

Evidence of carriage of antibiotic resistant salmonella species of public health and veterinary significance in the intestines of house crows(*CorvusSplendens*) in Tanzania

ABSTRACT

Background

The Indian house crow, *Corvus splendens* (Vieillot) was introduced in Zanzibar in Tanzania by the British and immigrants from India in 1897 to help clean the town. The crow is 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 *Salmonella* infections in Indian house crows, in particular, to determine if the isolates were similar to those associated with disease in livestock or humans.

Methods

Indian house crows crossing the Mabibocompound of the National Institute for Medical Research (NIMR) in Dar es Salaam city in Tanzania were lured with meat and blood baits to land into the Crow live-trap. A total of 100 house crows were captured. The birds were humanely sacrificed and small and large intestines were obtained by using aseptic techniques for microbiological investigations. Preliminary identification of salmonella 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 including fermentation of glucose, lactose and sucrose, hydrogen sulfide production, urease activity, phenylalanine deamination, lysine decarboxylation, citrate, methyl red and indole tests. Antibiotic susceptibility testing was done by using the disc diffusion method of Mueller Hinton agar. Results were recorded as susceptible or resistant based on the diameter of the zone of inhibition for each antimicrobial disc used according to the guidelines

34 provided by manufacturers.

35

36 **Results and discussion**

37 Eight isolates were identified by standard microbiological techniques as *Salmonella*
 38 spp. (6% suggestive of *Salmonella gallinarum* and 2% suggestive of *S. typhi*). All
 39 isolates were found to be susceptible to ciprofloxacin but resistant to amoxicillin.
 40 Lower levels of susceptibility were noted for chloramphenicol and ceftriaxone. Our
 41 results demonstrate the presence of antibiotic resistant *Salmonella* spp. in the Indian
 42 house crows' population and provide an assessment of potential public and poultry
 43 health risks to the high-density human and poultry populations within the coastal area.

44

45 **Conclusion**

46 The reduced antibiotic susceptibility of *S.typhi* and *S.gallinarum* is veterinary,
 47 clinical and public health consequences associated with transmission of antimicrobial
 48 drug resistant *Salmonella* in poultry and humans. The risk of transmission of antibiotic
 49 resistant *Salmonella* to humans and poultry provides further rationale for the public
 50 action on eradicating the house crows.

51 **Keywords:** *Salmonella*, antibiotic resistance, susceptibility, Tanzania

52

53 **1. INTRODUCTION**

54 The Indian house crow, *Corvus splendens* (Vieillot), is one of the world's most
 55 invasive bird species, causing disturbance to more than 25 countries throughout the
 56 Indian Ocean, Arabian Peninsula and South East Asia [1]. Bijlsma and Meininger
 57 reported that the crow originated from Asia, but adaptively colonized many other
 58 parts of the world ranging from Malaysia to South Africa [2, 3]. It was first reported
 59 on the island of Zanzibar in East Africa in 1897 whereby it was introduced by the
 60 British and immigrants from India to help clean the town [4]. The Indian house crow
 61 presents great ecological flexibility and by the 1950s, it had spread to the large part of
 62 the East African coast and it continues to expand its range. This bird species has been
 63 causing problems to the natural biodiversity of the regions, as well as impacting upon
 64 human health, tourism, infrastructure, and general development [1]. The crows have
 65 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 enterica* 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 and human and livestock associated strains. The present study was therefore aimed at investigating the occurrence of *Salmonella* infections in Indian house crow and, in particular, to elucidate if the isolates were similar to those associated with disease in livestock or humans.

2.0 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. Indian house crows crossing the compound were lured with meat and blood baits to land into the crow live-trap. 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.

99

100 **2.2 Isolation of Salmonella spp.**

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

114

115 **2.3 Identification of Salmonella isolates**

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

126

127 **2.4 Antibiotic susceptibility testing**

128 Antibiotic susceptibility testing s done by using the disc diffusion method
129 on  Mueller Hinton (MH) Agar (Oxoid Ltd, Basingstoke, UK) using *C. jejuni* NCTC
130 11168 for quality control purposes. Bacterial suspensions were prepared in a sterile
131 normal saline and adjusted to a turbidity equivalent to 0.5 McFarland standards

(1.5×10^8 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 and incubated at 37°C for 24 hours. The isolates were tested for susceptibility to ceftriaxone, amoxicillin, ciprofloxacin and chloramphenicol (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.

2.5 Ethics statement

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. Permission to use the captured crows for research was provided by the Department of veterinary Medicine and Public Health 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 the Sokoine University of Agriculture (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* in house crows.

