INVESTIGATION AND CONTROL OF AN ATTACHING AND EFFACING \textit{Escherichia coli} OUTBREAK IN A COLONY OF CAPTIVE BUDGERIGARS (\textit{Melopsittacus undulatus})

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INVESTIGATION AND CONTROL OF AN ATTACHING AND EFFACING *ESCHERICHIA COLI* OUTBREAK IN A COLONY OF CAPTIVE BUDGERIGARS (*MELOPSITTACUS UNDULATUS*)


**Abstract:** An increase in mortality in a captive flock of budgerigars (*Melopsittacus undulatus*) coincided with the isolation of attaching and effacing *Escherichia coli* from postmortem samples. Common histologic lesions included hepatitis, enteritis, and in one case attaching and effacing lesions along the intestinal tract. Retrospective review of necropsy records and increased sampling led to the identification of several cases of *E. coli* with the attaching and effacing (*eae*) virulence gene. Factors such as environment, nutrition, and concomitant pathogens were thought to contribute to mortality in the flock. Although it is not clear whether *E. coli* was a primary pathogen during the period of increased mortality, the presence of the *eae* gene combined with associated histologic lesions supports the conclusion that this organism was a significant contributor to mortality. Manipulation of diet, environment, and the addition of probiotic supplementation resulted in a decline in mortality rate and decreased shedding of *E. coli* based on negative follow-up cultures of intestines, liver, and feces.

**Key words:** Budgerigar, enteritis, *Escherichia coli*, *Melopsittacus undulatus*, probiotics.

**INTRODUCTION**

Budgerigars (*Melopsittacus undulatus*) are popular exhibit animals in public display facilities and are often managed as flocks in open aviaries. Management of budgerigars in these situations provides many challenges, including the potential for disease outbreaks. Environmental hygiene, disease surveillance, and rapid response during periods of increased mortality help minimize losses and reduce the risk of zoonotic disease.

*Budgerigars* are susceptible to a wide range of bacterial, viral, fungal, and parasitic diseases that pose threats in a flock situation.

*Escherichia coli* are gram-negative, rod-shaped bacteria in the family *Enterobacteriaceae*. There are hundreds of serotypes of *E. coli* that are classified using a numbering system based on the type of outer membrane lipopolysaccharide (O-antigen), the flagella that exist in some motile strains (H-antigen), and the polysaccharides that form either a discrete capsule or amorphous layer (K-antigen).

Several serotypes of *E. coli* are nonpathogenic commensal organisms of the gastrointestinal tract in many species. Pathogenic strains of *E. coli* are determined by specific virulence factors and their effect in susceptible species.

One virulence factor found in pathogenic strains of *E. coli* is the attaching and effacing (*eae*) gene that leads to intimate bacterial adherence to the host epithelium, creating characteristic attaching and effacing lesions. The *eae* gene is encoded on a pathogenicity island called the locus of enterocyte effacement (LEE). The *eae* gene produces the protein intimin that, in coordination with additional receptors encoded on the LEE pathogenicity island, forms a pedestal that allows for intimate attachment of the bacteria to the epithelium and destruction of the underlying host tissue.

Attaching and effacing *E. coli* (AEEC) have a tropism for the small intestine. Unlike cattle, birds are not usually considered significant reservoirs of *eae*-positive *E. coli*. This report describes enteritis in a population of captive budgerigars at Zoo New England’s Franklin Park Zoo (Boston, Massachusetts 02121, USA), in which the lesions resembled those of AEEC in other species.

**CASE REPORTS**

**Background**

The flock entered quarantine as a single group of 400 budgerigars in April 2009, and the birds were quarantined for 45 days. During the quarantine period, a fecal sample yielded *E. coli*, but there were no clinical signs in the group so...
treatment was not initiated. The birds were held in a temporary off-exhibit holding for approximately 11 mo before introduction to their exhibit in April 2010. The exhibit provided access to an indoor building and an outdoor enclosure. Birds had full exhibit access during warmer months and were housed indoors during the winter. The floors of the indoor holding were concrete, and the walls were covered in polyvinyl chloride board. Daily cleaning was done using a quaternary ammonium based cleaner (ZEP PDC, Zep Inc., Atlanta, Georgia 30318, USA). The exhibit space was shared with a pair of Cape Barren geese (Cereopsis novaehollandiae). The enclosure was covered with wire mesh, creating potential for contact between the captive flock and wild animals, both physically and through their excrement.

