DIAGNOSTIC EXERCISE

From The Davis-Thompson Foundation*

Case #:**224**; Month: **November**; Year: **2023**

Answer sheet

Title: Influenza pneumonia in a pig

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Clinical History: A 26.2 kg (57.8 lb), 3-month-old, male mixed breed show pig had increased respiratory effort one-week after purchase. The clinical signs progressed to abdominal breathing, lethargy and lateral recumbency. The pig was kept in isolation from the herd. On clinical presentation, the pig was pyrexic with continued increased respiratory effort despite medical management. Thoracic radiographs showed bilateral lung consolidation.

Gross Findings: The pig was in good body condition. All lung lobes contained multifocal, dark red, firm areas (consolidation), resembling a checkerboard pattern (Fig. 1). The affected percentage of each lung lobe were as follows: 30% cranial portion of the left cranial, 50% caudal portion of the left cranial, 20% left caudal, 70% right caudal, 30% right cranial, 50% right middle, and 40% accessory. The remainder of the lungs were diffusely wet, heavy, and failed to collapse (edema). The lumen of the trachea and mainstem bronchi contained mucus admixed with hemorrhage and foam. The tracheobronchial and inguinal lymph nodes were mildly to moderately enlarged and homogeneously pale tan on cut surface (reactive lymphadenopathy).

Gross and Histological Images:

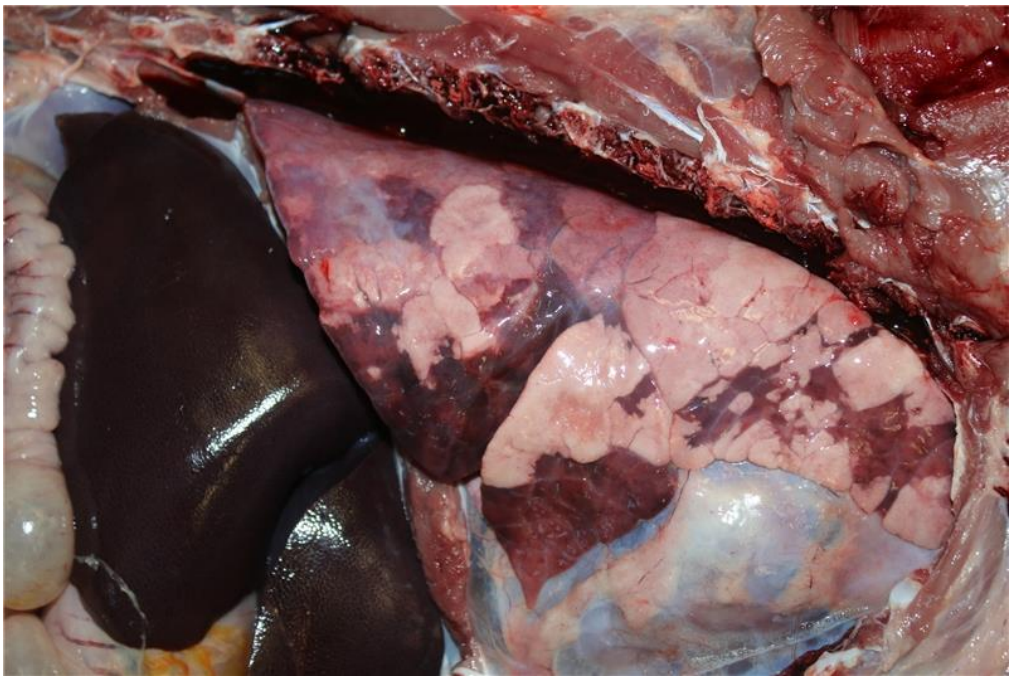

Figure 1. Throughout all lung lobes are multiple dark red, firm areas (consolidation), resembling a checkerboard pattern, with the right caudal and middle lung lobes being most affected. The remainder of the lungs are diffusely wet, heavy, and failed to collapse (edema).

