Diagnostic Exercise
From The Davis-Thompson Foundation*

Case: **218**; Month: **August**; Year: **2023**
Answer sheet

Title: Coxiella placentitis in a goat
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Clinical History:
An adult female Boer goat gave birth to two weak kids, one of which died within one hour after birth. Hard, firm caruncles were reportedly palpated in the uterus of this doe. The dead neonate and associated placenta were submitted to the Kansas State Veterinary Diagnostic Laboratory for necropsy and ancillary diagnostic testing.

One doe on the farm had recently given birth to an apparently healthy kid as well as a mummified fetus. Two other does on the farm had kidded that season with no apparent issues.

Gross Findings:
The placenta was diffusely and moderately thickened and gelatinous (Figure 1). Most of the cotyledons had multiple, randomly distributed, pinpoint, tan foci. The intercotyledonary areas had multifocal to coalescent pale tan plaques. Moderate amount of thick, tan, cloudy, exudate was adherent along the chorionic surface of the placenta.

No gross lesions were observed on postmortem examination of the neonate.

Gross Image:

Figure 1: Necrosuppurative placentitis. Fibrinopurulent exudate was adherent to the intercotyledonary region, and multiple pale tan foci of necrosis were present in the cotyledons.

Microscopic Images:

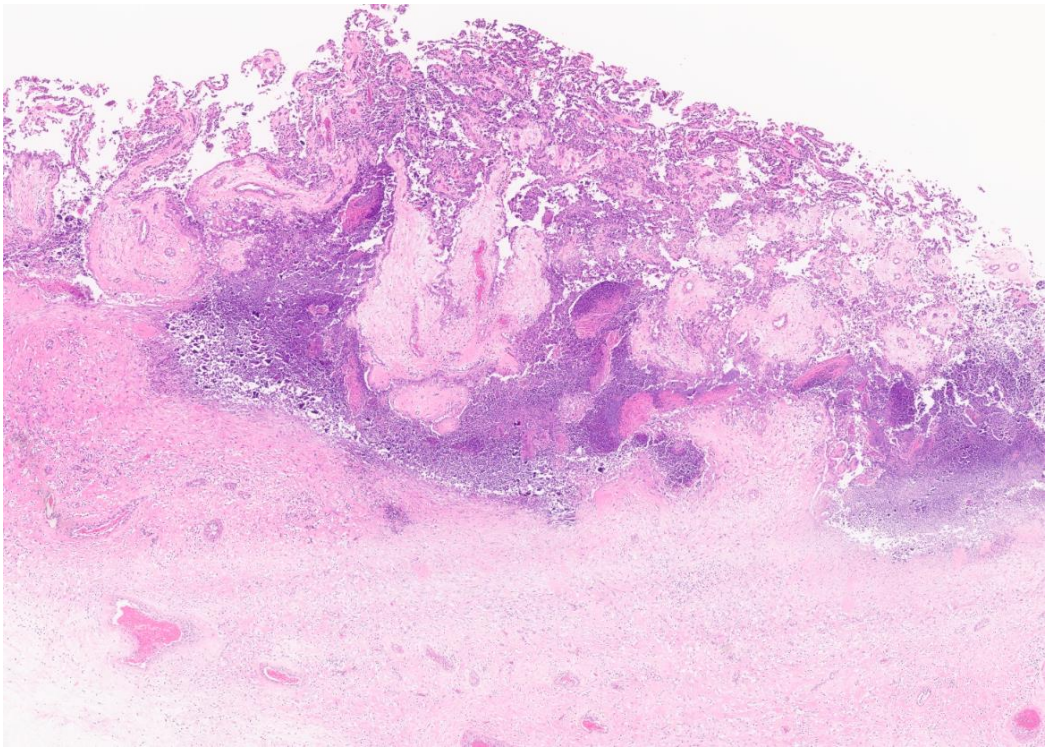

Figure 2: Necrosuppurative placentitis. The chorionic villous architecture is distorted with multifocal to coalescent areas of necrosis and dystrophic mineralization. H&E.