The flock was fed commercial pellets (Premium Daily Diet for Parakeets, Labeled Co., Cornell, Illinois 61319, USA) supplemented with kale greens. Birds were allowed ad libitum access to food. During the summer, from Memorial Day to Labor Day, the birds had access to millet seed fed by the public. Fresh water was provided ad libitum through an automated valve system.

Case 1

On 20 July 2010, a 21-g adult female budgerigar was found agonal on exhibit. It was brought to the hospital for treatment but died shortly after arrival. Gross necropsy was performed using standard avian techniques and revealed an emaciated body condition with feces and food caked around the cloaca. The left liver lobe contained a yellow-brown mass consistent with an abscess. Associated body condition, maggot eggs in the nares and choana, and mild fecal staining around the cloaca. Histology revealed severe atrophy of fat and acute pulmonary shock. Cultures of the liver and intestines were submitted to IDEXX, and both were gram negative. The bird was separated from the flock and improved initially but did not gain weight or condition. Due to lack of clinical recovery, the bird was humanely euthanized on 8 July 2010.

Case 2

A second bird, a 20-g adult male, was found dead on exhibit the same day as Case 1, with no premonitory signs. Gross necropsy showed emaciated body condition, maggot eggs in the nares and choana, and mild fecal staining around the cloaca. Histology revealed severe atrophy of fat and acute pulmonary congestion and hemorrhage. Mild-to-moderate lymphocytic inflammation at the isthmus of the proventriculus was associated with M. ornithogaster. Death was attributed to acute pulmonary shock. Cultures of the liver and intestines were submitted to IDEXX, and both samples were positive for E. coli. Further genotyping at the ECRC identified the strain as an eae- positive O8:H51.

Case 3

A 25-g adult female budgerigar was noted to be weak and lethargic on 24 June 2010. Fecal examination revealed no evidence of parasitism, but Gram stain showed that 30% of the bacteria were gram negative. The bird was separated from the flock and improved initially but did not gain weight or condition. Due to lack of clinical recovery, the bird was humanely euthanized on 8 July 2010. Histology showed moderate periportal hepatic cell necrosis, marked atrophy of fat, parakeratosis of the crop, and mild interstitial nephritis with adenovirus inclusions. Mild-to-moderate lymphocytic inflammation at the isthmus of the proventriculus was associated with Macrorhabdus ornithogaster.
20 July 2010. Gross necropsy showed emaciated body condition and white gelatinous material coating the left abdominal air sac. Histology revealed moderate chronic proventriculitis with intralesional *M. ornithogaster*, granulomatous air sacculitis with intralesional bacteria and fungi, lymphoid depletion of the spleen, mild chronic enterocolitis, and moderate periportal hepatitis with biliary hyperplasia and hepatocellular necrosis. Culture of the intestines submitted to IDEXX was positive for *E. coli*. No additional genotyping was performed in this case.

**Case 4**

On 28 July 2010, a 22-g adult male budgerigar was found dead on exhibit. The bird was emaciated and had fecal staining around the cloaca. Necropsy showed fibrin adhered to the pericardial sac and evidence of hemorrhage on the calvarium. Histology showed moderate periportal to random mixed cell hepatitis with focal necrosis of the liver, mild chronic proventriculitis, and mild hemosiderosis of the spleen and liver. Culture of the liver was sent to IDEXX and was positive for *E. coli*. No genotyping was performed in this case.

