

Intestine microbiota of Cattle Egret (*Bubulcus ibis*) and Pied Crow (*Corvus albus*) in Kinshasa city: a risk factor for public health?

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Abstract

Purpose: Our study focused on Pied Crow and Cattle Egret, two commensal and ubiquitous birds feeding in dumps and frequenting our homes. Our aim was to identify the bacteria that birds bring to our homes and could be a potential risk to Congolese health.

Method: We have done bacteriological analyses of bird feces for to explore its gut microbiota composition. The feces were collected in the uricotelic cloaca by using a swab in 52 Cattle Egrets (*Bubulcus ibis*) and 23 Pied Crows (*Corvus albus*) from Kinshasa city.

Results: Bacteriological analyses revealed the presence of *Proteus vulgaris* (3.8%) and *Klebsiella pneumoniae* (5.8%) on Cattle Egret, *Salmonella sp.* (8.7%), *Klebsiella pneumoniae* (8.7%), *Pseudomonas aeruginosa* (21.7%), *Proteus vulgaris* (30.4%) on Pied Crow, *Citrobacter spp* and *Escherichia coli* (100%) on both Cattle Egret and Pied Crow respectively.

Conclusion: The presence of these pathogenic germs, suggesting these commensal and ubiquitous birds may be potential vectors of various Diseases which pose serious health problems in the region.

Keywords: Intestine microbiota, Cattle Egret, Pied Crow, Kinshasa.

Introduction

In Kinshasa, the capital of the Democratic Republic of Congo (DRC), recent studies by Punga and Ifuta (2015) documented about 131 species of birds grouped into 40 families, representing 11% of species of the all country. Despite this avian diversity, rare studies carried out throughout the country concern only their systematics and habitats (Kisasa and Aloni, 2011). Information about the role of these birds in the emergence of epidemics in the region is lacking. A preliminary study by Kisasa et al., 2020 revealed the presence of lice mallophages (Phthiraptera) *Myrsidea balati*, *Columbicola columbae* and *Menacanthus stramineus*, *Ardeicola ciconiae*, *Goniodes sp.*, *Ciconiphilus decimfasciatus*, *Ardeicola ciconiae* and *Cuculiphilus sp* on some birds from seven bird species of Kinshasa city.

M. stramineus, in domestic hens, *Gallus domesticus* can cause the death of wild turkey chicks, *Meleagris gallopavo* (Djelil, 2012). It is also implicated in the transmission of encephalomyelitis viruses (Amir, 2006). The role of birds as vectors of pathogenic germs to humans has been amply documented. This is the case, for example, with some waterbirds. Potential pathogenic genera were identified in the gut microbiota of the different species, suggesting that waterbirds may disseminate pathogenic species like *Clostridium*, *Helicobacter*, *Campylobacter*, *Vibrio*, etc., between waterbodies (Laviad-Shitrit, 2019).

The gut microbiota of an organism is established immediately after birth and changes due to the host life-style, diet, environmental conditions, genome, etc. (Nicholson et al., 2012; Sommer and Bäckhed, 2013).

The aim of this study is to estimate the impact of environmental conditions on the composition of microbiota of the birds. We chose the Cattle Egret and Pied Crow, two commensal birds feeding in dumps and in our immediate environment. We hypothesized that the bacteriological composition of the gut microbiota of these two birds is a good indicator of the potential risk to public health and bacteria distribution on its hosts is random in the dumps explored.

Materials and Methods

Sampling sites and birds capture

Two different species wild birds (*Bubulcus ibis*, *Corvus albus*) were captured using self-made traps; a noose of nylon wire attached to a piece of wood. The birds were attracted to each trap by palm oil-soaked pouches and pieces of fish. They were caught by surprise from different areas of University of Kinshasa student residences; Home X, Home XX, Home Village Maluku, Triangle of market gardeners, and University clinics (Table1). All

of these sites are the dumps include various hospital and industrial waste thrown away without any treatment (Fig.1).

Our sample consisted of the feces of birds caught in five dumps in the city of Kinshasa. Sterile cotton swabs wetted with sterile normal saline water were inserted in the cloacae of the wild birds, and placed in sterile vials. After collection of cloacal swabs, the birds were then freed. The samples were transported immediately to the laboratory in an ice box.

Isolation and identification of Gram-negative bacilli

Swab samples were enriched in buffered peptone water at 37 °C for 24 h. Subsequently, the cultures were streaked on MacConkey agar and incubated at 37 °C overnight. Different single colonies were then collected and purified. Gram negative bacilli were identified using microbiological conventional methods including Gram staining, oxidase tests, indole and urease production, citrate utilization, hydrogen sulphide, gas production and fermentation of sugars, phenylalanine deaminase, lysine decarboxylase (L.D.C.), ornithine decarboxylase (O.D.C.), arginine dihydrolase (A.D.H.) tests, nitrate reduction, casein hydrolysis, methyl red and Voges-Proskauer reactions.

Distribution of bacteria and their hosts in the five dumps.

