pH 7.4 and incubated for 10 min. Each filter was removed from the solutions and air dried before baking at 80°C for 2 h, to filter bind the denatured bacterial chromosomal DNAs.

Nick translation procedure

A *Salmonella*-specific DNA probe fragment was prepared for use in DNA-DNA colony hybridization by labelling the fragment with radio-isotopes ^{32}P-d-ATP and 32P-d-CTP, using the Nick translation procedure of Rigby *et al.*, (1977), with slight modification. The reaction mixtures contained 1.0 µg of probe DNA, 2.5 µl of 10 x nick translation buffer [50 mmol $MgCl_2$, 500 µg/ml bovine serum albumin, 0.5 M tris-HCl, pH 7.5), 2 µl 0.2 mmol d-GTP, 2 µl 0.2 mmol d-TTP, 4 µl ^{32}P-d-CTP (400-2 000 mCi/mmol; 4 µl ^{32}P-d-ATP (400 mCi/mmol); 1.0 µl DNase I (stock solution stored at 20°C) and 1.0 µl of DNA polymerase 1] were incubated at 16°C for 2 h. The reactions were terminated by the addition of 25 µl of 0.5 M EDTA. The unincorporated radionucleotides were separated form the radioactive by labelled probe DNA by chromatography, using sephadex G-50 mini column. The columns were prepared by plugging pasteur pipettes with siliconized glass wool and pouring in sephadex G-50. The columns were equilibrated with 5 bed-volumes of tris-EDTA buffer (10 mmol tris-HCl 0.1 mmol EDTA pH 7.5). The probe DNA which was excluded from the sephadex matrix and eluted ahead of the unincorporated deoxyribonucleotides, was col-

lected in 200 µl fractions of tris-EDTA. The level of radioactivity was determined by cerenchov radio-isotope counter. The fractions containing the DNA were pooled together and stored at 4°C for DNA-DNA hybridization.

Hybridization

Hybridizations were carried out following the methods of Maniatis & Sambrook (1982) with some modification. The baked nitrocellulose filters were prepared by pre-soaking them in 3 × SSC (0.045 M $NaCl_2$, 0.01 M trisodium citrate buffer) for 10 min. The filters were then transferred to hybridization boxes containing prehybridization solution (3 × SSC, 10%(SDS), 5 × Denhardt's solution [50 × Denhardt's: (5g Ficol, 5g polyvinyl pyrroudione, 5g bovine serum albumin and 500 ml distilled water)] which had been stored at –20°C. The solutions were incubated at 65°C for 4 h. The prehybridization solutions were removed after the 4 h of incubation and replaced with hybridization solutions (3 × SSC, 0.01 M EDTA), and the ^{32}P labelled *Salmonella* DNA probe, which had been denatured by incubation at 100°C for 3 min. The hybridization mixes in the boxes were incubated at 65°C for 8 h, with gentle shaking in a shaker water bath. At the end of the hybridization period, the solutions were removed and stored for further use. The filters were washed three times with low stringency buffer (3 × SSC, 0.1% SDS). The levels of radioactivity on the filters were checked at the end of each wash. A higher stringency wash (0.3 × SSC, 1% SDS) was carried out only if the radioactivities were found to be higher, reflecting high background radioactivities on the filters. The filters were removed from the hybridization boxes and air dried. In order to ensure complete dryness, the air dried filters were placed between two 3 MM filter papers and incubated at 37°C for 1 h. The filters were prepared for autoradiography by exposing them to X-ray films in autoradiographic cassettes for 6–12 h depending on the strength of the signal. The exposed films were developed, washed and processed for hybridization analyses.

Probe sensitivity test

In order to estimate the approximate concentration of *Salmonella* bacterial cells in each positive sample, dot-blot sensitivity tests were carried out. *Salmonella typhimurium* was cultured by stab inoculating at 37°C overnight. A maxi-culture was made by transferring 2 ml from the mini-culture to the 500 ml of LB broth (10.0 g bactotryptone, 5.0 g bacto-yeast, 10.0 g NaCl and 1000 ml distilled water, pH 7.5). Cell growth was monitored by reading the O.D. at 650 nmol hourly until an O.D. of 0.8 was reached using uninoculated LB broth as a control. One millilitre of this culture was removed and a cell count was made using a haemocytometer method, which established the number of cells per ml at an O.D. of 0.8, to be 1×10^8. Cell concentrations of 1×10^8, 1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3, 1×10^2, 1×10^1 per ml were prepared and the O.D. of each preparation was measured before the bacterial cells were pelleted by centrifugation in a microfuge at 12 000 rpm for 5 min. The supernatants were discarded and the bacterial pellets were resuspended in 20 µl of distilled water. Each whole 20 µl bacterial suspension was dot-spotted onto a nitrocellulose filter. The dot-blots were lysed as above, and processed for DNA-DNA hybridization (Figure 3A–B).

