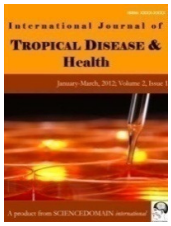

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Evidence of Carriage of Antimicrobial Resistant *Salmonella* species of Public Health and Veterinary Significance in the Intestines of House Crows (*Corvus splendens*) in Tanzania

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SJK, SLL, EMS and HMM designed the study, wrote the protocol and performed experiments. Authors SJK, EVGK and AM performed statistical data analysis, literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Background: The Indian house crow, *Corvus splendens* (Vieillot) was introduced in Zanzibar, Tanzania by the British and immigrants from India in 1897 to help clean the town. The crow is

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responsible for polluting the environment, water sources and human surroundings by their droppings and the rubbish they carry. This behavior has led to concern that, the crows may be responsible for the spread of certain pathogens including *Salmonella* and their persistence in the environment.Given the zoonotic potential of *Salmonella*, the main aim of this study was to investigate the occurrence of antimicrobial resistant *Salmonella* infections in Indian house crows and to determine if the isolates were similar to those associated with disease in livestock or humans.

Methods: Indian house crows were lured with meat and blood baits to land into the crow live-trap set at the Mabibo compound of the National Institute for Medical Research (NIMR) in Dar es Salaam city in Tanzania. A total of 100 house crows were captured, humanely sacrificed, and their small and large intestines were obtained by using aseptic techniques for microbiological investigations. Culture technique was employed to detect the presence of *Salmonella* in intestinal contents; and preliminary identification of the isolates was based on colonial characteristics on selective media and microscopic examination of smears following Gram staining. Confirmation of *Salmonella* species was done by biochemical tests. Antimicrobial susceptibility testing was done by using the disc diffusion method on Mueller Hinton agar.

Results and Discussion: Eight isolates were identified by standard microbiological techniques as *Salmonella* spp. (6 suggestive of *Salmonella gallinarum* and 2 suggestive of S. Typhi). All isolates were found to be susceptible to ciprofloxacin but resistant to amoxicillin. Lower levels of susceptibility were noted for chloramphenicol and ceftriaxone. Our results demonstrate the presence of antimicrobial resistant *Salmonella* spp. in the Indian house crows' population and provide an indication of potential public and poultry health risks associated with these birds in the coastal area.

Conclusion: The occurrence of antibiotic resistant S. Typhi and *S. gallinarum* among Indian house crows has both veterinary and public health consequences as they may be transmitted to poultry and humans. This therefore provides further rationale for the public action on eradicating the house crows.

Keywords: Salmonella; antibiotic resistance; crows; Tanzania.

1. INTRODUCTION

The Indian house crow, *Corvus splendens* (Vieillot), is one of the world's most invasive bird species, causing disturbance to more than 25 countries throughout the Indian Ocean, Arabian Peninsula and South East Asia [1]. Bijlsma and Meininger reported that the crow originated from Asia, but adaptively colonized many other parts of the world ranging from Malaysia to South Africa [2,3]. It was first reported on the island of Zanzibar in East Africa in 1897 whereby it was introduced by the British and immigrants from India to help clean the town [4]. The Indian house crow presents great ecological flexibility and by the 1950s, it had spread to the large part of the East African coast and it continues to expand its range. This bird species has been causing problems to the natural biodiversity of the regions, as well as impacting upon human health, tourism, infrastructure, and general development [1]. The crows have been found to be responsible for the reduction of small reptiles and amphibians and indigenous crows [5]. In addition they kill poultry, prey on the chicks and eggs of native birds and harass other birds, animals and insects [5].

The population of house crows in Tanzania mainland has increased significantly since 1980's as the species adapted well in the new environment and has no natural enemies [6]. The birds are called the world most destructive crows and are frequently observed in close contact with water sources, reserve water tanks and they keep picking unattended human food and crops. Their abundance is closely linked to human population size due to the expanding amount of rubbish generated [7]. The crows are responsible for polluting the environment, water sources and human surroundings by their droppings and the rubbish they carry. This behavior has led to concern that, the crows may be responsible for the spread of certain pathogens including *Salmonella* and their persistence in the environment [4].

