Diagnostic Exercise
From the Davis-Thompson Foundation

Case #: **222;** Month: October; Year: **2023**
Answer sheet

Title: An outbreak of bluetongue in sheep

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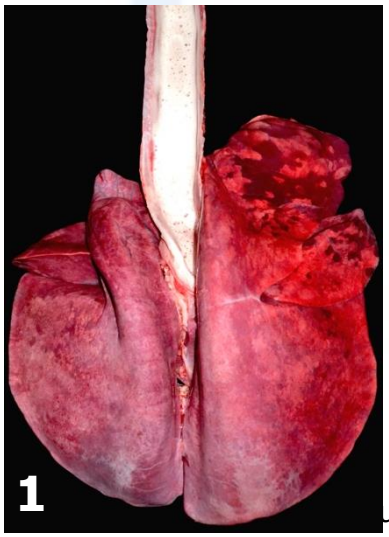
Clinical history:
During a 2-week period between mid-October and November 2022, approximately 20 out of 470 Rambouillet sheep from a flock in southern California died; the flock had been moved into irrigated hay fields recently. Some of the affected animals were initially underweight and most had nasal discharge, labored breathing and frothy discharge from the mouth. Two adult sheep (1 and 2) were submitted to the San Bernardino laboratory of the California Animal Health and Food Safety laboratory system (CAHFS) for necropsy and diagnostic work up.

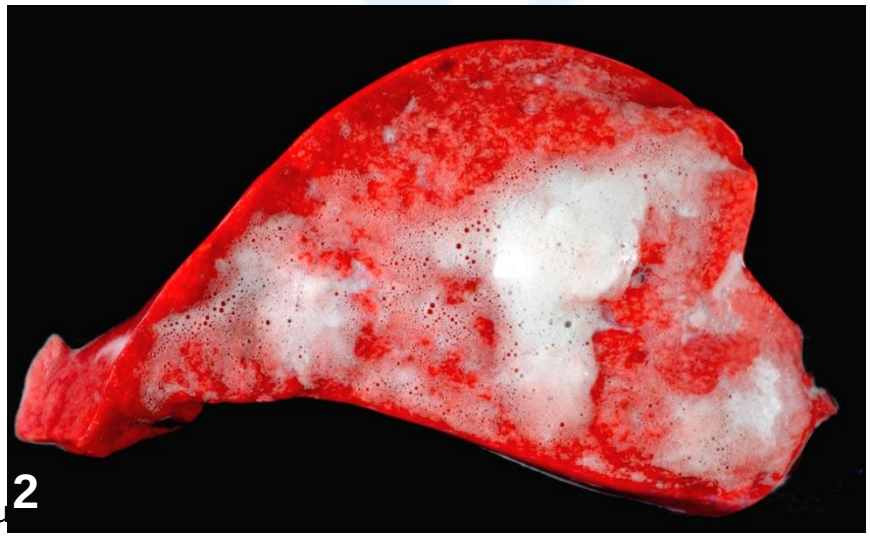
Necropsy findings:

On necropsy, both carcasses were in fair nutritional condition, with scant fat reserves. Sheep 1 had severely swollen and congested lungs, abundant froth in the trachea and bronchi, hydrothorax, and hydropericardium (Figures 1-2). Sheep 2 had multifocal ulcers in the alimentary tract (Figures 3-6).

Gross images:

Sheep 1:





Sheep 2:

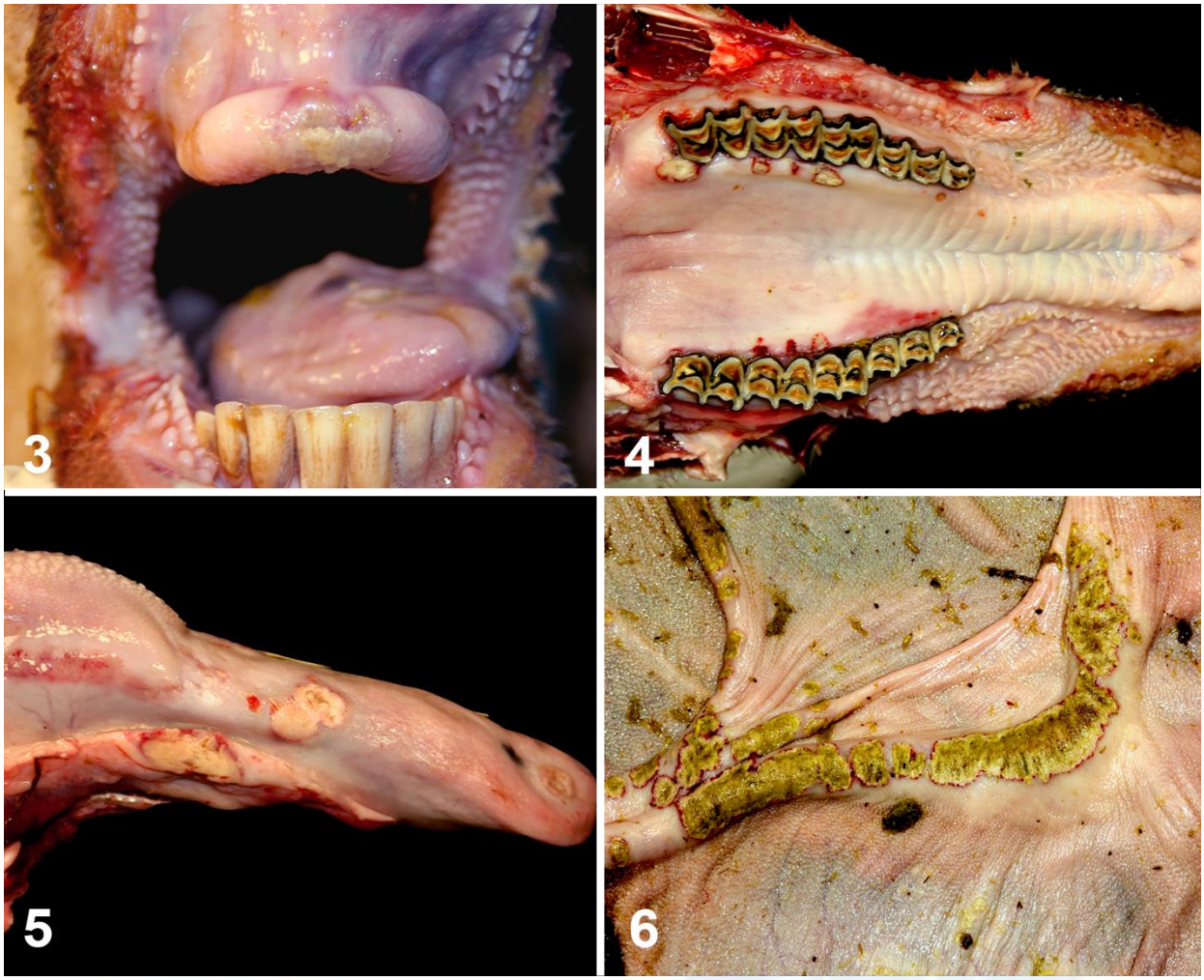


Figure 3. Dental pad. **Figure 4.** Hard palate and gums. **Figure 5.** Tongue. **Figure 6.** Rumen.

Follow-up questions:

1. Morphological diagnoses for figures 1-6.

Figures 1-2. Lung: edema and congestion, diffuse, severe, acute.

Figure 3. Dental pad: severe, focal extensive, acute, ulcerative and fibrinonecrotizing stomatitis.

