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Diagnostic Exercise
From the Latin Comparative Pathology Group and the Davis-Thompson Foundation

Mycoplasmosis infection with secondary heterophilic and necrotizing blepharoconjunctivitis and sinusitis in a chicken

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History:

A 3-months-old male Belgian Bearded d'Uccle chicken was presented together with two other juvenile chickens for progressive ocular and respiratory disease. The birds had been previously treated with oxytetracycline and topical ophthalmic antibiotics, including Tricin® ointment and chloramphenicol eye drops, without clinical improvement. Clinical signs progressively worsened. At presentation, all birds exhibited ocular lesions. The Belgian d'Uccle chick showed bilateral periocular distension and deformation, with complete obscuration of the left eye. Respiratory effort was increased, with audible crackles consistent with air sac involvement. The oral cavity was malodorous. Feces were light brown and loose, and the bird was markedly emaciated. Two of the affected chickens, including the Belgian d'Uccle chick, were bred on the owner's property from a newly introduced rooster. The third bird was an unrelated Showgirl cockerel purchased as a day-old chick that subsequently developed similar clinical signs.

Necropsy findings:

The left eye and adjacent periocular soft tissues are markedly distended by abundant yellow, friable, caseous exudate, resulting in pronounced mass effect with compression and displacement of the left globe (Figures 1A-C and 2A-D). The same material was also detected histologically in the infraorbital sinus and choanal cleft (Figure 2E, 2F).

Follow-up questions:

- *Histopathological descriptions*
- *Histopathological morphological diagnoses*

**The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation (DTF). These exercises are contributed by members and non-members from any country of residence. Consider submitting an exercise! A final document containing this material with answers and a brief discussion will be posted on the DTF website: https://davisthompsonfoundation.org/diagnostic-exercise/*

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Fugure. 1. Gross images of severe, locally extensive periocular and infraorbital lesions in a Belgian Bearded d'Uccle chicken. **(A–B)** The left eye and adjacent periocular soft tissues are markedly distended by abundant yellow, friable, caseous exudate, resulting in pronounced mass effect with compression and displacement of the left globe. **(C)** Cut section of the left eye and eyelid, showing accumulation of dry caseous material within the conjunctiva and eyelid. (white arrows).