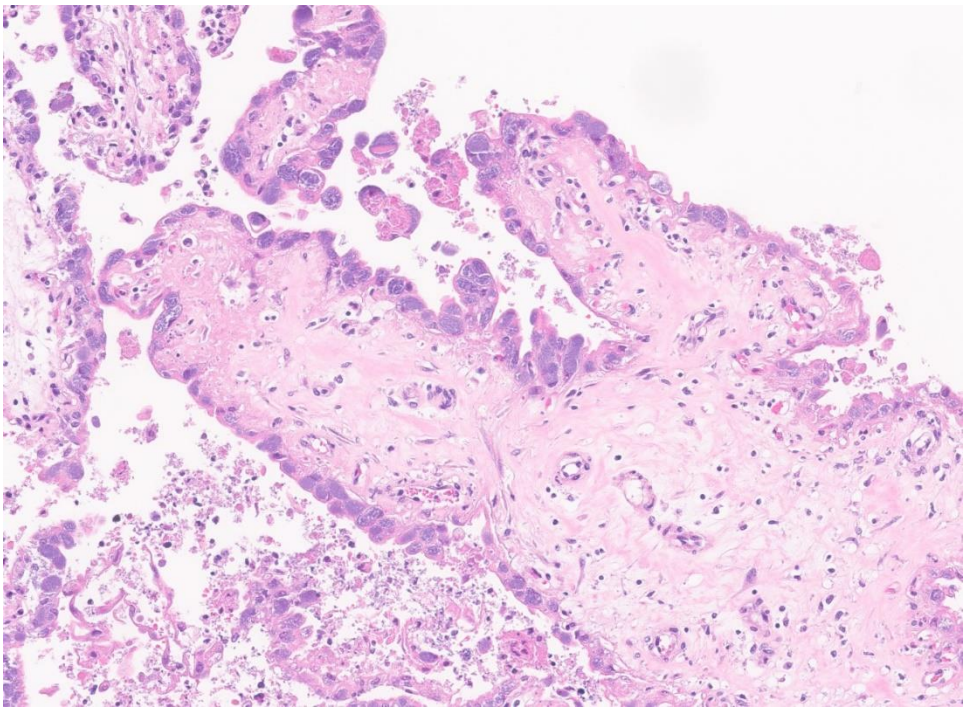

Figure 3: Numerous trophoblasts are distended with intracytoplasmic coccobacilli. H&E.

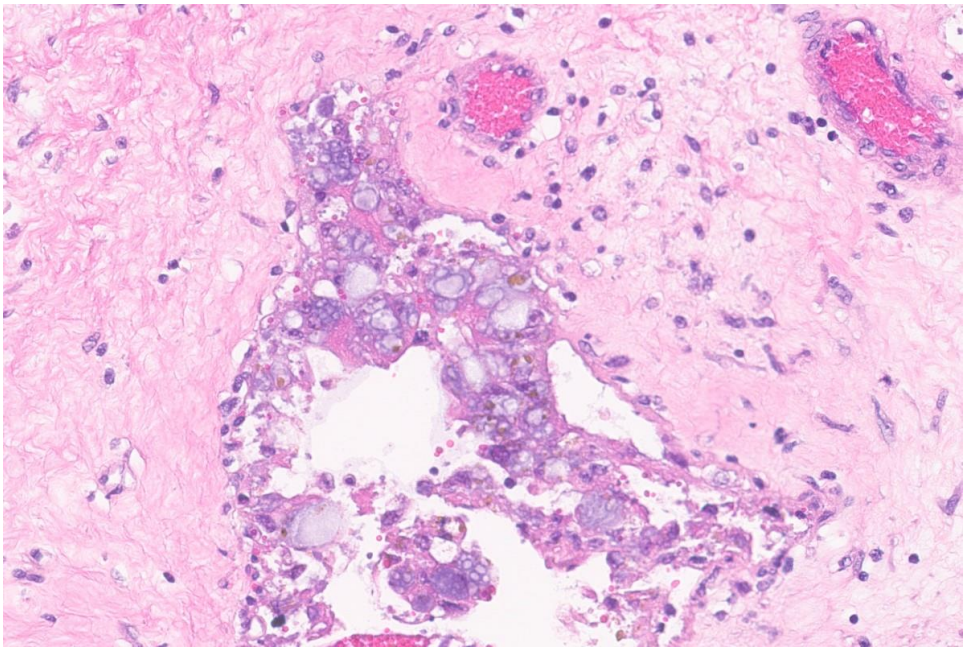

Figure 4: There are frequent intratrophoblastic vacuoles and bacteria characteristic of *Coxiella* species. H&E.

