Salmonella enterica in internal organs of cattle and comparative growth of *Fusobacterium necrophorum* subspecies and *Fusobacterium varium* in lactate- and/or lysine-based enrichment media

by

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Abstract

This report provides a review of prevalence and distribution of *Salmonella enterica* in internal organs of cattle and two experimental studies on *Fusobacterium* species associated with liver abscesses. The review highlights that *S. enterica* primarily colonizes the gastrointestinal tract of cattle and can also spread to internal organs such as the lymph nodes, liver, and lungs, where it can persist as hidden reservoirs, often causing asymptomatic infections that contribute to beef contamination during slaughter. Among the major serotypes of *S. enterica*, *S.* Dublin, *S.* Typhimurium, and *S.* Newport are frequently linked to systemic infections and foodborne outbreaks. The review includes the zoonotic significance of *Salmonella*, its persistence in internal organs, and the challenges of controlling contamination in beef production systems. Key preventive strategies include improving farm hygiene, strengthening biosecurity, vaccination, and adopting proper processing and handling practices to reduce bacterial transmission and protect public health.

Chapter 2 focuses on *Fusobacterium necrophorum and F. varium* involved in bovine liver abscesses and includes two experimental studies. The study 1 was on the detection and quantification *of F. necrophorum* subsp*. necrophorum and F. necrophorum* subsp*. funduliforme* in bovine tissues (healthy livers, abscessed livers, ruminal epithelial and colonic epithelial) and comparison of three enrichment media, Peptone-Yeast extract with lactate, lysine or both; PY-La, Py-Ly, and PY-La-Py). The enrichment containing both lactate and lysine yielded the highest recovery of *F. necrophorum* subspecies, especially from ruminal and colonic tissues, while lysine broth was most effective for liver abscesses. The study 2 was on comparative growth of *F. necrophorum* subsp*. necrophorum, F. necrophorum* subsp*. funduliforme,* and *F. varium* in PY-La, PY-Ly, and PY-Ly-La broths, which examined bacterial growth under anaerobic conditions and found that both *F. necrophorum* subspecies grew best in PY-Ly-La broth, while *F. varium* exhibited the highest growth in PY-Ly broth, indicating distinct substrate utilization patterns.

Together, these findings demonstrate that *Salmonella* and *Fusobacterium* are major pathogens impacting cattle health and in addition, *Salmonella* is a major foodborne pathogen with implications in beef safety. Understanding the persistence of *Salmonella* in internal organs

provides insight for developing more effective control and monitoring programs, while identifying the most effective enrichment medium capable of detecting and quantifying low concentrations of *Fusobacterium* provides a more accurate insight into its distribution in cattle tissue samples.

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Chapter 1- *Salmonella enterica* in Internal Organs of Cattle: A Review

Introduction

Salmonella enterica, a common enteric pathogen, causes a variety of infections in humans and animals, including cattle (Demirbilek, 2017a; Gast and Porter Jr, 2020). The species is a Gram-negative, flagellated, facultatively anaerobic bacterium that can grow in both aerobic and anaerobic conditions (Oludairo et al., 2022). The organisms are facultative intracellular pathogens capable of rapid adaptation to environmental changes (Ibarra and Steele-Mortimer, 2009; Wallace et al., 2016). Low-oxygen environments favor *Salmonella* binding to host cells and are associated with greater virulence and invasiveness (Pradhan and Negi, 2019; Spector and Kenyon, 2012). *Salmonella* can be transmitted from animals to humans and represent a significant zoonotic and epidemiological concerns (Adem, 2022). Transmission from animals to humans occurs mainly via the fecal–oral route, although the respiratory tract and conjunctiva may also act as entry sites (Kallapura et al., 2014; Liu et al., 2023).

The genus *Salmonella* consists of two recognized species: *S. enterica* and *S. bongori* (Grimont et al., 2000). *S. enterica* is divided into six subspecies, of which subsp**.** *enterica* is the most important in animals and humans (Brenner et al., 2000). This subspecies includes over 2,500 identified serotypes, classified based on their antigenic composition, which includes somatic (O), flagellar (H), and capsular (VI) antigens (Grimont and Weill, 2007). These serotypes vary in their pathogenicity, host specificity, and prevalence in different environments (Jajere, 2019).

In the beef cattle industry, salmonellosis is a major health concern that leads to significant economic losses, causes serious disease in cattle, and poses zoonotic risks for farm workers, calf handlers, and their families. (Yada, 2023). Beyond direct contact with animals, most human salmonellosis cases are foodborne, as *Salmonella* can contaminate beef carcass during slaughter and enter the food supply (Giaccone et al., 2012).

Salmonellosis in Cattle

Salmonellosis can lead to significant morbidity and mortality, especially in young calves. It is a common bacterial disease in cattle caused, more often, by certain serotypes of *Salmonella* such as Dublin, Typhimurium, and Newport. The disease appears in different forms depending on the animal's age, health, and environment. The main forms of salmonellosis are enteric, septicemic, and carrier infections. The enteric form is the most common and usually affects calves. It causes fever, watery or bloody diarrhea, dehydration, loss of appetite, and weakness. Calves between 2 and 12 weeks of age are more likely to be affected as their immune system is still developing. The bacteria damage the intestine and cause inflammation and fluid loss. In adult cattle, diarrhea may be mild or chronic and often related to stress or farm conditions. The septicemic form is more severe and may cause death, especially in young or weak calves. The bacteria spread into the bloodstream and cause high fever, depression, and breathing problems. The infection can reach the liver, spleen, and lymph nodes. *Salmonella* Dublin is the serovar most often associated with this form. Some cattle recover but continue to shed *Salmonella* in their feces for a long time without showing any signs of illness, which allows the bacteria to spread to others in the herd. Stress, transportation, or diet changes can increase shedding. Infection may also involve the lungs or mammary glands and resulting in pneumonia or mastitis. Chronic infections can reduce milk production and body weight. Salmonellosis causes economic losses due to death, reduced productivity, and treatment costs. It is also a zoonotic disease that can spread to humans through contact with animals or contaminated meat and milk. Proper management and regular monitoring are important to control the disease in cattle and protect human and animal health. Early and accurate diagnosis is essential for effective control. Diagnosis is based on bacterial isolation from feces, tissues, or lymph nodes using selective media, followed by biochemical and molecular confirmation. PCR and serotyping are commonly used for accurate identification of *Salmonella* strains.

Serogroups and Serotypes in Cattle

Classification of *Salmonella* serotypes is based on somatic (O) antigens, with serovars grouped into serogroups designated by the letters A, B, C, D, and E (Holschbach and Peek, 2017). In routine practice, diagnostic laboratories have serotyped *Salmonella* isolates by

agglutination, targeting O lipopolysaccharide and H flagellar antigens. For many years, serotyping methods have been the main method for diagnosing salmonellosis in humans and animals; however, they are time-consuming and resource-intensive (Wattiau et al., 2011). New molecular methods have replaced traditional ones, and genomic markers or specific targets are used to identify serotypes (Kitchens et al., 2024). The *rfb* gene cluster, which is responsible for the synthesis of O somatic group antigens, and the *fliC/fliB* genes, encoding flagellar antigens, are frequently utilized in genetic-based serotyping. (Fitzgerald et al., 2003; Tang et al., 2019). In cattle, clinically significant isolates are mainly linked to serogroups B, C, and E, together with the host-adapted *Salmonella* Dublin from serogroup D (Nielsen, 2013; Velasquez-Munoz et al., 2024). Recent epidemiological data show that *S.* Cerro (16%), *S.* Newport (14%), *S.* Montevideo (8%), *S.* Kentucky (8%), and *S.* Typhimurium (4%) are the serotypes most often isolated in cattle (Valenzuela et al., 2017)

Common Sites of Colonization in Cattle

The gastrointestinal tract is the main site where *Salmonella* colonizes in cattle (Rukambile et al., 2019). Within this tract, the colon, cecum, and small intestine are the main sites where *Salmonella* can proliferate (Li, 2022). The ileum, located at the distal end of the small intestine, provides favorable conditions for *Salmonella* colonization due to its dense microbial community and close interaction with the host immune system (Galyov et al., 1997). Cattle can carry *Salmonella* without showing signs of disease and shed it in their feces. The amount of shedding increases under stress conditions such as transport. Shedding is usually higher in summer and early fall than in winter (Koyun et al., 2023).

In severe infections, *Salmonella* can colonize lymphatic tissues surrounding the gastrointestinal tract (Gragg, 2012). This facilitates systemic dissemination of the bacteria, resulting in widespread inflammation and tissue damage (Voedisch et al., 2009). Additionally, the respiratory tract and reproductive organs in infected cattle can sometimes serve as secondary sites of colonization (Yoo, 2010). Identifying common sites of colonization is important for improving diagnosis and controlling the spread of *Salmonella* in cattle, as these reservoirs within the animal can contribute to disease transmission.

