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The Prevalence and Antibiotic Susceptibility of *Salmonella* sp. in Poultry and Ostrich Samples from Slaughter Houses in Gaborone, Botswana

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Abstract: We determined the prevalence and antibiotic susceptibility of *Salmonella* species in poultry and ostrich samples from slaughter houses in Gaborone, Botswana. We collected a total of 128 chicken and 124 ostrich tissue samples and cultured them for *Salmonella*. The chicken samples consisted each of 32 livers, gall bladders, small intestines and the large intestine while the ostrich samples consisted of each of 31 livers, small intestines, large intestines and cloacae. The chicken livers had the highest *Salmonella* prevalence of 50% followed closely by the large intestine with a prevalence of 43.8%. The small intestine and the gall bladder had *Salmonella* prevalence of 37.5 and 35.5%, respectively. The ostrich cloacae had the highest *Salmonella* prevalence of 51.6% followed by the large intestine with a prevalence of 29%. The ostrich small intestine and the liver had *Salmonella* prevalence of 16 and 12.9%, respectively. All of the rinse/cooling water samples from the chicken slaughterhouse were positive for *Salmonella* species. Isolates from poultry and those from the ostriches were serologically confirmed to be *Salmonella muenchen* and *Salmonella arizonae*, respectively. All the *Salmonella muenchen* and *Salmonella arizonae* were sensitive to Gentamycin but were all resistant to Tetracycline, Ampicillin and Sulphatriad. More studies in the area of *Salmonella* in poultry and antibiotic susceptibility of these organisms to antibiotics are essential for future effective control measures.

Key words: Antimicrobial, ostrich, poultry, *Salmonella*

INTRODUCTION

Non-typhoidal *Salmonella* sp. from poultry and poultry products are commonly implicated as cause of human *Salmonellosis* worldwide. Almost all of the ca 2400 different serovars are believed to be able to cause illness in humans (Acheson and Homan, 2001; Bangtrakulnonth *et al*., 2004; Cogan and Humphrey, 2003; Cooper, 1994; Herikstad *et al*., 2002; Lim *et al*., 2001).

Very little is known about the prevalence of viral, bacterial and parasitic diseases in Botswana's wild and captive ostriches (*Struthio camelus)*. While in quarantine (before import and export), it is mandatory that ostrich cloacal swabs be negative for *Salmonella* sp. as well as Newcastle disease by viral isolation (Rutina, 1993). It was of interest to investigate if slaughter ostriches, just like chicken, could be contaminated with *Salmonella*.

The wide spread use of antibiotics in human and veterinary medicine has lead to an increase in the number of resistant *Salmonella* strains isolated from human and environmental sources. Antimicrobial agents are added to animal feeds for their growth promoting effects. Poultry, especially those fed with antibiotics as growth enhancer, often exhibit multiple resistances to antibiotics (Kessling *et al*., 2002; Logue *et al*., 2003). The techniques of molecular epidemiology have enabled the tracing of resistance plasmids involved in outbreaks of *Salmonella* gastroenteritis from contaminated food back to the food processing plant and then to the originating farm (Acheson and Homann, 2001).

Salmonellosis can be a very serious disease especially in people with compromised immune system like those with HIV/AIDS. Persons with human immunodeficiency virus (HIV) have an estimated 20-100 fold increased risk of *Salmonellosis* when compared with the general population (Acheson and Homann, 2001).

In order to formulate effective preventative and control strategies and to understand the epidermiology, information on the prevalence of *Salmonella* in poultry is indeed necessary. In this study we investigated the prevalence of *Salmonella* sp. in different chicken and ostrich tissue samples at slaughter and determined the antibiotic susceptibility of *Salmonella* isolates.

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MATERIALS AND METHODS

Samples: We collected 128 chicken and 124 ostrich tissue samples at slaughter, in Gaborone, Botswana.

The chicken tissue samples included the 32 each of livers, small intestine gall bladder and the large intestines while those from the ostrich comprised each of 31 livers, small and large intestines and cloacae. The samples were collected immediately after evisceration into sterile stomach bags (Seward medical). About 200 mL of the rinse/cooling water was collected into sterile bottles during the slaughtering and processing period on 6 occasions. All samples were transported to the laboratory under ice.

