Prevalence and concentrations of major liver abscess pathogens in the lungs of feedlot cattle: Assessment of lungs as a potential source for liver abscesses

by

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Abstract

Liver abscesses are an economically significant disease in feedlot cattle, which are estimated to cost $250 million annually because of decreased weight gain, feed efficiency, and carcass weight and liver and viscera condemnations. The incidence of liver abscesses in feedlot cattle ranges from 12 to 32%. The primary pathogens are the two subspecies of *Fusobacterium necrophorum* (subsp. *necrophorum* and *funduliforme*), which are part of bacterial community of the rumen and hindgut of cattle, and two other pathogens, *Trueperella pyogenes* and *Salmonella enterica* are considered as secondary pathogens. Recent studies have also found *Fusobacterium varium* present in the rumen epithelium and contents, indicating a possible role in the development of liver abscesses. The widely accepted pathogenesis is that the pathogens translocate across the gut epithelium to enter portal circulation to reach the liver to cause abscesses. The translocation is facilitated by chronic ruminal acidosis and subsequent rumenitis in the rumen. Rumenitis and acidosis are the consequences of feeding feedlot cattle a high-grain diet, which because of digestion of rapidly fermentable carbohydrates leads to increased acidity and decreased pH of the rumen. The *Fusobacterium* in the ruminal contents decreases but the bacteria adhered to the epithelium escape destruction from the acidity which eventually breaks down the ruminal wall and allows for the translocation of the bacteria to enter the portal circulation. In addition to the gastrointestinal tract, liver abscesses pathogens are also prevalent in the respiratory tract and there is evidence of a potential positive association between liver abscesses and pulmonary lesions. We hypothesized that lungs harbor liver abscess pathogens and serve as a source to enter peripheral circulation and seed the liver to cause liver abscesses. A total of 157 healthy liver tissues and 120 liver abscesses, each with a matched lung tissue, were collected from feedlot cattle at slaughter. Samples were homogenized and subjected to culture

methods, direct and enriched, to detect and isolate, and quantitative PCR (qPCR) to detect and quantify *F. necrophorum* subsp. *necrophorum* and *funduliforme, F. varium, T. pyogenes*, and *S. enterica.* Data were analyzed using R statistical software with tissue type (lungs, healthy liver and liver abscesses) serving as a fixed effect. Fisher's exact test was used to assess the difference in prevalence and ANOVA was used for the analysis of the total concentrations after qPCR confirmation. $P \leq 0.05$ was considered as significant, while meaningful tendencies were discussed when P was > 0.05 and ≤ 0.10. The qPCR assay detected a greater number of lung samples as positive for *F. necrophorum,* either of the two subspecies, subsp. *necrophorum*, and subsp. *funduliforme* than the culture method (43.1 vs 27.7%; 29.2 vs 14.7%; 23.2 vs 15.0%, respectively; $P < 0.01$). The qPCR assay detected greater prevalence of *F. varium* than the culture method (22.7 vs 5.8%; $P < 0.01$). None of the 277 lung tissues were positive for *T. pyogenes* by either the culture or qPCR method. The prevalence of *S. enterica* in lung tissue was also greater by the qPCR method than the culture method (21.3 vs. 18.8%; $P = 0.04$). Liver abscesses were positive for the two subspecies of *F. necrophorum, T. pyogenes,* and *S. enterica* by both detection methods with subsp. *necrophorum* being the most dominant and prevalent at high concentrations (6 to 7 $\log_{10}$ CFU per g). Healthy liver tissues were also positive for the four pathogens, but at a much lower prevalence rates than the liver abscesses. There was a significantly greater prevalence of *F. necrophorum* in the lung tissues of cattle with liver abscesses than cattle that had healthy livers by both culture method and qPCR assay (34.6 vs 22.6%, $P = 0.02$; 50.2 vs 37.1%; $P = 0.03$). A positive association between the prevalence of *F. necrophorum* in the lungs of cattle with livers abscesses versus cattle with healthy livers suggests that lungs may serve as a source. These findings have implications in the development of novel interventions towards the pathogenesis of liver abscesses.

Table of Contents

List of Tables

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Chapter 1 - Literature Review: *F. necrophorum* and *F. varium* in the Respiratory Tract of Animals

Introduction

Fusobacterium is a genus of Gram-negative, anaerobic, non-spore-forming bacilli that comprises part of the normal microbial flora of animals and humans. These bacteria are commonly found in the oral, intestinal, respiratory, and genital tracts of animals and have been associated with several disease processes, particularly in cattle. Although the genus contains 13 recognized species, the species most associated with cattle are *Fusobacterium necrophorum* and *Fusobacterium varium* (Tan et al., 1996; Deters et al., 2025). One of the most common economically significant diseases is liver abscesses in feedlot cattle caused by *F. necrophorum*. Liver abscesses are the primary cause of liver condemnations in feedlot cattle harvested in the U.S. However, major economic impact is because of decreased weight gain, feed efficiency, and overall carcass yield (Nagaraja & Lechtenberg, 2007).

Fusobacterium necrophorum is considered a commensal bacterium because it normally lives in the gastrointestinal tract, specifically in the rumen and hindgut, the oral cavity, the respiratory tract, and the genitourinary tract in different animals (Creemers-Schild et al., 2014). There are two major subspecies, *F. necrophorum* subsp. *necrophorum,* and *fundeliforme* (Tadepalli et al., 2009) They differ in morphological and biochemical characteristics that can make them easily identifiable. Subsp. *necrophorum* has a colony appearance of smooth, opaque, with irregular edges that give it a "fried egg" appearance. Subsp. *fundeliforme* has a colony appearance of a yellowish pinpoint mound with a raised center. *Fusobacterium varium*, another major species of *Fusobacterium,* is described to have "nonhemolytic, semitranslucent, umbonate, with erose edges" colony morphology (Legaira, 2005). In broth cultures, subtle differences in growth

patterns can be observed. *Fusobacterium necrophorum* subsp. *necrophorum* and *Fusobacterium varium* grow with uniform turbidity in broth culture with no sedimentation of cells, while *F. necrophorum* subsp. *funduliforme* exhibits noticeable sedimentation. In addition to these phenotypic differences in culture characteristics, *F. necrophorum* and *F. varium* differ in the virulence factors associated with their pathogenic mechanisms.

The major virulence factors that contribute to *F. necrophorum's* pathogenesis include leukotoxin and the endotoxin or the lipopolysaccharide (LPS) of the cell wall. Leukotoxin protects the bacteria from phagocytosis and is cytotoxic to neutrophils, macrophages, hepatocytes, and ruminal epithelial cells, and is the major differing factor between *F. necrophorum* and *F. varium* (Oelke et al., 2005; Schwarz et al., 2023). Endotoxin initiates inflammation and disseminated intravascular coagulation, which causes the necrosis in the infected tissues or organs. Manson McGuire et al. (2014) classified *F. necrophorum* as a species that does not invade host cells like *F. nucleatum,* and causes disease through tissue necrosis and destruction, rather than direct epithelial invasion. The authors also discovered MORN2 proteins, which are present in the more virulent *Fusobacterium* species including *F. nucleatum*, *F. periodonticum*, and *F. varium,* that may facilitate adhesion, host-cell invasion, adaptions to new environments, and pathogenicity. Manson McGuire et al. (2014) classified *F. necrophorum* as a passive invader, meaning it lacks many of the invasion-associated features found in active tissue invaders, like the MORN2 proteins. Virulence factors of *F. varium* have not been well-characterized, but the species does possess the LPS and outer membrane proteins that mediate adhesion (Brooks et al., 2014). Both *F. necrophorum* and *varium* are known human and animal pathogens and their virulence factors have a primary role in the pathogenesis of many diseases, especially in cattle.

Fusobacterium in Cattle

The *Fusobacterium* genus consists of bacteria present in the normal gastrointestinal tract, the rumen, respiratory tract, genitourinary tract, and the oral cavity of cattle; however, disruption of normal barriers and colonization can allow these bacteria to become opportunistic pathogens (Narayanan & Nagaraja, 2005). *Fusobacterium* is also in the feces and can spread through contamination to other animals (Dornbach et al., 2025). In cattle, *Fusobacterium* species functions as part of the normal microbiota, but is also associated with inflammatory and necrotic diseases, such as liver abscesses, foot rot, calf diphtheria, and metritis.

Although *F. necrophorum* is recognized as the primary etiologic agent of liver abscesses in cattle, recent studies have identified *F. varium* as a potential contributor because of its abundance in the bovine rumen and its presence under culture conditions selective for *F. necrophorum* (Schwarz et al., 2023). However, further research is needed to determine the direct role of *F. varium* in liver abscess pathogenesis (Deters et al., 2025). Liver abscesses can occur in all ages and breeds; however, they are more prevalent in feedlot cattle and rarely exhibit clinical signs (Amachawadi & Nagaraja, 2016). Typically, liver abscesses are considered a polymicrobial infection with *F. necrophorum* as the primary etiologic agent, and two others often associated are *Truperella pyogenes* and *Salmonella enterica* (Amachawadi & Nagaraja, 2016; Fuerniss et al., 2022; Pinnell et al., 2023). This disease occurs when the organisms translocate into the epithelium and enter portal blood and colonize the liver to cause abscesses. The predisposing factors are important, including ruminal acidosis, leading to rumenitis, caused by a high-grain diet. The high-grain diet is associated with the rapid fermentation of carbohydrates, leading to an increase in volatile fatty acids and lactic acids (Ogata et al., 2019). The ruminal acidosis caused by the high-grain diet disrupts the protective barrier of the ruminal epithelium, allowing entrance

of the bacteria into the epithelium and then into the portal blood to reach the liver. This, along with traumatic reticulo-peritonitis, or sharp objects penetrating the reticulum, or foreign objects in the feed, will damage the ruminal epithelium, making it susceptible to the entrance of bacteria like *F. necrophorum* and *varium* (Amachawadi & Nagaraja, 2016; Deters et al., 2025).

F. necrophorum is also associated with metritis, a common disease of dairy cows, which is a uterine wall infection that usually takes place 7 to 10 days after calving (Várhidi et al., 2024). Calving causes a major disruption in the uterine environment, allowing for the introduction of many opportunistic pathogens that cause inflammation. Cows with metritis are found to have a fever, larger than normal uteruses, and watery red-brown vaginal discharge, and is considered an economically significant disease because of its impact on reproductive performance and milk production (Várhidi et al., 2024).