Significance of Salmonella as a Foodborne Pathogen

Consumption of contaminated foods, such as raw or uncooked meat, eggs, and dairy products, is one of the main ways people become infected with *Salmonella* (Mughini-Gras et al., 2014). As a major foodborne pathogen, *Salmonella* is a common cause of bacterial gastroenteritis, with clinical signs such as diarrhea, abdominal cramps, fever, and vomiting. In severe cases, particularly in children and the elderly, infection may progress to bacteremia and systemic complications that require hospitalization (Crump et al., 2015). Cattle are an important reservoir for *Salmonella*, and the pathogen can enter the food chain through beef and dairy products (Marshall, 2018). Although cattle often carry *Salmonella* asymptomatically, fecal shedding can contaminate the environment, including feed, water, and surfaces encountered during slaughter and processing (Holschbach and Peek, 2018). The grinding of beef can enhance the spread of the bacteria throughout the product, making ground beef a major source of foodborne infection (Omer et al., 2018). *Salmonella* can also survive in unpasteurized dairy products (Gast et al., 2021; LeJeune and Rajala-Schultz, 2009). Cattle-associated *Salmonella* represents a major public health concern due to its role in foodborne infections (Holschbach and Peek, 2018). In the United States, *Salmonella* causes an estimated 1.35 million human infections each year, highlighting its significant impact on human health (Hoffmann et al., 2012). The primary route of transmission to humans is through the consumption of undercooked or contaminated beef, milk, and other cattle-derived products (Bentum et al., 2025). Studies show that about 30- 40% of cattle skin surfaces are contaminated at slaughter, which can lead to cross-contamination during carcass processing (Arguello et al., 2013; Young et al., 2016). *Salmonella* can persist in the gastrointestinal tract of cattle and survive in the environment and on food-contact surfaces, which makes decontamination efforts in slaughterhouses and food processing plants difficult (Zacharski et al., 2018). The bacteria can also be found on dairy farms where animals are stressed or immunocompromised, and raw milk consumption can be a serious risk for humans (LeJeune and Rajala-Schultz, 2009; Oliver et al., 2005). Proper pasteurization lowers the risk but cannot completely remove it if the milk is handled poorly after pasteurization (Olsen et al., 2004; Silva and Gibbs, 2012). The importance of *Salmonella* is not limited to direct contamination, as cattle can carry and spread the bacteria even when they are asymptomatic (Hoelzer et al., 2011). Surveillance programs, strict biosecurity, improved sanitation,

vaccination, and regular testing are important for reducing contamination and keeping food safe (Demirbilek, 2017b; Renault et al., 2021). Educating consumers about proper cooking, handling, and storage is also important for reducing risk of infection (Redmond and Griffith, 2003). Human salmonellosis affects more than 150 million people each year worldwide, leading to serious illness, high healthcare expenses, and economic losses (Popa and Papa, 2021; Teklemariam et al., 2023). In response, many countries have adopted stricter rules and improved monitoring to reduce *Salmonella* levels in cattle and limit its spread through the food chain (Bentum et al., 2025). Effective control requires teamwork in research, surveillance, prevention, and public awareness.

Salmonella in Liver

Studies show that the bovine liver, in addition to its role in metabolism and detoxification, can serve as a site of *Salmonella* colonization (Apte and Krishnamurthy, 2011; Sheppard et al., 2003). Control strategies for *Salmonella* have traditionally emphasized gastrointestinal and lymphatic tissues; however, new evidence suggests that the liver may represent an additional reservoir promoting long-term persistence and dissemination (Gal-Mor, 2018).

The liver's high vascularization allows *Salmonella* to disseminate via the bloodstream following initial intestinal invasion. Once in the hepatic tissues, the bacteria can persist, posing a threat to cattle health and creating a potential source of zoonotic transmission through liver products *Salmonella* survival in the liver is facilitated by its ability to evade host immune responses, colonize hepatocytes, and resist antimicrobial interventions. Although the liver lacks surrounding adipose tissue, its intrinsic structure and vascular network provide an environment where *Salmonella* can maintain infection, highlighting the importance of surveillance and hygienic handling in preventing contamination of liver-derived foods (Harrell et al., 2021; Li, 2022).

Studies have shown that the prevalence of *Salmonella* in bovine abscessed livers can vary between 5% and 35%, depending on the detection technique used and herd management factors (Herrick et al., 2022; Olsen et al., 2015). Compared with non-host adapted strains, such as *S.* Typhimurium, the host-adapted serovar *Salmonella* Dublin is more frequently detected in

systemic sites, including the liver (Paudyal et al., 2019). Risk factors influencing liver colonization include stress, immunosuppression, and poor biosecurity, particularly in calves and chronically infected carriers (Morisse and Cotte, 1994; Velasquez-Munoz et al., 2024). The liver can act as a hidden reservoir for *Salmonella*, supporting persistence and zoonotic transmission, which shows the need for better monitoring and control in cattle production systems (Dróżdż et al., 2021).

The ability of *Salmonella* to infect the liver in cattle is linked to its virulence factors, especially those that allow the bacteria to survive inside cells and evade the host's immune system (Wang et al., 2020). Following ingestion, *Salmonella* invades the intestinal lining, mainly through M cells of Peyer's patches, where it is engulfed by macrophages (Hurley et al., 2014; Owen, 1994; Siebers and Finlay, 1996). *Salmonella* survives within macrophages, allowing it to spread to systemic sites such as the liver. In the liver, it infects Kupffer cells and hepatocytes and relies on virulence factors like the Type III secretion system (T3SS) to alter host signaling and promote intracellular survival (Dai et al., 2024; Kurtz et al., 2017). By altering autophagy and resisting oxidative stress, *Salmonella* can survive and multiply within hepatic macrophages, leading to chronic infection, granuloma formation, and ongoing bacterial shedding (Pham et al., 2020).

The growth of *Salmonella* in the liver shows the effect of environmental and physiological factors. Anaerobic culture on blood agar produces large, whitish, rounded colonies, resembling the oxygen-limited conditions of liver tissues (Amachawadi and Nagaraja, 2015). Under aerobic conditions, *Salmonella* forms smaller, round, gray colonies, showing a change in appearance. These changes show metabolic and physiological adaptation to oxygen, helping the bacteria survive in different tissues. (Yamamoto and Droffner, 1985). Zonal oxygen gradients in the liver create conditions that allow *Salmonella* to change its metabolism and survive. This ability makes it harder to control the bacteria in liver tissues with conventional antimicrobials (Li et al., 2021).

Examination cattle livers infected with *Salmonella* Dublin shows paratyphoid granulomas mainly made of monocytes, Kupffer cells, and macrophage clusters (Pecoraro et al., 2017). These granulomatous lesions act as localized sites for bacterial persistence, facilitating chronic

infection and prolonged carrier states. Immunohistochemical staining confirms the presence of *Salmonella* inside these cellular structures, suggesting that the bacteria may evade immune clearance by residing within phagocytic cells (Bettke et al., 2022). The results show that *Salmonella* can stay in the liver as a hidden infection, leading to periodic shedding into bile and contamination of feces and the environment (Urdaneta and Casadesús, 2017).

Hepatic colonization by *Salmonella* not only affects cattle but also increases the risk of human exposure through the consumption of animal products (Balasubramanian et al., 2019). Consumption of cattle liver in different regions provides an opportunity for *Salmonella*-contaminated products to reach the human food chain (Sanchez et al., 2002). Unlike gastrointestinal contamination, which can often be reduced by surface decontamination, *Salmonella* embedded in liver tissues can remain even after processing steps (Kurtz et al., 2020).

Salmonella in Lymph Nodes

Peripheral and mesenteric lymph nodes are important sites where *Salmonella* can localize in cattle. Multiple studies have confirmed its presence in these tissues; for example, (Arthur et al., 2008) reported that 10–20% of cattle carried *Salmonella* in mesenteric and peripheral nodes, illustrating the contamination risk posed by subclinical carriers. Additionally, Studies have shown that *Salmonella* can be found in bovine lymph nodes, indicating that lymphatic tissues may act as reservoirs of infection even in clinically healthy animals. Lymph nodes (LNs) are small, bean-shaped structures found throughout the bodies of mammals (Singh et al., 2023). They act as filters that collect and process tissue fluids and cellular debris from surrounding areas (Elmore and Bouknight, 2017). Lymph nodes are key parts of the immune system and can asymptomatically carry pathogens such as *Salmonella* (Demirbilek, 2017a). Biofilm formation, survival under low-oxygen conditions, and immune modulation are key strategies used by *Salmonella* in lymph nodes. These adaptations support its persistence as a chronic infection (Harrell et al., 2021). Entry of *Salmonella* into lymph nodes occurs through the gastrointestinal tract or the bloodstream, where it can persist and remain in the tissue after entry (Worley, 2023).

As they are protected from common surface decontamination processes, peripheral lymph nodes (PLNs) such as mandibular, subiliac, and cervical nodes may serve as sites where *Salmonella* persists in meat products (Webb et al., 2017). Montevideo, Lille, Cerro, Anatum, and

Dublin are the serotypes most often detected in peripheral lymph nodes (Webb et al., 2017). In contrast to bacteria such as *Escherichia coli*, which are frequently present on meat surfaces and can be more readily eliminated by standard decontamination practices (Edwards and Fung, 2006; Webb et al., 2017), *Salmonella* are able to persist in peripheral lymph nodes and can enter ground beef when contaminated lymph node tissue is included in fat trim during processing (Gragg et al., 2013).

Improper meat processing practices, including the failure to remove lymph nodes from fat trim, can result in *Salmonella* contamination (Niyonzima et al., 2015). Fat trim from cattle may contain lymph nodes that harbor *Salmonella*, posing a potential source of contamination during ground beef production (Arthur et al., 2008). Lymph nodes that are not removed during processing can be present in beef products such as hamburger patties (Gragg et al., 2013). A USDA-ARS study detected *Salmonella* in subiliac lymph nodes, showing that these nodes in fat trim can be an important source of ground beef contamination (Gragg et al., 2013). As ground beef combines meat from multiple animals, the presence of contaminated lymph nodes may elevate the risk of salmonellosis for consumers (Bentum et al., 2025). Detection of *Salmonella* in the lymphatic system of cattle at slaughter emphasizes the potential for contamination of beef products such as hamburgers (Arthur et al., 2008). To reduce contamination risks, effective pre-harvest interventions are required before cattle enter processing facilities, and additional research on lymph node removal during processing could provide further mitigation (da Costa et al., 2023).

Cross-contamination in meat processing occurs through the spread of *Salmonella* from mandibular lymph nodes or oral tissues to carcasses, equipment, or meat products (Arguello et al., 2013). *Salmonella* commonly colonizes mesenteric lymph nodes (MLN) after entering the intestinal tract, where immune cells transport it through lymphatic vessels. In contrast, bacteria introduced intradermally usually remain in nearby lymph nodes, as the skin has no direct lymphatic connections to deeper nodes such as the MLN. This difference reflects the anatomy and immune function of the gut, which directs pathogens to central lymphoid tissues, while the skin drains mainly to peripheral nodes (Bravo-Blas et al., 2019) (Li, 2022). In rare cases, bacterial clones isolated from intradermal injection sites are genetically the same as strains from

the gastrointestinal tract. This indicates that some bacteria can move internally from the gut, particularly when injections are given in the abdominal or dorsal regions, likely due to their link to internal lymphatic pathways (Koh et al., 2006). *Salmonella* may persist in mesenteric lymph nodes and can also disseminate from mandibular lymph nodes and other tissues, serving as potential sources of contamination in meat products (Harvey et al., 2020).

