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Case Report—

Isolation of *Salmonella* Typhimurium from Dead Blue and Gold Macaws (*Ara ararauna*)

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SUMMARY. Two blue and gold macaw (*Ara ararauna*) chicks died of fatal salmonellosis in Buenos Aires Province, Argentina. The birds were histopathologically and microbiologically examined. *Salmonella enterica* subspecies *enterica* serovar Typhimurium was isolated from the liver, spleen, heart, lung, kidney, and intestine of both birds. All strains were susceptible to ampicillin, cephalothin, cefotaxime, enrofloxacin, nalidixic acid, gentamicin, streptomycin, chloramphenicol, fosfomycin, tetracycline, nitrofurantoin, and trimethoprim-sulfamethoxazole. The *Xba*I-PFGE profile of the *Salmonella* Typhimurium isolated from the two animals, which shared the same cage, was identical and showed a unique pattern compared with 301 isolates included in the PulseNet national database of *Salmonella* pulsed-field gel electrophoresis patterns. This is the first report that describes fatal cases of salmonellosis from blue and gold macaws.

RESUMEN. *Reporte de Caso*—Aislamiento de *Salmonella typhimurium* a partir de guacamayas azul y amarillo (*Ara ararauna*) muertas.

Dos polluelos de guacamayas azul y amarillo (*Ara ararauna*) murieron por una salmonelosis fatal en la Provincia de Buenos Aires, Argentina. Las aves se examinaron por histopatología y microbiología. La *Salmonella enterica* subespecies *enterica* serovar Typhimurium fue aislada de hígado, bazo, corazón, pulmón riñón e intestino de ambas aves. Todas las cepas fueron susceptibles a ampicilina, cefalotina, cefotaxima, enrofloxacina, ácido nadilixico, gentamicina, estreptomicina, cloranfenicol, fosfomicina, tetraciclina, nitrofurantoina y la combinación trimetoprim-sulfametoxazol. El perfil *Xba*I de la *Salmonella* Typhimurium aislada de los dos animales que compartían la misma jaula fueron idénticos mediante electroforesis en gel de campos pulsantes (por sus siglas en inglés PFGE), y mostraron un patrón único comparado con 301 aislamientos que están incluidos en la base de datos nacional de patrones de PFGE de Salmonella, denominada PulseNet. Este es el primer reporte que describe casos fatales de salmonelosis en guacamayas azul y amarillo.

Key words: *Ara ararauna*, birds, blue and gold macaws, *Salmonella* Typhimurium, salmonellosis

Abbreviations: PAS = periodic acid-Schiff; PFGE = pulsed-field gel electrophoresis

CASE REPORT

Salmonella spp. have been isolated from a variety of mammals and birds, including Psittaciformes (14,15,18). *Salmonella* Typhimurium may manifest as a primary pathogen, or as a subclinical infection, in young or debilitated birds (27). The stressor of low nutrition, the higher concentrations of birds around available feed, and the resultant ease in spread of disease from one bird to another contribute to large-scale outbreaks (28). This organism is a concern in poultry producers because of its ability to cause foodborne infection in humans via unpasteurized shell eggs (7). The following is a report of two cases of blue and gold macaws that died of *Salmonella* Typhimurium.

In October 2006, two 6-week-old blue and gold macaw (*Ara ararauna*) chicks were found dead in their cage. These birds had been placed in the quarantine room at the age of 45 days old, lived in the same cage (the floor of which was cleaned on a daily basis), and had been fed with specific commercial pelleted rations supplemented with cereal and the entire contents of cooked chicken eggs. Fresh water was provided daily in a plastic container that was washed daily with soap and water. The plastic cage was cleaned with disinfectant before use. The quarantine room had an air conditioner and a sealed window; the floor, roof, and walls were painted with epoxy paint. Temperature was kept at 25 C. Feed was stored in a rodent-free, covered container. No other birds were found dead in the facility. The clinical signs were depression, anorexia, delay in the emptying of ingluvies, labored breathing, and diarrhea. These symptoms were present for about 3–4 days before any treatment was attempted.