Results

The study was conducted to detect, identify and determine the incidence and status of *Salmonella* during the processing of ostriches to ostrich meat and products, at an ostrich abattoir in Zimbabwe. Positive and negative samples were determined by the analysis of autoradiograms as shown in Figure 2.

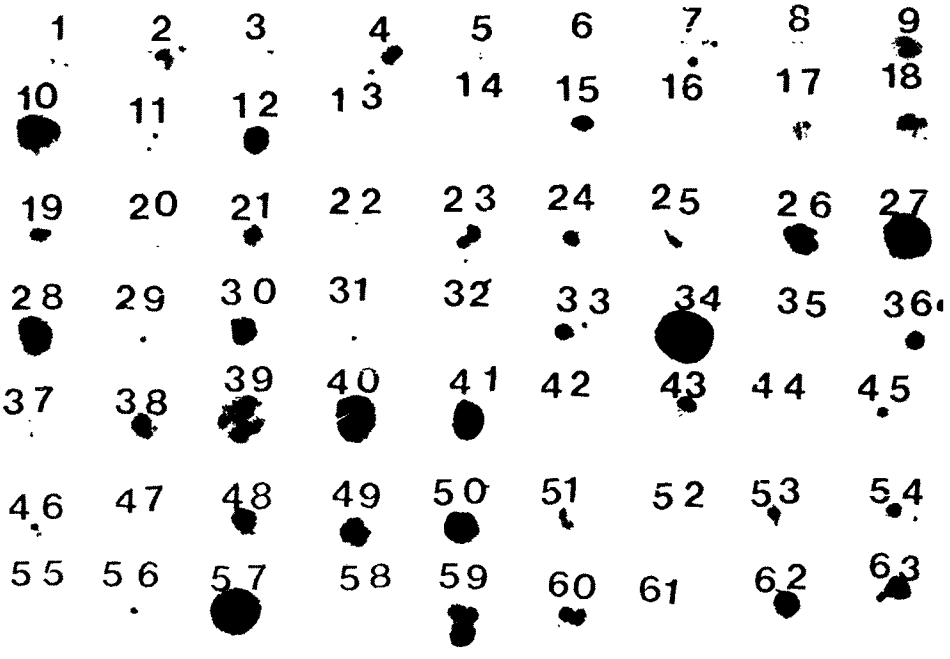

Figure 2 Colony hybridization results using *Salmonella* DNA probe. Determination of positive and negative samples using DNA–DNA hybridization of 63 representative samples taken from feaces, skins, large intestines and wash waters. Samples number 34 and 57 are controls (*S. typhimurium* 1×10^8 cells/ml). Samples 6, 13, 14, 32, 35 and 52 are negative while samples 56, 45, 29 and others represent samples with very low levels of *Salmonella* (+). Samples 29 and 40, have high levels of *Salmonella* (++++). The rest range between ++ and +++.

When a total of 1429 samples, collected from raw water, treated wash-water before wash, feather wash-water, carcass wash-water, large intestines, small intestines, gizzards, heart tissue, liver, skin, bloodmeal, meat and bonemeal, fillet and faeces, were screened for presence of *Salmonella* spp, using a *Salmonella*-specific DNA probe, (16.9%) of all the samples, were positive for *Salmonella*. (Table 1). The results showed that 61 out of 120 (50.8%) of the ostriches surfaces (feathers) were *Salmonella* positive, upon arrival at the abattoir. This may indicate that at least 51% of the ostriches were infected with *Salmonella* at the rearing farm environment, during transportation to the abattoir or in the pre-slaughtering handling process. A number of ostrich products were *Salmonella* positive such as: gizzards 5%, the skins 8.3%, bloodmeal 4.2%, large intestines 26.2%, small intestines 16.1%, and faeces 44.2%. When raw water was tested 17 out of 512 samples tested (33.3%) were positive for *Salmonella*. These results also showed that when treated water (wash-water before wash) was tested for *Salmonella*, no *Salmonella* presence was detected (Table 1.) The method used in the treatment of the wash-water was not revealed by the abattoir. The carcass wash-water was 33.3% *Salmonella* positive. The heart tissues, liver tissues, the fillet and meat and bonemeal products were *Salmonella* negative (Table 1).