**Case 5**

On 29 July 2010, a 22-g adult male budgerigar was found dead on exhibit. Necropsy showed mucus in the oral cavity, a soiled vent, and liquid fecal material throughout the small intestine. Histology showed acute moderate-to-marked necrotizing hepatitis, proliferative enteritis, and proventriculitis. There was congestion, hemorrhage, edema, and atelectasis of the lung and marked atrophy of fat and pancreatic tissue. Changes to the liver were consistent with bacterial infection, but no organisms were noted on histology. A culture of the liver sent to IDEXX was positive for *E. coli*. No genotyping was performed.

**Case 6**

A 26-g adult male budgerigar was found dead on 7 August 2010. The bird was in thin body condition with dried feces around the cloaca and liquid digesta in the intestines. Histology showed Kupffer cell activation in the liver, acute pulmonary hemorrhage, and proventricular macrorhabdiosis. Attaching and effacing lesions were noted in the small intestines, including mild crypt hyperplasia and rare crypt abscesses, villous blunting and fusion, and hypertrophy and exfoliation of apical villous epithelial cells. On some villi, several contiguous apical epithelial cells had prominently thickened brush borders, and small bacteria were intimately associated with the surface of the enterocytes. A tissue Gram stain revealed that the bacteria were gram-negative bacilli (Fig. 1). Culture of the liver was sent to New York State Veterinary Diagnostic Laboratory: Animal Health Diagnostic Center (Cornell University College of Veterinary Medicine, Ithaca, New York 14853, USA) and was positive for *E. coli*. Genotyping by the ECRC identified the strain as an *eae*-positive ON:H51.

**Case 7**

On 10 August 2010, a 28-g adult male budgerigar was noted to be lethargic on exhibit with a soiled vent, and died shortly after discovery. Necropsy revealed soiling of the vent and liquid feces in the intestine. Histology showed pulmonary ascariasis and proventricular macrorhabdiosis. There was chronic enteritis with bacterial overgrowth, moderate mixed cell periportal hepatitis, mild acute myocardial necrosis, and an adrenal stress response. Enteritis was suspected to be the primary cause of death. Liver culture performed at Cornell University was positive for *E. coli*. Genotyping by ECRC identified an *eae*-positive ON:H51 strain.

**Case 8**

On 29 November 2010, a 30-g adult female budgerigar was found dead. Gross necropsy showed fecal staining around the cloaca and serous discharge from the eyes. The air sacs had a cloudy appearance and there was a white precipitate on the pericardium. The contents of the small intestine were discolor dark reddish-brown, and the lungs, liver, and kidney were pale. Histology showed severe atrophy of fat, mild atrophy of pancreatic tissue, renal tubular mineralization, epicardial gout, and mild splenic hemosiderosis. There was mild periportal to random hepatitis, refluxed bile in the intestines, severe proventriculitis with intralesional *M. ornithogaster*, and an adrenal stress response. Fecal culture performed by Cornell University was positive for *E. coli*, which was genotyped at ECRC as *eae*-positive H48.

**Diagnostic evaluation and flock management**

Pulsed gel field electrophoresis (PFGE) was used to evaluate the relatedness of the *E. coli* that were isolated. According to laboratory standards requiring \(>94\%\) similarity, none of the tested samples were considered the same genetic strains,
although some degree of similarity did exist, with 88.5 and 89.5% homology seen in two of the strains.

Despite the presence of *E. coli*, the decision was made not to treat the flock or any individual birds with antibiotics. This choice was made in an effort to prevent resistant infections from developing in the group. In lieu of antibiotics, a probiotic (Bird and Reptile Bene-Bac Plus powder, PETAG, Inc., Hampshire, Illinois 60140, USA) formulated for birds was added to the feed. Probiotics improve intestinal microbial balance, and the bacteria in this product are frequently used as supplementation for avian species, competitively inhibiting pathogenic bacteria and supporting gastrointestinal health.10,21,27 It was first added to the food on 27 July 2010, three times a week for 1 mo at a ratio of 4 g probiotic powder for every 100 g of pelleted diet. The mortality rates in August and September subsequently decreased. The probiotic was continued until a full diet change was instituted the following summer.