Salmonella in the Lungs

Pulmonary *Salmonella* infections in cattle are often underdiagnosed, as signs resemble those of *Mannheimia haemolytica* and *Pasteurella multocida* (Rice et al., 2007). The prevalence of *Salmonella* in cattle lungs collected at slaughter varies, but it is generally not a high incidence finding (Holschbach and Peek, 2017). Sibhat et al. (2011) collected multiple sample types (hide swabs, mesenteric lymph nodes, cecal contents, and carcass swabs) in Ethiopian slaughterhouses and found that 2% of carcass swab samples were positive for *Salmonella*, whereas higher rates were observed in hides (31%) and mesenteric nodes (8%). Srednik et al., 2021. characterized *S. enterica* serovar Dublin isolates from clinical bovine cases in the United States and reported that a considerable proportion (44/140) were obtained from lung tissue, supporting previous observations that this serotype is not restricted to the intestinal tract but may also involve the respiratory system. Another study has also reported that serovar Dublin, in addition to causing enteritis and septicemia, can be associated with respiratory disease in calves, with pneumonia and lung lesions observed in infected animals (Sekhwal et al., 2025).

Salmonella can involve the lungs during systemic infection by hematogenous spread from the intestine. A less frequent route is aspiration of infected secretions, which may lead to complications such as pneumonia, pleuritis, and respiratory distress (Pardon and Buczinski, 2020; Velasquez-Munoz et al., 2024). Inhalation of infected aerosolized particles or secretions could potentially lead to pulmonary complications, although this pathway has not been widely documented (Kallapura et al., 2014). Once established in the lungs, the extensive blood supply facilitates bacterial dissemination, and alveolar macrophages provide intracellular niches that protect *Salmonella* from host defenses and antimicrobial agents (Molossi et al., 2021; Watson et al., 2000). Such pulmonary persistence contributes to more severe respiratory disease and increases the risk of bacterial shedding and zoonotic transmission (Holschbach and Peek, 2017).

Pulmonary *Salmonella* in cattle is rarely reported, and prevalence is likely underestimated, influenced by variation in diagnostics, management, and regional factors (Gutema et al., 2019). Studies suggest that systemic *Salmonella* infections, including those affecting the lungs, occur in due to diagnostic limitations (Demirbilek, 2017b). *Salmonella* Dublin has a strong association with systemic infection and has been detected in the lungs in up to 30% of septicemic cases (Pullinger et al., 2007). Key risk factors contributing to pulmonary *Salmonella* infections include immunosuppression, stress, and co-infections that weaken pulmonary defenses. Calves and chronically infected carriers are particularly vulnerable, with some developing asymptomatic lung colonization that may contribute to bacterial persistence within herds (Hodgson et al., 2012).

Histopathological examination of cattle lungs with *Salmonella* infection revealed fibrinous pneumonia, multifocal necrosis, and alveolar infiltration of neutrophils and macrophages (Molossi et al., 2021; Pecoraro et al., 2017). Detection of *Salmonella* in alveolar macrophages by immunohistochemistry suggests that intracellular survival, mediated in part by the Type III secretion system (T3SS), plays a key role in persistence (Coburn et al., 2007; De Souza Santos and Orth, 2019). As in hepatic infection, *Salmonella* in the lungs adapts its metabolism to oxygen availability, enabling survival in oxygen-rich alveoli as well as hypoxic lung tissue (Jennewein et al., 2015). Intracellular and biofilm-associated *Salmonella* are more resistant to antimicrobials, which makes treatment less effective (Aleksandrowicz et al., 2023). Shedding of *Salmonella* through respiratory secretions and aerosols highlights the need for stronger biosecurity in cattle production (Wathes et al., 1988). Pulmonary salmonellosis can be reduced through better monitoring, faster diagnostics, and targeted antimicrobial use to improve cattle health and productivity (Bentum et al., 2025).

Control and Prevention Strategies

A comprehensive approach that covers both pre-harvest and post-harvest stages is important for controlling *Salmonella* in cattle. Farm-level interventions can help lower the bacterial load before slaughter, given the ability of *Salmonella* to colonize lymph nodes, skin, and the gastrointestinal tract (Koohmaraie et al., 2005). Pre-harvest control includes improving farm hygiene, strengthening biosecurity, supporting gut health through proper nutrition, and

using vaccines to reduce *Salmonella* shedding (Edrington and Brown, 2022). Studies have shown that targeted vaccines can reduce bacterial colonization in lymphatic tissues, which helps lower the risk of contamination in beef products (Cernicchiaro et al., 2016). Additionally, probiotics and prebiotics have been studied as a mean to improve gut microbiota and inhibit the growth of *Salmonella* strains, helping to reduce their prevalence in cattle populations (Markowiak and Śliżewska, 2018).

Post-harvest interventions focus on minimizing bacterial transfer during processing. Since *Salmonella* present on cattle hides can be a major source of carcass contamination, effective hide-cleaning procedures, such as chemical washes and steam vacuum treatments, have been implemented in many slaughter facilities (Kochevar et al., 1997). However, traditional decontamination methods are often not effective at removing *Salmonella* that reside inside lymph nodes; therefore, improving detection and removal techniques is important (Brichta-Harhay et al., 2012). Current research is exploring alternative approaches, such as antimicrobial treatments that reach deeper tissues and genetic modifications in cattle that enhance resistance to *Salmonella* colonization (Kochevar et al., 1997).

In addition to industry-level interventions, educating consumers about proper food handling and cooking is an important part of *Salmonella* prevention (Ehuwa et al., 2021). Cooking beef products to an internal temperature of 71 °C (160 °F) effectively destroys *Salmonella* and reduces the risk of foodborne illness. Proper food storage, avoiding cross-contamination in kitchens, and maintaining good hygiene practices also play an important role in reducing *Salmonella* exposure in humans (Jarvis et al., 2016). Continuous monitoring programs at both the farm and processing stages are essential for tracking *Salmonella* prevalence and assessing the effectiveness of control measures (Jarvis et al., 2016). Since *Salmonella* remains a persistent challenge in cattle production systems, ongoing research and technological progress will play a key role in reducing its impact on cattle health and the beef industry (Galán-Relaño et al., 2023).

Research and Future Directions

Research on *Salmonella* in cattle keeps improving our understanding of its epidemiology, transmission dynamics, and persistence in various tissues. While the main reservoirs of

Salmonella, including the gastrointestinal tract, lymph nodes, hides, and internal organs such as the liver and lungs, have been identified, the ways *Salmonella* adapts and interacts with the host are still not fully understood (Holschbach & Peek, 2017). Future studies should focus on developing better pre-harvest control methods, improving detection techniques, and finding new ways to reduce *Salmonella* contamination in beef products.

Recent studies focus on developing new vaccines that reduce *Salmonella* shedding in cattle and target specific serovars that cause systemic infections, such as *Salmonella* Dublin and *Salmonella* Newport *(*Bentum et al*., 2025)*. Current vaccines show different levels of effectiveness; therefore, improving delivery methods, such as using recombinant antigens or nanoparticle-based approaches, could enhance their efficacy (Lamontagne et al., 2022). Additionally, studies on cattle microbiota suggest that altering gut microbial populations through probiotics, prebiotics, and competitive exclusion strategies may help provide a natural barrier against *Salmonella* colonization (Uyeno et al., 2015).

At the processing level, improvements in pathogen detection and decontamination are essential. Traditional culture-based methods for detecting *Salmonella* are time-consuming, whereas molecular techniques such as PCR and whole-genome sequencing provide faster and more accurate identification of bacterial strains (Awang et al., 2021). New biosensor technologies and machine learning tools are also being explored to improve real-time pathogen monitoring in meat processing facilities (Nastasijevic et al., 2025). Furthermore, new antimicrobial approaches, such as bacteriophage therapy and biofilm-disrupting compounds, could help reduce *Salmonella* persistence in processing environments and on carcasses (Jordá et al., 2023). Environmental and management studies are receiving more attention, as farm-level factors such as diet, stress, stocking density, and antimicrobial use can influence *Salmonella* prevalence (Dessale et al., 2023). Modern livestock farming uses real-time monitoring systems to track animal health and bacterial shedding and providing data-based insights for improved *Salmonella* control (Berckmans, 2014). Additionally, improving waste management and water sanitation on farms may help reduce the spread of *Salmonella* in cattle herds. (Jimenez et al., 2023).

In the long term, applying a "One Health" approach that integrates animal health, food production, and human health will be essential for addressing *Salmonella* challenges (Chen et al., 2024). Collaboration among veterinarians, microbiologists, food safety specialists, and policymakers will be essential to implement science-based strategies that ensure safer beef products and help reduce the global burden of salmonellosis (Qian et al., 2022). As antibiotic resistance in *Salmonella* strains continues to increase, future research should focus on developing alternative control strategies to reduce the risk of drug-resistant infections entering the food chain (VT Nair et al., 2018).

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Chapter 2 :Comparative Growth Analysis of *Fusobacterium necrophorum* Subspecies and *Fusobacterium varium* in Peptone Yeast Extract-Based Enrichment Media

Abstract

Liver abscesses in feedlot cattle cause significant economic losses to the beef cattle industry. They are polymicrobial infections in which *Fusobacterium necrophorum*, particularly subsp. *necrophorum*, is the dominant etiologic agent, often accompanied by *Trueperella pyogenes* as a secondary invader. Although *Salmonella enterica* has occasionally been isolated from abscesses, its contribution to lesion development remains unclear. The condition commonly follows ruminal acidosis or rumenitis resulting from high grain feeding, which damages the ruminal epithelium and enables bacterial translocation to the liver. This chapter includes two studies that assess the growth responses of *F. necrophorum* subspecies and *F. varium* in different enrichment media and substrate formulations, focusing on strains associated with liver abscesses and ruminal tissues in feedlot cattle.