In addition to the liver abscesses in feedlot cattle, *F. necrophorum* also causes calf diphtheria and foot rot, which are also significant economically important diseases of cattle. Calf diphtheria is caused by necrosis of the oral, laryngeal, and pharyngeal areas following damage to the oral cavity. *Fusobacterium necrophorum* is a normal inhabitant of the oral cavity, and when there is damage, the organism gains into the mucosal and submucosal areas of the laryngeal region causing necrotic infection, and the buildup of necrotic material can lead to aspiration into the lungs and pneumonia, especially in younger cattle (Pardon et al., 2018). With foot rot, cattle located in areas with damp soil are prone to developing necrotic tissue in the hooves. The soil provides an anaerobic environment, along with damage to the interdigital tissue, which provides a suitable environment for *F. necrophorum* growth (Narayanan and Nagaraja, 2005). *Fusobacterium varium* diseases are less common in cattle, however, are still known to causes abscesses, gangrene, and colitis in humans and animals (Shinjo et al., 1990; Ohkusa et al., 2002;

Legaria et al., 2005; Minami et al., 2009; Manson McGuire et al., 2014; Pett et al., 2014; Shamriz et al., 2015; Rachana et al., 2019; Lee et al., 2022). Deters et al. (2025) reported the isolation of *F. varium* from liver abscesses of beef cattle, suggesting that the species may contribute to liver abscess pathogenesis either independently or synergistically with *F. necrophorum*. In addition, prevalence studies have identified *F. varium* in ruminal fluid, feces, and respiratory-associated tissues, indicating that the organism may be broadly distributed throughout the bovine microbiota (Dornbach et al., 2025). Deters et al. (2024) also found that *F. varium* was highly prevalent in ruminal samples, 77.1% being positive in the ruminal epithelial tissues and 86.5% ruminal contents samples were positive for *F. varium*, which suggest the presence during rumenitis. However, only 3.1% (3/96) of liver abscesses yielded *F. varium* by culture, and only 10.4% were positive by qPCR suggesting that it is unlikely to be a major etiological agent of liver abscesses, unlike *F. necrophorum* (Deters et al., 2024). Overall, *F. necrophorum* and *varium* cause several forms of tissue damage due to their ability to take advantage of their virulence factors and the anaerobic environment, which contributes to their importance in several economically significant diseases of cattle, particularly liver abscesses, calf diphtheria, and foot rot.

Fusobacterium **in the Lungs**

Although *F. necrophorum* is commonly found in the gut, the organism also plays an important role in respiratory and upper airway disease. *Fusobacterium necrophorum* causes a respiratory disease called calf diphtheria, otherwise known as necrotic laryngitis. This disease commonly affects young cattle following injury to the oral or laryngeal mucosa, allowing bacterial invasion and subsequent tissue necrosis (Pardon et al., 2018). *F. necrophorum* is suspected to be a contributory pathogen to cattle pneumonia and has also been isolated in lung

tissue samples from cattle that have contracted both acute and chronic pneumonia (Chirino-Trejo & Prescott, 1983). Chirino-Trejo and Prescott (1983) reported that 45 of 144 (31.2%) of the lungs showing macroscopic pathological pneumonia tested positive for anerobic bacteria, with six of the isolates being *F. necrophorum*. It was also found to be absent in healthy lungs, further suggesting that there is link between the presence of bacteria and the disease. This study also highlighted the potential bacterial synergistic interactions between *T. pyogenes* and *F. necrophorum* that needs to be further studied to imply a cause for cattle pneumonia (Chirino-Trejo & Prescott, 1983). However, there is the potential that instead of being the primary pathogen, *F. necrophorum* could serve as a secondary pathogen, after aerobic bacteria present initiate tissue damage and inflammation. Shanthalingam et al. (2016) reported that out of 70 pneumonic lung samples that were examined by PCR assay, 37% contained *Fusobacterium*, and that the secreted leukotoxin was highly damaging to bighorn sheep leukocytes. However, the authors did not provide evidence that *Fusobacterium* could cause pneumonia in big horn sheep alone, because *Fusobacterium* was also detected in healthy lung samples as well, indicating that there might be a necessary predisposing step, such as the presence of aerobic bacteria. Unlike all the others, Miao et al. (2023) showed that *Mannheimia haemolytica* and *F. necrophorum* subsp. *necrophorum* most discriminating predictors of the severity of pneumonia in sheep, suggesting that they are primary etiological agents of pneumonia in sheep. In pneumonia cases with severe lesions, *Fusobacteria* was found to be in higher concentrations for these samples. Miao et al. (2023) have suggested that rumination could potentially introduce the ruminal bacteria to the lungs and could be the reason for differences in lung microbiota (Miao et al., 2023; Glendinning et al., 2017). There have also been cases in humans where *F. necrophorum* was isolated from the respiratory tract. *Fusobacterium* is a normal inhabitant of the human oropharynx and can

proliferate to the alveoli to cause necrosis and, and Liu et al. (2024) have reported that, *F. necrophorum* was isolated from a human patient diagnosed with lung abscesses with pleural effusion. Also, in another case study, Sonti and Fleury (2015) have described that *F. necrophorum* can produce pulmonary lesions without a prior pharyngitis infection, indicating the potential for *F. necrophorum* to cause disease or lesions in the lung. *Fusobacterium varium* does not have any known respiratory involvement in cattle; however, there are studies that show the presence of *F. varium* in the oral cavity in other animals, such as deer. Necrobacillosis, characterized by purulent necrotic lesions that most commonly affect the mouth, pharynx, lung, liver, or feet of wild ruminants, is associated with the isolation of *F. necrophorum* and *F. varium* (Chirino-Trejo et al., 2003; Handeland et al., 2010; Hattel et al., 2004; Ramos-Vara et al., 2003). Brooks et al. (2014) showed that when looking at isolates from white-tailed deer respiratory tracts, such as the lungs or larynx, *F. varium* accounted for 78.3% of the isolates, making up a large proportion of the isolates, and indicating a potential role in the development of necrobacillosis. Dornbach et al. (2025) also identified both *F. necrophorum* and *F. varium* within the nasal cavity, ruminal fluid, and feces of finishing beef steers, suggesting that these organisms may be more widely distributed throughout the bovine respiratory and gastrointestinal systems than previously recognized. However, Raabis et al. (2021) found little evidence that *Fusobacterium* is a major pathogen of the calf nasopharyngeal microbiota associated with pneumonia, suggesting that *F. necrophorum* is unlikely to be a primary upper respiratory pathogen. This does raise an important question whether the *Fusobacterium* comes from other sites within cattle, like liver or liver abscesses and are spreading to the lungs systemically.

Several studies have demonstrated a relationship between liver abscesses and pulmonary lesions in cattle, suggesting that systemic spread of infection or shared predisposing factors may

contribute to concurrent disease processes. Wypych et al. (2019) describe that healthy lungs harbor dominant microbiota, such as genera *Prevotella*, *Streptococcus*, *Veillonella*, *Fusobacterium* and *Haemophilus.* These bacteria were low in healthy lungs but can proliferate changing the microbiota and causing respiratory diseases. The gut-lung axis describes the relationship between the microbiota in the lungs versus the gut, whereby intestinal microbial communities influence the respiratory microbiota through microbial metabolites, immune-cell trafficking, and systemic inflammatory pathways (Wypych et al., 2019). This concept could introduce the association between liver abscess and lung lesions observed in the same cattle and provide a potential mechanism for an interaction between the gastrointestinal tract and respiratory tract. Based on these reviews a common hypothesis is to examine the relationship between hepatic and pulmonary lesions in cattle. Rezac et al. (2014) investigated the relationship between liver, lung and rumen lesions and found that among the cattle with severe liver abscesses, 4.9% had severe lung lesions and 28.3% had mild lung lesions, while this does not directly prove the spread of the bacteria, it does indicate a potential positive association. Herrick et al. (2024) looked further into this issue and reported that cattle with major liver abscesses had significantly greater rates of lung consolidation than cattle without liver abscesses. In cattle with major abscesses, 40.6% had pleural adhesions, and cattle with severe liver abscesses involving adhesions to the diaphragm also showed high lung consolidations scores, indicating the presence of pleural lesions. All the data indicate a possible connection between lung and liver health (Herrick et al., 2024). In contrast, however, Fernandes et al. (2025) found no association between lung consolidation at weaning and liver condemnation, liver abscesses, or altered liver microbial diversity at slaughter, suggesting that respiratory infection is not related to the development of liver abscesses.

Summary

In summary, *F. necrophorum* and *F. varium*, are common members of the bovine microbiota but can act as opportunistic pathogens under conditions that disrupt normal host protective barriers and allow the introduction of the bacteria. *F. necrophorum* is well recognized as the primary etiologic agent of liver abscesses, calf diphtheria, and foot rot in cattle, whereas the F. *varium* remains less clearly defined despite being identified in the rumen, gastrointestinal tract, and other body sites. Although research has examined the role of *F. necrophorum* in liver abscess development, less is known regarding the prevalence of *Fusobacterium* in lungs. This information highlighted possible correlations between pulmonary lesions and the presence of liver abscesses in cattle. However, conflicting findings regarding the association between respiratory lesions and liver abscess development highlight the need for further investigation. Understanding whether *Fusobacterium* species can contribute to these processes may provide new insights into the pathogenesis, transmission, and systemic impacts of these economically important infections in feedlot cattle. Therefore, a study to determine the prevalence and distribution of *F. necrophorum* and *F. varium* in the lungs of feedlot cattle with and without liver abscesses is warranted.

Chapter 1 References

Amachawadi, R. G., & Nagaraja, T. G. (2016). Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle. *Journal of Animal Science, 94*(4), 1620–1632. https://doi.org/10.2527/jas.2015-0261

Amachawadi, R. G., & Nagaraja, T. G. (2022). Pathogenesis of liver abscesses in cattle. *Veterinary Clinics of North America: Food Animal Practice, 38*(3), 335–346. https://doi.org/10.1016/j.cvfa.2022.08.001

Bista, P. K., Pillai, D., Roy, C., Scaria, J., & Narayanan, S. K. (2022). Comparative genomic analysis of *Fusobacterium necrophorum* provides insights into conserved virulence genes. *Microbiology Spectrum, 10*(6), e00297-22. https://doi.org/10.1128/spectrum.00297-22

Brooks, J. W., Kumar, A., Narayanan, S., Myers, S., Brown, K., Nagaraja, T. G., & Jayarao, B. M. (2014). Characterization of *Fusobacterium* isolates from the respiratory tract of white-tailed deer (*Odocoileus virginianus*). *Journal of Veterinary Diagnostic Investigation*. https://doi.org/10.1177/1040638714523613

Chirino-Trejo, M., Woodbury, M. R., & Huang, F. (2003). Antibiotic sensitivity and biochemical characterization of *Fusobacterium* spp. and *Arcanobacterium pyogenes* isolated from farmed white-tailed deer (*Odocoileus virginianus*) with necrobacillosis. *Journal of Zoo and Wildlife Medicine, 34*(3), 262–268.

