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# Septic shock associated with complex infection by crop *Candida* and bacteria in two blue-fronted amazon parrots: a case report

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**ABSTRACT**: Infectious disease is frequently associated with morbidity and mortality in companion birds. The clinical features of these bacterial and fungal diseases may be influenced by stress factors and the condition of the patient. We report a case of sepsis induced by complex infection by *Candida* fungi and gram-negative bacteria originating from the crop in two blue-fronted amazon parrots several days after import. On gross necropsy, severe cachexia and vomit within the oesophagus were found. White foci were observed in the kidney, liver, lung, and spleen of one parrot. In both patients, the crop contained cheese-like lesions within a white layer along the mucosa. Histopathologically, the crop mucosa was ulcerated with fungal hyphae and spores (*Candida* spp.), and bacteria within the ulcerated lesions. The mucosal epithelium of the digestive organs, including the crop, proventriculus, ventriculus, and intestines, were severely exfoliated and lysed in both patients. The solid organs, including the spleen, kidney, and liver, showed necrotic and/or apoptotic lesions. Rod-shaped, gram-negative bacteria were observed within the heart ventricles, lung, and liver of one parrot, along with white foci in the solid organs grossly. Based on staining analysis of the crop mucosa, histopathological findings, and clinical history, we surmise that these two birds died due to sepsis originating from complex infection with crop fungi (candidiasis) and gram-negative bacteria.

**Keywords**: blue-fronted amazon parrot; sepsis; candidiasis; bacterial infection; histopathology; diagnosis

**List of abbreviations**

**CITES** = Convention on International Trade in Endangered Species of Wild Fauna and Flora, ***E. coli*** = *Escherichia coli*, **GMSN** = Gomori's methenamine silver nitrate, **H&E** = haematoxylin and eosin, **PAS** = periodic acid-schiff

Infectious diseases, including those caused by bacteria, fungi, and viruses, cause significant economic losses in avian species. Clinically important bacterial pathogens include *Alcaligenes/Bordetella*, *Campylobacter*, *Clostridium*, *Erysipelothrix*, *Escherichia coli (E. coli)*, *Haemophilus*, *Listeria*, *Mycobacterium*, *Pasteurella*, *Pseudomonas*, *Aeromonas*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Yersinia* species (Gerlach 1994). Common fungal diseases include Candidiasis, Aspergillosis, and Cryptococcosis (Gerlach 1994). To control these infectious avian diseases, it is important to prevent and treat not only the initial infection, but also subsequent transmission and relapse through accurate diagnosis, especially for threatened birds such as those referenced by the

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Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

The blue-fronted amazon parrot (*Amazona aestiva*) is listed in the CITES Appendix II according to Korean law, but is not included in the CITES according to European law (Commission Regulation of EU). CITES is an international agreement that aims to ensure that the international trade in wild animals and plants does not threaten species survival. Appendix I includes species threatened with extinction. Trade in specimens of these species is permitted only in exceptional circumstances. Appendix II includes species not necessarily threatened with extinction, but those in which trade must be controlled to avoid utilisation incompatible with their survival.

In the present report, we describe two cases of blue-fronted amazon parrots that died due to sepsis originating from a complex infection by fungi (*Candida*) and bacteria (Gram-negative rods).

## Case description

Two blue-fronted amazon parrots (*Amazona aestiva*) were imported into Korea and several days later after a feed change, showed severe weight loss (from 350 to 220 g), fatigue, weakness, and diarrhoea. Radiographs revealed crop and intestinal distension, and systemic circulatory disturbance. Numerous yeasts (*Candida* spp.) were detected in cytological analysis of the crop mucosa. The parrots died several hours to days after medical examination and were subsequently necropsied at the Department of Veterinary Pathology, Kyungpook National University to determine the cause of death in each animal.

Gross necropsy was performed, and the solid organs were collected and fixed in 10% neutral buffered formalin (DC Chemical Co., Korea) for histopathological examination. Tissues were processed using standard methods, embedded in paraffin wax, and sectioned at 4 µm. The tissue samples were then deparaffinised in toluene (Duksan Pure Chemical, Korea) and rehydrated in a graded alcohol series. Haematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Gomori's methenamine silver nitrate (GMSN), and Gram staining were performed.