Study 1 demonstrated that enrichment enhanced the detection and isolation of *F. necrophorum* subsp. *necrophorum* and *F. necrophorum* subsp. *funduliforme* from bovine tissue samples. Before enrichment, subsp. *necrophorum* was primarily detected in liver abscesses, unhealthy rumen (ruminal tissue with visible lesions), and ruminal fluid, while its recovery from healthy liver and colonic tissues was limited. After enrichment, recovery increased markedly in ruminal and colonic samples, indicating that these tissues harbored viable *Fusobacterium* populations that were not detected previously (below the detection threshold of 10^3 CFU/g). Among the media tested, A pre-reduced, anaerobically sterilized peptone yeast extract medium containing both lysine and lactate as major carbon sources and supplemented with josamycin, vancomycin, and norfloxacin (PY-Ly-La-JVN broth) consistently yielded the highest recovery overall, particularly from ruminal and colonic tissues, followed by PY-La-JVN broth, whereas PY-Ly-JVN broth supported little additional growth. In liver abscesses, PY-Ly-JVN broth favored detection of subsp. *necrophorum*, while PY-Ly-La-JVN broth promoted isolation of subsp. *funduliforme*. In both healthy and unhealthy ruminal tissues, PY-Ly-La-JVN broth

showed the strongest enrichment effect for both subspecies, and in colonic tissue and ruminal fluid, the same medium improved recovery where other approaches yielded limited growth. Cattle were assigned into three groups: a control group (no feed additives), Treatment A (Clostat + Villitech), and Treatment B (Clostat + Villitech + clove oil). These additives were designed to reduce pathogenic bacterial load and inflammation in the gastrointestinal tract, promoting a healthier microbial balance. However, both treatments were associated with slightly lower pre-enrichment bacterial detection, suggesting a modest in vivo reduction of *Fusobacterium*. Collectively, these findings demonstrate that enrichment with PY-Ly-La-JVN broth enhances recovery of *Fusobacterium* from ruminal and colonic tissues regardless of dietary treatment.

Study 2 evaluated the growth of *F. n.* subsp. *necrophorum*, *F. n.* subsp. *funduliforme*, and *Fusobacterium varium* (*F. varium*) in PY-La, PY-Ly, and PY-Ly-La broths. After twenty-four hours of anaerobic incubation, both subsp. *necrophorum* and subsp. *funduliforme* exhibited the highest growth in PY-Ly-La broth, whereas *F. varium* grew best in PY-Ly broth. These results highlight the importance of media composition in influencing metabolic activity and growth performance among *Fusobacterium* species. PY-Ly-La broth provided optimal conditions for *F. necrophorum* subspecies and offers an approach for improving *Fusobacterium* detection and isolation in diagnostic and research applications.

Introduction

In feedlot cattle, liver abscesses represent a major challenge to both animal health and production efficiency and are mainly attributed to the anaerobic bacterium *Fusobacterium necrophorum (Nagaraja and Chengappa, 1998).* These abscesses compromise normal physiological function, resulting in slower growth and reduced efficiency in feed utilization. They also decrease carcass quality and result in liver condemnation at slaughter (Aguiar Veloso and Drouillard, 2020).

High-grain diets in feedlot cattle accelerate fermentation and acid production, which lowers ruminal pH and damages the epithelial lining, a condition known as ruminal acidosis and rumenitis (Joaquin et al., 2014). This disruption compromises the ruminal barrier and facilitates the translocation of opportunistic pathogens into the portal circulation (Garcia et al., 2017). Once

transported to the liver, these organisms proliferate within hepatic tissue, leading to localized inflammation and the development of abscesses (Nagaraja and Chengappa, 1998).

Multiple bacterial species are implicated in the pathogenesis of liver abscesses in cattle, with *Fusobacterium necrophorum* (*F. necrophorum*), *Trueperella pyogenes* (*T. pyogenes*), and *Salmonella enterica* (*S. enterica*) reported as the predominant isolates (Abbasi et al., 2025). Among these, *F. necrophorum* is recognized as the primary causative agent (Tadepalli et al., 2009). This species produces a leukotoxin that kills neutrophils and causes tissue necrosis, while *T. pyogenes* releases enzymes and toxins that lead to the formation of purulent material. *S. enterica* survives inside host cells as a facultative intracellular pathogen, thereby contributing to secondary infections.

Members of the genus *Fusobacterium* are obligate anaerobes that are Gram-negative, rod-shaped, non-motile, and non-spore-forming bacteria (Aguiar Veloso and Drouillard, 2020). *F. necrophorum is* divided into two subspecies: subsp. *necrophorum,* which is more frequently associated with liver abscesses due to its high leukotoxin production, and subsp. *funduliforme,* which exhibits lower virulence and is typically found in the rumen (Tadepalli et al., 2008). *F. varium* is also present in the rumen, though its role in liver abscess formation appears limited (Deters et al., 2025).

Although liver abscesses are well known for their health and economic impact, the microbial ecology of these lesions and associated tissues remains incompletely characterized (Pinnell and Morley, 2022). Detection of the implicated organisms has relied primarily on culture and molecular methods (Lechtenberg et al., 1988). While culture methods provide viable isolates, they often underestimate prevalence due to overgrowth by competing flora and limited sensitivity (Gruninger et al., 2025). Quantitative PCR (qPCR) improves detection and allows subspecies differentiation; however, its performance declines when bacterial concentrations fall below the quantification limit (10^3 CFU/g) (Shevtsov et al., 2024). Therefore, enrichment culture represents a critical step to improve detection of low-abundance pathogens (Vizzini et al., 2021).

Several enrichment media have been developed to enhance the detection of *Fusobacterium* species, often formulated with lactate or lysine as primary carbon sources and

supplemented, in some cases, with selective antibiotics such as josamycin, vancomycin, and norfloxacin (JVN). However, comparative evaluations of these formulations for isolating different *Fusobacterium* subspecies from bovine tissues remain limited (Kilama et al., 2025). Previous studies have shown that *F. necrophorum* can utilize lactate as a major energy source, supporting rapid early growth during ruminal acidosis or other metabolic disturbances (Elwakeel et al., 2013). Lysine and other amino acids can also sustain growth, though utilization efficiency varies among subspecies and isolates (Barker, 2002; Elwakeel et al., 2013). Together, these observations emphasize that both medium composition and metabolic preference influence the ecological fitness and detectability of *Fusobacterium* spp. in the rumen and liver.

Feed additives such as Clostat® (*Bacillus subtilis* PB6; Kemin Inc.,), Villitech® (a yeast-based product derived from *Saccharomyces cerevisiae;* Kemin Inc.), and clove oil (rich in eugenol) are used in feedlot cattle to support rumen health, stabilize fermentation, and reduce pathogenic bacterial loads. In study 1, samples were obtained from cattle belonging to three dietary treatment groups: a control group (no feed additives), Treatment A (Clostat + Villitech), and Treatment B (Clostat + Villitech + clove oil). These treatments were part of a broader nutritional study, and the present research utilized these samples to compare different enrichment media for the detection and recovery of *Fusobacterium* species across various bovine tissues. Therefore, the feed additive treatments were not evaluated as part of this study (study 1) but served as the source of experimental material for media comparison.

Given that microbial composition, nutrient availability, and inhibitory factors differ across the rumen, colon, and liver, the choice of enrichment medium is critical for accurate bacterial detection. This study, therefore, aimed to (1) evaluate tissue-specific enrichment media for the detection and isolation of *F. necrophorum* and *F. funduliforme* from bovine tissues, and (2) compare the growth performance of *F. necrophorum* subspecies, and *F. varium* in PY-La, PY-Ly, and PY-Ly-La broths to determine substrate preferences and optimal growth conditions.

Materials and Methods

Study 1: Tissue-Specific Enrichment Media for Detection and Quantification of Fusobacterium necrophorum Subspecies

Samples used in this study were obtained from a separate feeding trial that included dietary treatments. The present work focused exclusively on comparing enrichment media for the detection and recovery of *Fusobacterium* species from bovine tissues.

Experimental Design and Acidosis Induction Model

An acidosis induction model was used to predispose cattle to liver abscess formation. Steers were initially adapted to a low-grain basal diet and then subjected to an acidotic challenge by feeding a high-grain diet for three consecutive days, which reduced ruminal pH to ≤ 5.5, followed by two days on the basal diet. This five-day sequence was defined as one acidotic cycle, and the cycle was repeated three times. The model was designed to simulate feedlot conditions that predispose cattle to ruminal acidosis, rumenitis, and subsequent liver abscess development.

Treatment Groups and Bacterial Inoculation

Animals were randomly assigned to three groups.

- Positive control group (PCON): Two acidotic cycles with intraruminal inoculations of *F. n. subsp. necrophorum* and *F. n. subsp. funduliforme* on days 0, 5, and 11 (1×10^6 CFU/mL; 100 mL of each).
- Treatment A: Same acidotic cycles and inoculations as PCON, with dietary supplementation of Clostat (Kemin Industries, Des Moines, IA) and Villitech (Vilofoss, Graasten, Denmark).
- Treatment B: Same regimen as Treatment A, with the addition of clove oil.

Clostat is a probiotic containing *Bacillus subtilis* PB6; Villitech is a yeast-based additive derived from *Saccharomyces cerevisiae*; and clove oil contains eugenol as the primary bioactive compound. All feed additives were included at manufacturer-recommended concentrations.

Necropsy and Sampling

Necropsy was performed, and samples were collected from cattle with and without liver abscesses. Liver samples (n = 40) included both abscessed tissues from affected animals and healthy liver tissues from unaffected cattle. From each animal, a 5–10 cm section of ruminal epithelium was excised from the ventral sac of the rumen. For ruminal tissue, two samples were collected per animal, one from an area without visible lesions (healthy rumen) and one from an area showing lesions (unhealthy rumen), resulting in a total of 80 ruminal tissue samples. Additional samples included colonic tissues (n = 40), ruminal fluid (n = 40), and colonic contents (n = 40). Approximately 45 mL of ruminal and colonic contents was collected into sterile 50-mL screw-capped centrifuge tubes. All samples were tightly sealed, maintained under anaerobic conditions, and transported to the Anaerobe Laboratory for immediate processing.

Media Preparation

A peptone-yeast (PY) basal medium was prepared using equal quantities of peptone, trypticase, and yeast extract, supplemented with resazurin and a salt solution in distilled water. For the lactate formulation (PY-La), sodium lactate served as the main carbon source, whereas for the lysine formulation (PY-Ly), L-lysine hydrochloride was used as the major amino acid substrate. Both media were supplemented with hemin, vitamin K_1, and cysteine HCl to support bacterial growth under reducing conditions. The pH was adjusted to 7.2 ± 0.1 prior to autoclaving. After sterilization, the media were boiled under a cold-water condenser until the resazurin indicator changed from purple to light yellow, then cooled under an oxygen-free nitrogen gas to ensure anaerobiosis. For the combined medium (PY-Ly-La), both sodium lactate and L-lysine were included as energy sources, while maintaining identical basal and supplement compositions.