Table 1 Incidence of *Salmonella* at an ostrich abattoir. The table shows the numbers and types of ostrich products that were found to be *Salmonella* positive in this study. An overall positivity rate of 16.8% was shown. Over 50% of the ostriches with *Salmonella* were either infected at the rearing farm environment, or during transportation at the holding area. The main products (heart, liver, gizzards, fillet) were *Salmonella* negative

Sample type	Number tested	Number positive	Percent positive
1. Water			
(a) Raw Water	51	17	33.3
(b) Water before wash (treated water)	58	0	0
2. Water after wash (treated water)			
(a) Feathers	120	61	50.8
(b) Carcasses	120	40	33.3
3. Faeces	120	53	44.2
4. Heart	70	0	0
5. Liver	70	0	0
6. Gizzards	100	5	5
7. Fillet	120	0	0
8. Skin	120	10	8.3
9. Bloodmeal	120	5	4.2
10. Meat and bone meal	120	0	0
11. Small intestines	120	19	16.1
12. Large intestines	120	31	26.2
Total	1429	241	16.9%

When the *Salmonella* positive samples were analysed to determine the bacterial concentrations per positive samples, using Figure 3 as a point of reference, the results showed that 49.2% of the feathers' wash-water had bacterial concentrations of equal or greater that 1×10^8 cells per positive sample (++++); while 32.8% had bacterial concentrations ranging from 1×10^6–1×10^7 cells per positive sample (+++) and 18.0% had bacterial concentrations ranging from 1×10^3–1×10^5 (++). The carcass wash-water showed 65% ++++, 25% +++ and 10% ++ respectively. The skins showed 40% +++, 30% ++ and 30% +. The gizzards showed bacterial levels of 40% ++ and 60% +; while bloodmeal showed bacterial levels of 40% ++ and 60% +. Large intestines showed bacterial levels of 16.3% ++++, 32.3% +++, 19.4% ++ and 32.3% +. Small intestines bacterial levels were 5.3% ++++, 36.8%

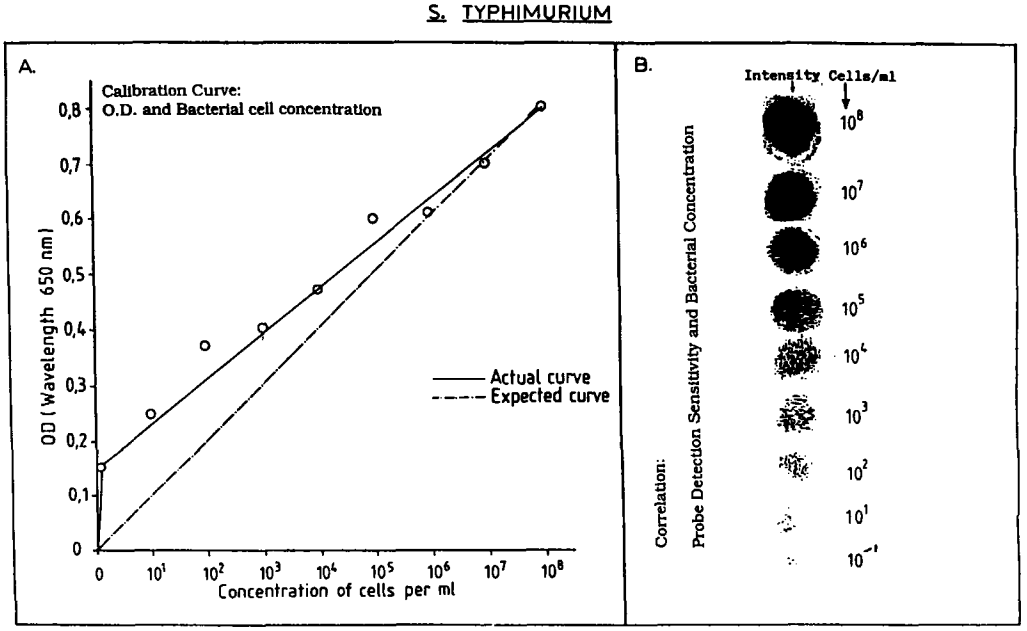

Figure 3 Relationship of exponential cell concentration of *S. typhimurium* with (A) optical density (OD), (B) population density and probe Sal-15 detection sensitivity.