In the first parrot, the gross necropsy revealed severe cachexia and thin skin. Diarrhoea staining was observed at the anus (Figure 1A), and vomit was present in the oesophagus. The coelomic cavity contained prominent yellow, fibrinous material. The crop contained cheese-like lesions within a white layer lining the mucosal surface (Figure 1B).

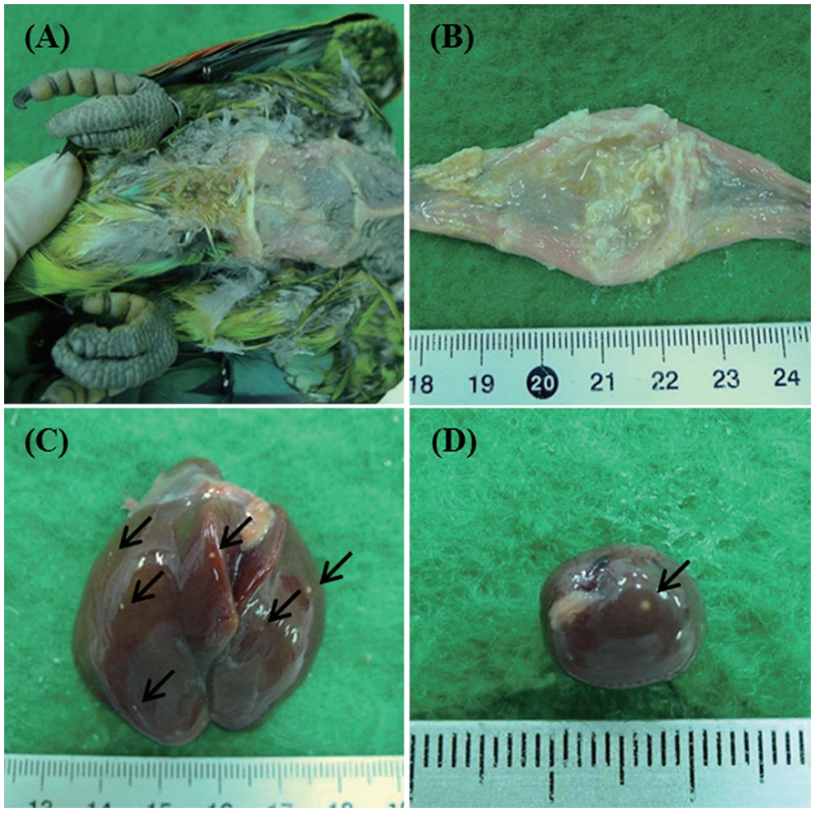


Figure 1. Gross necropsy findings in a blue-fronted Amazon parrot (*Amazona aestiva*). In the first parrot, (**A**) diarrhoea staining surrounding the anus, (**B**) cheese-like lesions caused by a white layer along the crop mucosa, and (**C**, **D**) white foci within the liver and spleen (arrows) were observed

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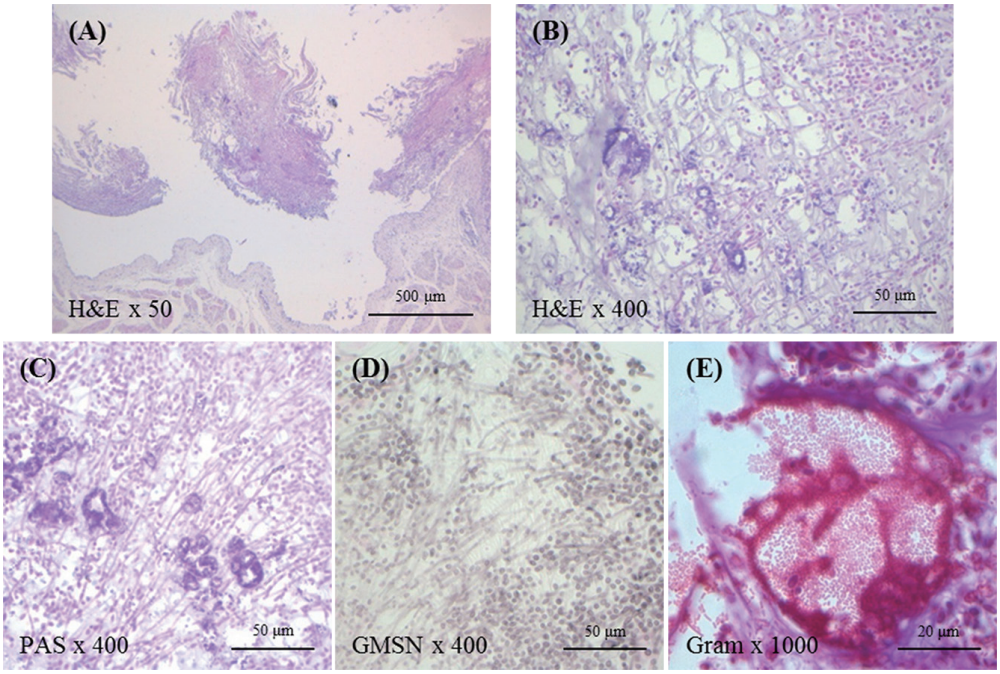


