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Aerobic Bacterial Flora of Addled Raptor Eggs in Saskatchewan

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ABSTRACT: In south-central Saskatchewan, Canada, in 1986, 1987 and 1989, the aerobic bacterial flora was evaluated from 75 unhatched raptor eggs of three species: 42 of the Swainson's hawk (*Buteo swainsoni*), 21 of the ferruginous hawk (*Buteo regalis*), and 12 of the great horned owl (*Bubo virginianus*). In addled Swainson's hawk eggs, the most common bacterial genera were *Enterobacter* (18 eggs), *Escherichia* (12), and *Streptococcus* (10). Seven great horned owl eggs and six ferruginous hawk eggs also contained *Escherichia coli*. *Salmonella* spp. were not isolated. These bacteria were interpreted as secondary contaminants and not the primary cause of reproductive failure.

Key words: Raptors, Swainson's hawk, *Buteo swainsoni*, ferruginous hawk, *Buteo regalis*, great horned owl, *Bubo virginianus*, addled eggs, bacteriology, field survey.

Our long-term study of unhatched eggs in Saskatchewan, Canada, raptor nests (Houston et al., 1987, 1991, 1993) is part of a raptor nestling banding program designed to measure reproductive success, and to identify causes of nesting failures. In a study of 2,031 Swainson's hawk (*Buteo swainsoni*) nest attempts, we observed a minimum average annual failure of 30%, ranging up to 60% (Houston and Schmutz, 1995).

Although the bacterial flora of captive raptors and their failed eggs has been evaluated in Great Britain (Needham, 1981), little has been published concerning the bacterial flora in eggs of free-living raptors. Our objective was to identify the aerobic bacterial flora in addled eggs and to determine if pathogenic organisms were contracted before the eggs were laid, as proposed by Cooper (1987). We predicted most microorganisms would be secondary infections by contaminant organisms normally present within the nest.

We recorded the frequency of addled eggs encountered during visits to 914 active nests of the Swainson's hawk in Saskatchewan between 1972 and 1987, and to another 132 nests (of which 57 failed) in 1989. The main study area was the Kindersley region, 51° to 52°N, 108° to 110°W. In the first 891 nests, aluminum bands were attached to 1780 nestlings; another 23 nests with adults in attendance contained addled eggs only. We also collected 21 unhatched eggs from nests of the ferruginous hawk (*Buteo regalis*), and 12 from the great horned owl (*Bubo virginianus*).

Eggs in nests with ≤1-wk-old young were not disturbed, since hatching is asynchronous in hawks (Newton, 1979); such eggs may have been fertile. If the smallest nestling was >1-wk-old, each egg was labelled and placed separately in aluminum foil. Most unhatched eggs had an audible sloshing sound, evidence that air and fluid was a probable consequence of embryonic death and bacterial action. A very few eggs with advanced embryos did not slosh.

Each Swainson's hawk and great horned owl egg was candled during the next working day. Shells of ferruginous hawk eggs were too thick to be candled. All eggs were refrigerated and subsequently cultured within 48 hr. Any dirty eggs were individually cleaned with paper towels dipped in a 1% solution of household detergent, rinsed with sterile water and allowed to air-dry. All eggs were dipped in 70% ethanol and flamed to kill surface contaminants.

In a laminar flow hood, with sterile scissors and forceps, each egg was cracked open at the air-cell end, the shell membrane removed, and the contents poured into a sterile beaker. Any embryo present was placed in a jar of 10% buffered formalin. In 1986 and 1987, 1.0 ml of well-

mixed egg content was added to 4.0 ml of sterile physiological saline solution (PSS), from which 0.1 ml was used to inoculate a 5% sheep blood agar (BA) (Kelran, Prince Albert, Saskatchewan, Canada), and a MacConkey (MAC) plate (Kelran) by the procedures of Carter and Cole (1990); 1.0 ml also was inoculated into a tube of Selenite F enrichment broth (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA for subsequent subculture onto Brilliant Green Agar Difco Company, Detroit, Michigan, USA) and Hektoen Enteric agar plates (Difco) for *Salmonella* spp. detection. The BA and MAC plates were incubated aerobically at 37 C for 48 hr. Isolates were identified by the methods of Carter and Cole (1990).

In the 1989 study, a 1.0 ml aliquot of well-mixed egg content was used to make eight 10-fold dilutions in tubes containing 9.0 ml PSS. Dilutions 5 through 8 were plated out in three regimes: 0.1 ml was added onto each of two BA and MAC plates for incubation at 37 C and 25 C for 48 hr; 1.0 ml was added to each of two sterile petri plates containing cooled molten trypticase soy agar (BBL Microbiology Systems, Cockeysville, Maryland, USA) for incubation at 37 C and 25 C for 48 hr; 1.0 ml of undiluted egg content was added to selenite-F broth, for detection of salmonellae as described.

Microorganisms, isolated on BA and MAC plates, were identified, using techniques of Carter and Cole (1990) supplemented where appropriate by micro-identification tests with API 20E, API NFT, and API STAPH TRAC systems (Analytab Products, Plainview, New York, USA).

With both series of eggs, some organisms (e.g., *Bacillus* spp. and *Micrococcus* spp.), were not identified to species level because they are usually regarded as contaminants rather than potential pathogens. In the case of *Pseudomonas* spp. isolates in 1986–1987, they were checked to determine if they were *Pseudomonas aeruginosa*, and, if not, were not identified further. Cultures for anaerobic bacteria and for fastidious bacteria such as *Mycoplasma* spp. or *Haemophilus* spp. were not tested further.