We used the MOMENTS method to describe the bacteria-bird interactions (hosts) in the five dumps. You remember, of course, that the Law of Fish is characterized by a variance equal to the average: $\sigma^2 = \mu$ where a σ^2 / μ ratio worth exactly 1 (Bowman & Shenton, 1998). It is this characteristic that ideally should serve as a null hypothesis for testing. Indeed, if we summarize, we have:

- $\sigma^2 / \mu < 1$, Regular distribution: Positive binomial distribution;
- $\sigma^2 / \mu = 1$, Random Distribution: Fish Distribution;
- $\sigma^2 / \mu > 1$, Aggregation Distribution: Positive Binomial Distribution.

We hypothesized H_0 as the distribution of bacteria and their hosts in the dumps ($n=5$) explored is random. In this case, the variable "number of birds per dump" follows a fish law with $\sigma^2 / \mu = 1$. By multiplying σ^2 / μ by $(n - 1)$, we obtained a random test variable called a Dispersion Index(I), which is approximately a law of the χ^2 with $n - 1$ degrees of freedom under this hypothesis H_0 . This χ^2 test reads in a rather peculiar way: the highest 2.5% of the distribution corresponds to a gap to H_0 by excess of heterogeneity (conclusion: aggregation distribution) while the lowest 2.5% correspond to a gap to H_0 by an excess of homogeneity (conclusion: regular distribution). Between these two terminals, we do not reject H_0 (random distribution until proven otherwise).

Results

In total 52 Cattle Egret and 23 Pied Crow were captured. *Proteus vulgaris* (3.8%) and *Klebsiella pneumoniae* (5.8%) were identified on Cattle Egret, *Salmonella sp.* (8.7%), *Klebsiella pneumoniae* (8.7%), *Pseudomonas aeruginosa* (21.7%), *Proteus vulgaris* (30.4%) on Pied Crow, *Citrobacter spp* and *Escherichia coli* (100%) on both Cattle Egret and Pied Crow respectively. All intestinal microbiota are Gram-negative bacilli more important in the dump of University Clinics followed by Home Maluku (Table2). However, the distribution of these bacteria on their hosts in the five dumps is aggregative (Dispersion Index Test, for $\chi^2 \geq 8$; 5 df, $P < 0,025$).

Discussion

Escherichia coli is *Enterobacteriaceae*s in the immediate environment of animals by feces (Fremaux, 2007). Its presence does not surprise us in the various dumps around student residential homes and markets. Indeed, the residential dwellers often defecate in the nature where rains facilitate feces dissemination. This bacteria in mutualist symbiosis in mammals and birds' colonists becomes pathogenic in the environment and responsible for the few pathologies as enteric diseases, Urinary tract infections (Hooton and Walter, 1997) and Neonatal meningitis (Ku et al, 2015).

Other bacteria were more found in birds frequenting the dump of Kinshasa University Clinics (E) or nearby (D). These are *Citrobacter spp.*, *P. vulgaris*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. They are responsible for various known diseases in humans such as Abdominal Sepsis, Urinary Tract Infection and Bacteremia (Bryan and Reynolds, 1984), Brain Abscess (Brouwer et al., 2014), infection of skin and tissue (Holms and Korman, 2007; Akihisa, 1997), Hospital- Acquired infection (Bryan and Reynolds, 1984), Chronic Infection in Cystic Fibrosis patients (Konings et al., 2013), Sepsis Form and Epizootic Enteritis, Febrile Form (Hsiu-Lin Chen, 2008). Indeed, these bacteria can be spread by direct contact with hospital staff, vertical mother-to-child transmission, or ingestion of contaminated material, direct transmission between humans remains widespread (Doran, 1999). The gut microbiota of an organism is established immediately after birth and changes due to the

host life-style, diet, environmental conditions, genome, etc. (Nicholson et al., 2012; Sommer and Bäckhed, 2013). In Canada, for example, Tshape et al. (1995) report that an outbreak of *Citrobacter* infection was associated with the consumption of parsley contaminated with pork purin; 8 urinary tract infections and 1 death were observed.

Conclusion

Here we identified the gut bacterial community composition of the Cattle Egret and Pied Crow from Kinshasa city. Our results demonstrate that the distribution of these bacteria on the two bird species in the five dumps is aggregative. Potential pathogenic genera were identified in its gut microbiota, suggesting these commensal and ubiquitous birds may disseminate pathogenic species like *Citrobacter sp.*, *Klebsiella pneumoniae*, *Salmonella sp.*, *E. coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. This gut microbiota of two commensal birds is established by unsanitary environmental conditions. To improve the health of Congolese population in the future, would require a serious investment in the disposal and treatment of waste, infestation sources of birds and emerging diseases.