Figure 2. Microscopic findings of the crop in the first parrot. (**A**) Mucosal ulceration was observed in the crop. *Candida* hyphae and spores and bacterial colonies were observed in these ulcerated portions. **(B–D)** Severe fungal infection was observed. (**E**) Rod-shaped, Gram-negative bacteria were confirmed on Gram stain

White foci were observed in the liver and spleen (Figure 1C, D). The kidney was weakened and exhibited green discolouring. On microscopic examination, the crop mucosa was ulcerated, with hyphae, fungal spores (*Candida* spp.), and bacterial colonies within the ulcerated portions. Severe

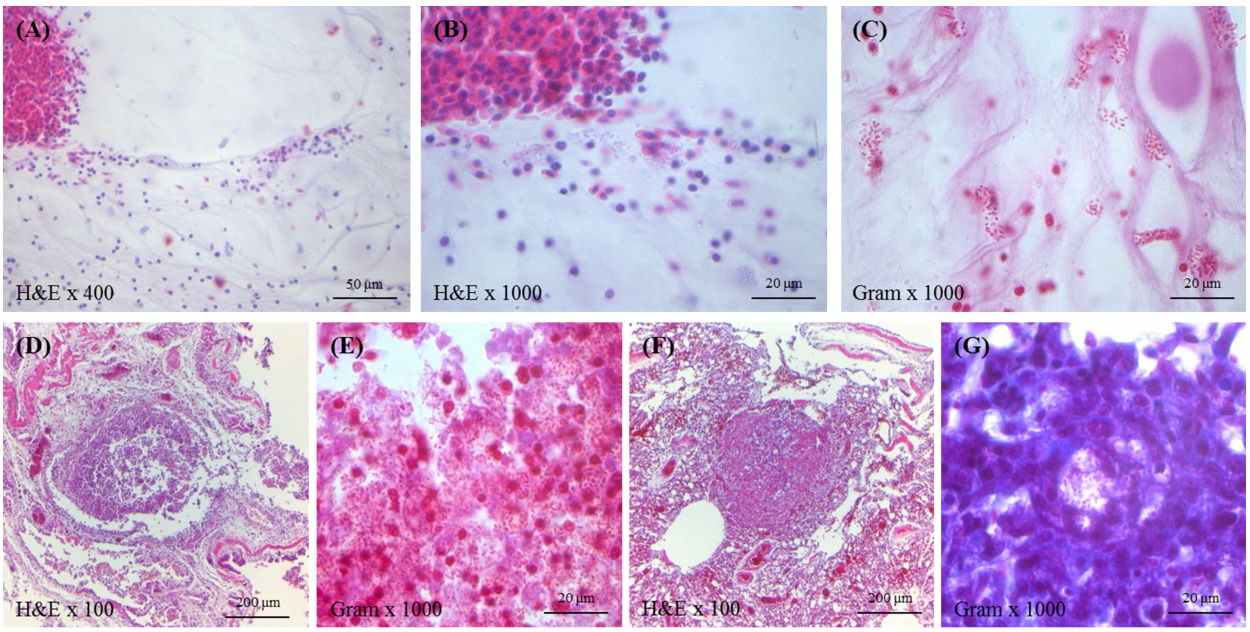


Figure 3. Microscopic findings of the heart and lung in the first parrot. (**A**, **B**) Rod-shaped bacteria and significant fibrinous material were found in the cardiac ventricles. **(C)** Rod-shaped, Gram-negative bacteria were confirmed by Gram stain. (**D**, **E**) Macrophages containing engulfed bacteria formed nodules within the pulmonary interstitial tissue. (**F**, **G**) Nodules comprising inflammatory cells and rod-shaped, Gram-negative bacteria were observed in lung parenchyma, and the alveolar capillaries were congested