Figure 4. Hard palate and gums: multifocal, moderate, acute, necro-ulcerative stomatitis

Figure 5. Tongue: multifocal, severe, acute, necro-ulcerative glossitis

Figure 6. Rumen, pillars: multifocal to coalescing, severe, acute, necro-ulcerative rumenitis

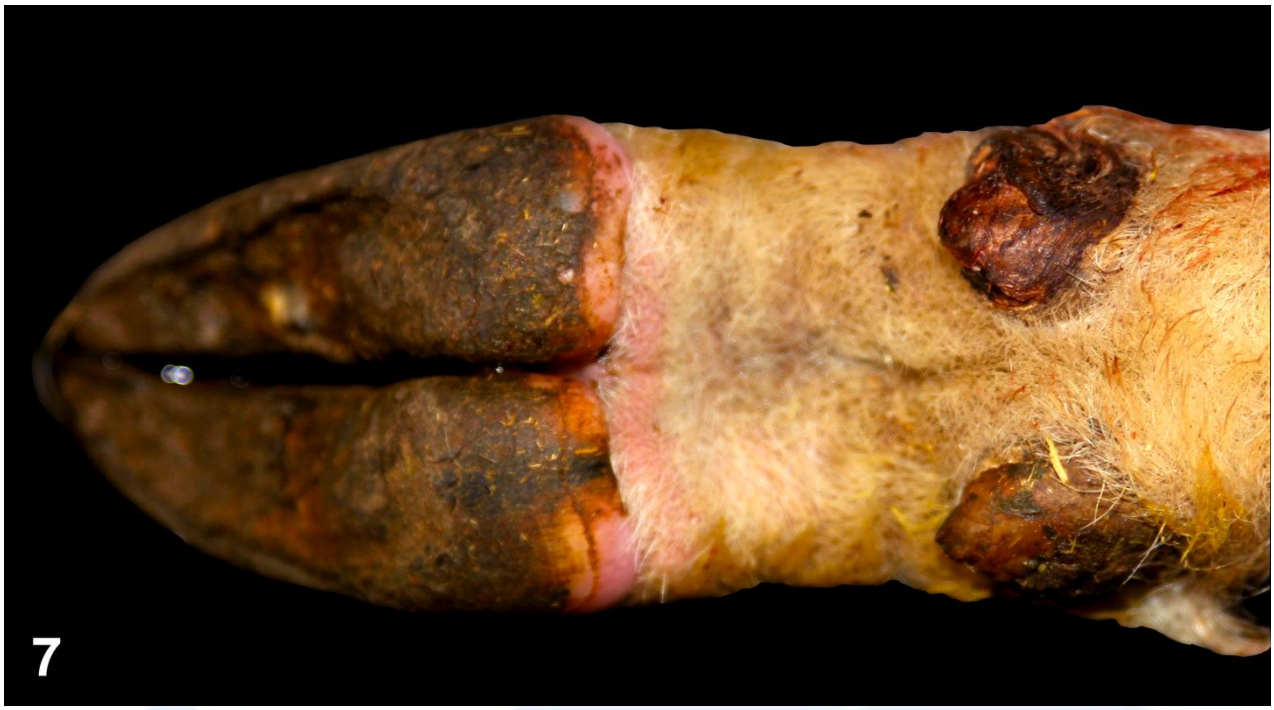


Figure 7. Hoof of sheep 2.