No ectoparasites or skin lesions were seen at necropsy. Postmortem examination revealed pectoral muscle atrophy, hepatomegaly, splenomegaly, renomegaly, fibrinous exudate on intestinal mucosa, small whitish nodules on intestinal serosa, white-grayish nodules of approximately 5 mm on myocardium (Fig. 1), white-grayish nodules of 2 mm in diameter on the surface and in depth in lungs (Fig. 2), and white nodules were observed on ingluvies mucosa. Samples of heart, lung, liver, spleen, kidney, and intestine were taken at necropsy, fixed in 10% buffered formalin, embedded in paraffin, sectioned at 3 μm, and stained with hematoxylin and eosin (20) and periodic acid-Schiff (PAS) (21). Smears of tissues were stained using Gram (12) and modified Ziehl-Neelsen (13) methods. Tissue samples were also inoculated onto Hektoen enteric agar (Becton Dickinson, France S.A.,

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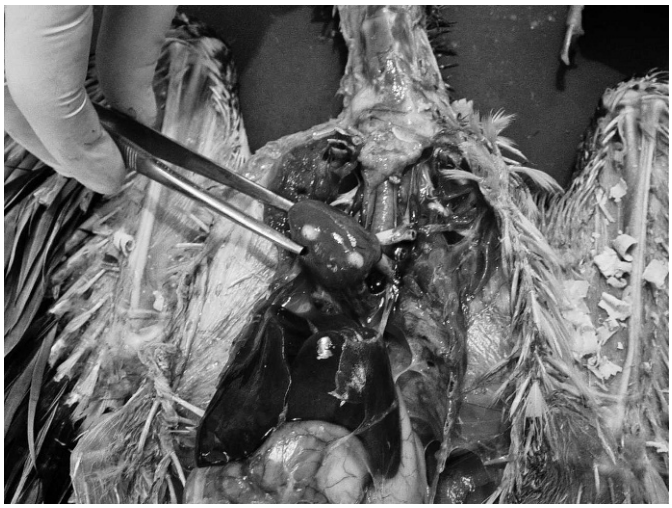

Fig. 1. White-grayish nodules of 5 mm on myocardium.

Le Pont de Claix, France), MacConkey agar (Becton Dickinson), and trypticase soy agar (Becton Dickinson) with 5% sheep's blood and were incubated at 37 C for 48 hr under aerobic conditions. In addition, these samples were inoculated onto Sabouraud agar (Merck Química, Buenos Aires, Argentina) and incubated at 28 C for 1 wk under aerobic conditions. Presumptive *Salmonella* colonies were chosen and biochemically tested. Species and subspecies differentiation of the isolates was performed according to the method described by Popoff (19). Serotyping was performed according to the Kauffman-White scheme (24). The susceptibility of the *Salmonella* isolates to ampicillin, cephalothin, cefotaxime, enrofloxacin, nalidixic acid, gentamicin, streptomycin, chloramphenicol, fosfomycin, tetracycline, nitrofurantoin, and trimethoprim-sulfamethoxazole was examined according to the Clinical and Laboratory Standards Institute for *Enterobacteriaceae* (4). The *Salmonella* isolates were subtyped by *Xba*I-pulsed-field gel electrophoresis (PFGE) according to the PulseNet standardized protocol (3). The *Xba*I-PFGE was performed in a counter CHEF DR III System (BioRad, Hercules, California), and the gel images obtained by Gel-Doc 2000 (BioRad) were analyzed using BioNumerics version 4.5 (Applied Maths, Kortrijk, Belgium). The relationship among the PFGE patterns was estimated with the same software by applying the parameters defined in PulseNet for PFGE profiles comparisons, i.e., Dice's coefficient with a 1.5% band-position tolerance, 1.5% optimization, and unweighted pair group method with arithmetic mean for the dendrogram construction. The PFGE pattern from the macaw *Salmonella* Typhimurium isolates was compared to 121 unique PFGE patterns corresponding to 301 *Salmonella* Typhimurium isolates of different sources (human, animal, and environmental) obtained from 1969 to 2007 in Argentina, isolates that are included in the PulseNet national database of *Salmonella* pulsed-field gel electrophoresis patterns.