Creemers-Schild, D., Gronthoud, F., Spanjaard, L., Visser, L. G., Brouwer, C. N., & Kuijper, E. J. (2014). *Fusobacterium necrophorum*, an emerging pathogen of otogenic and paranasal

infections? *New Microbes and New Infections, 2*(3), 52–57. https://doi.org/10.1002/nmi2.39

Deters, A., Shi, X., Lawrence, T., & Nagaraja, T. G. (2024). First report of isolation of *Fusobacterium varium* from liver abscesses and ruminal and colonic epithelial tissues of feedlot cattle. *Applied Animal Science, 40*(3), 244–249. https://doi.org/10.15232/aas.2023-02512

Deters, A. K., Abbasi, M., Wilson, R., Schneid, K. N., & Nagaraja, T. G. (2025). *Fusobacterium varium* in cattle: Prevalence and concentrations in healthy livers, liver abscesses, and ruminal and colonic epithelial tissues. *Journal of Animal Science, 103*, skaf194. https://doi.org/10.1093/jas/skaf194

Dornbach, C. W., Broadway, P. R., Wells, J. E., Childress, K. D., Thompson-Smith, A. C., Canterbury, L. G., Burdick Sanchez, N. C., Mathieu, J., Schwarz, C., Laverde Gomez, J., Tikhonova, M., Nagaraja, T. G., Galyean, M. L., & Hales, K. E. (2025). Longitudinal assessment of the prevalence of *Fusobacterium necrophorum*, *Fusobacterium varium*, and *Salmonella enterica* in the nasal cavity, ruminal fluid, and feces of finishing beef steers with and without liver abscesses. *Frontiers in Microbiology, 16*, 1565303. https://doi.org/10.3389/fmicb.2025.1565303

Fernandes, I. L. B., Cantor, M. C., Fonseca, A., Ganda, E., & Felix, T. L. (2025). Effects of lung consolidation at weaning on feedlot performance, carcass characteristics, liver health, and liver microbiota of beef × dairy cattle. *Journal of Animal Science, 103*(1).

Handeland, K., Boye, M., Bergsjø, B., Bondal, T., Isaksen, K., & Agerholm, J. S. (2010). Digital necrobacillosis in Norwegian wild tundra reindeer (*Rangifer tarandus tarandus*). *Journal of Comparative Pathology, 143*(1), 29–38. https://doi.org/10.1016/j.jcpa.2009.12.005

Herrick, R. T., Rogers, C. L., Jones, T. A., McEvers, T. J., Brown, T. R., Maxwell, C. L., & Lawrence, T. E. (2024). Association of liver-abscess presence and severity with trim loss, slaughter yield, carcass grading performance, lung lesions, and value of fed-Holsteins. *Applied Animal Science, 40*(4), 376–385. https://doi.org/10.15232/aas.2023-02471

Hofstad, T. (2006). The genus *Fusobacterium*. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The Prokaryotes*. Springer. https://doi.org/10.1007/0-387-30747-8_51

Lee, S. J., Baek, Y. J., Kim, J. N., Lee, K. H., Lee, E. H., Yeom, J. S., Choi, J. Y., Ku, N. S., Ahn, J. Y., Kim, J. H., et al. (2022). Increasing *Fusobacterium* infections with *Fusobacterium varium*, an emerging pathogen. *PLoS ONE, 17*, e0266610. https://doi.org/10.1371/journal.pone.0266610

Legaria, M. C., Lumelsky, G., Rodriguez, V., & Rosetti, S. (2005). Clindamycin-resistant *Fusobacterium varium* bacteremia and decubitus ulcer infection. *Journal of Clinical Microbiology, 43*(8), 4293–4295. https://doi.org/10.1128/JCM.43.8.4293-4295.2005

Liu, Y., Li, Z., Fu, H., Ruan, W., Wang, H., Ding, Y., & Zhang, M. (2024). The first case report: Diagnosis and management of necrotizing *Fusobacterium* lung abscess via BALF next-generation sequencing. *BMC Infectious Diseases, 24*, 218. https://doi.org/10.1186/s12879-024-09087-5

Manson McGuire, A., Cochrane, K., Griggs, A. D., Haas, B. J., Abeel, T., Zeng, Q., Nice, J. B., MacDonald, H., Birren, B. W., Berger, B. W., et al. (2014). Evolution of invasion in a diverse set of *Fusobacterium* species. *mBio, 5*(6), e01864-14. https://doi.org/10.1128/mBio.01864-14

Miao, Y., Zhao, X., Adam, F. E. A., Xie, Q., Feng, H., Ding, J., Bai, X., Wang, J., & Yang, Z. (2023). Characterization of the lung microbiota in sheep with varying degrees of pneumonia severity. *Animals, 13*(17), 2763. https://doi.org/10.3390/ani13172763

Nagaraja, T. G., & Lechtenberg, K. F. (2007). Liver abscesses in feedlot cattle. *Veterinary Clinics of North America: Food Animal Practice, 23*(2), 351–369. https://doi.org/10.1016/j.cvfa.2007.04.002

Narayanan, S. K., & Nagaraja, T. G. (2005). *Fusobacterium necrophorum* infections in animals: Pathogenesis and pathogenic mechanisms. *Anaerobe, 11*(4), 239–246. https://doi.org/10.1016/j.anaerobe.2005.01.007

Oelke, A. M., Nagaraja, T. G., & Wilkerson, M. J. (2005). The leukotoxin operon of *Fusobacterium necrophorum* is not present in other species of *Fusobacterium*. *Anaerobe, 11*(3), 123–129. https://doi.org/10.1016/j.anaerobe.2004.10.003

Pardon, B., Hostens, M., Duchateau, L., & Deprez, P. (2018). Use of a national identification database to determine the prevalence of necrotic laryngitis in cattle in Belgium from 2009 through 2016. *Journal of Veterinary Internal Medicine, 32*(4), 1454–1461. https://doi.org/10.1111/jvim.15202

Raabis, S., Ollivett, T. L., Caswell, J. L., Cerioli, M., Liman, M., & Buczinski, S. (2021). Association of nasopharyngeal bacterial communities with lung ultrasonographic scores in preweaned dairy calves. *Journal of Dairy Science, 104*(3), 3386–3403.

Ramos-Vara, J. A., Ganjam, I., & Fales, W. (2003). Pulmonary necrobacillosis in a white-tailed deer. *Veterinary Record, 152*(13), 401–403. https://doi.org/10.1136/vr.152.13.401

Rezac, D. J., Thomson, D. U., Bartle, S. J., Osterstock, J. B., Prouty, F. L., & Reinhardt, C. D. (2014). Prevalence, severity, and relationships of lung lesions, liver abnormalities, and rumen health scores measured at slaughter in beef cattle. *Journal of Animal Science, 92*(6), 2595–2602. https://doi.org/10.2527/jas.2013-7222

Schwarz, C., Mathieu, J., Gomez, J. L., Miller, M. R., Tikhonova, M., Nagaraja, T. G., & Alvarez, P. J. J. (2023). Unexpected finding of *Fusobacterium varium* as the dominant *Fusobacterium* species in cattle rumen: Potential implications for liver abscess etiology and interventions. *Journal of Animal Science, 101*, skad130. https://doi.org/10.1093/jas/skad130

Shanthalingam, S., Narayanan, S., Batra, S. A., Jegarubee, B., & Srikumaran, S. (2016). *Fusobacterium necrophorum* in North American bighorn sheep (*Ovis canadensis*) pneumonia. *Journal of Wildlife Diseases, 52*(3), 616–620. https://doi.org/10.7589/2015-02-039

Sonti, R., & Fleury, C. (2015). *Fusobacterium necrophorum* presenting as isolated lung nodules. *Respiratory Medicine Case Reports, 15*, 80–82. https://doi.org/10.1016/j.rmcr.2015.05.011

Tadepalli, S., Narayanan, S. K., Stewart, G. C., Chengappa, M. M., & Nagaraja, T. G. (2009). *Fusobacterium necrophorum*: A ruminal bacterium that invades the liver to cause abscesses in cattle. *Anaerobe, 15*(1–2), 36–43. https://doi.org/10.1016/j.anaerobe.2008.05.005

Várhidi, Z., Csikó, G., Bajcsy, Á. C., & Jurkovich, V. (2024). Uterine disease in dairy cows: A comprehensive review highlighting new research areas. *Veterinary Sciences, 11*(2), 66. https://doi.org/10.3390/vetsci11020066

Wypych, T. P., Wickramasinghe, L. C., & Marsland, B. J. (2019). The influence of the microbiome on respiratory health. *Nature Immunology, 20*(10), 1279–1290. https://doi.org/10.1038/s41590-019-0451-9

Chapter 2 - Prevalence and concentrations of major liver abscess pathogens in lungs of feedlot cattle: Assessment of lungs as a potential source for liver abscesses

Introduction

Liver abscesses are a significant health and economic concern in the beef cattle industry, resulting in an estimated $256 million in annual losses due to reduced performance, carcass trimming, organ condemnation, and processing inefficiencies (Taylor et al., 2025). They are the primary reason for liver condemnations in feedlot cattle harvested in the U.S. However, the major economic impact is because of decreased weight gain, feed efficiency, and overall carcass yield (Nagaraja and Lechtenberg, 2007). The disease is a polymicrobial infection caused by *Fusobacterium necrophorum*, which is regarded as the primary etiologic agent, along with two other bacterial species, *Trueperella pyogenes* and *Salmonella enterica*. The genus *Fusobacterium* is a Gram-negative, anaerobic, non-spore-forming bacilli that includes 13 recognized species, and the two species most associated with cattle *are Fusobacterium necrophorum and F. varium* (Tan et al., 1996; Nagaraja et al., 2005; Deters et al., 2025). These bacteria are commonly found in the gastrointestinal, respiratory, and genital tracts of animals and have been associated with several disease processes, particularly in cattle (Shinjo et al., 1990; Nagaraja, 2022; Umaña et al., 2019; Foster et al., 2009). *Trueperella pyogenes* is a Gram-positive, facultatively anaerobic, opportunistic pathogen, while *S. enterica* is a Gram-negative, facultative anaerobe.

The presence of *Fusobacterium* in the gastrointestinal tract, particularly the rumen and the hindgut of cattle, translocation of the pathogens across the gut epithelium, and entry into the portal circulation to reach the liver to cause abscesses is well established (Nagaraja and

Lechtenberg, 2007; Abbasi et al., 2025a). Besides the gut, *Fusobacterium* is also part of the normal bacterial community of the respiratory and genito-urinary tracts of cattle (Amachawadi & Nagaraja, 2022; Kilama et al., 2025). Wypych et al. (2019) have described that healthy lungs harbor microbiota, such as *Prevotella*, *Streptococcus*, *Veillonella*, *Fusobacterium* and *Haemophilus.* These bacteria were low in healthy lungs but can proliferate changing microbial community composition and contribute to respiratory diseases. The gut-lung axis describes the interactions between the microbiota in the lungs and the gut, whereby intestinal microbial communities influence the respiratory microbiota through microbial metabolites, immune-cell trafficking, and systemic inflammatory pathways (Wypych et al., 2019). This concept suggests possible association between liver abscesses and lung diseases in cattle and provides a potential mechanism for an interaction between the gastrointestinal tract and respiratory tract. Rezac et al. (2014) investigated the relationship between liver, lung and rumen lesions and reported that among cattle with severe liver abscesses, 14.9% had severe lung lesions, 28.3% had mild lung lesions, and 56.8% had no detectable pulmonary lesions. Herrick et al. (2024) have reported that cattle with major liver abscesses had significantly greater rates of lung consolidation than cattle without liver abscesses. In cattle with major abscesses, 40.6% had pleural adhesions, and cattle with severe liver abscesses involving adhesions to the diaphragm also showed high lung consolidations scores, indicating the presence of pleural lesions. All the data indicate a possible connection between lung and liver health (Herrick et al., 2024). In a study reported by Shanthilingam et al. (2016) *F. necrophorum* was isolated from 37% of pneumonic lungs, 40% of healthy lungs and 31% of pharyngeal swabs in big horn sheep. A further indication of a correlation between liver abscesses and the lungs is that the secondary pathogens of liver abscesses, *T. pyogenes* and *S. enterica*, also cause lung diseases in several animal species (Jensen

et al., 1976; Johnston et al., 2017). *Trueperella pyogenes* is also a normal inhabitant of the respiratory tract and causes chronic lower airway pathogens including suppurative bronchopneumonia in cattle, creating extensive lung abscesses (Quinn et al., 2011).