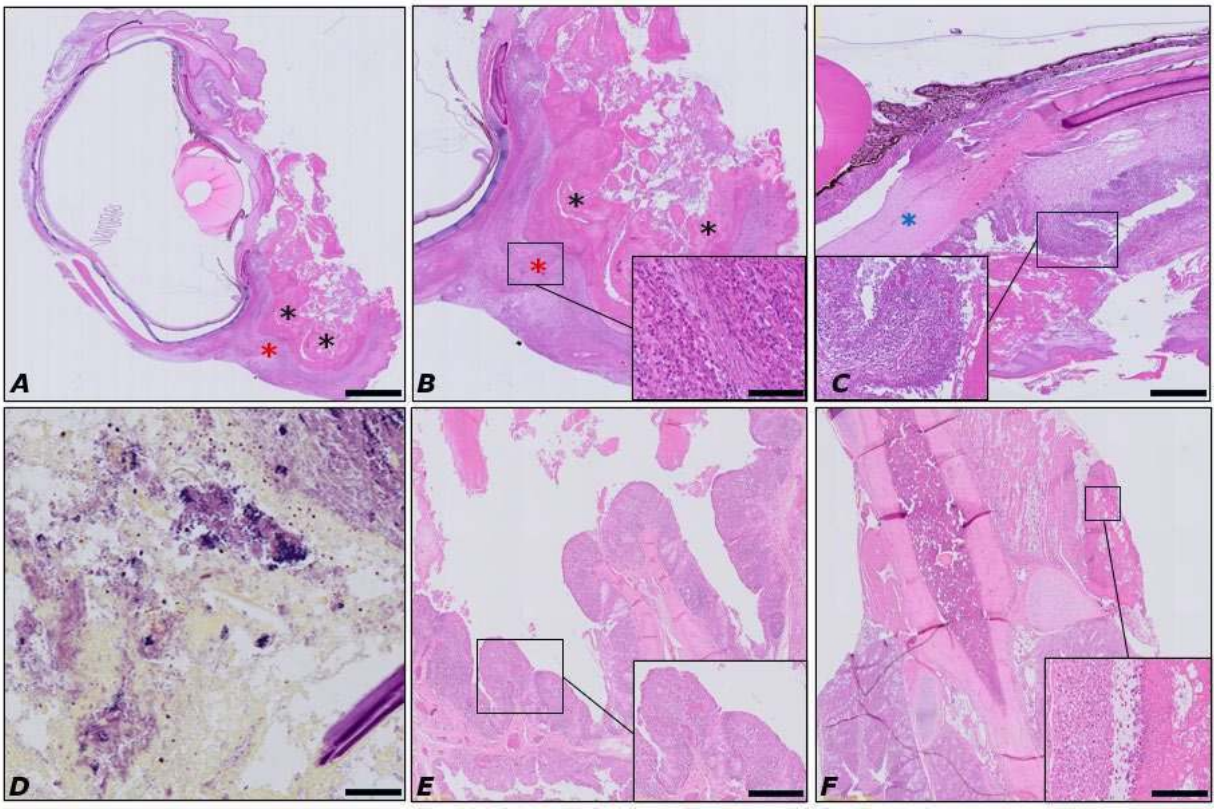

Figure 2. Histological sections (Hematoxylin and Eosin) of the left eye, eyelid, and infraorbital sinus from a Belgian Bearded d'Uccle chicken. **(A–B)** The conjunctival submucosa and eyelid dermis are markedly expanded and partially effaced by coalescing lakes of granular eosinophilic necrotic and cellular debris (black asterisks) and numerous intact and degenerate heterophils (red asterisks), admixed with granulation tissue, consistent with severe heterophilic and necrotizing blepharoconjunctivitis. Inset (Figure B): Higher magnification of heterophilic infiltrates. **(C)** The conjunctiva is infiltrated by numerous lymphocytes and plasma cells close to the areas of necrosis. Inset: Higher magnification of lymphoplasmacytic conjunctivitis. **(D)** Gram stain showing necrotic debris admixed with gram-negative and gram-positive bacterial aggregates. **(E)** The lamina propria and subepithelial connective tissue of the infraorbital sinuses are expanded 4-fold by numerous plasma cells, occasionally forming nodular aggregates. Inset: Higher magnification showing plasmacytic infiltrates **(F)** The infraorbital sinus mucosa is bilaterally and diffusely expanded by numerous plasma cells, occasionally forming nodular aggregates. Multifocally, the mucosal epithelium is attenuated to ulcerated, with loss of cilia and sloughing of necrotic epithelial cells into the lumen. Unilaterally (left), the sinus is markedly expanded and partially effaced by dense aggregates of heterophils surrounding central eosinophilic necrotic debris. Inset: Higher magnification of heterophilic infiltrates and necrosis. *Original magnification* ***200x*** *and high magnification* ***400x****. Scales bar* ***20 µm***.

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- *Differential diagnoses and ancillary tests*
- *Etiologies (primary and secondary)*
- *Name the disease*

ANSWERS

Histopathological description:

Left eye, eyelid and periorbital connective tissues: Focally expanding and effacing 70% of the submucosa of the conjunctiva and the dermis of the eyelid are lakes of granular eosinophilic material (necrosis and cellular debris) surrounded by numerous intact and degenerate heterophils (blepharoconjunctivitis, heterophilic and necrotizing), admixed with myriads of 1.5x0.3-micron rod bacteria that stain red on Gram stain and less frequently cocci that stain blue (gram-negative bacilli and gram-positive cocci). Adjacent to the necrotic areas, there is increased collagen matrix with numerous fibroblasts admixed with multifocal vascular profiles (granulation tissue), and multifocally dissected by clear spaces (edema). Multifocally within the lamina propria of the conjunctiva and in the granulation tissue are perivascular to interstitial infiltrates of plasma cells, histiocytes, and lymphocytes, which multifocally infiltrate the conjunctival epithelium (conjunctivitis, lymphoplasmacytic and histiocytic).

Infraorbital sinus: Bilaterally and diffusely, the lamina propria and subepithelial connective tissue of the infraorbital sinuses are expanded 4-fold by numerous plasma cells, occasionally forming nodular aggregates (sinusitis, plasmacytic). Bilaterally, multifocally, the mucosal epithelium is attenuated or superficially ulcerated, with loss of cilia, and necrotic cells sloughing off in the lumen. Unilaterally the left infraorbital sinus is expanded 3-fold and 60% effaced by numerous heterophils surrounding lakes of eosinophilic necrotic centers (sinusitis, heterophilic and necrotizing), The necrotic material admixed with degenerated heterophils, and myriads of bacteria is obstructing 40% of the lumen of the choanal cleft. In the left infraorbital sinus, the subepithelial connective tissue and surrounding skeletal muscle fibers are dissected 2 to 5-fold by clear spaces (edema).