Fig. 2. White-grayish nodules of 2–5 mm in diameter on the lung surface.

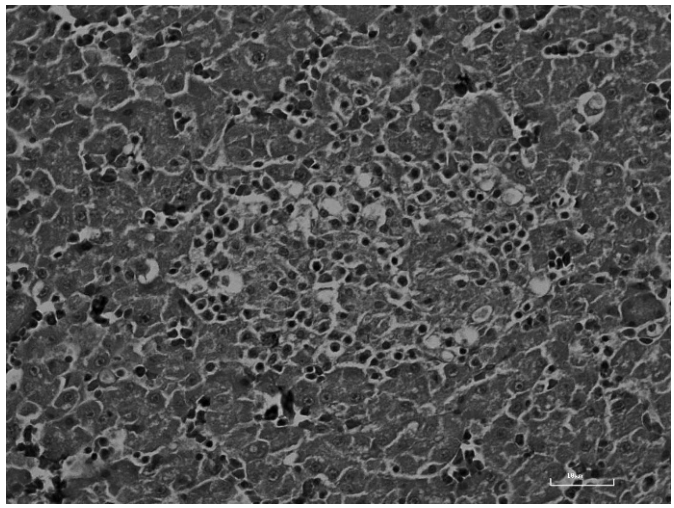

Fig. 3. Small areas of coagulative necrosis with scarce diffuse infiltration of mainly mononuclear inflammatory cells in the hepatic parenchyma.

A microscopic examination of the tissues revealed small areas of coagulative necrosis with scarce, diffuse infiltration of mainly mononuclear inflammatory cells in the hepatic parenchyma (Fig. 3). Vessel and capillary congestion, edema, and areas of coagulative necrosis surrounded by an infiltration of mononuclear and polymorphonuclear inflammatory cells with giant cells in lungs were seen (Fig. 4). Necrosis of villi, with an infiltration of mononuclear and polymorphonuclear inflammatory cells on intestine mucosa, was observed. Myocardium surrounded by mononuclear and polymorphonuclear inflammatory cells with giant

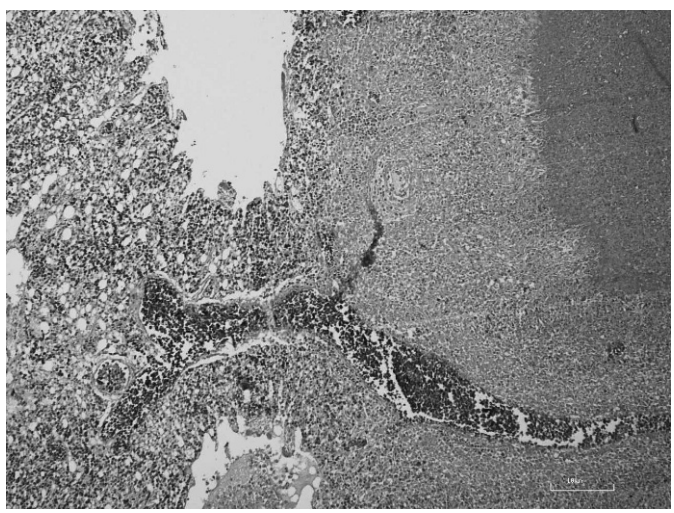

Fig. 4. Vessel and capillary congestion, edema, areas of coagulative necrosis surrounded by an infiltration of mononuclear and polymorphonuclear inflammatory cells with giant cells in the lung.