Several studies have shown that a number of serovars of *Salmonella enteric* exist in wild birds, and it has been suggested that wild birds might be playing a significant role in the epidemiology of human and livestock salmonellosis [8]. However, little is known about the existence of *Salmonella* strains in Indian house crows; particularly the human and livestock associated

strains.The present study was therefore aimed at investigating the occurrence of antimicrobial resistant *Salmonella* infections in Indian house crows and to elucidate if the isolates were similar to those associated with disease in livestock or humans.

2. MATERIALS AND METHODS

2.1 Indian House Crow Collection

Indian house crows were captured at the Mabibo compound of the National Institute for Medical Research (NIMR) in Dar es Salaam city in Tanzania. This specific site was chosen based on convenience as most of the researchers involved in this study are based there. The crows were lured with meat and blood baits to land into the crow live-trap set within the compound. A total of 100 house crows were captured and used in this study. This sample size considered both the available funds and the study duration.

2.2 Isolation of *Salmonella* spp.

The birds were euthanized by an overdose of pentobarbitone sodium (50 mg/kg body weight) and then dissected immediately. While occluded by forceps small and large intestines were aseptically removed from the abdominal cavity for microbiological investigations. Intestinal samples from each bird were placed in a separate container. About 1cm of the opened intestine of each sampled bird was placed in bijou bottle containing 10 ml of sterile biological peptone water and incubated overnight at 37°C. A loopful of intestinal contents from the biological peptone water was streaked on solid differential and selective media (MacConkey) agar (Oxoid Ltd, Basingstoke, UK) and incubated at 37°C for 24 hours. Colonies suggestive of *Salmonella* (colorless, translucent to opaque, irregular edge colonies which are non-lactose fermenters) were sub-cultured on *Salmonella*-Shigella agar (Oxoid Ltd, Basingstoke, UK) in duplicate. Pure *Salmonella* colonies from *Salmonella*-Shigella agar (colorless edge colonies with black centre) were then subjected to identification.

2.3 Identification of *Salmonella* Isolates

Preliminary identification of *Salmonella* was based on colonial characteristics on selective media and microscopic examination of Gram stained smears. Presumptive *Salmonella* colonies were picked using a sterile wire loop, added to a drop of normal saline and smeared on a microscopy glass slide. Gram stain was performed and gram-negative rod shaped organisms were identified as suggestive of *Salmonella* species. Confirmation of *Salmonella* species was done by biochemical tests using the Analytical Profile Index (API-20E) system according to manufacturer's instructions (bioMérieux, inc, Darham, USA). Nutrient agar slant and butt were utilized for determination of production of specific metabolites such as gas, acid or alkaline solution.

2.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done by using the disc diffusion method on Mueller Hinton (MH) Agar (Oxoid Ltd, Basingstoke, UK) using *C. jejuni* NCTC 11168 for quality control purposes. Bacterial suspensions were prepared in a sterile normal saline and adjusted to a turbidity equivalent to 0.5 McFarland standards ($1.5x10^8$ cells/ml). Sterile swab was used to inoculate the suspension by evenly streaking on prepared Mueller Hinton agar plate and allowed to stay for 3-5 minutes to allow drying. Sterile forceps were used to place discs on the inoculated plates which were then incubated at 37°C for 48 hours. The isolates were tested for susceptibility to ceftriaxone, amoxicillin, ciprofloxacin and chloramphenical (Oxoid Ltd, Basingstoke, UK), which are drugs of choice for treatment of *Salmonella* infections. After 48 hours of incubation, the diameters of inhibition zones were measured. Interpretation of results was guided by both standardized tables supplied by the National Committee on Clinical Laboratory Standards (currently known as Clinical and Laboratory Standards Institute) (NCCLS, 2002) and manufacturer's instructions.

3. RESULTS

3.1 Occurrence of Enteric *Salmonella* in Sampled House Crows

Salmonella spp. were found in eight out of the 100 intestinal samples of captured Indian house crows tested, which represents a prevalence of 8.0%. Using the API-20E system (Table 1) the isolates exhibited biochemical profiles suggestive of two different *Salmonella* serotypes i.e. *S.* Gallinarum being the predominant accounting for 75.0% of the isolates and S. Typhi which accounted for 25.0% samples of Indian house crows.