Fusobacterium necrophorum and *F. varium* are common members of the bovine microbiota and can be opportunistic pathogens under conditions that disrupt normal host protective barriers and allow the introduction of the bacteria. *Fusobacterium necrophorum* is well recognized as the primary etiologic agent of liver abscesses, calf diphtheria, and foot rot in cattle (Tan et al., 1996; Nagaraja et al., 2005), whereas *F. varium* remains less clearly defined despite being identified in the rumen, gastrointestinal tract, and other body sites (Deters et al., 2025). There are two major subspecies of *F. necrophorum,* subsp. *necrophorum,* and *funduliforme* (Tadepalli et al., 2009). They differ in morphological and biochemical characteristics that can make them easily identifiable, and subsp. *necrophorum* is more virulent subspecies. Because of the evidence available to suggest a gut-lung axis in cattle, we hypothesized that bovine lungs harbor *F. necrophorum* and lungs could be a source and pathway for pathogens to enter peripheral circulation and seed the liver to cause liver abscesses**.** The objectives of the study were to determine prevalence and concentrations of the pathogens, the two subspecies of *F. necrophorum, F. varium, T. pyogenes* and *S. enterica,* in lungs of feedlot cattle, collected at slaughter, and relate the prevalence to healthy or abscessed livers of feedlot cattle.

Materials and Methods

Sample collection

A total of 277 beef carcasses were sampled at random at a packing plant located in the High Plains of Texas. Samples were collected on 11 random days, approximately 2 to 3 weeks apart, during a 5-month period in 2024. From each carcass, the following samples were collected: a

large piece of lung tissue from the caudal lobe, a large piece of healthy liver tissue from the caudate lobe close to the portal vein entry, and the large slice of liver with an intact abscess. Samples were placed individually in freezer bags and shipped in a cooler with ice or ice packs and shipped overnight to the Anaerobe Laboratory in the College of Veterinary Medicine at Kansas State University.

Sample processing

The samples were processed within 24 h after collection. Each tissue sample was placed on a clean cutting board. A wide metal spatula was flamed in a Bunsen burner till it became red hot and was pressed onto the surface of the lung and healthy liver tissues to sterilize. Using a sterile scalpel, the tissue was sliced through on the sterilized surface to cut out approximately 20 g of internal tissue. Similarly, the surface of the abscess capsule was flame sterilized, incised with a sterile scalpel to open the abscess, and a sterile cotton swab was used to swab the inner capsule. In addition, approximately 20 g of the internal capsule wall mixed with minimal purulent material were collected. The tissue samples were placed in a Nutribullet Pro Blender (NutriBullet, Los Angeles, CA) cup and 80 mL of phosphate buffered saline (PBS), pH 7.4, was added and the lid was secured. Each sample was homogenized for DNA extraction for qPCR assay and for direct plating onto blood agar plates (for isolations of *Fusobacterium* spp. and *Trueperella pyogenes*), Hektoen Enteric (HE) agar for isolation of *Salmonella* and inoculated into enrichment broths, pre-reduced and anaerobically sterilized peptone-yeast extract (PY) broth containing 100 mM of sodium lactate (Sigma-Aldrich Co., St. Louis, MO; PY-La) and lysine hydrochloride (Sigma-Aldrich Co.) as major carbon sources and supplemented with josamycin (3 µg/ml), vancomycin (4 µg/ml), and norfloxacin (1 µg/ml) (PY-La-Ly-JVN; Deters et al., 2024a; Abbasi et. al 2025a) for *F. necrophorum,* and tetrathionate and Rappaport-Vassiliadias R10

broths for *S. enterica* (Abbasi et al., 2025a). In addition to the culture method, two qPCR assays were used. A four-plex qPCR assay that targeted the two subspecies of *F. necrophorum, T. pyogenes,* and *S. enterica*, and a three-plex assay that targeted *F. varium* which both were used to determine prevalence and concentration (Abasi et al., 2025a).

Culture Method

For direct plating of purulent materials of abscesses and homogenized samples onto blood and HE plates, sterile swabs were used to transfer the sample, followed by streaking with sterile inoculating loops for isolation. The plates for isolations of *F. necrophorum, F. varium* and *S. enterica* were incubated in an Anaerobic Glove Box (Bactron Anaerobic Chamber, Cornelius, OR) at 37°C for 48 hours. Another set of inoculated HE plates was incubated aerobically at 37 C for 48 h. Plates for *T. pyogenes* were incubated for 48 h at 37°C in 5% CO_2 atmosphere. The tubes were incubated for 24 hours at 37°C. If the direct plating of the purulent material did not yield putative *Fusobacterium* or *Salmonella* colonies, then the enriched samples were streaked onto blood agar and PY-Ly-La-JVN agar for isolation of *Fusobacterium* and HE agar for isolation of *Salmonella.* In addition, 1 mL of the enrichment broths was transferred to 2 mL microcentrifuge tubes for GeneClean DNA extraction and purification. The extracted DNA was used for the 3-plex and 4-plex qPCR assays (Deters et al., 2024a; Abbasi et al., 2025a). The putative colonies on blood agar or HE agar were streaked onto blood agar plates and species, and subspecies confirmations were performed by 4-plex or 3-plex PCR assay. The qPCR confirmed *Fusobacterium* isolates were stored in both anaerobic Brain Heart Infusion (BHI) agar slants and anaerobic BHI broth with 20% glycerol, and species confirmed *Salmonella* and *Trueperella* isolates were stored in aerobic BHI broth with 20% glycerol at -80°C.

qPCR for Detection and Quantification of F. necrophorum

The MagMax-96 DNA Multi-sample Kit (Applied Biosystems, Foster City, CA) was used according to the manufacturer's protocol for the isolation of DNA from liver and lung tissue homogenates for use in the qPCR assay. The GeneClean Turbo Kit (MP Biomedicals, Solon, OH) was used to extract and purify the DNA from samples that were enriched in Py-Ly-La-JVN or TT-RV broth.

A qPCR assay that targeted the promoter region of the leukotoxin gene, *lktA*-n and *lktA*-f, for subsp. *necrophorum* and subsp. *funduliforme*, respectively, was used to detect and quantify the two subspecies of *F. necrophorum*. The *hgdA* gene, which encodes for 2-hydroxyglutaryl dehydratase, was used for species confirmation of *F. varium.* The assay running conditions were as follows: 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 40 s, using the BioRad CFX96 Real-Time System (BioRad, Hercules, CA). If tissue homogenates were negative for *F. necrophorum*, samples enriched in PY-La-JVN or PY-Ly-JVN were tested by qPCR to determine the presence or absence of the two subspecies.

Statistical Analysis

The tissue prevalence within each detection method, culture method, qPCR assay, and culture method or qPCR assay, which is the total prevalence, a generalized linear model [*glm*()] with a binomial error distribution and logit link function from the stats package (R Core Team, 2024) was fitted. Tissue type served as the fixed effect. Initially, collection date was used as a random effect but was not significant ($P > 0.05$*).* The bacterial prevalence in cattle with healthy or abscessed liver was analyzed using a generalized linear model with a binomial error distribution and logit link. When appropriate, pairwise comparisons of estimated marginal probabilities were conducted using a Tukey adjustment for multiple comparisons. For comparison of bacterial concentrations, liver abscess status served as a fixed effect. Bacterial

counts were log (base 10) transformed and an ANOVA was computed using the aov function from the stats package. Tukey *P*-value adjustment was applied for pairwise comparisons (Healthy liver vs. liver abscess) using cld function from multcomp package (Hothron et al., 2008) with the emmeans function from the emmeans package (Lenth, 2024). Kenward-Rogers degrees of freedom approximation were applied to all pairwise comparisons. A P value of ≤ 0.05 was considered significant, and meaningful tendencies were discussed when P was > 0.05 and ≤ 0.10.

Results

Prevalence of F. necrophorum, F. varium, T. pyogenes and S. enterica in lung tissues

The total prevalence, which includes before and after enrichment of homogenized lung tissues, based on the culture method, qPCR assays or either of the two methods are shown in table 1. The qPCR assay detected a greater ($P < 0.01$) number of samples as positive for *F. necrophorum,* either of the two subspecies (43.1% vs 27.7%), and the two subspecies, subsp. *necrophorum* (29.2% vs 14.7%) and subsp. *funduliforme* (23.2% vs 15.0%) than the culture method. The prevalence of subsp. *necrophorum* (29.2% vs 14.7%) in lung tissues were greater than the subsp. *funduliforme* by qPCR (29.2% vs. 23.2%) or by either of the two methods (33.2% vs. 22.4%). None of the 277 lung tissues were positive for *T. pyogenes* by either the culture or qPCR method. The qPCR assay also detected more samples as positive for *S. enterica* than the culture method (21.3% vs. 18.8%; $P = 0.04$).

Culture method-based prevalence of F. necrophorum and F. varium in healthy livers, liver abscesses, and lung tissues

Of the 157 healthy liver samples, 31.6 % of the livers were positive for either *F. necrophorum* subsp. *necrophorum* or subsp. *funduliforme*, and 1.3% were positive for both

subspecies based on the culture method. All liver abscesses (n=120) were positive for either *F. necrophorum* subsp. *necrophorum* or subsp. *funduliforme* (100%), and 10% were positive for both subspecies. The differences in prevalence of *F. necrophorum,* either of the two subspecies or both together, between the healthy and liver abscesses were significant ($P < 0.01$). In liver abscesses, a majority of either of the two subspecies were isolated by direct plating of the homogenized tissues and two samples yielded subsp. *funduliforme* after enrichment. In contrast, the number of healthy liver tissues that yielded the two species were similar or greater after than before enrichment. The prevalence of subsp. *necrophorum,* either alone (no subsp. *funduliforme*) or total (with or without subsp. *funduliforme*) were greater ($P < 0.01$) in abscesses compared to healthy livers (76.1% vs 15.9% and 90% vs. 19.2%, respectively). In contrast, the prevalence of subsp. *funduliforme,* either alone (no subsp. *necrophorum*) or total (with or without subsp. *necrophorum*) were similar ($P > 0.10$) between liver abscesses and healthy livers (8.7% vs 11.7% and 20.0% vs. 13.1%, respectively). Only a few healthy livers and liver abscesses yielded *F. varium* and almost all were from samples that were subjected to enrichment (Table 2). There was a significant difference between *F. varium* alone between healthy and abscessed livers (7% vs 0%; $P < 0.01$). However, there was no difference between the total number of isolations between healthy and abscessed livers for *F. varium* ($P = 0.55$).