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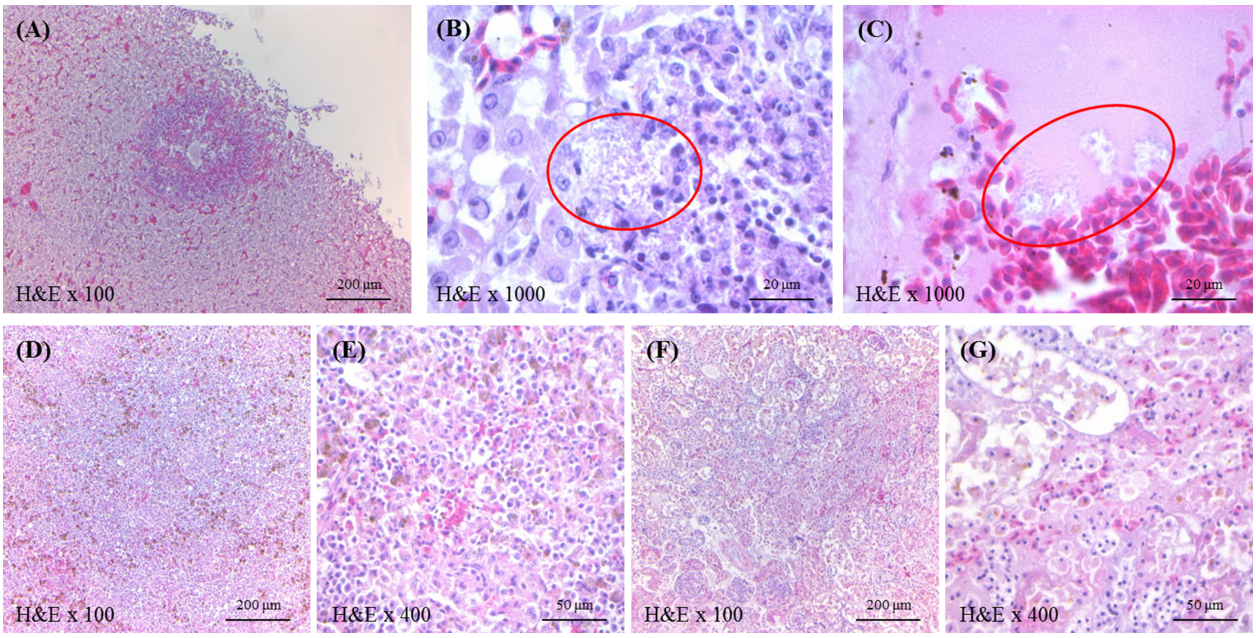


Figure 4. Microscopic findings of the liver, spleen, and kidney in the first parrot. (**A**–**C**) Small nodules comprising macrophages were found in the liver parenchyma. Bacteria were observed in the hepatic blood vessels and parenchyma. (**D**, **E**) Apoptosis, indicated by a starry sky appearance, and necrosis were observed in the spleen. (**F**, **G**) Lysed renal tubular epithelial cells and foamy macrophages were found in the kidney

fungal infection was confirmed by PAS and GMSN staining, and Gram-negative rod bacteria were confirmed on Gram staining (Figure 2). Rod-shaped, Gram-negative bacteria were also observed in much of the fibrinous debris in the cardiac ventricles (Figure 3A–C). The tracheal epithelium was sloughed off the underlying basement membrane, and the epithelial cells were mostly lost. The car-