Table 1. API- Biochemical indications of *Salmonella* strains

Test	*Salmonella* Gallinarum	*Salmonella* Typhi
ONPG	-	-
ADH	-	-
LDC	+	+
ODC	+	-
CIT	+	-
H_2S	+	+
URE	-	-
TDA	-	-
IND	-	-
VP	-	-
GEL	-	-
GLU	+	+
MAN	+	-
INO	-	-
SOR	-	-
RHA	-	-
SAC	-	-
MEL	+	+
AMY	-	-
ARA	+	-

Key: ONPG = β-galactosidase, ADH = arginine dihydrolase, LDC = lysine decarboxylase, ODC = ornithine decarboxylase, CIT = Citrate, H_2S = hydrogen sulfide, URE = urea agar, TDA *= tryptophan deaminase, IND = indole, VP = acetoin production test, GEL = gelatinase test, GLU = glucose assimilation test, MAN = mannitol assimilation test, INO = inositol assimilation test, SOR = sorbitol assimilation test, RHA = rhamnose assimilation test, SAC = saccharose assimilation test, MEL = melibiose assimilation test, AMY = amygdaline assimilation, ARA = arabinose assimilation test*

3.2 Antimicrobial Susceptibility Profiles of Isolated Enteric *Salmonella*

Table 2 shows all the 8 *Salmonella* isolates detected were susceptible to ciprofloxacin. More than half (75%) of isolates (6 out of 8) were resistant to chloramphenicol, of which 5 were *S.* Gallinarum and 1 was *S.* Typhi. High resistance was also noted for Ceftriaxone, whereby 7 out of 8 isolates (87.5%) were resistant, of which 5 were *S.* Gallinarum and 2 were *S.* Typhi. All the 8 *Salmonella* isolates were resistant to amoxicillin.

4. DISCUSSION

In this study we have isolated *Salmonella* species, most probably *S.* Gallinarum and *S.* Typhi, from 8.0% of the intestines of collected apparently healthy Indian house crows. Our results are comparable to the findings reported in 2004 by Millán et al. [9] who observed that 8.5% of wild birds including crows were harboring *Salmonella* spp. Furthermore, our results correlate with those of Agasi et al. [10] who reported the isolation of *Salmonella* spp. from 6.7% of the cloacal swabs taken from apparently healthy crows in Japan(n=30). Contrary to these findings, other studies conducted elsewhere [11,12] couldn't isolate *Salmonella* from the crows. The isolation of the two serovars of *Salmonella* (*S.* Gallinarum and *S.* Typhi) from the intestines of Indian house crows in this study confirms the role of these birds as potential carriers of enteric *Salmonella*for both humans and animals [13,14]. S. Typhi is the causative agent of human typhoid fever [15] while *S.* Gallinarum causes fowl typhoid in birds [16]. Occurrence of *S.* Gallinarum in chickens [17,18] and S. Typhi in humans [19-21], in the country, has been reported in previous studies. The risk of enteric *Salmonella* infections originating from crows is heightened due to the fact that their population is expanding both in the country and in neighboring countries [17].

One of the important symptoms of *Salmonella* infection in humans is the occurrence of fever. However, fever is among the most common syndromes prompting persons to seek healthcare in Tanzania and other countries. It follows true that, the numerous causes of fever (febrile illness) are often difficult to distinguish by mere clinical presentation. Although malaria is easily ruled out by blood film examination or a malaria rapid diagnostic test, salmonellosis requires serological testing. Recently, increasing *Salmonella* infection in humans has been detected by blood culture in different parts of Tanzania and other countries [19,20]. The outbreaks described in northern Tanzania, the complications of intestinal perforation and neurologic manifestations provided clue of typhoid fever as the possible cause whereby in the follow up analysis a much larger group of patients with undifferentiated fever was found positive for typhoid fever [19,20].