In lung tissues, the prevalence of *F. necrophorum* and *F. varium,* determined by culture method, are presented in relation to whether cattle had healthy or abscessed livers (Table 2). In cattle with liver abscesses, lung tissue had greater ($P = 0.02$) prevalence of *F. necrophorum* compared to cattle with healthy livers (34.6% vs. 22.6%). Of the two subspecies, the prevalence of total subsp. *necrophorum* (either with or without subsp. *funduliforme*) was greater ($P = 0.03$) in lung tissues from cattle with liver abscesses compared to cattle with healthy livers (20.0% vs.

10.7%). More lung tissues yielded subsp. *necrophorum* by direct plating of lung tissue from cattle with liver abscesses compared to cattle with healthy livers (10.8% vs. 2.5%). The prevalence of subsp. *funduliforme,* either alone or total, in lung tissues were not different between cattle with liver abscesses or healthy livers (14.6% vs. 13.3% and 17.1% vs. 13.3%, respectively). Similar to liver tissue, healthy or abscessed, the prevalence of *F. varium* in lung tissue was low (< 10%) and were not different between cattle with healthy liver or with liver abscesses.

qPCR assay-based prevalence of F. necrophorum and F. varium in healthy livers, liver abscesses, and lung tissues

The prevalence of *F. necrophorum* and *F. varium,* determined by the qPCR assay, are presented (Table 3). Of the 157 healthy liver samples, 51.3% of the livers were positive for either *F. necrophorum* subsp. *necrophorum* or subsp. *funduliforme*, and 15.7% were positive for both subspecies based on the qPCR. In liver abscesses, 100% were positive for either *F. necrophorum* subsp. *necrophorum* or subsp. *funduliforme*, and 33.7% were positive for both subspecies. The differences in prevalence of *F. necrophorum,* either of the two subspecies or both together, between the healthy and liver abscesses were significant ($P < 0.01$). Similar to the culture method, a majority of liver abscesses were positive for subsp. *necrophorum* before enrichment. In contrast, more healthy liver tissues were positive after enrichment than before (32% vs 6.1). Almost half of the liver abscess (45.6%) was positive for subsp. *necrophorum* alone (without the subsp. *funduliforme*), The prevalence of subsp. *necrophorum* alone (45.6 vs. 16.5%) or with the subsp. *funduliforme* were greater ($P < 0.01$) in liver abscesses than healthy liver tissues (91.7% vs 35.7% and 45.6% vs 16.5%; respectively). The prevalence of subsp. *funduliforme* in liver abscesses before and after enrichment were similar (20.8% vs. 28.2%,

respectively). The prevalence of subsp. *funduliforme* alone, without the subsp. *necrophorum* was not different ($P = 0.59$) between healthy liver tissue and liver abscesses (10.4% vs. 8.4%). However, the total prevalence was significantly greater (P- 0.01) in liver abscesses than healthy liver tissues (47.2% vs. 33.1%). For *F. varium,* the qPCR method detected one sample before enrichment, and there was no significant difference between the prevalence of abscessed versus healthy livers after enrichment (21.3% vs 15.6%; $P = 0.21$). For the prevalence of *F. varium* alone, there was a greater prevalence for the healthy livers than the abscessed livers (5.2% vs 0%; $P < 0.01$). There was no significant difference between abscessed in healthy livers for the total prevalence of *F. varium* (21.5% vs 16.3%; $P = 0.27$).

In lung tissues, cattle with liver abscesses had greater ($P = 0.03$) prevalence of *F. necrophorum* (either subsp. *necrophorum* or *funduliforme*) than cattle with healthy livers (50.2% vs. 37.1%). There was no significant difference between lung tissues positive for both subspecies together for cattle with healthy livers and with abscessed livers (6% vs 8.2%; $P = 0.44$). All lung tissue samples were positive after enrichment, except for two samples, for subsp. *necrophorum,* and cattle with abscessed livers showed a greater prevalence in lung tissues than in cattle with healthy livers (34.7% vs 24.2%; $P = 0.04$). The prevalence of subsp. *necrophorum* alone was not different, however, the total prevalence (before and after enrichment) was greater in lung tissues from cattle with abscessed livers than cattle with healthy livers (36.5% vs 24.2%; $P = 0.02$). Similar to the subsp. *necrophorum,* subsp. *funduliforme* was detected by qPCR in all samples after enrichment, except for one sample, with no significant difference between cattle with healthy and abscessed livers. The detection of subsp. *funduliforme* alone nor total prevalence in lung tissues between cattle with healthy or with liver abscesses did not differ ($P = 0.21$ and $P = 0.57$, respectively).

For *F. varium* in the lungs, a majority was positive after enrichment and the prevalence alone (without *F. necrophorum)* or total prevalence (before and after combined were not different between cattle with healthy livers or with abscessed livers ($P = 0.48$ and $P = 0.65$, respectively).

Culture method-based prevalence of T. pyogenes and S. enterica in healthy livers, liver abscesses, and lung tissues

In healthy liver tissues, *T. pyogenes* was isolated only from 2 samples (1.3%) and in liver abscesses, 14 of 120 (11.7%) were positive and the difference was significant ($P < 0.01$). None of the healthy liver tissue yielded *S. enterica* by direct plating of the homogenized samples, but liver abscesses were positive for *S. enterica* before enrichment (Table 4). However, samples that were enriched were positive for *S. enterica,* and the total prevalence between cattle with healthy livers or with liver abscesses did not differ (15.2% vs. 16.6%; $P = 0.36$). In cattle with healthy livers or with liver abscesses, none of the lung tissues yielded *T. pyogenes*. One lung tissue in cattle with healthy liver and two lung tissues in cattle with liver abscesses yielded *S. enterica* before enrichment and the total prevalence in lung tissues after enrichment were 17.2% in cattle with healthy livers and 20.8% in cattle with liver abscesses and the difference was not significant ($P = 0.16$)

qPCR assay-based prevalence of T. pyogenes and S. enterica in healthy livers, liver abscesses, and lung tissues

In contrast to the culture method, qPCR assay detected *T. pyogenes* in one healthy liver tissue sample (0.6%) and 13 liver abscesses (10.8%) and the difference was significant ($P < 0.01$). For *S. enterica*, all the samples that were positive were after enrichment, and there was no significant difference between the prevalence of healthy livers and abscessed livers (17.7% vs 22.7%; $P = 0.29$).

In lung tissues, the prevalence of *T. pyogenes* and *S. enterica,* determined by qPCR, are presented in relation to whether cattle had healthy or abscessed livers (Table 5). None of the lung tissues were positive for *T. pyogenes,* regardless of whether cattle had abscessed or healthy livers. *Salmonella enterica* was positive by qPCR in one abscessed liver tissue sample before enrichment. In samples after enrichment, the prevalence was 19.7% and 22.5% in lung tissues of cattle with healthy livers and in cattle with liver abscesses (19.7% and 23.3%, respectively; Table 5).

Concentrations of F. necrophorum, T. pyogenes and S. enterica in healthy livers, liver abscesses, and lung tissues

The number of samples that were quantifiable and the mean concentrations ($\log_{10}$ CFU per g) of the two subspecies of *F. necrophorum, T. pyogenes,* and *S. enterica* in liver and lung tissues are shown in table 6 Only a few healthy liver tissues (≤ 11 of 157) had quantifiable concentrations of *F. necrophorum* and the concentrations ranged from 4.22 to 5.06 $\log_{10}$ CFU per g. In contrast, all liver abscess samples, except 3 (117 of 120; 97.5%) had quantifiable concentrations, which ranged from 6.40 to 7.22 $\log_{10}$ CFU per g. The concentrations of the two subspecies were similar. Only one healthy liver tissue had quantifiable concentration of *T. pyogenes* compared to 13 liver abscesses, but the difference in concentration was not significant (4.10 to 5.62 $\log_{10}$ CFU per g; $P = 0.14$). Neither healthy liver tissue nor liver abscesses had quantifiable concentration of *S. enterica.* Across all bacterial species, except *T. pyogenes* and *S. enterica*, cattle with abscessed livers had a greater percentage of samples quantifiable and concentration than cattle with healthy livers ($P < 0.01$).

In cattle with healthy liver tissue, none of the lung tissues had quantifiable concentrations of *F. necrophorum* (both subspecies) or *T. pyogenes.* Only one lung tissue in cattle with healthy

liver tissue had quantifiable concentration of *S. enterica* (5.73 $\log_{10}$ CFU/g) (Table 6). In cattle with liver abscesses, only 3 lung tissues out of 120 had quantifiable concentrations with a mean concentration of 4.25 $\log_{10}$ CFU/g. Two of the 3 had subsp. *necrophorum* and one had subsp. *funduliforme*. None of the lung tissues had quantifiable concentrations of *T. pyogenes* and *S. enterica* (Table 6).

Discussion

An important finding of the study was the prevalence of major bacterial pathogens associated with liver abscesses, particularly the two subspecies of *F. necrophorum,* in apparently healthy lung tissues, collected at slaughter, from feedlot cattle. The prevalence estimates of the pathogens were based on both culture method and qPCR assay. The prevalence of *F. necrophorum,* based on the qPCR method, was generally greater than the culture method. Because *F. necrophorum* is an anaerobe, although somewhat aerotolerant, and lung tissue is a highly aerobic organ, it is possible that the number of viable bacteria is lower than the nonviable bacteria. In a study of anaerobic bacterial flora of pneumonic lungs (n=144) in cattle determined by culture method, 45 lungs yielded a total of 73 anaerobic isolates and 6 of them were identified as *F. necrophorum* (Chirino-Trejo and Prescott, 1983). In another study conducted in big horn sheep, *F. necrophorum* was isolated from 37% of pneumonic lungs, 40% of healthy lungs and 31% of pharyngeal swabs (Shanthilingam et al., 2016). In both studies, there was no attempt at identifying the subspecies of *F. necrophorum* that were prevalent in lung tissues. In studies that have utilized DNA sequence-based analyses of the resident bacterial communities of the upper (nasopharyngeal) and lower (lung tissue) regions of the respiratory tract of healthy cattle or cattle with bovine respiratory disease, phylum *Fusobacteria* and genus *Fusobacterium* were consistently identified (Timsit et al., 2018; Amat and Alexander, 2025). In such DNA sequence-

based studies, the identification was only at the genus level, and not at the species or subspecies level. In the current study, the identification by the culture method or qPCR assay identified prevalence at the subspecies level, and the subsp. *necrophorum,* the primary pathogen in liver abscesses responsible for approx. 80% of liver abscesses, was greater than the subsp. *funduliforme,* which is the primary etiological agent only in approx. 15% of liver abscesses (Abbasi et al., 2025a). The other species of *Fusobacterium, F. varium,* was included in the study because the species is a known respiratory pathogen of ruminants, including deer (Brooks et al., 2014; Chirino-Trejo et al., 2003). Brooks et al. (2014) reported that a total of 23 clinical isolates of *Fusobacterium* spp. were recovered at necropsy over a 2-year period from the respiratory tract of white-tailed deer (*Odocoileus virginianus*). The majority of isolates were identified as *F. varium* (18/23), and the remaining were *F. necrophorum* subsp. *funduliforme* (3/23), and *F. necrophorum* subsp. *necrophorum* (2/23). The low prevalence of *F. varium* suggests that the species is not a major commensal and not likely to be contributing to respiratory infection in cattle