Antibiotic Supplementation

To suppress the growth of background flora in tissue samples, selective antibiotics were incorporated into the enrichment media. The antibiotic mixture consisted of josamycin (3 µg/mL), vancomycin (4 µg/mL), and norfloxacin (1 µg/mL) (collectively referred to as JVN). These antibiotics were used only in Study 1, as enrichment cultures derived from ruminal,

colonic, and liver tissues contained diverse bacterial populations that could otherwise outcompete *Fusobacterium* spp.

Sample Processing

Liver abscesses were surface sterilized with a flame-heated spatula, and the capsule was aseptically incised using a sterile scalpel. Approximately 5 g of purulent material, including sections of the abscess wall, or 5 g of ruminal and colonic epithelial tissues were each suspended in 45 mL of sterile phosphate-buffered saline (PBS) and homogenized using a Nutribullet Pro Blender (Nutribullet, Los Angeles, CA) for 1 minute. Rumen and colonic contents were processed similarly: rumen contents were filtered through sterile cheesecloth using a funnel to obtain 10 mL of filtrate, whereas colonic contents were first mixed 1:1 (v/v) with sterile PBS and then filtered through sterile cheesecloth to yield 10 mL of filtrate. From each homogenate or filtrate, a 1 mL aliquot was collected for pre-enrichment quantification of *Fusobacterium* species by real-time qPCR, while 1 mL of the remaining material was inoculated into Hungate tubes containing 9 mL of PY-La-JVN, PY-Ly-JVN, or PY-Ly-La-JVN broth. The inoculated tubes were incubated anaerobically at 37 °C for 24 h to allow selective enrichment prior to post-enrichment analysis (Deters et al., 2024).

Study 2. Comparative Growth of Fusobacterium necrophorum Subspecies and F. varium in PY-La, PY-Ly, and PY-Ly-La Broths

Culture Media and Materials

Study 2 was conducted using antibiotic-free formulations of PY-La, PY-Ly, and PY-Ly-La broths to evaluate the intrinsic growth performance without selective pressure. Additional materials included blood agar plates, anaerobic brain–heart infusion (BHI) broth, sterile inoculating loops, and an anaerobic workstation (VPI, USA) for culture handling and incubation.

Culture Preparation and Growth Assay

Frozen *Fusobacterium* strains (-80 °C) were revived on blood agar and incubated anaerobically at 37 °C for 48 hours. The morphology and culture purity were confirmed, and single colonies from each strain were transferred into 10 mL of brain heart infusion (BHI) broth

and incubated overnight at 37 °C. A 300 µL aliquot of the overnight culture was then inoculated into 10 mL of fresh BHI broth and incubated at 37 °C until an optical density (OD_{600}) of 0.6 was reached (approximately 4–6 hours). At this stage, 300 µL of medium in each test broths (PY-La, PY-Ly, PY-Ly-La, or BHI control) was replaced with 300 µL of bacterial culture. Controls consisted of uninoculated BHI medium. The optical density (OD_{600}) at 600 nm was measured at 0, 2, 4, 6, 18, and 24 hours using a spectrophotometer. All readings were normalized against the corresponding uninoculated medium.

Results

Study 1: Detection and Isolation of Fusobacterium necrophorum subsp. necrophorum and F. necrophorum subsp. necrophorum from Bovine Tissues

F. necrophorum* subsp. *necrophorum

Results are presented by tissue type, comparing detection and enrichment outcomes across the control, Treatment A, and Treatment B groups (Table 1).

Liver Abscess

In the control group, detection was observed in 3 of 4 samples (75%) before enrichment (7.39 Log_{10} CFU/g). The single undetected sample was recovered following enrichment in PY-Ly-JVN (100%) with 3.67 Log_{10} CFU/g, while PY-La-JVN and PY-Ly-La- JVN showed no additional recovery. In Treatment A, 7 of 8 samples (88%) were positive before enrichment (8.36 Log_{10} CFU/g); the remaining sample was recovered in PY-Ly-JVN (1/1; 100%) post-enrichment (3.67 Log_{10} CFU/g). No further recovery occurred in PY-La-JVN or PY-Ly-La-JVN. In Treatment B, all 7 samples (100%) were already positive prior to enrichment (8.07 Log_{10} CFU/g); therefore, no additional recovery was required.

Healthy Liver Tissue

In the control group, none of the 6 samples (0%) were positive before enrichment (ND). After enrichment, PY-La-JVN (1 sample; 17%, 3.82 Log_{10} CFU/g) and PY-Ly-La-JVN (1 sample; 17%, 3.65 Log_{10} CFU/g) each detected a single sample. In Treatment A, 2 of 7 samples

(29%) were positive before enrichment (4.46 Log_{10} CFU/g). Of the remaining undetected samples, 1 (20%) was recovered in PY-La-JVN (4.28 Log_{10} CFU/g) and 1 (20%) in PY-Ly-La-JVN (4.36 Log_{10} CFU/g). PY-Ly-JVN yielded no recovery. In Treatment B, all nine samples were initially negative (ND). Following enrichment, one sample (11%) was detected in PY-La-JVN (3.28 Log_{10} CFU/g), while no recovery was observed in other media.

Unhealthy Ruminal Epithelial Tissue (Rumen with lesions)

In the control group, 6 of 10 samples (60%) were positive before enrichment (6.52 Log_{10} CFU/g), while 4 remained negative. The four undetected samples were recovered equally across media: 25% in PY-La-JVN (3.98 Log_{10} CFU/g), 25% in PY-Ly-JVN (3.19 Log_{10} CFU/g), and 25% in PY-Ly-La-JVN (3.10 Log_{10} CFU/g). In Treatment A, 8 of 15 samples (53%) were positive before enrichment (5.60 Log_{10} CFU/g), and additional recovery was obtained in 29% of samples using PY-La-JVN (4.51 Log_{10} CFU/g) and 29% using PY-Ly-La-JVN (5.82 Log_{10} CFU/g); PY-Ly-JVN did not yield additional isolates. In Treatment B, 6 of 15 samples (40%) were positive before enrichment (5.29 Log_{10} CFU/g). After enrichment, 11% were recovered in PY-La-JVN (4.50 Log_{10} CFU/g) and 56% in PY-Ly-JVN (3.89 Log_{10} CFU/g), while PY-Ly-La-JVN showed no recovery.

Healthy Ruminal Epithelial Tissue

In the control group, 3 of 10 samples (30%) were positive before enrichment (5.43 Log_{10} CFU/g). Enrichment yielded one additional isolate each in PY-La (14%; 6.65 Log_{10} CFU/g), PY-Ly-JVN (14%; 4.10 Log_{10} CFU/g), and PY-Ly-La-JVN (14%; 7.64 Log_{10} CFU/g). In Treatment A, 4 of 15 samples (27%) were positive prior to enrichment (4.88 Log_{10} CFU/g). Additional recovery occurred in 2 isolates (18%) each for PY-La-JVN (4.68 Log_{10} CFU/g) and PY-Ly-JVN (3.19 Log_{10} CFU/g), and 3 isolates (27%) in PY-Ly-La-JVN (4.36 Log_{10} CFU/g). In Treatment B, only 1 of 15 samples (7%) was positive before enrichment (4.79 Log_{10} CFU/g). Enrichment markedly improved recovery, with 29% (5.33 Log_{10} CFU/g) in PY-La-JVN and 36% (5.88 Log_{10} CFU/g) in PY-Ly-JVN, while PY-Ly-La-JVN reached 79% (5.88 Log_{10} CFU/g).

Colonic Epithelial Tissue

In the control group, 2 of 10 samples (20%) were positive before enrichment (4.82 Log_{10} CFU/g). After enrichment, recovery was achieved in 50% using PY-La-JVN (5.02 Log_{10} CFU/g) and 38% using PY-Ly-La-JVN (4.75 Log_{10} CFU/g), whereas PY-Ly-JVN remained negative. In Treatment A, 5 of 15 samples (33%) were positive before enrichment (5.08 Log_{10} CFU/g), leaving 10 undetected. Following enrichment, detection occurred in 30% with PY-La (4.54 Log_{10} CFU/g) and 60% with PY-Ly-La-JVN (3.93 Log_{10} CFU/g), while PY-Ly- JVN showed no recovery. In Treatment B, all 15 samples were negative before enrichment (ND). After enrichment, detection was recorded in 13% with PY-La-JVN (6.33 Log_{10} CFU/g), 7% with PY-Ly- JVN (5.33 Log_{10} CFU/g), and 33% with PY-Ly-La- JVN (5.11 Log_{10} CFU/g).

Ruminal contents

In the control group, 3 of 10 samples (30%) were positive before enrichment (4.31 Log_{10} CFU/g), leaving 7 undetected. Enrichment recovered 14% in PY-La-JVN (4.24 Log_{10} CFU/g) and 29% in PY-Ly-La-JVN (6.06 Log_{10} CFU/g), while PY-Ly-JVN remained negative. In Treatment A, 4 of 15 samples (27%) were positive before enrichment (4.21 Log_{10} CFU/g), and additional recovery was achieved in 9% (3.33 Log_{10} CFU/g) and 18% (5.75 Log_{10} CFU/g) for PY-La-JVN and PY-Ly-La-JVN, respectively. In Treatment B, 7 of 15 samples (47%) were positive before enrichment (4.28 Log_{10} CFU/g). Enrichment further improved detection in 13% (4.51 Log_{10} CFU/g) in PY-Ly-JVN, while PY-La-JVN and PY-Ly-La-JVN showed no additional recovery.

Colonic Contents

In the control group, no samples were positive before enrichment (ND). After enrichment, 10% of samples were detected in PY-Ly- JVN (3.04 Log_{10} CFU/g), while PY-La-JVN and PY-Ly-La- JVN remained negative. In Treatment A, all 15 samples (0%) were negative before enrichment. After enrichment, isolation was observed in 7% with PY-Ly-La- JVN (5.21 Log_{10} CFU/g) only. In Treatment B, all 15 samples (0%) were negative before enrichment. Recovery occurred in 7% with PY-Ly-La- JVN (4.97 Log_{10} CFU/g), whereas PY-La- JVN and PY-Ly- JVN remained negative.