It has been reported by several authors that infections with *S.* Typhi are restricted to humans [22,23] and that there exists no animal or environmental reservoirs for the bacterium [24]. Experimental infections with *S.* Typhi have however been reported in higher primates [25]. The observation that this bacterium infected crows in the present study could be an attribute

Table 2. Antimicrobial susceptibility of isolated *Salmonella* strains

Antimicrobial agent	Concentration per disc (µg)	Susceptible strains (N)	Resistant strains (N)
1. Ciprofloxacin	10	*S.* Gallinarum= 6 *S.* Typhi= 2	*S.* Gallinarum = 0 *S.* Typhi= 0
2. Chloramphenicol	30	*S.* Gallinarum= 1 *S.* Typhi= 1	*S.* Gallinarum= 5 *S.* Typhi= 1
3. Ceftriaxone	30	*S.* Gallinarum= 1 *S.* Typhi= 0	*S.* Gallinarum= 5 *S.* Typhi= 2
4. Amoxycillin	30	*S.* Gallinarum= 0 *S.* Typhi= 0	*S.* Gallinarum= 6 *S.* Typhi= 2

of the use of an identification technique with low discriminatory ability thereby leading to misidentification. Another possibility could be that this study has hit upon a major discovery in *Salmonella* biology by identifying a new host for this particular species.

The present study revealed the highest antimicrobial sensitivity level of 100% shown by the isolates to ciprofloxacin which was in agreement with the previous report of Zahrei et al. [26]. This signifies continual use of ciprofloxacin as a drug of choice for treatment of infections caused by these organisms. However, the present study also revealed the prevalence of resistance to different antimicrobial agents among the obtained *Salmonella* isolates. The isolates were highly resistant to amoxicillin (100%), ceftriaxone (87.5%) and chloramphenicol (75%); suggesting that they are ineffective for the treatment of infections caused by these strains in the study area. The possible sources of antimicrobial resistant *Salmonella* species could be humans and animals as overuse and misuse of antimicrobials both in veterinary [27,28] and medical fields [29], which are key attributes in development of antibiotic resistant among different microbes, have been reported in the country. In turn, Indian house crows which are ubiquitous scavengers could bean important factor in spread of antimicrobial resistant salmonellosis both to humans and animals over the distances they cover by their droppings. In the One Health Initiative, the problem of antimicrobial resistance in which house crows now seem to take part, collective efforts from public health and veterinary professionals are needed for its mitigation.

The limitations of the current study included the following i) sampling of the crows was conducted in a limited area making the sample less representative of the city crow population ii) adoption of an identification technique with low discriminatory power which could lead to miss-classification of the isolates into serovars.

5. CONCLUSION

From our results we can conclude that, the Indian house crows could be the source of *Salmonella* (*S.* Gallinarum and *S.* Typhi) infections in men and animals. The observed antibiotic resistance among crow derived *Salmonella* isolates is of particular interest since it suggests that the crows might contribute to spread the antibiotic resistant Salmonellosis in men and animals thereby complicating treatment of such cases. Based on the findings from this study, it is advisable that people should avoid contacts with crows and maintain good hygienic practices, treat drinking water and improve sanitation so as to avoid acquiring infections originating from crows. Crow eradication programs should be strengthened so as to stem the risk of transmission of antibiotic resistant *Salmonella* from these wild birds to human and animals.

CONSENT

Not applicable.

ETHICAL APPROVAL

The trapping of Indian house crows was done using a live trap provided to NIMR by the Ministry of Natural Resources and Tourism under the crows' eradication program. The crows' eradication program has permission of the government of the United Republic of Tanzania through the Ministry of Natural Resources and Tourism to capture and humanely kill crows in Dar es Salaam city in Tanzania. Crows trapped in the program are being killed so that to reduce their population density. The research protocol was approved by the undergraduate studies committee of the Department of veterinary

Medicine and Public Health of the Sokoine University of Agriculture (SUA) in accordance with Tanzania Wildlife Research Institute (TAWIRI) *Guidelines for Conducting Wildlife Research* (2001) and in compliance with Tanzania Veterinary Act number 16 of 2003 (Veterinary regulations; Government notice no. 389 of 2005), following a mandate of SUA to conduct disease investigations in wildlife in different protected and unprotected areas in Tanzania. All biological samples were collected in order to perform research on presence of *Salmonella* species in house crows.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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