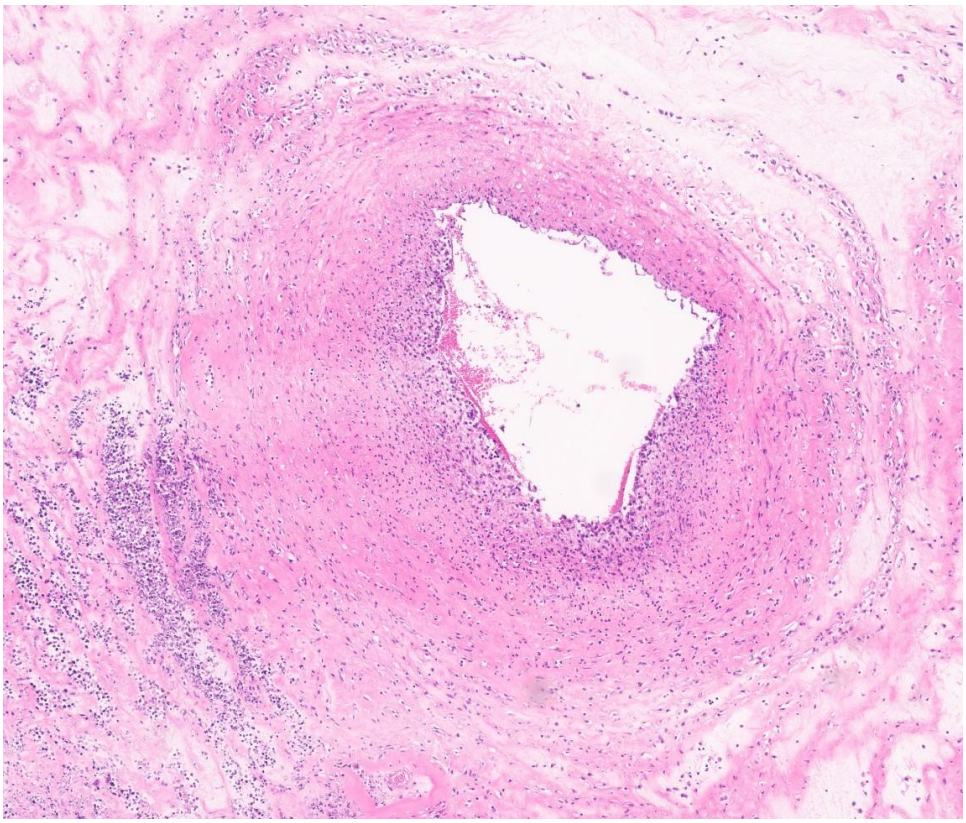

Figure 5A

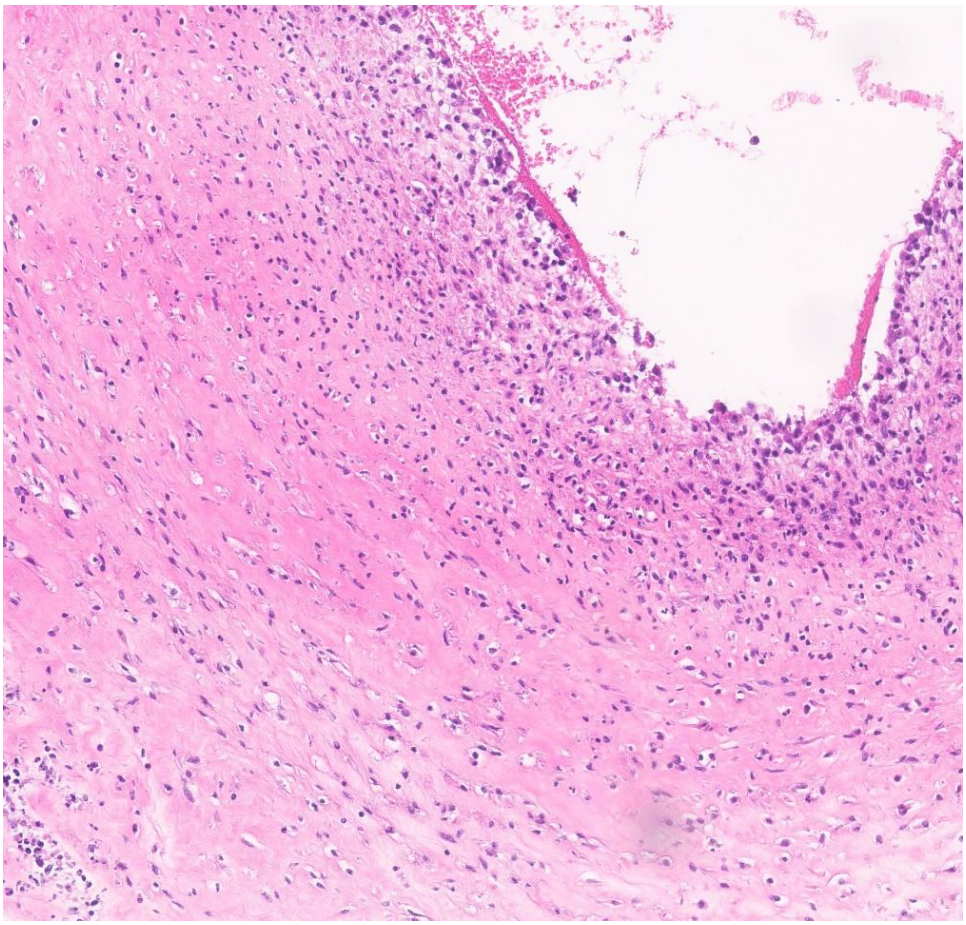

Figure 5B

Figure 5: The tunica adventitia, media, and intima of occasional placental arteries are partially replaced by hyalinized eosinophilic material (fibrinoid vascular necrosis) and infiltrated by low to moderate numbers of neutrophils and karyorrhectic cellular debris (vasculitis). Low (A) and high (B) magnification. H&E.

