Investigating *Salmonella* in Wildlife of Atlantic Canada

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Introduction

Salmonella is a gram-negative, rod-shaped, flagellated bacterium found in the gastrointestinal tracts of humans and animals worldwide. The nontyphoidal *Salmonella* (NTS) serovars of *Salmonella enterica* subspecies *enterica* are largely implicated in foodborne zoonoses[2, 3]. It consists of diverse serovars identified based on structural antigens, resulting in varying properties among each serovar[2] (Figure 1). Wildlife can act as reservoirs of *Salmonella*, leading to potential spillover events to domestic animals and humans [1].

The ability of *Salmonella* to become multidrug-resistant poses significant public health challenges. Moreover, the interactions between humans, the environment, and animals are intricate and not well comprehended, especially with animals adapting to anthropogenic ecosystems. Recent *Salmonella* outbreaks in Atlantic Canada linked to wildlife highlight the need to examine the occurrence of *Salmonella* among wildlife species of the Atlantic Canada region.

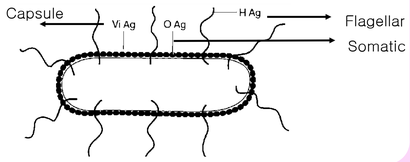

Figure 1. Surface antigens for serotyping

Hypothesis

The wildlife in Atlantic region of Canada is a potential carrier and propagator of various serovars of *Salmonella.*

Objectives

- ❑ To detect *Salmonella* in the deep rectal swab of wildlife
- ❑ To characterize the *Salmonella* isolates
  - ➢ Serotyping of *Salmonella* isolates
  - ➢ Antimicrobial susceptibility testing (AST) of isolates

Methods

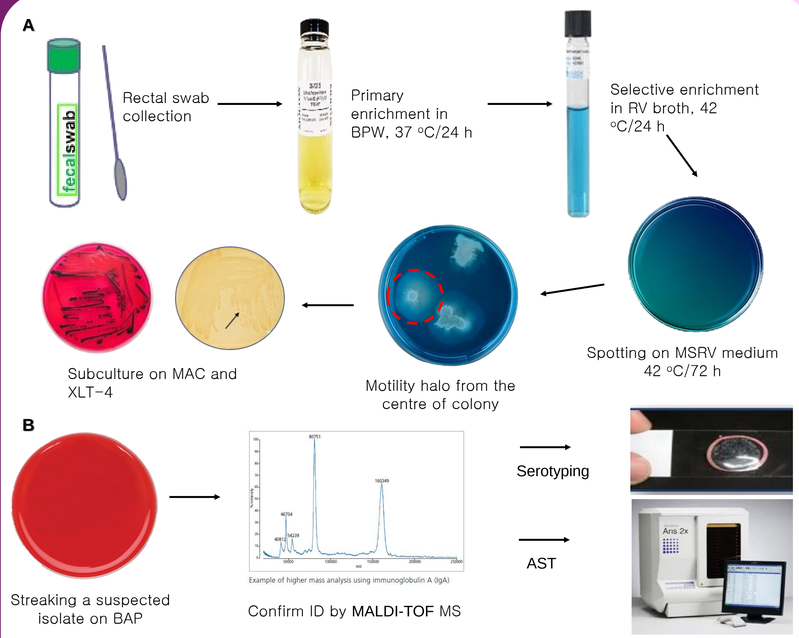

Figure 2. Methods of Collecting and Processing Samples for the Detection of Salmonella.
(A) Enrichment steps for the detection of Salmonella, and (B) characterization of confirmed Salmonella isolates. AST (B) was done using the micro broth dilution method on the NARMS plate carrying 14 antibiotics.

Abbreviations: BPW = Buffered Peptone Water; RV = Rappaport-Vassiliadis; MSRV = Modified Semi-solid RV; XLT 4 = Xylose-Lysine-Tergitol 4; BAP = Blood Agar Plate; MALDI-TOF MS = Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry; NARMS = National Antimicrobial Resistance Monitoring System;

Results

Sample distribution

- A total of 34 rectal swab samples from deceased wildlife animals were collected from May to August 2024 across Atlantic Canada over 12 weeks.
- The distribution of samples included 18 rectal swabs from Nova Scotia, 11 from Prince Edward Island, and 5 from New Brunswick (Figure 3B).
- The rectal swab samples from a variety of terrestrial and aquatic animals were subjected to microbiological procedures to detect *Salmonella* spp.
  - The samples comprised 68% mammals (23 samples) and 32% birds (11 samples) (Figure 3A).
  - The mammalian samples primarily included raccoons. Other mammals were white-tailed deer, squirrels, beavers, and a porcupine.
  - Three samples were from aquatic mammals, including a striped dolphin and two harbor porpoises.
  - Rectal swabs from avian species included samples from bald eagles, purple finches, an American crow, a willet, a red-winged blackbird, and a grackle.
- While the majority of carcasses sampled were from the years 2023 and 2024, one carcass submission was from 2021, and two were from 2022 (Figure 3C).