Morphologic diagnoses:

1.Left eyelid and conjunctiva:

Conjunctivitis, lymphoplasmacytic, chronic, multifocal, moderate, with

multifocal conjunctival epithelial hyperplasia, hydropic degeneration, goblet cell hyperplasia and multifocal orthokeratosis.

Blepharoconjunctivitis, heterophilic and necrotizing, acute to subacute, focally extensive, severe, with granulation tissue formation, edema, and with multifocal intralesional colonies of gram-positive cocci and gram-negative bacilli.

2. Infraorbital sinus

Sinusitis, plasmacytic, chronic, generalized, moderate.

Sinusitis, heterophilic and necrotizing, acute to subacute, multifocal, severe, with multifocal edema.

Ancillary testing:

<u>*Molecular tests - PCR*</u>
Mycoplasma gallisepticum: Positive
Mycoplasma synoviae: Negative
Avibacterium paragallinarum: Negative
Ornithobacterium rhinotracheale: Negative
Pasteurella: Negative
Gallid alphaherpesvirus 1 (ILTV): Negative
Influenza A virus: Negative
Avian Paramyxovirus-1 class I PCR: Negative
Avian Paramyxovirus-1 class II: Negative

<u>*Microbiological culture*</u>
Streptococcus bovis
Escherichia coli
Corynebacterium bovis

Etiologies:

<u>Primary pathogen:</u> *Mycoplasma gallisepticum*
<u>Secondary bacterial pathogens:</u> *Escherichia coli, Streptococcus bovis*
<u>Contaminant/commensal:</u> *Corynebacterium bovis*

Etiological diagnosis:

Mycoplasmal blepharoconjunctivitis and sinusitis with secondary bacterial infection.

Comments:

Upper respiratory tract (URT) infections commonly affect individual birds, frequently spreading within the flock (1,2). The etiologies implicated in infection of the URT and conjunctiva in birds are multiple, including viral, bacterial, protozoal, and fungal agents, most importantly *Mycoplasma gallisepticum*, *Avibacterium paragallinarum* (Infectious coryza), Gallid herpesvirus type 1 (laryngotracheitis – ILT), as well as chlamydiosis, and Avian metapneumovirus (AMPV) (1,2). Ancillary molecular tests confirmed mycoplasmosis as the likely primary cause of disease associated with secondary bacterial infection (1,2).

Mycoplasmosis due to *M. gallisepticum* is an important cause of disease in flocks of chickens (1). The infection occurs in birds of any age, but mostly in chickens

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older than 4-weeks of age (1). Transmission of the agent can occur through direct or indirect contact with susceptible birds, either clinically or subclinically infected, as well as vertical transmission through eggs, which often results in high infection/disease prevalence within flocks (1). *M. gallisepticum* lacks a cell wall, making it inherently resistant to beta-lactam antibiotics and requiring treatment with tetracyclines, macrolides, or fluoroquinolones. Clinical signs of disease are tracheal rales, nasal discharge, coughing, a decrease in body condition, and keratoconjunctivitis (1,2). Gross lesions in chickens consist of mucosal congestion, catarrhal exudate in the nasal and paranasal passages, trachea, bronchi, and air sacs (1,3). Histologically, lymphocytic tracheitis and sinusitis, and mucus gland hyperplasia infection are characteristic of *M. gallisepticum* in chickens (3). In this case interestingly plasma cells were the predominant cell type infiltrate causing sinusitis. Sinusitis with caseous exudate is a feature more commonly observed in turkeys but can occur in chickens (1). Caseous lesions in the air sacs are commonly reported, and in association with fibrinous pericarditis and perihepatitis, are reported to result in increased mortality (1,3). Commonly, primary viral or bacterial infections facilitate colonization and infection by secondary opportunistic agents, often mixed species of bacteria (1,2,4). In this case, microbiological culture from the left eye isolated a moderate growth of a mixed population of bacteria, such as *Escherichia coli*, *Corynebacterium bovis*, and *Streptococcus bovis*. Caution must be taken with the interpretation of microbiological culture results, as some agents may represent contamination from normal flora or post-mortem contamination. *Corynebacterium bovis* is primarily a commensal organism of mammalian skin and mucous membranes and has not been reported, to the best of our knowledge, as a cause of blepharoconjunctivitis and sinusitis in avian species (4). Its isolation likely represents environmental or post-mortem contamination. The gram-negative bacilli observed histologically are morphologically consistent with E. coli, supporting its role as a secondary pathogen. Isolated bacteria such as *E. coli* and *Streptococcus* spp. are well-recognized secondary pathogens in avian upper respiratory tract infections (5).

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