Surprisingly, none of the lung tissues were positive for *T. pyogenes* by either culture method or qPCR assay. The absence contradicts many previous studies that have reported *T. pyogenes* as a common commensal in lungs of cattle, healthy or with respiratory disease, and in wild deer, and swine (Dong et al., 2019; Ji et al., 2026; Magossi et al., 2025). This bacterium is an opportunistic pathogen that normally inhabits the epithelium of the gastrointestinal, respiratory and urogenital tracts, and the skin (Jost and Billington, 2005; Magossi et al., 2025; Rezewuska, 2019). The prevalence of *S. enterica* in apparently healthy lungs has been reported previously. In a study from Germany, Sinell et al. (1984) have reported that 31 of 45 (68.9%) lung samples collected from wholesale meat market contained were positive for *S. enterica.*

The prevalence of the two subspecies of *Fusobacterium* and *S. enterica* in apparently healthy lung tissues raises the question of the origin of the pathogens. It is possible that bacteremia in situations of infections caused by the pathogens such as necrobacillosis and Salmonellosis, could seed the lungs and other internal organs, which is not likely to happen in healthy cattle. *Fusobacterium necrophorum* is a normal inhabitant of the oral cavity, where it is known to cause necrotic laryngitis (Panciera et al., 1989; Amachawadi et al. 2026). Also, *F. necrophorum* being a ruminal bacterium will be brought to the mouth during regurgitation step of the rumination. Therefore, it is logical to assume that the organism in the mouth may find its way down to the lower respiratory tract. In addition, both *F. necrophorum* and *S. enterica,* being in the gut contents, are excreted in the feces (Dornbach et al., 2025; Fegan et al., 2004; Smith and Thornton, 1993), which when becomes dry can get aerosolized in the feedlot dust and inhaled by cattle. Dust in feed yards, especially during summer months, has been shown to carry bacteria, including pathogens, which get inhaled and enter the respiratory tract of cattle (Wilson et al., 2002; Miller et al., 2008).

In case of *Salmonella* in gut contents, because it is a facultatively intracellular pathogen, the organism can translocate into the subepithelial region of the gut and get engulfed by the phagocytic cells to be transported to the regional LN (mesenteric LN) and eventually into the blood for systemic distribution into internal organs (Li et al., 2002). In poultry, respiratory system is a recognized portal of entry and has been identified as a likely pathway for *S. enterica* transmission throughout the internal organs (Kallapura et al., 2014).

The prevalence and concentrations of the three pathogens reported in liver abscesses, with *F. necrophorum* subsp. *necrophorum,* being the most dominant, are in agreement with other recent studies from our laboratory that have utilized culture- and qPCR-based methods (Abbasi et al.,

2025a; Abbasi et al., 2025b; Salih et al., 2025; Schneid et al., 2026). However, the higher prevalence of the pathogens detected by the qPCR assay than the culture method in the current study was not consistently observed in other studies. More often, the two methods did not differ, and in some instances, culture method detected more liver abscess samples as positive compared to the qPCR assay. Similar to the current study, the other studies detected more samples as positive for *Salmonella* after enrichment than before enrichment, which also meant none of the samples had quantifiable concentrations of *Salmonella* (Abbasi et al., 2025b). The lower prevalence of the pathogens in healthy parenchymal tissue of the liver is not surprising, however the study does confirm our previous observation on the prevalence of the three pathogens in healthy liver tissue, particularly *F. necrophorum* and *Salmonella,* suggesting that liver abscesses are an outcome of successful evasion of the hepatic defense system by the pathogens (Abbasi et al., 2025b). Both culture and culture-independent methods (16S amplicon-sequence- and metatranscriptomic-based) have indicated that healthy liver harbor a resident bacteriome and most of the bacteria originate from the gut because of the bidirectional connection between liver and the gut via portal blood and bile (Stotz et al., 2021; Asakura et al., 2022; Park et al., 2023).

Liver abscesses are considered as part of a disease complex where ruminal lesions are the primary foci and the liver abscesses are the secondary foci of infection (Amachawadi and Nagaraja, 2022). The pathogenesis associated with rumenitis is because of the intake of diet rich in highly fermentable carbohydrates in a typical high-grain finishing diet. The resulting increased acidity of ruminal and hindgut contents causes breakdown in the epithelial integrity of the epithelial barrier, which allows *F. necrophorum,* and possibly, secondary pathogens to colonize and enter the portal blood to reach the liver to induce liver abscesses. Besides the rumen, the other fermentative compartment, the hindgut, also harbors *F. necrophorum* in the contents and

the epithelium (Abbasi et al., 2025a; Salih et al, 2025), which is another source of the pathogens that reach the liver. (Abbasi et al., 2025a). However, based on the prevalence and the concentration, rumen is the major source of *F. necrophorum,* and hindgut is the major source of *S. enterica* (Abbasi et al., 2026). Abbasi et al., (2025a) analyzed *F. necrophorum* prevalence in ruminal epithelium and colonic epithelium, detected by both culture and qPCR method, collected at slaughter from 252 cattle with liver abscesses, 40 of them had ruminal epitheliums that were negative for both subspecies by both detection methods of *F. necrophorum* but still found that 125 of the cattle had liver abscesses that tested positive for *F. necrophorum*. The hindgut has also been proposed as a potential source for *F. necrophorum* which also showed similar results in the 141 cattle that were negative for subspecies in the colonic epithelium, 125 of them still had *F. necrophorum* detected in their liver abscesses. This absence in the rumen and colon but still the continued ability to develop liver abscesses indicates that the *F. necrophorum* could be coming from a different source. The authors of the study hypothesized that lungs could harbor liver abscess pathogens and serve as a source to enter the liver, via blood circulation, to cause liver abscesses.

An interesting finding of the study was a significantly greater prevalence of *F. necrophorum* subsp. *necrophorum,* and not the subsp. *funduliforme,* in lung tissues of cattle with liver abscesses than in cattle that had healthy livers in both culture method and qPCR. Also, a few of the lung tissues from cattle with liver abscesses, and none from cattle with healthy tissues, had quantifiable concentrations of *F. necrophorum*. Of the two subspecies, subsp. *necrophorum* is the primary pathogen prevalent in approx. 80% of liver abscesses, and subsp. *funduliforme* alone is responsible for approx. 15% of liver abscesses (Abbasi et al., 2025a). The subsp. *F. funduliforme* is the normal inhabitant of the GI tract and is prevalent in all cattle and the subsp.

subsp. *necrophorum* is prevalent in some (approx. 50%), but not in all feedlot cattle (Deters et al., 2024b). The finding of a positive association raises the possibility that lungs may be another source of *F. necrophorum,* besides the gut, to reach the liver to cause liver abscesses. However, causation that lung tissue is a source requires further confirmation based on whole genomic sequence analysis to prove clonal identity between lung and liver abscess isolates from the same animal.

In conclusion, the study provides data to show that lungs harbored two of the three pathogens implicated or associated with liver abscesses of cattle. The greater prevalence of subsp. *necrophorum,* the primary pathogen of liver abscesses, in the lung tissue of cattle with liver abscesses compared to cattle with healthy livers by culture- and qPCR-based methods, suggest that lungs may serve as a source of the pathogen. Interestingly, the prevalence of the secondary pathogens, subsp. *funduliforme* and *S. enterica* were not different between cattle with healthy livers or abscessed livers. The positive association of subsp. *necrophorum* between lung tissue and liver abscesses needs to be confirmed by clonal identity by whole genome sequencing. If confirmed, any novel interventions to prevent liver abscesses should consider lungs as a possible target.

Table 1. Prevalence of major liver abscess-pathogens, based on culture method or quantitative PCR assay(qPCR), in lung tissues (n=277) of feedlot cattle collected at slaughter

	Prevalence, % (No. of samples positive)[a]			Culture vs. qPCR *P* value
	Culture method	qPCR	Culture method or qPCR assay	
Fusobacterium necrophorum	27.8 (77)	43.0 (119)	48.7 (135)	< 0.01
Subsp. *necrophorum*	14.7 (41)	29.2 (80)	33.2 (92)	< 0.01
Subsp. *funduliforme*	15.0 (38)	23.2 (57)	22.4 (62)	< 0.01
Fusobacterium varium	5.8 (16)	22.7 (63)	24.2 (67)	< 0.01
Trueperella . pyogenes	0	0	-	-
Salmonella enterica	18.8 (52)	21.3 (59)	21.7 (60)	0.04

[a] Prevalence includes samples positive before and after enrichment of homogenized tissue samples

Table 2. Culture-based prevalence of the two subspecies of *Fusobacterium necrophorum* and *Fusobacterium varium* in healthy liver tissue and liver abscesses, and in lung tissues from cattle with healthy or abscessed livers

	Prevalence, % (No. of samples positive)			Prevalence, % (No. of samples positive)		
	Liver			Lung tissue		
Bacterial species	**Healthy**	**Abscesses**	***P*-value**	**Cattle with healthy livers**	**Cattle with abscessed livers**	***P*-value**
No. of samples cultured	157	120		157	120	
subsp. *necrophorum* or *funduliforme*	31.6 (47)	100 (120)	< 0.01	22.6 (36)	34.6 (41)	0.02
subsp. *necrophorum* and *funduliforme*	1.3 (2)	10 (12)	< 0.01	0 (0)	1.7 (2)	0.10
Subsp. *necrophorum*						
Isolation by direct plating of homogenized tissues	10.2 (15)	91.5 (108)	< 0.01	2.5 (4)	10.8 (13)	< 0.01
Isolation by plating homogenized tissues enriched in PY-La-Ly-JVN[1] broth	8.7 (14)	0	< 0.01	7.8 (13)	9.1 (11)	0.70
Subsp. *necrophorum* alone	15.9 (25)	76.1 (91)	< 0.01	10.5 (17)	18.4 (22)	0.06
Total number of isolations	19.2 (29)	91.5 (108)	< 0.01	10.7 (17)	20.0 (24)	0.03
Subsp. *funduliforme*						
Isolation by direct plating of purulent material	3.2 (5)	18.6 (22)	< 0.01	3.3 (5)	8.3 (10)	0.07
Isolation by plating homogenized tissues enriched in PY-La-Ly-JVN[1] broth	9.7 (15)	1.2 (2)	< 0.01	9.2 (14)	7.9 (9)	0.68
Subsp. *funduliforme* alone	11.7 (18)	8.7 (11)	0.42	13.3 (19)	14.6 (16)	0.73
Total number of isolations	13.1 (20)	20.0 (24)	0.11	13.3 (19)	17.1 (19)	0.34
F. varium						

Isolation by direct plating of purulent material	0.6 (1)	0	0.38	1.3 (2)	0	0.22
Isolation by plating homogenized tissues enriched in PY-La-Ly-JVN[1] broth	9.7 (12)	8.5 (7)	0.70	4.0 (7)	5.9 (7)	0.47
F. varium alone	7 (11)	0	< 0.01	5.4 (9)	5.4 (6)	1.00
Total number of isolations	9.9 (13)	8.0 (7)	0.55	5.3 (9)	6.2 (7)	0.74

[1]Peptone yeast extract with sodium lactate (100 m*M*) and lysine hydrochloride (100 m*M*) and supplemented with josamycin (3 µg/ml), vancomycin (4 µg/ml), and norfloxacin (1 µg/ml) antibiotics.