Overall, enrichment substantially increased detection of F. *necrophorum* subsp. *necrophorum* across most tissues and treatments, with PY-Ly-La-JVN consistently yielding the highest recovery efficiency, followed by PY-La- JVN, while PY-Ly- JVN was the least effective.

F. necrophorum* subsp. *funduliforme

Results are presented by tissue type, comparing detection and enrichment outcomes across the control, Treatment A, and Treatment B groups (Table 2).

Liver Abscess

In the control group, none of the four samples (0%) were positive before enrichment. Following enrichment, no medium recovered any isolates. In Treatment A, all eight samples (0%) were negative prior to enrichment; PY-Ly-La- JVN detected 13% with 4.82 Log_{10} CFU/g, and no recovery occurred in PY-La-JVN or PY-Ly-JVN. In Treatment B, none of the six samples (0%) were positive before enrichment; PY-Ly recovered 17% (4.35 Log_{10} CFU/g) and PY-Ly-La- JVN recovered 33% (3.71 Log_{10} CFU/g), while PY-La- JVN yielded no isolates.

Healthy Liver Tissue

In the control group, none of the six samples (0%) were positive before enrichment. After enrichment, PY-Ly-La- JVN broth recovered 17% with 3.55 Log_{10} CFU/g. In Treatment A, one of seven samples (14.3%) was positive before enrichment (4.31 Log_{10} CFU/g), and no further recovery was obtained post-enrichment. In Treatment B, all nine samples (0%) were negative before enrichment. Following enrichment, PY-Ly- JVN broth and PY-Ly-La- JVN broth each recovered 44% (4.62 and 4.27 Log_{10} CFU/g, respectively), while PY-La- JVN broth showed no recovery.

Unhealthy Ruminal Epithelial Tissue (Rumen with Lesions)

In the control group, six of ten samples (60%) were positive before enrichment (6.38 Log_{10} CFU/g). The remaining four negatives were recovered as follows: 25% in PY-La- JVN

(3.31 Log_{10} CFU/g), 50% in PY-Ly-JVN (4.03 Log_{10} CFU/g), and 100% in PY-Ly-La-JVN (7.03 Log_{10} CFU/g). In Treatment A, nine of fifteen samples (60%) were positive before enrichment (5.88 Log_{10} CFU/g). Additional recovery occurred in 83% with PY-La-JVN (5.86 Log_{10} CFU/g) and 100% with PY-Ly-La-JVN (6.86 Log_{10} CFU/g); PY-Ly-JVN did not yield additional isolates. In Treatment B, seven of fifteen samples (46.7%) were positive before enrichment (5.41 Log_{10} CFU/g). After enrichment, 38% were recovered in PY-La-JVN (6.20 Log_{10} CFU/g), 20% in PY-Ly-JVN (4.24 Log_{10} CFU/g), and 50% in PY-Ly-La-JVN (7.58 Log_{10} CFU/g).

Healthy Ruminal Epithelial Tissue

In the control group, three of ten samples (30%) were positive before enrichment (5.26 Log_{10} CFU/g). Enrichment improved detection among negatives: PY-La-JVN 57% (6.78 Log_{10} CFU/g), PY-Ly-JVN 14% (4.49 Log_{10} CFU/g), and PY-Ly-La-JVN 86% (7.91 Log_{10} CFU/g). In Treatment A, five of fifteen samples (33.3%) were positive prior to enrichment (5.25 Log_{10} CFU/g). Additional recovery occurred in 60% with PY-La-JVN (5.33 Log_{10} CFU/g), 30% with PY-Ly-JVN (5.89 Log_{10} CFU/g), and 100% with PY-Ly-La-JVN (6.99 Log_{10} CFU/g). In Treatment B, one of fifteen samples (6.7%) was positive before enrichment (4.44 Log_{10} CFU/g). Enrichment markedly improved recovery among negatives: 36% in PY-La-JVN (6.29 Log_{10} CFU/g), 29% in PY-Ly-JVN (6.94 Log_{10} CFU/g), and 79% in PY-Ly-La-JVN (7.86 Log_{10} CFU/g).

Colonic Epithelial Tissue

In the control group, none of the ten samples (0%) were positive before enrichment. After enrichment, recovery occurred in 20% with PY-Ly-JVN (4.21 Log_{10} CFU/g) and 60% with PY-Ly-La-JVN (5.71 Log_{10} CFU/g), while PY-La-JVN showed no recovery. In Treatment A, all fifteen samples (0%) were negative before enrichment; post-enrichment, detection occurred in 13% with PY-La-JVN (3.59 Log_{10} CFU/g), 13% with PY-Ly-JVN (4.63 Log_{10} CFU/g), and 13% with PY-Ly-La-JVN (4.05 Log_{10} CFU/g).In Treatment B, all fifteen samples (0%) were negative before enrichment; post-enrichment, 20% were detected in PY-La-JVN (5.58 Log_{10} CFU/g) and 20% in PY-Ly-La-JVN (7.54 Log_{10} CFU/g), with no recovery in PY-Ly-JVN.

Rumen Contents

In the control group, six of ten samples (60%) were positive before enrichment (5.05 Log_{10} CFU/g). No additional recovery occurred following enrichment. In Treatment A, twelve of fifteen samples (80%) were positive before enrichment (4.68 Log_{10} CFU/g). Among negatives, additional recovery was 33% with PY-La-JVN (3.71 Log_{10} CFU/g), 33% with PY-Ly-JVN (3.37 Log_{10} CFU/g), and 67% with PY-Ly-La-JVN (5.78 Log_{10} CFU/g). In Treatment B, eight of fifteen samples (53.3%) were positive before enrichment (4.92 Log_{10} CFU/g). Enrichment further improved detection among negatives in 14% with PY-La-JVN (4.44 Log_{10} CFU/g) and 14% with PY-Ly-La-JVN (3.21 Log_{10} CFU/g), with no recovery in PY-Ly-JVN (0%).

Colonic Contents

In the control group, one of ten samples (10%) was positive before enrichment (3.88 Log_{10} CFU/g). After enrichment, 11% were recovered in PY-Ly-La-JVN (5.72 Log_{10} CFU/g). In Treatment A, all fifteen samples (0%) were negative before enrichment; following enrichment, recovery occurred in 7% with PY-La-JVN (4.85 Log_{10} CFU/g), 14% with PY-Ly-JVN (3.26 Log_{10} CFU/g), and 7% with PY-Ly-La-JVN (5.70 Log_{10} CFU/g). In Treatment B, one of fifteen samples (6.7%) was positive before enrichment (3.88 Log_{10} CFU/g); post-enrichment, recovery occurred in 7% with PY-La-JVN (5.32 Log_{10} CFU/g), 7% with PY-Ly-JVN (3.06 Log_{10} CFU/g), and 14% with PY-Ly-La-JVN (5.68 Log_{10} CFU/g).

Study 2. Comparative Growth of F. necrophorum subsp. necrophorum, F. necrophorum subsp. funduliforme, and F. varium in PY-La, PY-Ly, and PY-Ly-La Broths

The following sections describe the growth performance for each species individually in PY-La, PY-Ly, and PY-Ly-La media.

F. necrophorum* subsp. *necrophorum

At 24 hours of incubation, all growth curves showed a similar pattern for *F. n* subsp. *necrophorum* strains 2024-3-1081, 2024-3-1219, 2024-3-1221, 2024-3-1228, and 2024-3-1229. Optical density increased rapidly during the first 6 hours and continued to rise gradually until 24 hours, indicating active bacterial growth throughout the incubation period. Among the three media tested, PY-Ly-La consistently supported the highest absorbance values across all

strains, followed by PY-La, while PY-Ly supported the lowest growth. The same pattern was observed across replicates. These findings (Figure 1) demonstrate that *F. necrophorum* subsp. *necrophorum* grows in all tested media but achieves the highest biomass accumulation in PY-Ly-La, indicating synergistic utilization of lactate and lysine.

F. necrophorum* subsp. *funduliforme

All *F. necrophorum* subsp. *funduliforme* strains (2024-3-926, 2024-3-936, 2024-3-921, and 2024-3-1374) displayed similar growth kinetics. Rapid increases in OD_{600} were observed between 2 and 6 h, followed by stabilization between 18 and 24 h. PY-Ly-La consistently produced the highest absorbance values, followed by PY-La, with PY-Ly supporting the least growth. As shown in Figure 2, *F. necrophorum* subsp. *funduliforme* reached the greatest cell density in PY-Ly-La broth, suggesting that the combination of lactate and lysine enhances metabolic activity compared with single-substrate media.

F. varium

All *F. varium* strains (2024-3-775, 2024-3-776, 2024-3-774, 2024-3-1236, and 2024-3-1093) exhibited comparable growth patterns, characterized by a rapid increase in OD_{600} during the first 6 h, followed by stabilization. Among the media tested, PY-Ly consistently supported the highest absorbance values across all strains, followed by PY-Ly-La, while PY -La produced the lowest growth. As illustrated in Figure 3, *F. varium* grew steadily in all media but achieved maximum cell density in PY-Ly, indicating that lysine serves as its preferred growth substrate.

Discussion

Study 1: Detection and Quantification of Fusobacterium necrophorum Subspecies from Bovine Tissues

Detection of *Fusobacterium necrophorum* subspecies varied by tissue, treatment, and enrichment medium, and enrichment improved recovery across most samples, especially in the rumen and colon. In liver abscesses, *F. n.* subsp. *necrophorum* was detected in most samples prior to enrichment in all groups, and the few culture-negative samples in the control and Treatment A became positive only in PY-Ly-JVN broth; PY-La-JVN broth and PY-Ly-La-JVN broth did not add abscess recoveries. In contrast, *F. n.* subsp. *funduliforme* was not detected prior

to enrichment in any group; after enrichment, the control remained negative in all media, Treatment A yielded a single recovery in PY-Ly-La-JVN broth, and Treatment B was recovered in PY-Ly-JVN broth and PY-Ly-La-JVN broth, with PY-La-JVN broth not contributing to that niche.