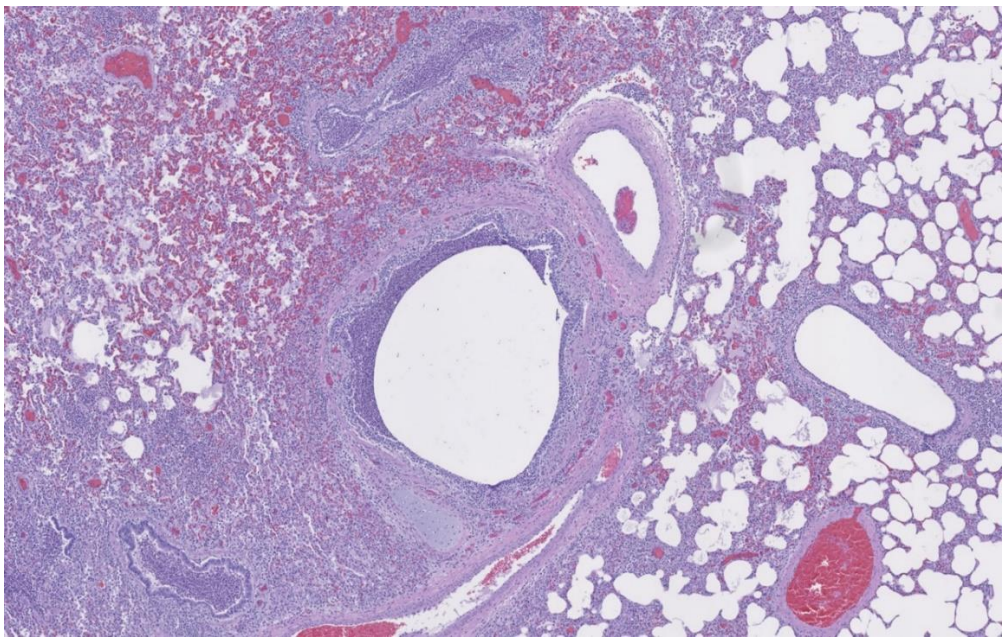

Figure 2. The consolidated areas of the pulmonary parenchyma contain an inflammatory infiltrate centered on or around bronchi and bronchioles and extends to adjacent alveoli.

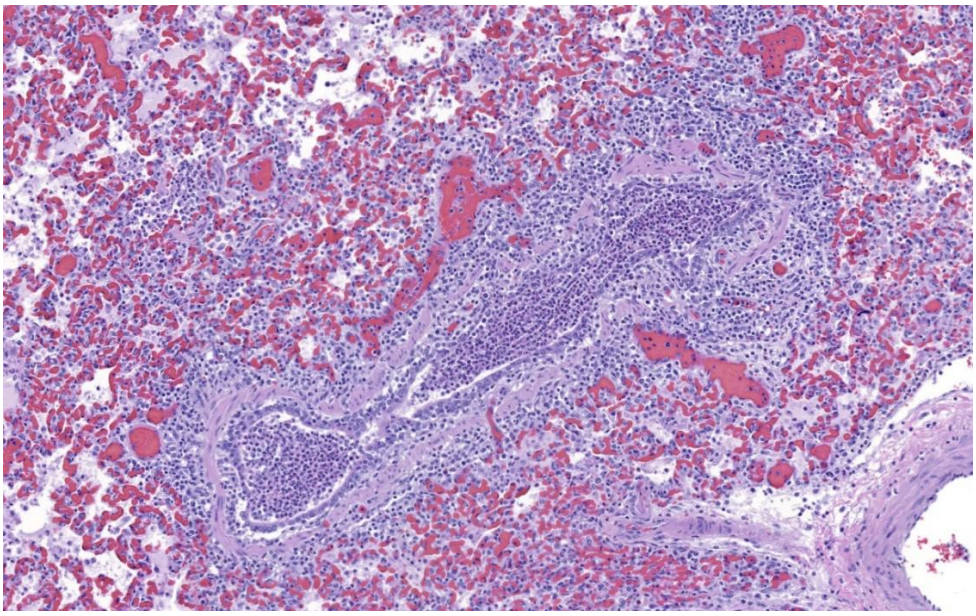

Figure 3. The bronchiolar lumen is obliterated by degenerate neutrophils and necrotic debris. Multifocally, the bronchiolar epithelium is degenerate and/or necrotic. Adjacent alveolar spaces exhibit atelectasis.