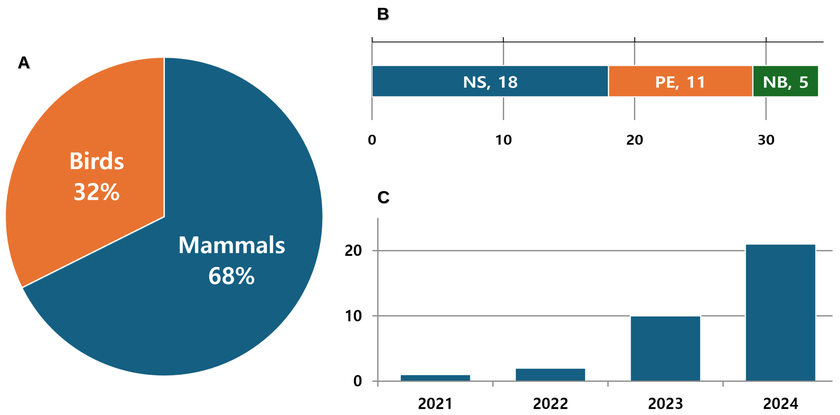

Figure 3. Wildlife sample distribution. Rectal swabs of varied wildlife animal species subjected to culture-based *Salmonella* detection. (A). Percentage of Animal Classes Submitted, (B) Number of Animal Submissions by Province, and (C) Year of Animal Carcass Submissions.

Salmonella Detection

- One sample tested positive for *Salmonella* spp., representing a prevalence rate of 2.94% (95% CI: 0.07% to 8.62%). The positive sample was from a bald eagle found dead in 2024 in Nova Scotia.
- The serotyping identified it as *Salmonella enterica* subspecies *enterica* serovar Muenchen of O:8 serogroup
- The serotype was susceptible to several classes of antimicrobials except aminoglycosides. The susceptibility to ciprofloxacin could not be interpreted.
- In addition to *Salmonella*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Escherichia coli* were identified as the dominant enteric bacteria in some of the samples tested.

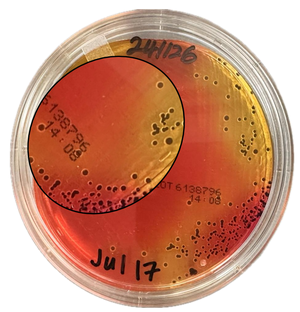

Figure 4. *Salmonella* colonies on XLT-4 selective culture medium. Black-centered colonies are due to hydrogen sulfide production.

Human-Animal Interaction

- Only 4 out of 34 samples were handled by humans for extended periods through wildlife rehabilitation centers or by being raised by the founders.
- The positive sample had no reported human contact during history-taking.

Discussion

- The serovar Muenchen is among the top ten serovars implicated in foodborne zoonoses. The isolate identified in this study is susceptible to several point-of-care antimicrobials, except aminoglycosides. However, in a recent retrospective study, multi-drug resistant Canadian poultry isolates of serovar Muenchen were reported to be resistant to aminoglycosides, sulfa drugs, and tetracyclines.[3]
- Among several samples, *Proteus mirabilis, Klebsiella pneumoniae, and Escherichia coli* were identified as the dominant enteric bacteria.
- The reasons for the lack of Salmonella detection in several samples are suggested to be: (1) the swabs sampled a very small amount of diagnostic material, (2) the presence of very low numbers of detectable Salmonella, (3) the carcasses were frozen; the freezing and thawing could adversely impact the viability of Salmonella, and (4) the resuscitation procedures for Salmonella are not optimal, especially concerning the small volume of starting fecal material and freeze-thaw injury to salmonellae.

Conclusions and Outlook

- The low prevalence of *Salmonella* spp. in wildlife of Atlantic Canada suggests a limited risk of transmission to humans and domestic animals.
- Given the increasing adaptation of wildlife in human ecosystems, continued surveillance is recommended, which is fundamental to understanding the potential pathways and wildlife-associated *Salmonella* transmission dynamics.
- Besides *Salmonella*, wildlife can potentially shed large numbers of zoonotic pathogens, such as *Klebsiella pneumoniae* and *Escherichia coli*.
- Future research should focus on confirming *Salmonella*-negative culture samples using PCR-based methods to ensure detection sensitivity.
- Methods to enhance the viability of injured salmonellae from frozen feces can be further identified.

References

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