References

- Amir, A. (2006) Détermination du Microbisme en Élevage Avicole. Thèse, Dép. des Sciences Vétérinaires, Université Mentouri de Constantine, Constantine.
- Akihisa M., Chikao N., Nobuyasu E. & Shinkichi T. (1997) Antibacterial activity and mode of action of plant flavonoids against *Proteus vulgaris* and *Staphylococcus aureus*. *Phytochemistry*, Vol.26(8): pages 2231-2234.
- Bowman, K.O & Shenton, LR (1998) Estimateur : Méthode des Moments. Encyclopédie des sciences statistiques, pp 2092-2098
- Brouwer MC, Tunkel AR, Mckhan GM & Diederik van de Boek (2014) Brain Abscess. *N. Emgl. J. Med.* Vol.371: pages 447-456.
- Bryan C.S & Reynolds K. (1984) Hospital-Acquired bacteremic urinary tract infection: Epidemiology and Outcome. *Journal of Urology*, Vol. 132(3): pages 494-497.
- Djelil, H. (2012) Ectoparasitisme et parasitémie du poulet de ferme (*Gallus gallus domesticus*, Linnaeus, 1758) dans la région d'Oran. These, Dép. de Biologie, Université d'Oran, Oran.
- Doran, T.I. (1999) *Citrobacter* in Clinical disease of Children: Review Clinical. *Clinical Infectious Diseases*, Vol 28(2); 384-394.
- Holms NE & Korman TM. (2007) *Corynebacterium Kutscheri* Infection of Skin and Soft Tissue. *Journal of Clinical Microbiology*, Vol. 45(10): pages 3468-3469.
- Hooton TM & Walter Stamm, WE. (1997) Diagnosis and Treatment of uncomplicated urinary tract infection. *Infections Disease Clins.* Vol.11(3): pages 551-581.
- Hsiu-Lin Chen, Chih-Hsing Hung, Hsing-I Tseng & Rei-Cheng Yang (2008) Soluble Form of Triggering Receptor Expressed on Myeloid Cells-1 (sTREM-1) as a Diagnostic Marker of Serious Bacterial Infection in Febrile Infants Less Than Three Months of Age. *Jpn.J Infect. Ds.*, Vol.61: pages 31-35.
- Kisasa, K.R. and Aloni, K.J. (2011) The impact of soil texture on the selection of nesting sites by the Malachite Kingfisher (Alcedinidae: *Alcedocristata* PALLAS 1764). *Ostrich journal* 82 (3): 243-246.
- Kisasa, K.R., Banshomire, C. & Malekani, D. (2020) Ectoparasites (Phthiraptera) of some birds of Kinshasa city. *Malimbus* Vol.42: pages 1-6.
- Konings AF, Martin LW, Sharples KJ., Roddam LF., Lathan R., Reid DW & Lamont IL. (2013) *Pseudomonas aeruginosa* Uses Multiple Pathways to Acquire Iron during Chronic Infection in Cystic Fibrosis lung. *Infection and Immunity*, Vol.81(8): pages 2697-2704.
- Ku LC, Boggess KA & Cohen-Wolkowicz M. (2015) Bacterial meningitis. *Clin. Perinatal.* Vol.42(1): pages 29-45.

Laviad-Shitrit S., Izhaki I., Lalar M. and Halpern M. (2019) Comparative Analysis of Intestine Microbiota of Four Wild Waterbird Species. *Front microbiol.*, vol. 10 (1911): pages 1-13.

Nicholson J. K., Holmes E., Kinross J., Burcelin R., Gibson G., Jia W., et al. (2012). Host-gut microbiota metabolic interactions. *Science* 336 1262–1267

Punga, J.K. & Ifuta, B.N. (2015) Recent data on birds of Kinshasa in Democratic Republic of Congo. *J. agr. Sci. Technol. A5*: pages 218–233.

Sommer F., Bäckhed F. (2013). The gut microbiota-masters of host development and physiology. *Nat. Rev. Microbiol.* 11 227–238.

Tschape H., Prager R., Streckel W., Fruth A., Tietze E. & Bohme G (1995) Verotoxinogemie citrobacter preundii associated with severe gastroenteritis and cases of haemolytic uraemic syndrome in a nurse school: green batter as the infection source. *Epidemiology and Infection*, vol. 114(3): pages 441-450.

Author contributions

Robert Kisasa is the project designer and editor of this manuscript. Clarisse Nduaya is my assistant, she was in charge of catching birds. Liesse Iyamba is the head of the microbiology lab that allowed us to do our analyses. It was he who determined the laboratory protocol for bacteriological analyses of microbiota. Cyprien Mbundu is the laboratory technician and assistant to Liesse Mbundu, he was the one who did the bacteriological analyses assisted by Clarisse Nduaya.

Conflict of Interest Statement and Ethics

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. However, as an ecologist, we used ecological methods to collect feces without harming the birds (we fed them and then released them afterwards). The field study involving sample collection from birds was approved by the Ethical Committee of the Department of Biology (Faculty of Kinshasa) under the registration number N/Ref.005-2020/CE-CDB.

Acknowledgements

We are grateful to Serge Kabongo wa Ntita and Dr. J.P Ngbolua K. Niwa. Their comments were relevant and appreciated for presentation of this paper.