Isolation and identification of *Salmonella*: Following surface sterilization of the liver surface, duplicate sterile swabs were used to swab the interior of all samples. The interior lumens of the small and large intestines as well as the cloacae and gall bladders were swabbed with sterile swabs. All swab samples were then immersed in 10 mL of sterile buffered peptone water (Oxoid) with a pH 7 and incubated for 37°C for 24 h. For the rinse/cooling water samples, 1 mL in duplicate was used to inoculate 10 mL of buffered peptone water and incubated as for tissue samples. The peptone water culture (0.1 mL) was transferred to 10 mL of Rappaport-Vassiliadis medium (Oxoid) and the incubation was carried out at 42°C for 24 h. From each broth culture, a swab was used to subculture on xylose lysine deoxycholate (XLD) agar (Oxoid). The plates were incubated at 37°C for 24 h. The presence of colonies with morphologies typical of *Salmonella* on XLD (pink colonies with black centres due to hydrogen sulphide gas production) was recorded as a presumptive positive result. Randomly chosen colonies were tentatively confirmed to be *Salmonella* by reaction in tubes of Triple sugar iron (Oxoid) and Urea agar (Oxoid). Isolates that fitted the biochemical profile of *Salmonella* (TSI-alkaline slant with an acid butt, positive for gas production; Urea agar-negative for ammonia liberation, therefore no colour change in the media) were subcultured on nutrient agar (Oxoid). The isolates were Gram stained and further characterized biochemically using API 20E system (Biomerieux, France). Isolates confirmed to be *Salmonella* on API 20E were confirmed serologically with *Salmonella* somatic O and flagella antisera following the International Standard ISO 6579E (1993).

The antibiotic susceptibility test were done following the disc diffusion method using the M43 specification discs (Mast diagnostics), Gentamycin 10 μg, Tetracycline 10 μ, Ampicillin 10 μg and Sulphatriad 200 μg. After the incubation (37°C for 24 h), the inhibition zones were measured and the susceptibility of the organisms to the antibiotics were determined by reference to a chart of zones sizes as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (1998). *Salmonella typhimurium* ATCC 13311 was used for reference purposes.

RESULTS AND DISCUSSION

Fifty percent 16/32 of chicken livers samples were positive for *Salmonella* sp. (Table 1). The chicken gall bladder, small intestine and large intestine had *Salmonella* prevalence of 35.5, 37.5 and 43.8%, respectively. All the 6 rinse/cooling water samples analysed were positive for *Salmonella* (Table 1).

The Ostrich cloacae had the highest *Salmonella* prevalence of 51.6% (16/31).

The ostrich liver, small intestine and large intestines had Salmonella prevalence of 12.9, 16 and 29%, respectively (Table 1). Figure 1 shows a comparison of the prevalence or *Salmonella* species in chickens and ostrich tissue samples. The ostrich samples of small and large intestines and liver were less contaminated with *Salmonella* sp. as compared to the chicken ones.

Only one serotype *Salmonella muenchen* was confirmed from the chicken samples while *Salmonella arizonae* was the sole serotype obtained from the ostrich samples. Twenty *S. muenchen* and 15 *S. arizonae* isolates were tested for antibiotic susceptibility. All the chicken and ostrich *Salmonella* species were sensitive to Gentamycin (10 μg). However, all The *Salmonella* serovars were resistant to Tetracycline (10 μg). Ampicillin (10 μg) and Sulphatriad (200 μg).