Husbandry changes included alterations to the physical structure of the enclosure and modifications to cleaning and handling protocols to reduce contact and spread of *E. coli* shed in feces. In summer 2010, the grading within the outdoor portion of the enclosure was increased to improve drainage and decrease water pooling. The staff was increasingly diligent about identifying potentially sick birds early and separating them from the flock into individual cages within the indoor enclosure or at the veterinary hospital. For more compromised birds, supportive care with subcutaneous fluid therapy was provided.

A diet assessment was performed after lesions consistent with hypovitaminosis A in several birds with concomitant *E. coli* infections were identified in November and December 2010. Although the complete pelleted diet (Premium Daily Diet for Parakeets) was adequately balanced to meet the nutritional needs of a budgerigar, there were times during the summer when it was possible that the birds could have chosen to eat mostly or solely the millet offered by the public, leading to vitamin A deficiency. From May to July 2011, a gradual switch to a diet consisting entirely of Nutri-Berries (Lafeber) was executed. Public feeding of millet was discontinued, while substituting with a nutritionally balanced feed stick (Stick-A-Roos, Lafeber). Probiotic supplementation was discontinued after the diet transition was completed since Nutri-Berries contain more grass seeds and plant material that are similar to food items they would eat in the wild.8 The goal was not to eliminate *E. coli* but to reduce shedding in the flock.

Follow-up screening

Following the last documented case of *eae*-positive *E. coli* in November 2010, the flock was monitored by taking intermittent liver and intestinal cultures from deceased birds as well as group fecal cultures from the enclosure. From January 2011 to December 2012, a total of eight cultures were done. Seven of the eight cultures had no growth or had growth of other organisms but were *E. coli* negative. One of the eight cultures had moderate growth of *E. coli*, but no typing was performed, and this was the only time *E. coli* was detected in the group after the aforementioned changes were made.

DISCUSSION

The apparent spike in mortality rate in July 2010 prompted a review of the necropsy records and creation of a budgerigar mortality chart (Fig. 2). The average monthly mortality rate during the quarantine period was 2.1% and ranged from 0.2% to 2.7% while the animals were kept in the off-exhibit holding area. Monthly mortality rates increased to 6.7% beginning in July 2010. Although culturing was performed during necropsy on the majority of the cases, further genotyping of the *E. coli* was not initiated until the histopathology results from Case 6 indicated attaching and effacing lesions associated with *E. coli*. In some cases, banked tissue samples that had been stored at −80°C were submitted for culture, but typing was not always possible as some of the culture lines failed before submission to ECRC. In total, from July to November 2010, eight birds that died cultured positive for *E. coli*; five of the eight cultures were positive for the *eae* gene. In July 2010, of the 21 birds that died, 24% were culture positive for *E. coli*, and an additional 33% had lesions consistent with *E. coli* infection (Table 1). The high percentage of deaths during July 2010 that were either culture positive or had lesions consistent with *E. coli* indicates that this bacterium was contributing to the increased mortality in the flock.

There are several possible sources of the *E. coli*; given the PFGE results, more than one source is likely. This flock of birds came in as a group and no budgerigars were added during their time at the facility. A fecal culture performed during quarantine was positive for *E. coli*
and frozen tissue samples were retrospectively sent for culture and genotyping. A liver sample from a bird in quarantine cultured positive for *E. coli* but was not positive for the *eae* gene. Nearly 1 yr elapsed between that culture and the increased mortality associated with *eae*-positive cultures. It is possible that the budgerigars may have been asymptomatic carriers and began to show clinical disease when factors such as inappropriate diet, overcrowding, or public interaction caused stress and potentially led to immunosuppression. Alternately, because the enclosure was open to the environment, *E. coli* may have been introduced through contact with wild birds on exhibit or through contact with the public. Finally, the budgerigars shared the exhibit with two Cape Barren geese, which potentially could have transmitted *E. coli* to the budgerigars. These geese cultured positive for *E. coli* on 22 April 2010, during their quarantine period, as well as 27 July 2010, during the disease outbreak. Neither of these samples was genotyped, and neither of the birds ever showed clinical signs of infection. Because the budgerigars also entered quarantine positive for *E. coli* before arrival of the geese, it can not be determined whether either source is related to the described cases without availability of banked samples for genotyping.