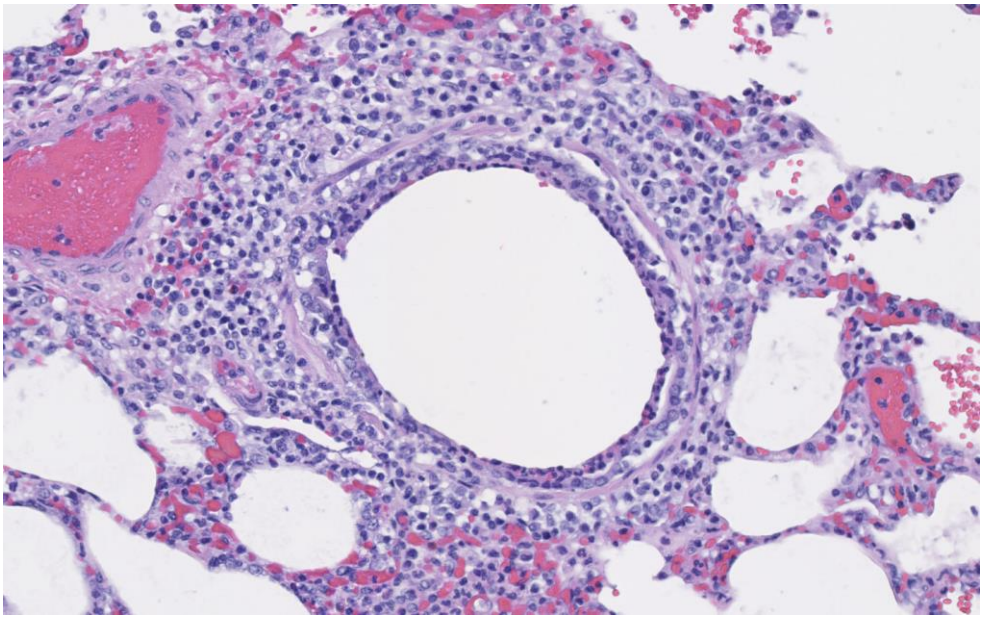

Figure 4. The bronchiolar epithelium is degenerated/necrotic and replaced by cellular debris. The bronchiole is surrounded by a rim of lymphocytes and macrophages.

Histological Description: Multifocally, the lung is consolidated by inflammatory cells centered on or around bronchi and bronchioles and often extending into adjacent alveoli with subsequent atelectasis (Fig. 2 and Fig. 3). The bronchial and bronchiolar epithelium is affected as follows: shrunken and hypereosinophilic with pyknotic nuclei and loss of cilia (degeneration and necrosis), or sloughed into the lumen and replaced by eosinophilic proteinaceous to karyorrhectic cellular debris

(Fig. 3 and Fig. 4). Bronchial and bronchiolar lumina and alveolar spaces are variably distended by neutrophils, necrotic debris (Fig. 3), edema, and occasionally hemorrhage and fibrin. Bronchi and bronchioles are multifocally surrounded by a rim of lymphocytes and macrophages extending into the alveolar interstitium (Fig. 4). Type I pneumocytes are replaced by cuboidal type II pneumocytes (type II pneumocyte hyperplasia). Multifocally, there is moderate peribronchial, peribronchiolar, septal, and perivascular edema.

Morphologic diagnosis: Lung. Moderate, multifocal, subacute, necrotizing bronchitis and bronchiolitis with suppurative bronchointerstitial pneumonia.

Ancillary testing: PCR from a lung sample was performed for the following infectious agents: Influenza A virus (positive), *Mycoplasma hyopneumoniae* (negative), porcine circovirus 2 (PCV2) (negative), PCV3 (negative), porcine deltacoronavirus (negative), porcine parainfluenza virus 1 (negative), multiplex PCR for porcine respiratory and reproductive syndrome virus (PRRSV) US strain (negative) and EU strain (negative).