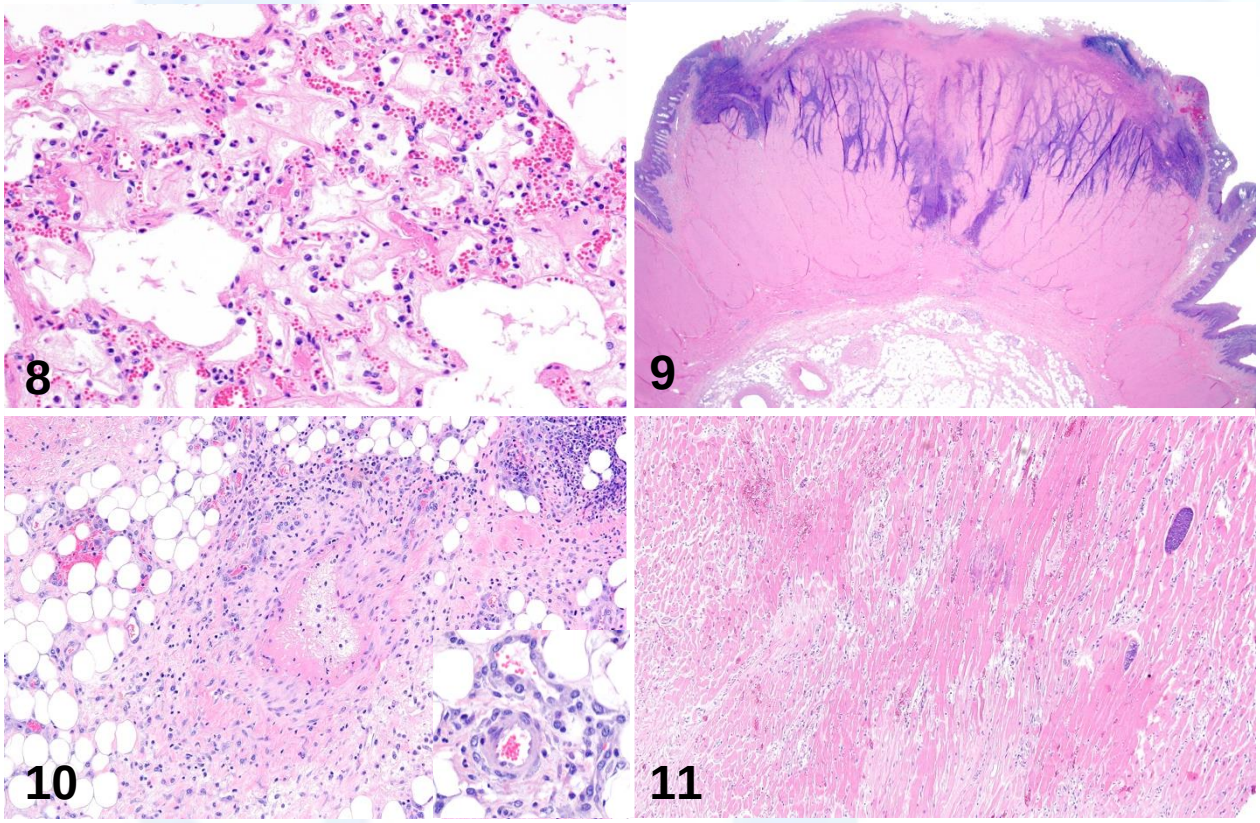


Figure 8. Microvascular thrombosis and alveolar edema in the lung, sheep 1. **Figure 9.** Focal ulceration of ruminal mucosa, sheep 2. **Figure 10**. Fibrinoid vasculitis with mononuclear cell infiltrates and capillary endothelial cell hypertrophy/hyperplasia (inset) nearby an ulcer, sheep 2. **Figure 11.** Myocardial necrosis, sheep 1.

2. Most likely cause based on clinical history and necropsy findings.

Bluetongue virus (BTV). A collection of concomitant facts is suggestive of bluetongue (BT) disease:

(1) A previous history of access to irrigated outdoor pastures.
(2) Occurrence during fall in California.
(3) Multiple, non-proliferative, non-vesicular, ulcerative and necrotizing lesions in the digestive tract, in combination with fatal cases of severe pulmonary edema.
(4) Mortality rates of <10%.

4. Name at least 1 associated microscopic lesion.
3. Name at least 1 other associated gross lesion you should expect in the hoof and other in the heart.

Hoof - coronitis with prominent hyperemia of the periople and swelling of the coronary band (Figure 7).

Expected microscopic lesions include microvascular thrombosis, edema and hemorrhages in sites with gross lesions; necrosis and ulceration oral mucosae; and skeletal and cardiac muscle necrosis (Figure 8,9,10,11).

Most of them are important notifiable viral diseases. On the top of the list would be foot-and-mouth disease (caused by an *Aphthovirus*) and peste des petits ruminants (*Morbillivirus*), followed by vesicular stomatitis (*Vesiculovirus*) and sheeppox (*Capripoxvirus*). Others could be photosensitization and contagious ecthyma (*Parapoxvirus*).

Discussion
BTV serotype 11 (BTV-11) was detected by RT-qPCR from spleen in both sheep. FMD virus, PPR virus, parapoxvirus, pestivirus, malignant catarrhal fever virus, respiratory syncytial virus, type D enterotoxemia, *Salmonella* sp, and *Mycoplasma* sp. were ruled out via different ancillary tests during the diagnostic work up.