In healthy liver, subsp. *necrophorum* was absent in control prior to enrichment and detected in Treatment A; enrichment produced recoveries in PY-La-JVN broth and PY-Ly-La-JVN broth across groups, while PY-Ly-JVN broth did not contribute. For subsp. *funduliforme*, the control showed recovery in PY-Ly-La-JVN broth and not in PY-Ly-JVN broth; Treatment A showed limited pre-enrichment detection, and Treatment B showed post-enrichment detection in both PY-La-JVN and PY-Ly-JVN broths. In healthy rumen, subsp. *necrophorum* was detected at low levels before enrichment in all groups, and recovery increased after enrichment with PY-Ly-La-JVN broth, yielding the highest positivity, followed by PY-Ly-JVN broth and PY-La-JVN broth. For subsp. *funduliforme*, control samples increased substantially after enrichment in PY-Ly-La-JVN broth; Treatment A reached complete recovery among negatives in PY-Ly-La-JVN broth with additional smaller gains in PY-La-JVN and PY-Ly-JVN broths, and Treatment B rose markedly post-enrichment in PY-Ly-La-JVN broth.

Colonic tissue showed low pre-enrichment detection across groups. For subsp. *necrophorum*, post-enrichment increases were driven mainly by PY-Ly-La-JVN broth, with PY-La-JVN broth contributing in some cases and PY-Ly-JVN broth often not contributing. For subsp. *funduliforme*, the control increased post-enrichment in PY-Ly-La-JVN broth and, to a lesser extent, PY-Ly-JVN broth; Treatment A recovered in all three media with the smallest gain in PY-Ly-La-JVN broth, and Treatment B recovered in PY-La-JVN and PY-Ly-La-JVN broths but not in PY-Ly-JVN broth.

In rumen fluid, subsp. *necrophorum* showed moderate pre-enrichment detection. Enrichment resulted in additional recoveries in PY-La-JVN and PY-Ly-La-JVN broths for the control and Treatment A, and only in PY-Ly-La-JVN broth for Treatment B. For subsp. *funduliforme*, the control did not gain additional recovery after enrichment; Treatment A showed recovery in PY-La-JVN, PY-Ly-JVN, and PY-Ly-La-JVN broths, with the highest

recovery in PY-Ly-La-JVN broth; and Treatment B showed recovery in PY-La-JVN and PY-Ly-La-JVN broths but not in PY-Ly-JVN broth.

Colonic content was not uniformly negative prior to enrichment. For subsp. *necrophorum*, the control became positive only in PY-Ly-JVN broth, while Treatments A and B showed recovery only in PY-Ly-La-JVN broth. For subsp. *funduliforme*, post-enrichment detection occurred in the control in PY-Ly-La-JVN broth, in Treatment A across all three media at low levels, and in Treatment B with PY-La-JVN, PY-Ly-JVN, and PY-Ly-La-JVN broths, with the highest recovery observed in PY-Ly-La-JVN broth.

These results confirm that *F. necrophorum* subsp. *necrophorum* is the main agent associated with liver abscesses, consistent with previous studies. In contrast, *F. necrophorum* subsp. *funduliforme* was more common in the rumen and colon, and its detection depended strongly on the type of enrichment medium. These findings show that both tissue type and medium composition influence the isolation of *Fusobacterium* species.

Study 2. Comparative Growth of Fusobacterium necrophorum subspecies, and F. varium in PY-La, PY-Ly, and PY-Ly-La Broths

Under anaerobic conditions, *Fusobacterium* generates energy by fermenting organic acids and amino acids rather than sugars. Lactate is converted mainly to propionate and acetate, producing ATP by substrate-level phosphorylation; NH_3 formation is minimal under lactate fermentation. Lysine is catabolized via deamination to butyrate and acetate, releasing NH_3 and yielding ATP, consistent with amino-acid fermentation. When lactate and lysine were provided together in the lactate and lysine combination (PY-Ly-La), cultures typically reached higher biomass and left less residual lactate and free NH_3, indicating co-utilization of carbon and nitrogen and more efficient growth. The strong growth observed in the PY-Ly-La medium supports previous findings that *Fusobacterium necrophorum* utilizes lactate as a major carbon source (Russell, 2005) and further shows that the addition of lysine to lactate enhances this metabolic activity under anaerobic conditions.

Fusoacterium necrophorum subsp. *funduliforme* showed consistent growth under anaerobic conditions in all PY-based media. The highest cell density occurred in the PY-Ly-La

broth, indicating that the combination of lactate and lysine supported efficient metabolism. These results agree with previous findings that lactate serves as a key energy source for many *Fusobacterium* isolates (Elwakeel et al., 2013; Tan et al., 1994) and suggest that the availability of both carbon and nitrogen substrates enhances overall growth performance.

Fusobacterium varium exhibited active growth in all media, with the greatest OD_{600} values recorded in PY-Ly, indicating that lysine is its preferred energy and nitrogen source under anaerobic conditions. This observation supports recent findings showing that *F. varium* can degrade amino acids such as lysine as part of its energy metabolism (Schwarz et al., 2023, Deters et al., 2024).

Overall, the findings from this experiment demonstrate that substrate composition strongly influences the growth of *Fusobacterium* subspecies under in vitro conditions. *F. necrophorum* subsp. *necrophorum* showed flexibility by utilizing both PY-La and PY-Ly, achieving the greatest growth in PY-Ly-La. *F. necrophorum* subsp. *funduliforme* also responded best to PY-Ly-La, indicating efficient use of both substrates. In contrast, *F. varium* preferred PY-Ly, showing higher optical density values in this medium. These results highlight key metabolic distinctions among subspecies and suggest that growth behavior in *Fusobacterium* is both substrate- and strain-dependent.

Conclusion

This research investigated the detection, isolation, and growth characteristics of *Fusobacterium* species from bovine tissues under anaerobic conditions, focusing on the influence of tissue type, enrichment media, and substrate composition. The findings collectively highlight the importance of selecting appropriate enrichment and culture conditions for the accurate identification and study of *Fusobacterium* subspecies associated with liver abscesses and ruminal health.

Study 1: Detection and Isolation of Fusobacterium Subspecies from Bovine Tissues

Enrichment culture significantly improved the recovery of *F. necrophorum* subspecies from various bovine tissues, particularly the rumen and colon. *F. necrophorum* subsp.

necrophorum was confirmed as the dominant species in liver abscesses, aligning with its established role as the primary etiological agent. It was frequently detected without enrichment, though PY-Ly-JVN broth improved recovery in culture-negative samples. In contrast, *F. necrophorum* subsp. *funduliforme* was more prevalent in ruminal and colonic tissues, and its detection was highly dependent on the enrichment medium. Among the tested media, PY-Ly-La-JVN broth consistently provided the highest recovery efficiency, followed by PY-La-JVN broth. In contrast, PY-Ly-JVN broth was most effective for abscess isolates. These findings are consistent with previous reports (Nagaraja and Chengappa, 1998; Tadepalli et al., 2009; Elwakeel et al., 2013), confirming the tissue-specific distribution of *Fusobacterium* subspecies. Therefore, medium selection should be tailored to both the target subspecies and tissue type:

- PY-Ly-JVN broth is recommended for confirming *F. necrophorum* subsp. *necrophorum* in liver abscesses.
- PY-Ly-La-JVN broth should be the preferred medium for rumen and colon samples.
- PY-La-JVN broth may serve as a supplemental medium where additional sensitivity is desired.

Study 2. Comparative Growth of Fusobacterium necrophorum subspecies and F. varium in PY-La, PY-Ly, and PY-Ly-La Broth.

Growth assays conducted under anaerobic conditions revealed distinct substrate preferences among *Fusobacterium* species. Both *F. necrophorum* subsp. *necrophorum* and *F. necrophorum* subsp. *funduliforme* demonstrated the highest growth in PY-Ly-La broth, indicating synergistic utilization of lactate and lysine as combined carbon and nitrogen sourcesIn contrast, *F. varium* exhibited optimal growth in PY-Ly broth, suggesting its reliance on amino acid catabolism for energy production.These results highlight key metabolic differences among *Fusobacterium* subspecies, where lactate–lysine co-supplementation enhances biomass production in *F. necrophorum*, while lysine alone supports the metabolic activity of *F. varium*.

Overall, all *Fusobacterium* strains grew well under anaerobic conditions, but the level of growth depended on the type of substrate. These differences show that each subspecies uses

nutrients in different ways, which should be considered when choosing media for their detection and isolation.

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Table 1. Comparative Analysis of Various Enrichment Media (JVN-La broth, JVN-Ly broth, and Combination) for Detecting, and Quantifying *Fusobacterium necrophorum* subsp. *necrophorum* in Healthy and Abscessed Animal Tissue Samples.

		qPCR							
		Before Enrichment		After Enrichment[1]					
				PY-La JVN Broth		PY-Ly JVN Broth		PY-Ly-La JVN Broth	
	No. of Samples Tested	No. of Positive Samples (%)	Concentration (Log_{10} CFU/g)	Detection (%)	Concentration (Log_{10} CFU/g)	Detection (%)	Concentration (Log_{10} CFU/g)	Detection (%)	Concentration (Log_{10} CFU/g)
Control									
Liver Abscess	4	3 (75)	7.39	0	ND	1 (100)	3.67	0	ND
Unhealthy Rumen[2]	10	6 (60)	6.52	1 (25)	3.98	1 (25)	3.19	1 (25)	3.1
Healthy Liver	6	0	ND	1 (17)	3.82	0	ND	1 (17)	3.65
Healthy Rumen	10	3 (30)	5.43	1 (14)	6.65	1 (14)	4.1	1 (14)	7.64
Colon Tissue	10	2 (20)	4.82	4 (50)	5.02	0	ND	3 (38)	4.75
Rumen Fluid	10	3 (30)	4.31	1 (14)	4.24	0	ND	2 (29)	6.06
Colon Content	10	0	ND	0	ND	1 (10)	3.04	0	ND
Clostat + Villitech									
Liver Abscess	8	7 (88)	8.36	0	ND	1 (100)	3.67	0	ND
Unhealthy Rumen	15	8 (53)	5.6	2 (29)	4.51	0	ND	2 (29)	5.82
Healthy Liver	7	2 (29)	4.46	1 (20)	4.28	0	ND	1 (20)	3.9

Healthy Rumen	15	4 (27)	4.88	2 (18)	4.68	2 (18)	3.19	3 (27)	4.36
Colon Tissue	15	5 (33)	5.08	3 (30)	4.54	0	ND	6 (60)	3.93
Rumen Fluid	15	4 (27)	4.21	1 (9)	3.33	0	ND	2 (18)	5.75
Colon Content	15	0	ND	0	ND	0	ND	1 (7)	5.21
Clostat + Villitech + clove oil									
Liver Abscess	7	7 (100)	8.07	N/A[4]	N/A	N/A	N/A	N/A	N/A
Unhealthy Rumen	15	6 (40)	5.29	1 (11)	4.5	0	ND	5 (56)	3.89
Healthy Liver	9	0	ND[3]	1 (11)	3.28	0	ND	0	ND
Healthy Rumen	15	1 (7)	4.79	0	ND	4 (29)	5.33	5 (36)	5.88
Colon Tissue	15	0	ND	2 (13)	4.33	1 (7)	6.33	5 (33)	5.11
Rumen Fluid	15	7 (47)	4.28	0	ND	0	ND	1 (13)	4.51
Colon Content	15	0	ND	0	ND	0	ND	1 (7)	4.97

[1]Samples that were negative prior to enrichment were re-examined after enrichment to assess positivity.