Our study indicates a high prevalent rate of *Salmonella* sp. in poultry products and cooling/ washing water during slaughter. This means that care should be taken during and after evisceration, to prevent carcase contamination with bacterial pathogens in the viscera. Other possible measures to prevent *Salmonellosis* and dissemination of *Salmonella* through the food chain include keeping the initial number of salmonellae in the foods as low as possible and making use of Hazard Analysis Critical Point (HACCCP), from primary product throughout further processing. The application of and strict adherence to hazard analysis critical control points (HACCP) protocols may help to reduce the prevalence of *Salmonella* and other bacterial pathogens in slaughterhouses and abattoirs. The prevalence of *Salmonella* in poultry products collected at federally inspected establishment, was lower after the implementation of Pathogen Reduction; Hazard Analysis

Table 1: Prevalence of *Salmonella* in chicken and Ostrich samples from slaughter houses

Salmonella serotype (isolates)		Number and sample type					
		Liver	Gallbladder	Small intestine	Large intestine	Rinse water	Cloacae
	Chicken						
	Total samples tested	32	31	32	32	6	N/A
S. muechen	Samples testing positive	16	11	12	14	6	N/A
	Samples testing positive (%)	50	35.5	37.5	43.8	100	N/A
	Ostrich						
	Total samples tested	31	N/A	31	31	N/A	31
S. arizonae	Samples testing positive	4	N/A	5	9	N/A	16
	Samples testing positive (%)	12.9	N/A	16	29	N/A	51.6

N/A: Not applicable; *Two swabs taken from each sample

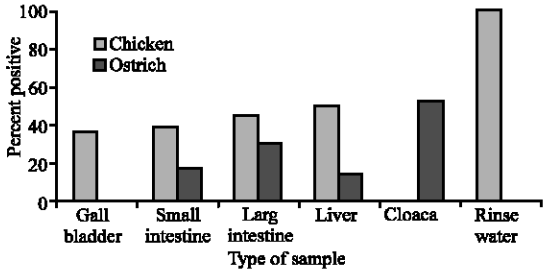

Fig. 1: Prevalence of *Salmonella* sp. in different samples

and Critical Control Point (PR/HACCP) (Rose *et al*., 2002). The consumer should be made aware of the dangers involved in consuming contaminated food products. Preventive measures in food preparation e.g. proper heating and cooking, should be brought to the attention of the consumer. This is important especially in rural areas of Africa.

All the *Salmonella* serovars were sensitive to gentamycin but were resistant to tetracycline, ampicillin and sulphatriad. Kiessling *et al.* (2002), obtained similar results of *Salmonella* serotypes that were resistant to tetracycline and sulphamethoxazole, *S. muenchen* inclusive. High rates of resistance (>50%) to chloramphenicol, sulfamethoxazole and ampicillin have been reported from Africa, Asia and South America (Kiessling *et al*., 2002; Mandell *et al*., 2000). Studies in the USA and China showed that *Salmonella* isolates recovered from retail raw poultry were resistant to multiple antimicrobials. Eighty-two percent: of the *Salmonella* isolates were resistant to at least one antimicrobial. Resistance to the following antibiotics was common among USA isolates: Tetracycline (68% of the isolates were resistant), streptomycin (61%), sulphamethoxazole (42%) and ampicillin (29%) (Chen *et al*., 2004). Strains of food-borne pathogenic bacteria, like *Salmonella*, that are resistant to a variety of antimicrobial agents have become a major health concern. Drug resistance of pathogenic bacteria diminishes the effectiveness of antimicrobial treatment and can lead to the use of less safe, ineffective, or expensive alternatives (Mandell *et al*., 2000).

Use of antibiotics for therapeutic purposes in animals and as growth promoters in animal feeds have been implicated in promoting emergence of and spread of antimicrobial resistance among salmonellae (Holmberg *et al*., 1984; Humphrey, 2000; Spica *et al*., 1987). Cattle and poultry that consume feed supplemented with antimicrobial agents develop a resistant enteric flora that spreads throughout the herd/flock. As a consequence, many countries have banned or controlled addition to animal feeds of antimicrobial agents that are useful for systemic therapy in humans. However, some countries have not yet taken action because of opposition by animal husbandry industry that fear loss of profit. In many developing countries there may be lack of Information and/or resources to deal with the problem of antibiotic resistance contributed by prophylactic use of antimicrobial in the animal industry.

CONCLUSION

In the light of the findings of this investigation showing isolation of *Salmonella* from poultry products and resistance of the isolates to various antibiotics and the potential spreading of *Salmonella* by consignments of chicken products, it is apparent that efforts are required to improve the control of *Salmonella* contamination in poultry products in Botswana.

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