3.0 RESULTS

3.1 Occurrence of enteric Salmonella in sampled house crows

Salmonella spp. were found in 8 out of the 100 intestinal samples of captured Indian house crows tested, which represents a prevalence of 8%. Using the API-20E system (Table 1) the isolates exhibited biochemical profiles suggestive of two different serotypes i.e. *S. gallinarum* being the predominant *Salmonella* serotype and

165 accounting for 6 (6%) of the isolates, and *S.typhi* which was found in 2 (2%) samples
166 of Indian house crows.

167 *Table 1 here*

168 3.2 Antibiotic susceptibility of isolated enteric Salmonella

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

175 *Table 2 here*

176 4.0 DISCUSSION

177 In this study we have isolated two serovars of Salmonella, *S. gallinarum* and *S.*
178 *typhi* from the intestines of apparently healthy Indian house crows. Our results are
179 comparable to the findings reported in 2004 by Millán *et al.*, who observed that 8.5%
180 of wild birds including crows were harboring *Salmonella* spp [9]. Furthermore, our
181 results correlate with those of Agasiet *al.*, who reported the isolation of *Salmonella*
182 spp. from cloacal swabs taken from two out of 30 apparently healthy crows in
183 Japan [10]. Contrary to these findings, other studies conducted elsewhere [11, 12]
184 couldn't isolate salmonella from the crows.

185
186 The isolation of the two serovars of Salmonella (*S. gallinarum* and *S. typhi*) from the
187 intestines of Indian house crows in this study confirms the role of these birds as
188 potential carriers of enteric Salmonella for both humans and animals [13, 14]. *S. typhi*
189 is the causative agent of human typhoid fever [15] while *S. gallinarum* causes fowl
190 typhoid in birds [16].

191
192 Occurrence of *S. gallinarum* in chickens [17, 18] and *S. typhi* in humans [19-21], in the
193 country, has been reported in previous studies. The behavior of Indian house crows of
194 keeping in close contact and their interaction with human environment indicates their
195 potential as vehicles of transmission of Salmonella infections in Tanzania. The risk of
196 enteric salmonella infections originating from crows is heightened due to the fact that
197 their population is expanding both in the country and in neighboring countries [17].

198

199 It has been reported by several authors that infections with *S. typhi* are restricted to
 200 humans [22, 23] and that there exists no animal or environmental reservoirs for the
 201 bacterium [24]. Experimental infections with *S. typhi* have however been reported in
 202 higher primates [25]. The observation that this bacterium infected crows in the present
 203 study could be an attribute of the use of an identification technique with low
 204 discriminatory ability thereby leading to misidentification. Another possibility could
 205 be that this study has hit upon a major discovery in Salmonella biology by identifying
 206 a new host for this particular species.

207

208 The present study revealed the highest antibiotic sensitivity level of 100% shown by
 209 the isolates to ciprofloxacin which was in agreement with the previous report of
 210 Zahreiet *al.*, 2005 [26]. This signifies continual use of ciprofloxacin as a drug of
 211 choice for treatment of infections caused by these organisms. In addition, the present
 212 study also revealed the prevalence of antibiotic resistance among
 213 Salmonella isolates. The isolated strains were highly resistant to amoxycillin,
 214 ceftriaxone and chloramphenicol. The highest resistance level of 100% was observed
 215 for amoxycillin, followed by ceftriaxone (87.5%) and lastly, chloramphenicol (75%);
 216 rendering them ineffective for the treatment of infections caused by these strains. The
 217 possible sources of antibiotic resistant Salmonella species could be humans and
 218 animals as overuse and misuse of antibiotics both in veterinary [27, 28] and medical
 219 fields [29], which are key attributes in development of antibiotic resistance among
 220 different microbes, have been reported in the country. In turn, Indian house crows
 221 which are ubiquitous scavengers could be an important factor in spread of antibiotic
 222 resistant Salmonellosis both to humans and animals over the distances they cover by
 223 their droppings. In the One Health Initiative, the problem of antibiotic resistance in
 224 which house crows now seem to take part, collective efforts from public health and
 225 veterinary professionals are needed for its mitigation.

226

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

231

232 **5.0 Conclusion**

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

243

244

245 **References**

- 246 1. Ryall C. The pest status of the Indian house crow *Corvus splendens* in Mombasa
 247 and a survey of its expansion of range in coastal Kenya; In: Bennun, L. (ed. 1992).
 248 *Proceedings of the VIIth Pan African Ornithological Congress, Nairobi*, Aug
 249 1988.
- 250 2. Bijlsma GG and Meininger PL. Behaviour of the house crow (*Corvus splendens*), and
 251 additional notes on its distribution. *Le Gerfaut*. 1984. 74, 3-13.
- 252 3. Lever C. *Naturalised birds of the world*. T and A D Poyser. 2006, 352 .
- 253 4. Cooper JC. Health studies on the Indian house crow. *Avian Pathology*. 1996, 25:
 254 381-386.
- 255 5. GISD-2010. Global Invasive Species Database of the IUCN/ISSG (Invasive
 256 Species Specialist Group of the World Conservation Union). Fact sheet on
 257 *Corvus splendens*. Accessed online at <http://www.issg/database> on 12th January
 258 2010.
- 259 6. Suliman AS, Meier GG and Haverson PJ. Eradication of the house crow from
 260 Socotra Island, Yemen in *Island Invasives: eradication and management*. Veitch
 261 CR, Clout MN, and Town CR. (eds). *IUCN*, Grand, Switzerland. 2011. 361-363.