Only late embryonic death could be detected by candling. Whether an embryo was present or not, we found almost universal heavy contamination with secondary bacterial growth. In 1987, one great horned owl egg and one Swainson's hawk egg felt solid and contained a fully-formed embryo; in the case of the owl egg, malposition of the embryo prevented contact of the egg tooth with the shell. Apart from these two exceptions, all other owl eggs sloshed. All but three Swainson's and three ferruginous hawk eggs grew large quantities of bacteria.

Of 42 addled Swainson's hawk eggs examined, five, all in 1989, contained an identifiable embryo. Many eggs had a total decomposition of contents; we could not exclude the possibility of bacterial destruction of an earlier small disc embryo, nor overgrowth by secondary organisms masking an original infection. There were identifications of 37 microorganisms from the first 21 eggs and 41 from the second 21 eggs (Table 1). One Swainson's egg contained five identifiable organisms, while another egg with three organisms had the highest total colony count, 2.9×10^9 colony forming units/ml. In addled Swainson's hawk eggs the most common were *Enterobacter* spp. (in 18 eggs), *E. coli* (12 eggs), and *Streptococcus* spp. (10 eggs). Seven of twelve great horned owl eggs and six ferruginous hawk eggs also contained *E. coli*. *Salmonella* spp. were not encountered.

Pure cultures of a single organism were isolated from 14 Swainson's hawk eggs: *Bacillus* sp. ($n = 1$); *Enterobacter agglomerans* ($n = 4$); *Enterobacter cloacae* ($n = 1$); *Escherichia coli* ($n = 2$); *Enterobacter taylorae* ($n = 1$); *Aeromonas hydrophila* ($n = 1$); *Pseudomonas stutzeri* ($n = 1$); *Pseudomonas* sp. ($n = 1$); CDC-VE1 (Centers for Disease Control, Atlanta, Georgia; $n = 1$). Mixed cultures of two organisms were obtained in nine instances, of three organ-

TABLE 1. Bacteria isolated from 75 addled raptor eggs, Saskatchewan, Canada, 1986 to 1989.

	Swainson's hawk	Ferruginous hawk	Great horned owl	Total
Eggs examined:	42	21	12	75
Embryos present:	5	2	4	11
Acinetobacter calcoaeticus var. *anitratus*	2[a]	0	0	2
Acinetobacter calcoaeticus var. *wolfii*	0	0	0	2
Aeromonas hydrophila	5	1	0	6
Alcaligenes spp.	0	0	1	1
Aspergillus fumigatus	0	1	0	1
Bacillus spp.	1	5	0	6
Bacillus stearothermophilus	1	0	0	1
CDC group V E-1	1	0	0	1
Corynebacterium sp.	2	4	1	7
Enterobacter agglomerans	4	2	0	6
Enterobacter amigenus	0	0	1	1
Enterobacter cloacae	2	0	1	3
Enterobacter taylorae	1	0	0	1
Enterobacter spp.	11	3	0	14
Escherichia coli	12	6	7	23
Hafnia alvei	0	0	2	2
Klebsiella spp.	1	0	0	1
Lactobacillus spp.	1	0	0	1
Micrococcus spp.	1	2	2	5
Pasteurella spp.	0	1	0	1
Proteus mirabilis	3	1	0	4
Providencia rettgeri	2	0	0	2
Pseudomonas fluorescens group	1	0	1	2
Pseudomonas luteola	0	0	2	2
Pseudomonas putrefaciens	0	0	1	1
Pseudomonas stutzeri	3	1	1	5
Pseudomonas spp.	7	0	2	9
Rhizopus spp.	1	0	0	1
Serratia liquefaciens	3	0	0	3
Serratia odorifera	0	1	0	1
Serratia plymuthica	0	0	1	1
Staphylococcus epidermidis	1	1	2	4
Staphylococcus hemolyticus	1	1	0	2
Streptococcus faecalis	5	0	1	6
Streptococcus spp.	5	1	1	7
No growth	3	3	0	5

[a] Number of eggs containing the bacterial species.

isms in three eggs, and of five organisms in one egg. Three ferruginous hawk eggs and three Swainson's eggs had no growth.

Once death of the embryo occurs, there is immediate failure of the normal bacteriostatic and immunologic mechanisms of the live organism; diversified microorganisms already residing on or in the eggshell soon proliferate in the excellent culture medium within the egg (Romanoff and Romanoff, 1949). No clear-cut theory has emerged from studies on the mechanism of penetration of bacteria through the external egg structures, but there is a time lag of 7 to 20 days between exposure and the appearance of micro-organisms in the yolk (Burley and Vadehra, 1989). By the time we had visited the nests, bacterial proliferation in addled eggs had been under way for at least 3 wk, the average age of the nestlings present. Somewhat contrary to expectations there was no appre-

ciable difference in bacterial growth between great horned owl eggs in cool May weather with occasional night frosts, ferruginous hawk eggs in moderate June temperatures, and Swainson's hawk eggs in July heat (≤40 C).

We encountered no consistent infections by known pathogens that might have resulted from acquisition within the adult female oviduct before the egg was laid (Cooper, 1987). Most bacteria were common environmental microorganisms associated with putrefaction, especially to be expected after invasion or spoilage of infertile eggs or following embryo death. They were secondary infections and not a primary cause of reproductive failure. Similarly, there was no evidence that the organisms within an unhatched egg were in any way deleterious to the other eggs or nestlings, nor was there evidence of an adverse effect on clutches in subsequent years.

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