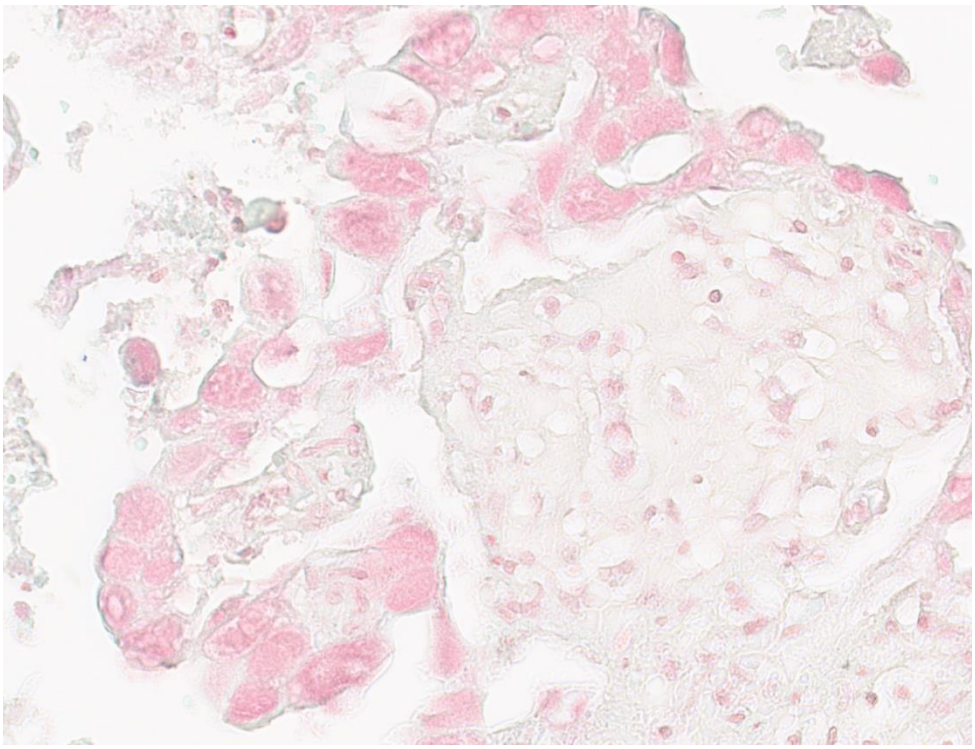

Figure 6: The trophoblasts contain numerous intracytoplasmic Gram-negative coccobacilli. Gram stain.

Gross Morphologic Diagnosis:
Placenta: Moderate multifocal to coalescent necrosuppurative placentitis with fibrinopurulent exudate

Potential Etiologies:
Coxiella burnetii
Chlamydia abortus
Brucella melitensis
Campylobacter fetus

Microscopic Findings:
Along the chorionic surface of the placenta are extensive areas of necrosis, characterized by distortion to loss of the chorionic villous architecture in the cotyledonary regions and by degeneration, necrosis, and loss of trophoblasts lining intercotyledonary and cotyledonary regions with replacement by abundant eosinophilic cellular debris (lytic necrosis). Intermixed with this necrotic material are often numerous degenerating neutrophils and several sloughed/degenerating trophoblasts containing numerous intracellular coccobacilli. Gram stain reveals that these intracellular coccobacilli are gram-negative.
Along the areas of the placenta with intact chorionic epithelium, there are several similar trophoblasts with numerous intracytoplasmic coccobacilli, and the underlying stroma is occasionally expanded by edema and/or infiltrated by low to moderate numbers of lymphocytes and plasma cells. In some areas, the tunica adventitia, media, and intima of the blood vessels are infiltrated by low to moderate numbers

of neutrophils (vasculitis), occasionally accompanied by hyalinized eosinophilic material replacing the tunica media (fibrinoid vascular necrosis). Rarely, the tunica media of the blood vessels within the subepithelial stroma contains deposition of basophilic granular material (mineral).

Histopathologic Diagnosis:
Placenta (chorioallantois): Severe subacute multifocal to coalescent necrosuppurative placentitis with myriad intratrophoblastic gram-negative coccobacilli and neutrophilic vasculitis

Additional Diagnostic Tests:
1. Gram stain was performed on histologic sections of the placenta, which identified the intracellular coccobacilli as gram-negative.
2. Small ruminant abortion tissue PCR panel (performed at Kansas State Veterinary Diagnostic Laboratory):
Coxiella burnetii: Cycle threshold value of 18.21 (positive result).
Cache Valley virus, *Toxoplasma gondii*, *Chlamydia abortus,* Border disease virus: all negative
3. Culture of the placenta:
Brucella: negative
Campylobacter: negative

Etiology:
Coxiella burnetii

Etiologic Diagnosis:
Coxiella placentitis

Comments:
The gross and histologic findings of necrosuppurative placentitis, the presence of myriad intracellular gram-negative coccobacilli, and the results of molecular diagnostics (Ct value of 18.21 for *Coxiella burnetii*) are consistent with coxiellosis (Q fever) as the cause of abortion and neonatal death in this case.

Q fever is a zoonotic disease caused by the intracellular gram-negative bacterium *Coxiella burnetii* and is a reportable disease in the United States. The replication of *C. burnetii* involves the formation of intracellular *Coxiella*-containing vacuoles that represent the fusion of phagosomes with lysosomes to form phagolysosomes (2, 6). These vacuoles are often prominent in H&E-stained sections with infected trophoblasts and macrophages and can be used to tentatively distinguish the organism from other intracellular bacterial causes of placentitis (i.e., *Chlamydia, Brucella*). *C. burnetii* induces a persistent infection that can be asymptomatic in non-pregnant animals but results in reproductive disease and abortion in pregnant animals (6).