Table 2 Levels of bacterial concentrations in positive samples. Determination of levels of *Salmonella* bacteria for the positive samples, show that for the feather wash-water, 82% of the positive samples had bacteria concentrations of greater than +++ category. Carcasses had 90% greater than +++ category; feaces had 75% greater than +++ category; skins had 40% +++ category; bloodmeal 40% ++ and 60% ++; gizzards; 60% + and 40% ++; large intestines 48.3% greater than +++ and small intestines 42% greater than +++ category

Positive sample type	Number positive	Level of positivity			
		+	++	+++	++++
Feathers	61	0	11	20	30
		0%	18.%	32.9%	49.2%
Carcass wash water	40	0	4	10	26
		0%	10%	25%	65%
Faeces	53	0	13	10	30
		0%	24.5%	18.9%	56.6%
Skin	10	3	3	4	0
		30%	30%	40%	0%
Bloodmeal	5	3	2	0	0
		60%	40%	0%	0%
Gizzards	5	3	2		
		60%	40%	0%	0%
Large intestines	31	10	6	10	5
		32.3%	19.4%	32.3%	16%
Small intestines	19	7	4	7	1
		36.8%	21.1%	36.4%	53%
Totals	224	26	45	61	92
		11.6%	20.1%	27%	41%

Key: + = $1 \times 10 - 1 \times 10^2$ Cells/sample tested

++ = $1 \times 10^3 - 1 \times 10^5$Cells/sampletested

+++ = $1 \times 10^6 - 1 \times 10^7$ Cells/sample tested

++++ $\geq 1 \times 10^8$ Cells/sample tested

+++, 21.1% ++ and 36.8% +; the bacterial levels in the faeces were 56.6% ++++, 18.9% +++ and 24.5% ++ (Table 2).

Discussions and Conclusions

Ostrich farming in Zimbabwe is a growing industry that is aimed at the supply of ostriches, ostrich meat and ostrich products for the local and international markets. Like any other fledgling industry, its future success depends on its ability to supply quality and disease-free products in order to improve its market competitiveness. The safety of the products in the ostrich industry can be guaranteed by the strict enforcement of decontamination measures that involve longitudinal integration of various steps, such as strict hygiene care of the raw materials, thorough pasteurization and prevention of adverse post-process handling of products. The industry must develop the capacity to rapidly, efficiently and cost-effectively detect and identify sources of contamination with pathogens such as *Salmonella.* In this study, 51% of the ostriches were infected either at the rearing farm environment, or during transportation from the farm to the abattoir. The industry must be able to rapidly, specifically and cost-effectively detect and identify the sources that result in the *Salmonella* infected ostriches. The use of a *Salmonella*-specific DNA diagnostic probe may provide such a procedure to the industry. Some of the sources not covered in this study could include feeds, farm water, rearing houses and cages or even the farm workers who handle the birds. The ostrich producers in Zimbabwe should be encouraged to practice, in addition to decontamination control measures, strict hygiene care through establishing separate handling facilities for product processing areas from the areas for animal handling. In the abattoir environment, there should also be separation between the animal handlers and product handlers among the abattoir workers. It was clear at this abattoir, that the enforcement of such measures was in practice. The main ostrich products for the Zimbabwe local market and international markets such as the ostrich meat, fillet meat, and meat and bonemeal, were found to be *Salmonella* negative. This was an important result because it showed that in spite of more than 50% of the ostriches which came to the abattoir with various degrees of surface *Salmonella* contamination, the important products were still *Salmonella* negative, after the handling and processing steps at the abattoir. The only product for the local and export markets which had a mild degree of *Salmonella* contamination were the skins. Further processing during tanning, could eliminate the *Salmonella* contamination of this product. Other *Salmonella* products which were positive, were the heart, liver tissue, bloodmeal and small and large intestines. All these products showed various levels of *Salmonella* positivity. These products are used as concentrates in the production of pet foods and poultry/ostrich supplementary feed. These low levels of *Salmonella* in these products could be eliminated during the processing and production of feed by-product. Their use may therefore constitute a source for infection of the ostriches at the rearing farm environment, and for the recycling of *Salmonella* spp in the ostrich industry. Further investigation to determine the survival rates of *Salmonella* during the production of concentrates, needs to be carried out. Such a study will establish whether or not the faeces (ostrich chicken droppings) used in acquaculture as fish feeds may constitute a direct source of infection with *Salmonella* to fish and products.

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