Table 3. Quantitative PCR-based prevalence of the two subspecies of *Fusobacterium necrophorum* and *Fusobacterium varium* in healthy liver tissue and liver abscesses, and lung tissues from cattle with healthy or abscessed livers

	Prevalence, % (No. of samples positive)			Prevalence, % (No. of samples positive)		
	Liver			Lung		
Bacterial species	**Healthy**	**Abscesses**	***P*-value**	**Cattle with healthy livers**	**Cattle with abscessed livers**	***P*-value**
No. of samples cultured	157	120		157	120	
subsp. *necrophorum* or *funduliforme*	51.3 (77)	100 (120)	< 0.01	37.1 (59)	50.2 (60)	0.03
subsp. *necrophorum* and *funduliforme*	15.7 (18)	33.7 (34)	< 0.01	6.0 (9)	8.2 (9)	0.44
Subsp. *necrophorum*						
No. of samples positive before enrichment	6.1 (8)	90.5 (107)	< 0.01	0	1.7 (2)	0.10
No. of samples positive after enrichment of homogenized tissues in PY-La-Ly- JVN[1] broth	32.0 (48)	2.5 (3)	< 0.01	23.7 (38)	34.7 (40)	0.04
subsp. *necrophorum* alone	16.5 (29)	45.6 (58)	< 0.01	16.0 (26)	22.1 (26)	0.20
Total no. of samples positive	35.7 (56)	91.7 (110)	< 0.01	24.2 (38)	36.5 (42)	0.02
Subsp. *funduliforme*						
No. of samples positive before enrichment	1.9 (3)	20.8 (25)	< 0.01	0	0.8 (1)	0.25
No. of samples positive after enrichment of homogenized tissues in PY-La-Ly- JVN[1] broth	31.4 (36)	28.2 (21)	0.14	20.2 (30)	22.0 (26)	0.70
subsp. *funduliforme* alone	10.4 (16)	8.4 (10)	0.59	8.8 (14)	13.5 (17)	0.21

Total no. of samples positive	33.1 (39)	36.7 (44)	< 0.01	20.1 (30)	22.7 (27)	0.57
F. varium						
No. of samples positive before enrichment	0.6 (1)	0	0.38	0.7 (1)	0.9 (1)	0.82
No. of samples positive after enrichment of homogenized tissues in PY-La-Ly- JVN[1] broth	15.6 (24)	21.3 (25)	0.21	24.4 (37)	20.4 (24)	0.42
F. varium alone	5.2 (8)	0	< 0.01	17.1 (25)	15.2 (17)	0.65
Total no. of samples positive	16.3 (25)	21.5 (25)	0.27	25.2 (38)	21.6 (25)	0.48

[1]Peptone yeast extract with sodium lactate (100 m*M*), lysine hydrochloride (100 m*M*) and josamycin (3 µg/ml), vancomycin (4 µg/ml), and norfloxacin (1 µg/ml) antibiotics.

Table 4. Culture-based prevalence of *Trueperella pyogenes* and *Salmonella enterica* in healthy liver tissue and liver abscesses, and in lung tissues from cattle with healthy or abscessed livers

	Prevalence, % (No. of samples positive)			Prevalence, % (No. of samples positive)		
	Liver			Lungs		
Bacterial species	**Healthy**	**Abscesses**	***P*-value**	**Cattle with healthy livers**	**Cattle with abscessed livers**	***P*-value**
No. of samples cultured	157	120		157	120	
T. pyogenes						
Isolation by direct plating of homogenized tissues	1.3 (2)	11.7 (14)	< 0.01	0	0	-
S. enterica						
Isolation by plating homogenized tissues before enrichment	0	1.7 (2)	0.07	0.6 (1)	1.6 (2)	0.41
Isolation by plating homogenized tissues after enrichment[1]	15.3 (24)	15.0 (18)	0.62	16.6 (26)	19.2 (23)	0.26
Total number of isolations	15.3 (24)	16.7 (20)	0.36	17.2 (27)	20.8 (25)	0.16

[1]Enriched in tetrathionate broth followed by Rappaport-Vassiliadis broth

Table 5. Quantitative PCR-based prevalence of *Trueperella pyogenes* and *Salmonella enterica* in healthy liver tissue and liver abscesses, and in lung tissues from cattle with healthy or abscessed livers.

	Prevalence, % (No. of samples positive)			Prevalence, % (No. of samples positive)		
	Liver			Lungs		
Bacterial species	Healthy	Abscesses	*P*-value	Cattle with healthy livers	Cattle with abscessed livers	*P*-value
No. of samples cultured	157	120		157	120	
T. pyogenes						
No. of samples positive before enrichment (%	0.6 (1)	10.8 (13)	< 0.01	0	0	-
S. enterica						
No. of samples positive before enrichment of homogenized tissues	0	0	-	0	0.8 (1)	0.20
No. of samples positive after enrichment of homogenized tissues	19.7 (31)	21.7 (26)	0.29	25.8 (31)	22.5 (27)	0.58
Total no. of samples positive (%)	19.7 (31)	22.7 (26)	0.29	25.8 (31)	23.3 (28)	0.14

Table 6. Number of samples quantifiable and concentrations of the two subspecies of *Fusobacterium necrophorum*, *Trueperella pyogenes*, and *Salmonella enterica* determined by qPCR assay, in healthy liver tissue and liver abscesses, and lung tissues from cattle with healthy/abscessed livers

	Cattle with healthy livers (n=157)		Cattle with abscessed livers (n=120)			
Sample type and bacterial species	**No. of samples quantifiable (%)**	**Concentrations, Log_{10} CFU/g**	**No. of samples quantifiable (%)**	**Concentrations, Log_{10} CFU/g**	**SEM**	***P*-value**
Liver tissues						
Subsp. *necrophorum* or *funduliforme,*	11 (7.0)	4.82	117 (97.5)	6.88	0.28	< 0.01
Subsp. *necrophorum* and *funduliforme*	1 (0.6)	4.50	15 (12.5)	6.40	0.82	< 0.01
Subsp. *necrophorum*	8 (5.1)	4.96	107 (89.2)	6.96	0.31	< 0.01
Subsp. *necrophorum* alone	7 (4.5)	5.06	92 (76.7)	6.99	0.32	< 0.01
Subsp. *funduliforme*	3 (1.9)	4.41	25 (20.8)	6.58	0.71	< 0.01
Subsp. *funduliforme* alone	2 (1.3)	4.22	10 (8.3)	7.22	0.57	< 0.01
Trueperella pyogenes	1 (0.6)	4.10	13 (10.8)	5.62	1.09	0.14
Salmonella enterica	NQ[1]	NQ	NQ	NQ	-	-
Lung tissues						
Subsp. *necrophorum* or *funduliforme,*	NQ	-	3 (2.5)	4.25	-	-
Subsp. *necrophorum* and *funduliforme*	NQ	-	NQ	-	-	-
Subsp. *necrophorum*	NQ	-	2 (1.7)	4.23	-	-
Subsp. *necrophorum* alone	NQ	-	2 (1.7)	4.23	-	-
Subsp. *funduliforme*	NQ	-	1 (0.8)	4.29	-	-

Subsp. *funduliforme* alone	NQ	-	1 (0.8)	4.29	-	-
Trueperella pyogenes	NQ	-	NQ	-	-	-
Salmonella enterica	1 (0.6)	5.73	NQ	-	-	-

[1]NQ: Not Quantifiable

Chapter 2 References

Abbasi M, Deters A, Salih H, Wilson RA, Schneid KN, Shi X, George LA, Bai J, Amachawadi RG, Woerner DR, Samuelson KL, Lawrence TE, and Nagaraja TG. Quantification of major bacterial pathogens in liver abscesses and matched ruminal and colonic epithelial tissues of feedlot cattle. *Microbiol Spect*. 2025a; 13(10):e0188825. https://doi.org/10.1128/spectrum.01888-25

Abbasi M, Wilson RA, Shi X, George LA, Woerner DR, Amachawadi RG, and Nagaraja TG. Liver abscess bacterial pathogens in healthy liver tissues, normal tissues of abscessed livers and corresponding ruminal epithelial tissues of beef-on-dairy cattle. *Appl Anim Sci*. 2025b: 41:405-417. https://doi.org/10.15232/aas.2025-02704

Abbasi M, Wilson RA, Schneid KN, Shi X, George LA, Woerner DR, Samuelson KL, Lawrence TE, Amachawadi RG, and Nagaraja TG. Prevalence and concentrations of major bacterial pathogens in liver abscesses and ruminal and colonic epithelial tissues in relation to abscess severity in feedlot cattle. J Anim Sci. 2026; 104:skag180. https://doi.org/10.1093/jas/skag180

Amachawadi RG and Nagaraja TG. Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle. *J Anim Sci*. 2016; 94(4):1620–1632. https://doi.org/10.2527/jas.2015-0261

Amachawadi RG and Nagaraja TG. Pathogenesis of liver abscesses in cattle. *Vet Clin North Am Food Anim Pract*. 2022; 38(3):335–346. https://doi.org/10.1016/j.cvfa.2022.08.001

Amachawadi RG, Miesner M, Beard W, Beard L, Thomson DU, Noffsinger T, Lukasiewicz K, Salih HM, Wang H, Nagaraja TG. *Fusobacterium necrophorum* in necrotic laryngitis of feedlot cattle. *J Vet Intern Med*. *In print*.

Amat S and Alexander TW. Respiratory microbiome and respiratory health: Association with gut microbiome. In: Rumen Health Compendium. Tedeschi LO and Nagaraja TG, editors. 2nd ed. Dubuque, IA: Kendall Hunt; 2025.

Asakura H, Yamamoto S, Sasaki Y, Okada Y, Katabami S, Fujimori A, Munakata K, Shiraki Y, Nishibu H, Hisamoto C, Kawase J, Ojima Y, Kiyoshima A, and Shiroma K. Bacterial distribution and community structure in beef cattle liver and bile at slaughter. *J Food Prot.* 2022; 85(3):424–434. https://doi.org/10.4315/JFP-21-288

Brooks JB, Kumar A, Narayanan S, Myers S, Brown K, Nagaraja TG, and Jayarao BM. Characterization of *Fusobacterium* isolates from the respiratory tract of white-tailed deer (*Odocoileus virginianus*). *J Vet Diagn Invest.* 2014; 26:213–220.