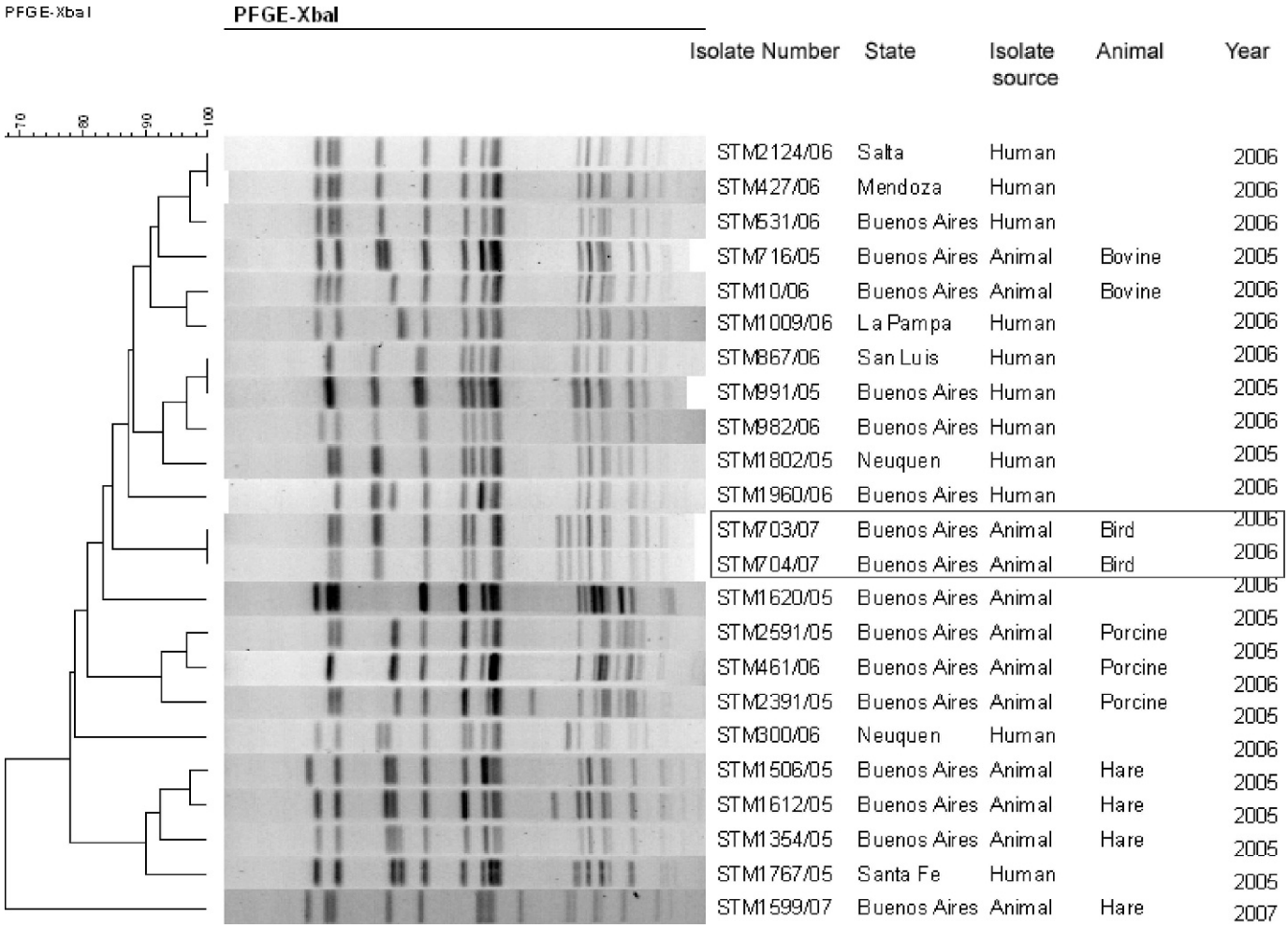

Fig. 5. Dendrogram showing the genetic relationship between the blue and gold macaw PFGE pattern (indicated in a box) and those from other *Salmonella* Typhimurium isolates of animal and human origin recovered in Argentina between 2005 and 2007.

cells and diffuse infiltration of mononuclear and polymorphonuclear cells among muscular fibers were also seen. In the kidneys, areas of basophil coloration surrounded by giant cells were observed. In PAS colorations, elements compatible with fungi were not observed, and Ziehl-Neelsen colorations were negative.