- 262 7. Nyari A, RyallC and PetersonA:Global invasive potential of the house crow based
263 on ecological niche modeling. *Journal of Avian Biology*.2006, 37: 306- 309.
- 264 8. HughesLA,ShoplandS,WigleyP,BradonH,LeatherbarrowAH,WilliamsNJ,Bennett
265 M,de PinnaE,LawsonB,CunninghamAA andChantreyJ:Characterisation of
266 *Salmonella enterica*serotype Typhimurium isolates from wild birds in northern
267 England from 2005 – 2006. *BMC Veterinary Research*. 2008,
268 4:4doi:10.1186/1746-6148-4-4.
- 269 9. Millán J, AdurizG, MorenoB, JusteRAand BarralM.Salmonella isolates from wild
270 birds and mammals in the Basque Country (Spain).*Rev. sci. tech. Off. int.*
271 *Epiz.*,2004, 23 (3), 905-911.
- 272 10. Agasi M, Oka C and SatoG.Isolation of *Salmonella typhimurium*var Copenhagen
273 from crows in the city of Otaru. *Japanese Journal of Veterinary Science* 1967,
274 38,521-522
- 275 11. Kapperud G, Rosef O.Avian wildlife reservoir of *Campylobacter fetus* subsp.
276 *jejuni*, *Yersinia* spp., and *Salmonella* spp. In Norway. *Appl. Environ.*
277 *Microbiol.*1983, 45, 375–380.
- 278 12. Ganapathy K, SalehaAA, JaganathanM, TanCG, ChongCT, TangSC, IderisA,
279 DareCMand BrandburyJM.Survey of *Campylobacter*, *Salmonella* and
280 mycoplasmas in house crows (*Corvus splendens*) in Malaysia. *Veterinary record*.
281 2007, 160, 622-624.
- 282 13. Willumsen B and HoleS.Presence of thermotolerant *Campylobacter* spp. and
283 *Salmonella* spp. from seagulls and crows and its association with human enteritis
284 in Bodo area (Norway). *Norsk. Vet.* 1987, **99**:277-282.
- 285 14. Refsum T, HandelandK, BaggesenDL, HolstadG and KapperudG.*Salmonellae* in
286 avian wildlife in Norway from 1969 to 2000. *Appl. Environ. Microbiol.* 2002,
287 68:5595-5599.
- 288 15. Farmakiotis D, VarugheseJ, SueP, AndrewsP, BrimmageM, DobroszyckiJC and
289 CoyleM.Typhoid Fever in an Inner City Hospital: A 5- Year Retrospective
290 Review. *Journal of Travel Medicine*.2012, 20(1): 17-21.
- 291 16. Barrow PA and FreitasNetoOC.Pullorum disease and fowl typhoid-new thoughts
292 on old diseases: A review. *Avian pathology*.2011, 40(1): 1-13.