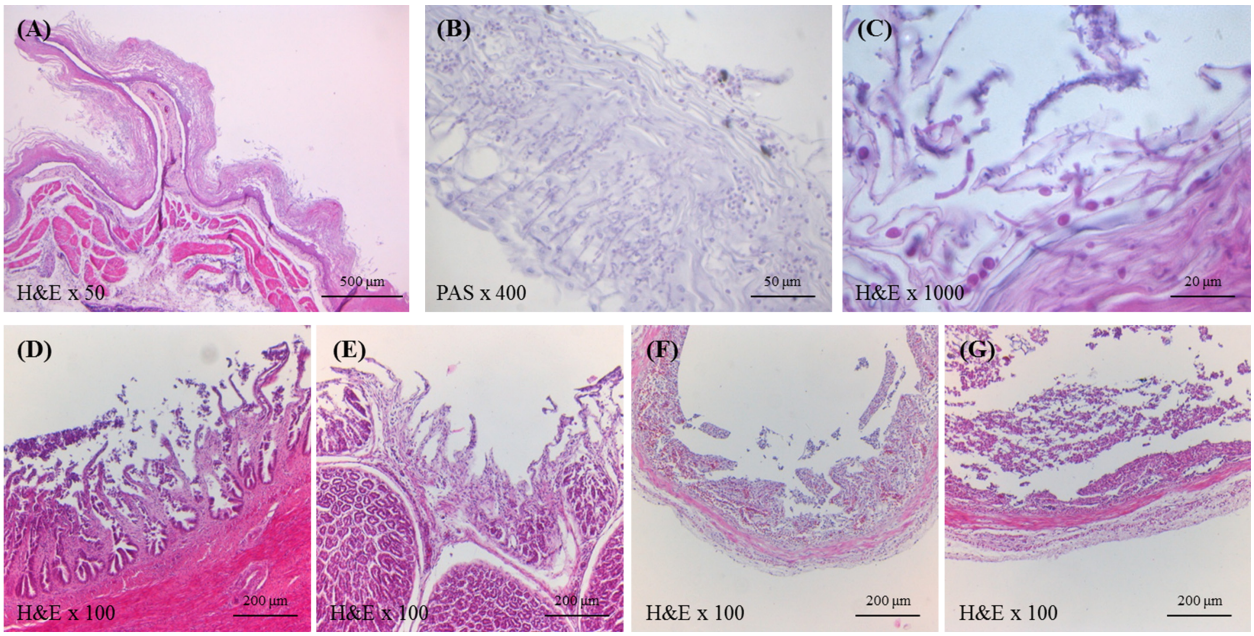


Figure 5. Microscopic findings of the digestive tract in the second parrot. (**A**–**C**) The crop epithelium was degenerated, and fungal hyphae and spores (*Candida* spp.) and rod-shaped bacterial colonies were present in these lesions. (**D**-**G**) The mucosal epithelium of the digestive tract, including the proventriculus, ventriculus, small intestine, and large intestine, was severely exfoliated and lysed