The decrease in mortality rate subsequent to the addition of the probiotic may support the idea that the animals were undergoing some degree of intestinal imbalance either leading to or caused by *E. coli* proliferation, or caused by other disease processes, diet, or a combination of these factors. However, because several other factors were altered simultaneously, including husbandry and diet change, it is impossible to determine conclusively whether the probiotics alone would have helped.

The detection of attaching and effacing *E. coli* in this population of budgerigars raised several points regarding the importance of this bacterium in bird species, flock management and potential zoonotic concerns. Like other vertebrate species, *E. coli* can be a normal component of the gut flora of some birds including raptors and galliformes. A survey of whooping cranes (*Grus americana*) and sandhill cranes (*Grus canadensis*) found it to be one of the three most common bacteria cultured out of the gastrointestinal tract. However, in most psittacine populations, it is considered pathogenic or transient in the gastrointestinal tract.

Pathogenic strains of *E. coli* have been cultured out of various bird populations after morbidity and mortality events. In Germany, 103 *E. coli* isolates were obtained from a variety of birds in the order Psittaciformes. *E. coli* from the following seven species contained the *eae* gene: a red winged parakeet (*Aprosmictus erythopterus*), rose cockatoo (*Cocatua moluccensis*), cockatiel (*Nymphicus hollandicus*), blue crowned amazon parrot (*Amazona farisona*), fairy lorikeet (*Charmosyna pulchella*), slender-billed parakeet (*Enicognathus leptorhynchus*), and masked lovebird (*Agapornis personata*). All seven of these birds had diarrhea or were septic. In the Scottish highlands, after a mass mortality of siskins (*Carduelis spinus*), greenfinches (*Carduelis chloris*), and chaffinches (*Fringilla coelebs*), 43 of the 46 dead birds cultured positive for *E. coli* containing the *eae* gene.

### Table 1. Monthly mortalities in a flock of captive budgerigars (*M. undulatus*) at Zoo New England’s Franklin Park Zoo in the 2 mo preceding the first documented case of *E. coli* and the 1 mo following the final documented case of *E. coli*, May 2010–December 2010. Total number of deaths is further subdivided into the number of birds that were necropsied and sent for histology, and the number of individuals that were *E. coli* positive or had lesions consistent with *E. coli* infection.

<table>
<thead>
<tr>
<th>Month</th>
<th>Total deaths</th>
<th>No. of birds with necropsies performed</th>
<th>No. of birds with histology done</th>
<th>% of deaths that were positive for <em>E. coli</em></th>
<th>% of deaths with lesions consistent with <em>E. coli</em> but no positive culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>0 (n = 0)</td>
<td>0 (n = 0)</td>
</tr>
<tr>
<td>Jun</td>
<td>18</td>
<td>18</td>
<td>2</td>
<td>0 (n = 0)</td>
<td>11 (n = 2)</td>
</tr>
<tr>
<td>Jul</td>
<td>21</td>
<td>20</td>
<td>13</td>
<td>24 (n = 5)</td>
<td>33 (n = 7)</td>
</tr>
<tr>
<td>Aug</td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>20 (n = 2)</td>
<td>10 (n = 1)</td>
</tr>
<tr>
<td>Sep</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>33 (n = 1)</td>
<td>0 (n = 0)</td>
</tr>
<tr>
<td>Oct</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0 (n = 0)</td>
<td>0 (n = 0)</td>
</tr>
<tr>
<td>Nov</td>
<td>13</td>
<td>9</td>
<td>6</td>
<td>7.7 (n = 1)</td>
<td>7.7 (n = 1)</td>
</tr>
<tr>
<td>Dec</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>0 (n = 0)</td>
<td>10 (n = 1)</td>
</tr>
</tbody>
</table>