Q fever is most common in ruminants, but other vertebrate and invertebrate hosts (mammals, birds, reptiles, ticks) can be infected with the pathogen and may play a role in the epidemiology of the disease and persistence of the pathogen in the

environment (1). Nevertheless, most infections in humans occur through aerosolized bacteria from secretions or placental membranes of infected cattle, sheep, and goats (3). Other routes of infection, including ingestion of contaminated milk and milk products, percutaneous inoculation, and blood transfusion, have also been reported (6, 7).

The most important clinical manifestations of the disease in ruminants are reproductive complications including abortion, weak neonates, and infertility. The pathogen is commonly shed in aborted fetal membranes, vaginal secretions, and milk products, and bacterial shedding can persist for up to several months. Outbreaks of coxiellosis can lead to abortion storms on farms, and appropriate preventative measures should be adopted to reduce both human and animal exposure.

Typical gross findings include necrosuppurative placentitis involving both the cotyledonary and intercotyledonary regions, as well as birth of weak animals or stillborn animals as evident in this case. Necrosuppurative placentitis, vasculitis (to a lesser extent), and large numbers of gram-negative intracellular coccobacilli are common microscopic findings in cases of abortion due to *Coxiella burnetii*.

There are vaccines available for protection against the disease in some countries, but there is currently no licensed vaccine for the prevention of coxiellosis in the United States (4, 5). Given that this disease is reportable, appropriate officials, submitting veterinarian, and owners were notified, and the recommended precautions and tissue disposal requirements were fulfilled in this case.

Other differential diagnoses of placentitis and abortion in small ruminants include brucellosis, toxoplasmosis, campylobacteriosis, and chlamydiosis. Similar to coxiellosis, lesions of brucellosis, campylobacteriosis, and chlamydiosis include necrotizing placentitis affecting both cotyledonary and intercotyledonary regions as well as intratrophoblastic bacteria; however, intracytoplasmic vacuoles as can be seen with coxiellosis are not observed with these other agents. An additional common and prominent feature of *Chlamydia abortus* infection of the placenta is severe vasculitis. *Toxoplasma gondii* typically affects only the cotyledons, in contrast to the previously discussed bacterial infections (8).

References:

1. Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J, Kersh GJ, Limonard G, Marrie TJ, Massung RF, McQuiston JH, Nicholson WL. Diagnosis and management of Q fever—United States, 2013: recommendations from CDC and the Q Fever
2. Working Group. MMWR: Recommendations and Reports 2013; 29:1-29.
3. Eldin C, Mélenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, Mege JL, Maurin M, Raoult D. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin Microbiol Rev 2017; 30(1):115-90.
4. Afonso A, Neubauer H, Needham H, Thiéry R, RODOLAKIS A, Roest HJ, Stark KD, Stegeman JA, Vellema P, van der Hoek W, More SJ. Q fever in humans and farm animals in four European countries, 1982 to 2010. Eurosurveillance 2013; 8(18)

5. Schmeer N, Müller P, Langel J, Krauss H, Frost JW, Wieda J. Q fever vaccines for animals. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Series A: Medical Microbiol, Infectious Dis, Virol, Parasitol 1987; 267(1):79-88.
6. España PP, Uranga A, Cillóniz C, Torres A. Q Fever (Coxiella Burnetii). In Seminars in Respiratory and Critical Care Medicine Thieme Medical Publishers 2020; 41:509-521.
Maurin M, Raoult DF. Q fever. Clin Microbiol Rev 1999; 12(4):518-53.
7. Fishbein DB, Raoult D. A cluster of *Coxiella burnetii* infections associated with exposure to vaccinated goats and their unpasteurized dairy products. Am J Trop Med 1992; 47(1):35-40.
8. Moeller RB. Disorders of sheep and goats. Kirkbride's Diagnosis of Abortion and Neonatal Loss in Animals 2012; 27;4:49-87.

*The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation. 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 CL Davis website (https://davisthompsonfoundation.org/diagnostic-exercise/).

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