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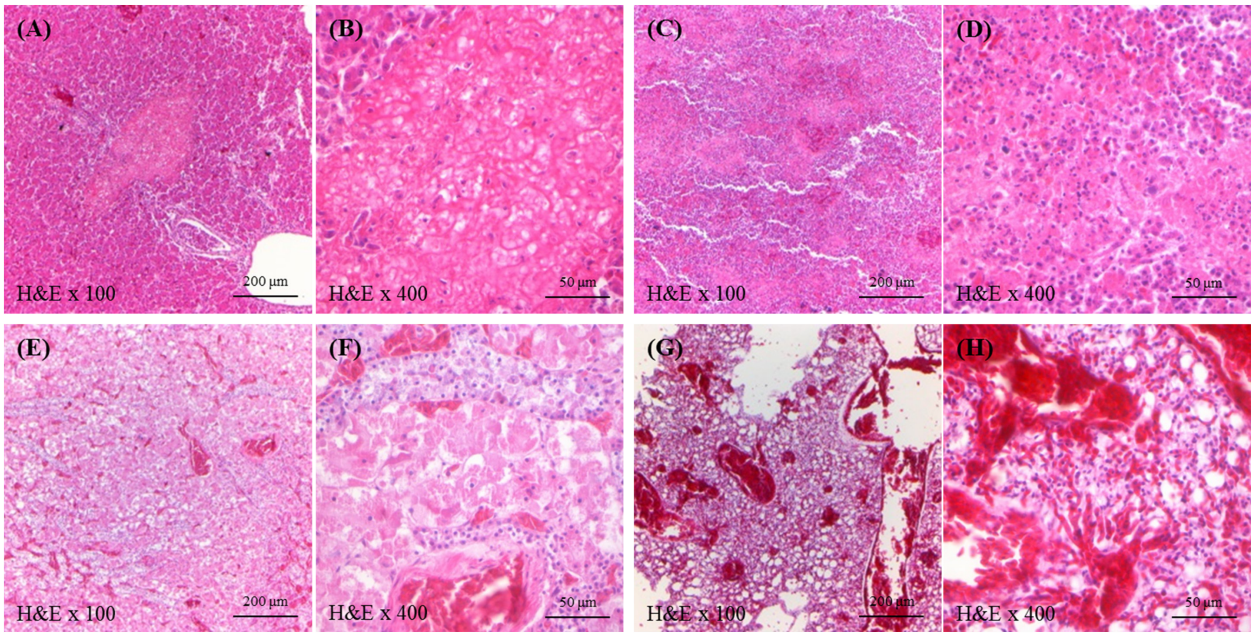


Figure 6. Microscopic findings of the solid organs in the second parrot. The solid organs, including the (**A**, **B**) liver, (**C**, **D**) spleen, and (**E**, **F**) kidney, showed necrotic and/or apoptotic lesions. (**G**, **H**) The lung parenchyma was swollen, and the alveolar capillaries were congested

tilaginous tracheal rings were mineralised. The lung parenchyma contained numerous nodules composed of inflammatory cells and bacteria. In the lung interstitium, collections of macrophages containing engulfed Gram-negative, rod-shaped bacteria were present (Figure 3D, E). The alveolar capillaries were congested, and pulmonary haemorrhage was present. As in the lung, small nodules of macrophages and bacteria were present in the liver parenchyma (Figure 3F, G). Rod-shaped bacteria were also observed within hepatic veins. The hepatocytes were necrotic and lysed (Figure 4A–C). Apoptosis, indicated by a starry sky appearance, and necrosis were found in the spleen (Figure 4D, E). The renal tubular epithelial cells were lysed, and foamy macrophages were present (Figure 4F, G). The keratinoid layer in the ventriculus was absent in some portions. Numerous glandular epithelial cells in the proventriculus and small intestine were lysed.

On gross necropsy, the second parrot showed vomit around the mouth and diarrhoea staining around the anus. The crop contained occasional cheese-like lesions, and hemorrhagic lesions were observed along the digestive tract (crop, proventriculus, ventriculus, and intestines). In particular, the small intestine wall exhibited severe petechial haemorrhage and thinning. The spleen was weakened and pale, and the mesenteric lymph node showed haemorrhage. Histopathologically, the crop epithelium was degenerated and contained hyphae and fungal spores (*Candida* spp.) as well as bacterial colonies. Fungal infection was confirmed by PAS staining (Figure 5A–C). The digestive tract mucosa, including the proventriculus, ventriculus, small intestine, and large intestine, was severely exfoliated and lysed (Figure 5D–G). Blood vessel dilation and moderate haemorrhaging were present in the intestine. Plasma cell and lymphocytic infiltration was observed in the intestinal *lamina propria*. The solid organs, including the liver, spleen, and kidney, contained necrotic and/or apoptotic lesions (Figure 6A–F). The lung parenchyma was swollen, and the alveolar capillaries were markedly congested (Figure 6G, H). The tracheal cartilage was mineralised, degenerated, and haemorrhagic.

## DISCUSSION AND CONCLUSIONS

Mycotic infections are common in birds and include Aspergillosis, Candidiasis, Dactylariosis, Cryptococcosis, Favus, Rhodotorulosis, Torulopsis, Mucormycoses and Histoplasmosis. Among these diseases, Candidiasis is a particularly important avian fungal disease (Hubalek 1978; Dhama et al. 2013).