Cause: Influenza A virus. Sequencing and analysis of the Influenza HA gene revealed US Clade – H1 delta 2 and Global Clade – 1B.2.1. These clades correspond to H1N2 subtype of influenza.

Pathogenesis: Infection through aerosolized or bodily secretions -> viral attachment and fusion of viral hemagglutinin to the host respiratory epithelium sialic acid -> viral neuraminidase cleaves host sialic acid -> entry into host respiratory cell -> viral polymerase cleaves host mRNA -> suppression of the host cell gene -> blockage of host cell protein synthesis -> viral replication and protein synthesis -> cytolysis of infected cell -> necrotizing bronchitis and bronchiolitis.

Comments: Influenza virus is a single-stranded RNA virus within the family *Orthomyxoviridae*, and is separated into nine genera: *Alphainfluenzavirus*, *Betainfluenzavirus*, *Deltainfluenzavirus*, *Gammainfluenzavirus*, *Isavirus*, *Mykissvirus*, *Quaranjavirus*, *Sardinovirus*, and *Thogotovirus* (8). Although pigs are infected with *Alphainfluenzavirus*, *Betainfluenzavirus*, *Deltainfluenzavirus*, and *Gammainfluenzavirus*, *Alphainfluenzavirus* (IAV) is the most important in the swine industry (8). The outer surface of the IAV virion contains hemagglutinin (HA) and neuraminidase (NA) which is responsible for binding to the host's cell surface sialic acid, while neuraminidase cleaves sialic acid to allow for viral entry into the host cells (2, 8). The respiratory tract of pigs contains both mammalian α-2,6 sialic acid and avian α-2,3 receptors. In pigs, α2,6-linked SA receptors predominate in the upper respiratory tract, but α2,3-SA receptors are present in the trachea and lower respiratory tract (8).

Transmission of IAV between pigs is primarily through direct contact with oronasal or aerosolized secretions, and typically transmission occurs most often in late fall to early winter (2, 3, 8). Increased morbidity and mortality are frequently seen with the introduction of new pigs, typically during times of increased stress and

decreased maternal antibody protection (8). IAV causes an acute infection with clinical signs occurring within 1 to 3 days, and clinical signs commonly include high fever, anorexia, lethargy, abdominal breathing, and coughing (2, 8). In acute outbreaks with high morbidity, the immune response is robust with elimination of the virus from the respiratory tract and recovery within 5 to 7 days in uncomplicated cases (2, 8). However, secondary bacterial and viral infections can result in severe clinical disease and mortality. Differentials in younger pigs should include porcine reproductive and respiratory syndrome (PRRSV), pseudorabies (Aujeszky's disease), porcine circovirus 2 (PCV-2), and porcine circovirus 3 (PCV-3) (4).

Lesions of IAV in pigs can vary widely from mild pneumonia with multifocal small red to dark red areas of lung consolidation resembling a checkerboard pattern within the cranioventral lung lobes, to severe cranioventral lung consolidation with diffuse pulmonary edema and congestion (2, 8). A sharp line of demarcation delineating the affected from the normal lung tissue is seen (7, 8). Histologically, the characteristic lesion of IAV is a necrotizing bronchitis and bronchiolitis with sloughing of epithelial cells, degenerate neutrophils and cellular debris within the lumen, often obstructing the larger airways (2, 4, 8). In response to the degree of inflammation, there is an influx of macrophages and lymphocytes surrounding the bronchi and bronchioles with attenuation of the epithelial lining and an eventual hyperplastic response (2, 8). In chronic cases, severe damage to the epithelial lining can result in increased fibrous connective tissue response seen as a polyp protruding into the lumen (bronchiolitis obliterans) (2, 8). Other changes associated with IAV include an influx of degenerate neutrophils, cellular debris, edema and hemorrhage into alveolar lumina and interstitium. Commonly, the alveolar septa are spared, however in severe cases or cases complicated by secondary infections, necrosis can be observed (2, 4, 8). Although necrotizing bronchitis and bronchiolitis is a characteristic histologic finding, these changes can be masked in cases complicated by secondary infections, and additional ancillary testing is imperative in the diagnosis of influenza as multiple other viral etiologies can appear clinically and grossly similar. Comparatively, PRRSv and PCV usually cause an interstitial pneumonia, while Aujeszky's disease causes a necrotizing bronchitis, bronchiolitis and alveolitis with intranuclear viral inclusion bodies in epithelial cells (4). Antemortem testing of IAV is through RT-PCR, and samples can be collected in one of two ways in a field setting. The first is through using a polyester nasal swab on an individual animal, or through placing a rope in a pen for multiple pigs to chew on and testing the oral fluids in a group setting (2, 7, 8). PCR can similarly be pursued by selecting fresh lung tissue from the affected cranioventral area. Prevention of influenza within swine herds continues to be problematic, however, vaccination remains to be the primary means of control (1, 2, 3, 8). In commercial settings, an inactivated whole viral vaccine with adjuvant is used in sows, with the hopes of providing maternal antibodies to piglets (1, 2, 3, 8). Vaccination has proven to be successful when the appropriate strain is protected against, however, it is not always effective.