Chirino-Trejo JM and Prescott JF. The identification and antimicrobial susceptibility of anaerobic bacteria from pneumonic cattle lungs. Can J Comp Med. 1983; 47:270-276.

Chirino-Trejo M, Woodbury MR, and Huang F. Antibiotic sensitivity and biochemical characterization of *Fusobacterium* spp. and *Arcanobacterium pyogenes* isolated from farmed white-tailed deer (*Odocoileus virginianus*) with necrobacillosis. J Zoo Wildlife Med. 2003; 34:262-268. doi.org/10.1638/02-019

Deters A, Shi X, Bai J, Kang Q, Mathieu J, and Nagaraja TG. A real-time PCR assay for the detection and quantification of *Fusobacterium necrophorum* and *Fusobacterium varium* in ruminal contents of cattle. *Appl Anim Sci.* 2024a; 40(3):250–259. https://doi.org/10.15232/aas.2023-02507

Deters A, Shi X, Lawrence T, and Nagaraja TG. First report of isolation of *Fusobacterium varium* from liver abscesses and ruminal and colonic epithelial tissues of feedlot cattle. *Appl Anim Sci.* 2024b; 40(3):244–249. https://doi.org/10.15232/aas.2023-02512

Deters AK, Abbasi M, Wilson R, Schneid KN, and Nagaraja TG. *Fusobacterium varium* in cattle: Prevalence and concentrations in healthy livers, liver abscesses, and ruminal and colonic epithelial tissues. *J Anim Sci.* 2025; 103:skaf194. https://doi.org/10.1093/jas/skaf194

Dong WL, Liu L, Odah KA, Atiah LA, Gao YH, Kong LC, and Ma HX. Antimicrobial resistance and presence of virulence factor genes in *Trueperella pyogenes* isolated from pig lungs with pneumonia. *Trop Anim Health Prod.* 2019; 51(7):2099–2103. https://doi.org/10.1007/s11250-019-01916-z

Dornbach CW, Broadwabay PR, Wells JE, Childress KD, Thompson-Smith AC, Canterbury LG, Burdick Sanchez NC, Mathieu J, Schwarz C, Laverde Gomez J, Tikhonova M, Nagaraja TG, Galyean ML and Hales KE. Longitudinal assessment of the prevalence of *Fusobacterium necrophorum, Fusobacterium varium,* and *Salmonella enterica* in the nasal cavity, ruminal fluid, and feces of finishing beef steers with and without liver abscesses. Front. Microbiol. 2025; 16:1565303. doi: 10.3389/fmicb.2025.1565303

Fegan N, Vanderlinde P, Higgs G, Desmarchelier P. Quantification and prevalence of *Salmonella* in beef cattle presenting at slaughter. *J. Appl Microbiol*. 2004; 97:892–898. https://doi.org/10.1111/j.1365-2672.2004.02380.x

Foster AP, Otter A, Naylor R, Wessels ME, and Veenstra B. Hepatitis in a six-month-old lamb with *Fusobacterium varium* infection. *Vet Rec.* 2009; 164(3):98 https://doi.org/10.1136/vr.164.3.98

Ji MJ, Kim Y, Kim BS, Kim J, and Choi KS. *Trueperella pyogenes*-associated acute pneumonia in Korean native calves. *Vet Med Sci.* 2026; 12(3):e70913. https://doi.org/10.1002/vms3.70913

Jost BH and Billington SJ. *Arcanobacterium pyogenes*: molecular pathogenesis of an animal opportunist. *Antonie Van Leeuwenhoek.* 2005; 88:87–102.

Herrick RT, Rogers CL, Jones TA, McEvers TJ, Brown TR, Maxwell CL, and Lawrence TE. Association of liver-abscess presence and severity with trim loss, slaughter yield, carcass grading performance, lung lesions, and value of fed-Holsteins. *Appl Anim Sci*. 2024; 40(4):376–385. https://doi.org/10.15232/aas.2023-02471

Kallapura G, Morgan MJ, Pumford NR, Bielke LR, Wolfenden AD, Faulkner OB, Latorre JD, Menconi A, Hernandez-Velasco X, Kuttappan VA, Hargis BM, and Tellez G. Evaluation of the respiratory route as a viable portal of entry for *Salmonella* in poultry via intratracheal challenge of *Salmonella Enteritidis* and *Salmonella Typhimurium*. *Poultry Sci*. 2014; 93(2):340-346. https://doi.org/10.3382/ps.2013-03602

Li H, Tsolis RM, Bäumler AJ, and Adams LG. Pathogenesis of *Salmonella* infections in cattle. *Vet Microbiol*. 2002; 89(1):1–14. https://doi.org/10.1016/S0378-1135(02)00175-2

Magossi G, Gzyl KE, Holman DB, Nagaraja TG, Amachawadi R, and Amat S. Genomic and metabolic characterization of *Trueperella pyogenes* isolated from domestic and wild animals. *Appl Environ Microbiol*. 2025; 91(1):e0172524. https://doi.org/10.1128/aem.01725-24

Miller MF, Loneragan GH, Harris DD, Adams KD, Brooks JC, Brashears MM. Environmental dust exposure as a factor contributing to an increase in *Escherichia coli* O157 and *Salmonella* populations on cattle hides in feedyards. *J Food Prot*. 2008; 71: 2078- https://doi.org/10.4315/0362-028X-71.10.2078

Nagaraja TG, Narayanan SK, Stewart GC, and Chengappa MM. *Fusobacterium necrophorum* infections in animals: Pathogenesis and pathogenic mechanisms. *Anaerobe*. 2005; 11(4):239–246. https://doi.org/10.1016/j.anaerobe.2005.01.007

Nagaraja TG. 2022. Gram negative anaerobes, In: *Veterinary Microbiology*. McVey DS, Kennedy M, Wilkes R, and Chengappa MM, editors. Ames, IA: John Wiley & Sons, Inc.; 2022.

Nagaraja TG and Lechtenberg KF. Liver abscesses in feedlot cattle. *Vet Clin North Am Food Anim Pract*. 2007; 23(2):351–369. https://doi.org/10.1016/j.cvfa.2007.04.002

Panciera RJ, Perino LJ, Baldwin CA, Morton RJ, Swanson JE. Observations on calf diphtheria in the commercial feedlot. *Agri-practice*. 1989; 10: 12-17.

Park T, Li W, and Zanton G. A meta-analysis of liver microbiome in beef and dairy cattle using metatranscriptome sequencing reads. *bioRxiv.* 2023. https://doi.org/10.1101/2023.03.28.534613

Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S, and Hartigan PJ. *Veterinary microbiology and microbial disease.* 2nd ed. Chichester, UK: Wiley-Blackwell; 2011.

Rezac DJ, Thomson DU, Bartle SJ, Osterstock JB, Prouty FL, and Reinhardt CD. Prevalence, severity, and relationships of lung lesions, liver abnormalities, and rumen health scores measured at slaughter in beef cattle. *J Anim Sci*. 2014; 92(6):2595–2602. https://doi.org/10.2527/jas.2013-7222

Rezewuska M, Kwiecien E, Chrobak-Chmiel D, Kizerwetter-Swida M, Stefańska I, and Gierynska M. Pathogenicity and virulence of *Trueperella pyogenes*: A review. *Int J Mol Sci.* 2019; 20:2727.

Salih HM, Deters AK, Amachawadi RG, Wang H, Mahmood T, Shi X, Abbasi M, George LA, Lawrence TE, and Nagaraja TG. Further delineation of the etiology of liver abscesses in cattle and indication of hindgut as a potential source of pathogens. *Microbiol Spectr.* 2025; 13(11):e0253925. https://doi.org/10.1128/spectrum.02539-25.

Schneid KN, Abbasi M, Young JD, Deters AK, Shi X, George LA, Nagaraja TG, Lawrence TE, Richeson JT, and Samuelson KL. Effects of dietary starch concentration and feeding management regimen on bacterial pathogens in liver abscesses and ruminal and colonic epithelial tissues in feedlot steers. *J Anim Sci*. 2026; 104:skag131. https://doi.org/10.1093/jas/skag131

Shanthalingam S, Narayanan S, Batra SA, Jegarubee B, and Srikumaran S. *Fusobacterium necrophorum* in North American bighorn sheep (*Ovis canadensis*) pneumonia. *J Wildl Dis*. 2016; 52(3):616–620. https://doi.org/10.7589/2015-02-039

Shinjo T, Hiraiwa K, and Miyazato S. Recognition of biovar C of *Fusobacterium necrophorum* (Flügge) Moore and Holdeman as *Fusobacterium pseudonecrophorum* sp. nov., nom. rev. (ex Prevot 1940). *Int J Syst Bacteriol.* 1990; 40(1):71–73. https://doi.org/10.1099/00207713-40-1-71

Sinell HJ, Klingbeil H, and Benner M. Microflora of edible offal with particular reference to *Salmonella. J Food Prot*. 1984; 47(6):481-48

Smith GR and Thornton EA. The prevalence of *Fusobacterium necrophorum* biovar A in animal feces. *Epidemiol Infect.* 1993; 110:327–331. https://doi.org/10.1017/S095026880006826

Stotz MK, Henry DD, and Crossland WL. Characterization of bacterial DNA identified in abscessed and non-abscessed bovine hepatic tissue at the time of harvest. *J Anim Sci.* 2021; 99:1–11. https://doi.org/10.1093/jas/skab280

Tadepalli S, Narayanan SK, Stewart GC, Chengappa MM, and Nagaraja TG. *Fusobacterium necrophorum*: A ruminal bacterium that invades the liver to cause abscesses in cattle. *Anaerobe*. 2009; 15(1–2):36–43. https://doi.org/10.1016/j.anaerobe.2008.05.005

Taylor DD, Groenendaal H, Morley PS, and Zagmutt FJ. Economic costs of liver abscesses in U.S. beef feedlot cattle: A comprehensive economic analysis. *J Anim Sci*. 2025; 103: skaf127. https://doi.org/10.1093/jas/skaf127

Timsit E, Workentine M, van der Meer F, Alexander T. Distinct bacterial metacommunities inhabit the upper and lower respiratory tracts of healthy feedlot cattle and those diagnosed with bronchopneumonia. *Vet Microbiol*. 2018; 221:105-113. https://doi.org/10.1016/j.vetmic.2018.06.007

Umaña A, Sanders BE, Yoo CC, Casasanta MA, Udayasuryan B, Verbridge SS, and Slade DJ. Utilizing whole *Fusobacterium* genomes to identify, correct, and characterize potential virulence protein families. *J Bacteriol.* 2019; 201(23):e00273. https://doi.org/10.1128/JB.00273-19

Wilson, SC, Morrow-Tesch J, Straus DC, Cooley JD, Wong WC, Mitlöhner FM, and McGlone JJ. Airborne microbial flora in a cattle feedlot. *Appl Environ Microbiol*. 2002; 68(7):3238–3242. https://doi.org/10.1128/AEM.68.7.3238-3242.2002

Wypych TP, Wickramasinghe LC, and Marsland BJ. The influence of the microbiome on respiratory health. *Nat Immunol*. 2019; 20(10):1279–1290. https://doi.org/10.1038/s41590-019-0451-9