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Candidiasis is caused by fungi of the genus *Candida*, which includes approximately 200 species. Among them, *C. albicans* is the most abundant and clinically significant species (Odd 1994; Dhama et al. 2013). While *Candida* species are a normal part of the avian gastrointestinal flora, fungal overgrowth and disease may occur in patients with certain risk factors that suppress the host immune system, including malnutrition, vitamin D deficiency, poor hygiene, prolonged antibiotic use suppressing normal bacterial flora, and stress. In the present cases, the parrots were imported from abroad and had their feed changed several days before they died. We surmise that the long travel period and sudden feed change were stressors that weakened the immune system and enabled *Candida* overgrowth.

Infected birds show poor growth, depression, anorexia, diarrhoea, and dehydration, and mortality may occur in severely affected birds (O'Meara and Witter 1971; Bauck 1994; Chute 1997; Oglesby 1997; Velasko 2000; Dhama et al. 2013;). Lesions are usually observed in limited areas of the upper digestive tract. Fungi proliferate, form hyphae or pseudo-hyphae on the surface of the upper digestive tract, and invade epithelial layers. This results in hyperplasia and pseudo-membrane formation, appearing grossly as a cheese-like material, usually in the crop (Mayeda 1961; Velasko 2000; Dhama et al. 2013;). False membrane formation along the upper digestive tract and mucosal erosion of the proventriculus and gizzard along with intestinal inflammation are commonly observed (O'Meara and Witter 1971; Chute 1997; Bethea et al. 2010; Dhama et al. 2013). In the parrots in the present two cases, crop candidiasis and ulceration with mucosal epithelial exfoliation in the digestive organs were observed. Bacterial proliferation was also presented in the affected crops. In one parrot, bacteria were found in additional organs, including the heart, lung, and liver. The bacterial growth within the crop followed by systemic dissemination explains the other systemic lesions, which included necrosis, haemorrhage, and congestion of solid organs. We suspect that *Candida* overgrowth and invasion destroyed the integrity of the mucosal layer and altered the crop environment, which allowed bacteria to proliferate and spread into systemic organs.

Gram-negative, rod-shaped bacteria were observed in both our cases. Among known avian pathogens, Gram-negative, rod-shaped bacteria include *E. coli*, *Salmonella*, *Klebsiella, Yersinia*, *Pseudomonas*, *Aeromonas*, *Campylobacter*, *Vibrio*, *Pasteurella*, *Haemophilus*, *and Acinetobacter* spp. Of these, *E. coli*, *Salmonella*, *Pseudomonas*, *Aeromonas*, and *Pasteurella* infect the gastrointestinal tract of birds; *E. coli* is considered a particularly important pathogen in many avian species. Birds infected by *E. coli* develop colisepticaemia, which is characterised by acute lethargy, anorexia, ruffled plumage, diarrhoea, polyuria, and weight loss. Catarrhal enteritis is common but non-specific. *E. coli* can cause fibrous polyserositis (mainly yellow), and grey foci (granulomas) of varying size in the liver, intestinal subserosa, spleen, or kidney are typical findings at necropsy. *E. coli* infection can also cause septicaemia and death (Bauck 1994). Although culture and identification of organisms in infected tissues are required for specific diagnosis, polyserositis and granuloma formation (grey foci) in organs are suggestive of *E. coli* infection.

Animals dying of septic shock typically show evidence of cavitary lung disease, pulmonary oedema, petechial haemorrhage, liver and intestine congestion, and dehydration. Common microscopic lesions include acute necrosis of renal tubules, centrolobular hepatocytes, cardiac myocytes, adrenal cells, and intestinal villi tips (Ackermann 2006).

Based on the histopathologic findings and clinical history, we suspect that the parrots in these cases died due to sepsis originating from complex infection of the crop by *Candida* spp. and Gram-negative, rod-shaped bacteria. Obviously, prevention is better than cure and effective prevention and control are the best policy for almost all diseases. In bird breeding, precautions are required to eliminate any risk factors, such as stress, poor diet, poor sanitation, or other diseases.

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