BTV causes BT, the most economically important, non-contagious and vector-borne arboviral hemorrhagic disease of domestic and wild ruminants (1–4). After being transmitted by midges or competent vectors of *Culicoides* spp., the virus replicates extensively in the endothelial cells of multiple organs, causing ischemic necrosis with ulceration, edema and hemorrhages (1,4). BTV is an *Orbivirus* within the family *Reoviridae* with a segmented RNA that has facilitated genetic shift and drift and the generation of up to 29 currently recognized serotypes not uniformly distributed around the world (5); some of these RNA segments have been associated with attenuated vaccine strains (1). The global distribution of serotypes has been limited to tropical/subtropical and temperate areas in the latitudinal band of 40ºN and 35ºS, according to the population dynamics of the *Culicoides* sp. vector (1,3,4,6). Generally, the disease is asymptomatic in sheep, goats and cattle in endemic areas (1,5). Lesions appear in sheep (specially in European breeds) following seasonal incursions of midges into cooler temperate zones adjacent to these latitudes, or in form of epizootics, when new serotypes contact with naïve enzootic populations, since there is limited cross-protection between serotypes (1,5,6). Exceptionally, BTV-8, BTV-6 and BTV-11 in Europe, have been reported in outbreaks as far as 50ºN in northern

countries, possibly due to the impact of global warming on the expansion of *Culicoides* sp. distribution (1,4). BTV-8 induced severe disease in sheep and also in cattle, goats and other ruminants, which are normally considered asymptomatic (1,4,7).

After inoculation, BTV replicates in regional lymph nodes (1,6), where there is dendritic cell necrosis, which contributes to delayed seroconversion and promotes ensuing viraemia with viral distribution throughout most tissues carried by leukocytes, platelets and red blood cells (1,8). Affected sheep may be asymptomatic or suffer a fulminant disease characterized by fever, nasal discharge, labored breathing, hyperemia of oral and nasal mucosa, drooling and edema of the head (1,4,6,7). Animals with more prolonged clinical courses have focal hemorrhages in muzzle, lips and gums, ulceration of dental pad and hard palate and inconsistent congestion/cyanosis of the tongue, which coins the name of the disease. Reluctance to move and lameness may be associated with swelling of the feet due to hyperemia and/or hemorrhage of the coronary band and also due to muscle necrosis (1,4,6,7). Some apparently recovered animals, can die suddenly due to severe progressive pulmonary edema that occur in later stages of fatal infections (1). Gross and microscopic lesions are secondary to direct virus-mediated endothelial damage and the indirect effect of vasoactive and proinflammatory mediators induced by host cells (1,2,7). There are ventral intermuscular and subcutaneous oedema and hemorrhagic effusions, pulmonary edema, hydrothorax, hydropericardium, hemorrhages of tunica media at the base of pulmonary artery, hyperemia, hemorrhages and/or ulceration of esophagus and pre-stomachs and skeletal and myocardial muscle necrosis, specially within the papillary muscle of the left ventricle (1,4,6). Abortion and fetal congenital defects, such as hydranencephaly in sheep and cattle and arthrogryposis, macroglossia, excessive gingiva or dwarf-like fetuses in cattle, have been associated with BTV, especially with vaccine attenuated strains and BTV-8 (1,6).

BTV-11 is one of the widely distributed serotypes in North America transmitted by *C. sonorensis*, together with BTV-10, BTV-13, BTV-17 (and, more recently, BTV-3) (4,5). BTV-1 and BTV-2 are transmitted by *C. insignis* and were considered restricted to the southeastern US, but in 2010, BTV-2 was detected in California (4,5). BT is endemic in ruminant livestock of California with high seasonality in late July to November and with a November-July interseasonality during which the virus survives in female, long-live adult midges (3). BTV can also induce severe disease in North American white-tailed deer, black-tailed deer, bighorn sheep, pronghorn antelope, and milder disease in elk and bison (1,6). Llamas and alpacas can also be affected by BTV, while African ruminants are highly resistant (1,5,6).

References

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*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.
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