Microscopically, gram-negative bacilli were observed in large numbers on liver smears and, in lesser numbers, on spleen, kidney, lung, and heart smears. *Salmonella* was isolated from the liver, spleen, lung, heart, kidney in pure culture, and the intestine. The isolates were identified as *Salmonella enterica* subspecies *enterica* to biochemical reaction results. In serotyping, the strains had the following formula: (1,4,5,12:i:1,2), corresponding to serovar Typhimurium. The isolates were susceptible to all the antimicrobial agents tested. The two isolates of *Salmonella* Typhimurium recovered from the blue and gold macaw (*Ara ararauna*) chicks were indistinguishable by *Xba*I-PFGE, showing an electrophoretic profile with 17 defined bands between 670 and 20 kilobases. This profile was found to be different from 121 unique patterns of the PulseNet national database of *Salmonella* pulsed-field gel electrophoresis patterns. A dendrogram showing the comparison of selected PFGE patterns is presented in Fig. 5; this selection comprised isolates of human and animal origin from the most recent years, 2005 to 2007. It can be observed that, compared with the macaw *Salmonella* isolates, even the strains with the most similar PFGE patterns showed at least four different bands (i.e., strains STM1802/05 and STM531/06), while the most divergent ones, like those in the lower branch of the dendrogram, had more than eight different bands.

DISCUSSION

Salmonellosis is a well-known cause of disease in many wild bird species (11,17) and in poultry (27). *Salmonella* Typhimurium has been reported in wild birds from Norway (10,22,23), Japan (25), the United States (9), and Canada (6) and is an intermittently reported disease in psittacine birds (14). This is the first report that describes fatal salmonellosis in blue and gold macaw chicks, although salmonellosis in macaws is mentioned in avian medicine textbooks. The isolation of the bacteria from the liver, spleen, heart, lung, kidney, and intestine indicates the presence of a septicemic infection in the birds, and the observed lesions were according to the acute course of the disease in psittacines (5,16). These lesions, and the absence of other etiologic agents, could indicate that *Salmonella* Typhimurium infection was the primary cause of death in the blue and gold macaws.

In the last decades, *Salmonella* Enteritidis and *Salmonella* Typhimurium have emerged as a major cause of human foodborne illnesses worldwide (1,26). *Salmonella* Typhimurium was the most commonly isolated serovar from animal infections in Argentina (2). *Salmonella* Enteriditis was prevalent in human infections until 2006,

but the most prevalent serovar in 2007 was *Salmonella* Typhimurium. However, there is no data available regarding the existence of the agent in blue and gold macaw pets, pets which may also act as an important reservoir for transmission of salmonellae to humans. Kapperud *et al.* (10) reported that sporadic cases of domestically acquired human salmonellosis, and cases of fatal salmonellosis among small passerines in Norway, were most often observed at the same time of year, thus indicating an epidemiologic link. In addition, a molecular epidemiologic study has shown that 32% of the isolates recovered from human patients belonged to clones detected in the small passerine fauna in Norway (8). The same clone of *Salmonella* Typhimurium was isolated in the two blue and gold macaws evaluated in this study. This could reinforce the hypothesis that this agent came from the same source. There could be two principal sources of infection: birds with subclinical infection and feed. There was not any control over dry feces, which could act as vehicle to spread this agent in the quarantine room; however, no clinical signs of salmonellosis were observed among the rest of the birds in the quarantine room, and they were tested for *Salmonella* and were negative. The blue and gold macaws might have acquired the infection by humans when they were fed improperly with cooked chicken eggs, or the humans might have been carriers, etc. Unfortunately, these possible sources of infection were not analyzed. The analysis by PFGE made it possible to confirm that both macaws were infected by the same *Salmonella* Typhimurium strain and, thus, probably acquired the infection from the same source. The *Xba*I-PFGE pattern of *Salmonella* Typhimurium isolated from blue and gold macaws had not been identified before among the *Salmonella* Typhimurium isolates subtyped at the National Reference Laboratory, Buenos Aires, Argentina. This new PFGE pattern has been incorporated into the PulseNet national database of *Salmonella* pulsed-field gel electrophoresis patterns; this database will be shared with other members of PulseNet International.

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