- 293 17. Minga UM, Kikopa R, Minja KSGZ, Mwashia JD. The prevalence and improved
294 serodiagnosis of fowl typhoid in Tanzania. Proceedings of the 5th Tanzania
295 Veterinary Association Scientific Conference. 325–338, 1987.
- 296 18. Mdegela RH, Yongolo MG, Minga UM, Olsen JE. Molecular epidemiology of
297 *Salmonella gallinarum* in chickens in Tanzania. Av. Path.29, 457-463, 2000.
- 298 19. Mtove G, Amos B, von Seidlein L, Hendriksen I, Mwambuli A, Kimera J,
299 Mallahiyo R, Kim DR, Ochiai RL, Clemens JD, Reyburn H, Magesa S, Deen JL:
300 Invasive salmonellosis among children admitted to a rural Tanzanian hospital and
301 a comparison with previous studies. *PLoSOne* 5(2): e9244, 2010.
- 302 20. Crump JC, Ramadhani HO, Morrissey AB, Saganda W, Mwako MS, Yang LY,
303 Chow SC, Morpeth SC, Reyburn H, Njau BN, Shaw AV, Diefenthal HC, Shao JF,
304 Bartett JA, Maro VP: Invasive bacterial and fungal infections among hospitalized
305 HIV-infected and HIV-uninfected adults and adolescents in northern Tanzania.
306 *CID* 52(3):341-348, 2011.
- 307 21. Ley B, Thriemer K, Ame SM, Mtove GM, von Seidlein L, Amos B, Hendriksen
308 IC, Mwambuli A, Shoo A, Kim DR, Ochiai LR, Favorov M, Clemens JD, Wilfing
309 H, Deen JL, Ali SM. Assessment and comparative analysis of a rapid diagnostic test
310 (Tubex®) for the diagnosis of typhoid fever among hospitalized children in rural
311 Tanzania. *BMC Infect Dis.* 11:147, 2011.
- 312 22. Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, Casadesus J, Platt
313 DJ, Olsen JE. Host adapted serotypes of *Salmonella enterica*. *Epidemiol Infect* 125:
314 229-255, 2000.
- 315 23. Parry C, Hien TT, Dougan G, White N, Farrar J. Typhoid fever. *N Engl J Med.*
316 347:1770-1782, 2002.
- 317 24. Wain, J., D. House, J. Parkhill, C. Parry, and G. Dougan. Unlocking the genome
318 of the human typhoid bacillus. *Lancet Infect. Dis.* 2:163–170, 2002.
- 319 25. Edsall G, Gaines S, Landy M, Tigertt WD, Sprinz H, Trapani RJ, Mandel AD,
320 Benenson AS. Studies on infection and immunity in experimental typhoid fever. I.
321 Typhoid fever in chimpanzees orally infected with *Salmonella typhosa*. *J. Exp.*
322 *Med.* 112:143, 1960.

- 323 26. Zahrei ST and Saeedzadeh MM. The isolation of antibiotic resistant *Salmonella*
 324 from intestine and liver of poultry in Shiraz province of Iran. *Int. J. Poult. Sci.*,
 325 4(5): 320-322, 2005.
- 326 27. Karimuribo ED, Mdegela RH, Kusiluka LJM and Kambarage DM. Assessment of
 327 drug usage and antimicrobial residues in milk on small holder farms in Morogoro,
 328 Tanzania, *Bulletin of Animal Health and Production*. 53: 234 – 241, 2005.
- 329 28. Katakweba AAS, Mtambo MMA, Olsen JE and Muhairwa AP. Awareness of human
 330 health risks associated with the use of antibiotics among livestock keepers and
 331 factors that contribute to selection of antibiotic resistance bacteria within livestock
 332 in Tanzania. *Livestock Research for Rural Development* 24 (10), 2012.
- 333 29. Aminov RI. The role of antibiotics and antibiotic resistance in nature. *Environ.*
 334 *Microbiol.* 11:2970-2988, 2009.

335

336

337

338

339

340 **Table 1:** API- Biochemical confirmation of *Salmonella* strains

341

<i>Test</i>	<i>Salmonella gallinarum</i>	<i>Salmonella typhi</i>
ONPG	-	-
ADH	-	-
LDC	+	+
ODC	+	-
CIT	+	-
H ₂ S	+	+
URE	-	-
TDA	-	-
IND	-	-
VP	-	-
GEL	-	-
GLU	+	+
MAN	+	-
INO	-	-
SOR	-	-
RHA	-	-
SAC	-	-
MEL	+	+
AMY	-	-
ARA	+	-

342 **Key:** ONPG = β -galactosidase, ADH = arginine dihydrolase, LDC = lysine decarboxylase, ODC = ornithine
 343 decarboxylase, CIT = Citrate, H₂S = hydrogen sulfide, URE = urea agar, TDA = tryptophan deaminase, IND =
 344 indole, VP = acetoin production test, GEL = gelatinase test, GLU = glucose assimilation test, MAN =
 345 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

Table.2 Antibiotic susceptibility of isolated *Salmonella* strains

Antibiotic	Concentration per disc (µg)	Susceptible strains (N)	Resistant strains (N)
1. Ciprofloxacin	10	<i>S. gallinarum</i> = 6 <i>S. typhi</i> = 2	<i>S. gallinarum</i> = 0 <i>S. typhi</i> = 0
2. Chloramphenicol	30	<i>S. gallinarum</i> = 1 <i>S. typhi</i> = 1	<i>S. gallinarum</i> = 5 <i>S. typhi</i> = 1
3. Ceftriaxone	30	<i>S. gallinarum</i> = 1 <i>S. typhi</i> = 0	<i>S. gallinarum</i> = 5 <i>S. typhi</i> = 2
4. Amoxycillin	30	<i>S. gallinarum</i> = 0 <i>S. typhi</i> = 0	<i>S. gallinarum</i> = 6 <i>S. typhi</i> = 2