Further analysis of the Influenza HA gene in this case corresponded to H1N2 subtype of influenza. IAVs are characterized into subtypes based on its

hemagglutinin (HA) and neuraminidase (NA) glycoproteins. In total, there are 16 HAs and 9 NAs, and the combination of the two defines the subtype (ex. H1N2) (2, 3, 8). Within the swine population, H1N1, H1N2, and H3N2 are reported, with broad circulation of two H1 subtypes (H1N1 and H1N2) (1, 2, 3, 8). Historically, reporting of H1 virus HA in swine relied on colloquial names. In Europe, there are 4 global HA H1 clades based on the host or regional introduction history (1, 3, 5). In the United States, a nomenclature system grouped viruses into 5 HA H1 clades, using Greek letters: alpha (H1-α), beta (H1-β), gamma (H1-γ) for classical H1 origin, and delta1 (H1-δ1) and delta2 (H1-δ2) for human seasonal H1 origin (1, 3, 5). Because IAVs can be dispersed globally, the regional clade names are not informative and a new Global nomenclature clade was proposed for H1 global swine IAVs according to the major 3 lineages: 1A, swine seasonal lineage, associated with the 1918 human influenza pandemic; 1B, human seasonal IAVs spilling into swine herds and subsequent evolution in pigs; and 1C, Eurasian avian-like IAV that resulted from the spillover of H1 viruses from wild birds in Europe with subsequent export to Asia (1).

In this pig, sequencing and analysis of the Influenza HA gene revealed US Clade – H1 delta 2 (H1-δ2) and Global Clade – 1B.2.1. A web-based platform, called ISU Fluture, has been developed to monitor the temporal genetic patterns of IAV in swine from samples submitted to a veterinary diagnostic laboratory (https://influenza.cvm.iastate.edu/) (6). Reported results take in consideration the US and Global nomenclature. In the United States, the H1 classical swine lineage emerged coincident with the 1918 Spanish flu in humans, and diversified to contain five distinct clades of viruses: H1-α (global nomenclature 1A.1 and 1A.1.1); H1-β (1A.2); H1-γ2 (1A.3.2); H1-pdm09 (1A.3.3.2); and the H1-γ (1A.3.3.3) (1, 3). A second H1 lineage was detected in the 2000s, the result of two separate human-to-swine spillovers: these include clades H1-δ1 (1B.2.2, 1B.2.2.1, 1B.2.2.2) and H1-δ2 (1B.2.1) (1, 3, 6). Zoonotic transmission of swine influenza to humans includes the subtypes H1N1, H1N2, and H3N2 within the gamma and delta clades (1, 3, 8). Importantly, in a recent study, it was shown that H1δ-2 was isolated from up to 92% of show pigs with IAV within the United States, which continues to circulate in swine populations and was isolated in this case (3). These are viruses that have previously circulated within people and therefore have both human and swine genes.

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