*Lesions consistent with *E. coli* are defined as histologic evidence of enteritis or hepatitis.*
These reports indicate that eae-positive *E. coli* can cause disease in bird populations. During the mortality peak, *E. coli* may have been the primary pathogen, or it may have been an opportunistic invader, allowed to flourish given conditions that may have been established by environment, diet, or presence of other potential pathogens. *Escherichia coli* was never found as a single pathogen. Of the birds that died, 62.5% had one concomitant pathogen and 37.5% had two concomitant pathogens. It should be noted however that several of these potential pathogens, *M. ornithogaster* in particular, can be routinely found in otherwise healthy budgerigars or as incidental histopathologic findings. The prevalence of various disease states within the flock is presented in Table 2.

The presence of potentially pathogenic *eae*-positive *E. coli* within the flock has management implications for public health. The birds are maintained as an interactive exhibit where there is contact between animals and visitors. In addition, the staff that cleans the indoor enclosure on a regular basis is at increased risk when hosing down the area and potentially aerosolizing fecal material. The zoonotic potential of this organism is not clear, but protective measures were instituted as a precaution. Personnel cleaning the indoor holding area and feeding the animals are now required to wear personal protective equipment, including facemasks and disposable gloves. Guests are encouraged by employees and with signage to use hand-sanitizing stations after contact with animals, similar to measures typically taken to prevent zoonotic transmission of *E. coli* in petting zoos or other public settings with human–animal contact.3

**CONCLUSIONS**

This case study demonstrates that the presence of pathogenic *E. coli* in a budgerigar colony can contribute to increased morbidity and mortality. Classification of pathogenic *E. coli* genotypes may assist in diagnosing whether the organism is contributing to clinically observed disease. General supportive measures, attention to husbandry practices, diet change, and supplementation with appropriate probiotics may help control outbreaks of pathogenic bacteria in lieu of antibiotic treatment.

<table>
<thead>
<tr>
<th>Disease or pathogen</th>
<th>No. of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Macrorhabdus ornithogaster</em></td>
<td>21 (38.18)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>8 (14.54)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>7 (12.73)</td>
</tr>
<tr>
<td>Gout</td>
<td>4 (7.27)</td>
</tr>
<tr>
<td>Hypovitaminosis A</td>
<td>4 (7.27)</td>
</tr>
<tr>
<td><em>Candida</em> spp.</td>
<td>3 (5.45)</td>
</tr>
<tr>
<td>Polyoma virus</td>
<td>2 (3.64)</td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td>1 (1.82)</td>
</tr>
<tr>
<td>Reovirus</td>
<td>1 (1.82)</td>
</tr>
<tr>
<td>Ascariasis</td>
<td>1 (1.82)</td>
</tr>
</tbody>
</table>

**Table 2.** Prevalence of potentially pathogenic organisms and disease processes among birds necropsied and sent for histopathology in a captive flock of budgerigars (*M. undulatus*) at Zoo New England’s Franklin Park Zoo from April 2009 to December 2010.

**Figure 1.** Budgerigar, small intestine. a) mucosal villi are blunted and fused, apical epithelium is hypertrophied and few exfoliated cells are present. HE, bar = 150 μm. Inset: higher magnification of apical epithelium showing prominent brush border. HE, bar = 20 μm. b) Tissue gram stain accentuating the gram negative bacteria in the brush border. Brown and Brenn, bar = 80μm.
Acknowledgments: The authors acknowledge Fred Beall, general curator, Ed O’Brien, assistant curator, and the Bird’s World keepers for assistance with management of these cases. The authors also acknowledge the veterinary technicians for expert assistance with sample handling and laboratory submissions.

LITERATURE CITED

Figure 2. A graphical representation of the monthly mortality rates in a flock of captive budgerigars (Melopsittacus undulatus) at Zoo New England’s Franklin Park Zoo (Boston, MA 02121) from April 2009 to December 2010. The arrow indicates the month of July during which the percentatge mortality peaked within the flock.
intestinal mucosa: role of intimin and tir in adherence.


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