[2]Rumen with lesions

[3]ND: Not Detectable

[4]N/A: Not Applicable

[5]PY-La JVN broth = Peptone–yeast extract broth + lactate + JVN antibiotics (josamycin, vancomycin, norfloxacin)

[6] PY -Ly JVN broth = Peptone–yeast extract broth + lysine + JVN antibiotics

[7]PY-Ly-La JVN broth = Peptone–yeast extract broth + lysine + lactate + JVN antibiotics

[8]CFU = Colony-forming units[1]

Table 2. Comparative Analysis of Various Enrichment Media (JVN-La broth, JVN-Ly broth, and Combination) for Detecting, and Quantifying *Fusobacterium necrophorum subsp. funduliforme* in Healthy and Abscessed Animal Tissue Samples.

		qPCR							
		Before Enrichment		After Enrichment[1]					
				PY-La JVN Broth		PY-Ly JVN Broth		PY-Ly-La JVN Broth	
	No. of Samples Tested	No. of Positive Samples (%)	Concentration (Log_{10} CFU/g)	Detection (%)	Concentration (Log_{10} CFU/g)	Detection (%)	Concentration (Log_{10} CFU/g)	Detection (%)	Concentration (Log_{10} CFU/g)
Control									
Liver Abscess	4	0	ND[3]	0	ND	0	ND	0	ND
Unhealthy Rumen[2]	10	6 (60)	6.38	1 (25)	3.31	2 (50)	4.03	4 (100)	7.03
Healthy Liver	6	0	ND	0	ND	0	ND	1 (17)	3.55
Healthy Rumen	10	3 (30)	5.26	4 (57)	6.78	1 (14)	4.49	6 (86)	7.91
Colon Tissue	10	0	ND	0	ND	2 (20)	4.21	6 (60)	5.71
Rumen Fluid	10	6 (60)	5.05	0	ND	0	ND	0	ND
Colon Content	10	1 (10)	3.88	0	ND	0	ND	1 (11)	5.72
Clostat + Villitech									
Liver Abscess	8	0	ND	0	ND	0	ND	1 (13)	4.82

Unhealthy Rumen	15	9 (60)	5.88	5 (83)	5.86	0	ND	6 (100)	6.86
Healthy Liver	7	1 (14.3)	4.31	0	ND	0	ND	0	ND
Healthy Rumen	15	5 (33.3)	5.25	6 (60)	5.33	3 (30)	5.89	10 (100)	6.99
Colon Tissue	15	0	ND	2 (13)	3.59	2 (13)	4.63	2 (13)	4.05
Rumen Fluid	15	12 (80)	4.68	1 (33)	3.71	1 (33)	3.37	2 (67)	5.78
Colon Content	15	0	ND	1 (7)	4.85	2 (14)	3.26	1 (7)	5.7
Clostat + Villitech + clove oil									
Liver Abscess	6	0	ND	0	ND	1 (17)	4.35	2 (33)	3.71
Unhealthy Rumen	15	7 (46.7)	5.41	3 (38)	6.2	1 (20)	4.24	4 (50)	7.58
Healthy Liver	9	0	ND	0	ND	4 (44)	4.62	4 (44)	4.27
Healthy Rumen	15	1 (6.7)	4.44	5 (36)	6.29	4 (29)	6.94	11 (79)	7.86
Colon Tissue	15	0	ND	3 (20)	5.58	0	ND	3 (20)	7.54
Rumen Fluid	15	8 (53.3)	4.92	1 (14)	4.44	0	ND	1 (14)	3.21
Colon Content	15	1 (6.7)	3.88	1 (7)	5.32	1 (7)	3.06	2 (14)	5.68

[1]Samples that were negative prior to enrichment were re-examined after enrichment to assess positivity.

[2]Rumen with lesions

[3]ND: Not Detectable

[4]N/A: Not Applicable

[5]PY-La JVN broth = Peptone–yeast extract broth + lactate + JVN antibiotics (josamycin, vancomycin, norfloxacin)

[6]Y-Ly JVN broth = Peptone–yeast extract broth + lysine + JVN antibiotics

[7]PY-Ly-La JVN broth = Peptone–yeast extract broth + lysine + lactate + JVN antibiotics

[8]CFU = Colony-forming units

Figures

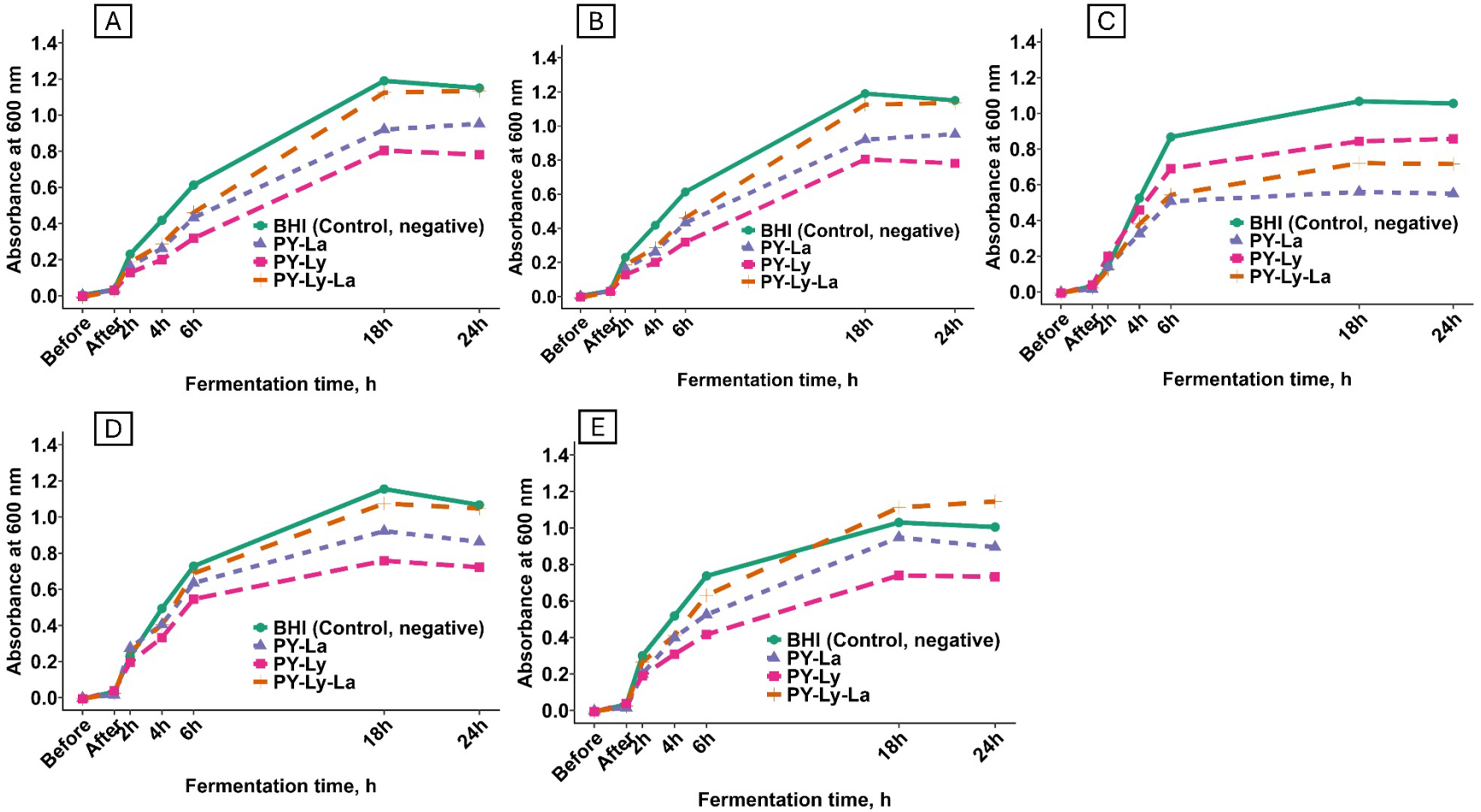

Figure 1. Absorbance of *Fusobacterium necrophorum* subsp. *necrophorum* in different enrichment media BHI, PY-La, PY-Ly, and PY-Ly-La
Absorbance (OD_{600}) was measured at 0 (uninoculated control)and **after inoculation at 2, 4, 6, 18, and 24 h** to evaluate the growth of *Fusobacterium necrophorum* subsp. *necrophorum* strains in different enrichment media.
Brain heart infusion (BHI) served as the control, while peptone–yeast (PY) enrichment media supplemented with lactate (La), lysine (Ly), or a combination of both substrates (Ly-La) were represented as PY-La, PY-Ly, and PY-Ly-La, respectively.The x-axis represents fermentation time (hours), and the y-axis indicates optical density at 600 nm (OD_{600}). Panels show results for individual strains: (A) strain 2024-3-1228, (B) 2024-3-1081, (C) 2024-3-1219, (D) 2024-3-1221, and (E) 2024-3-164.

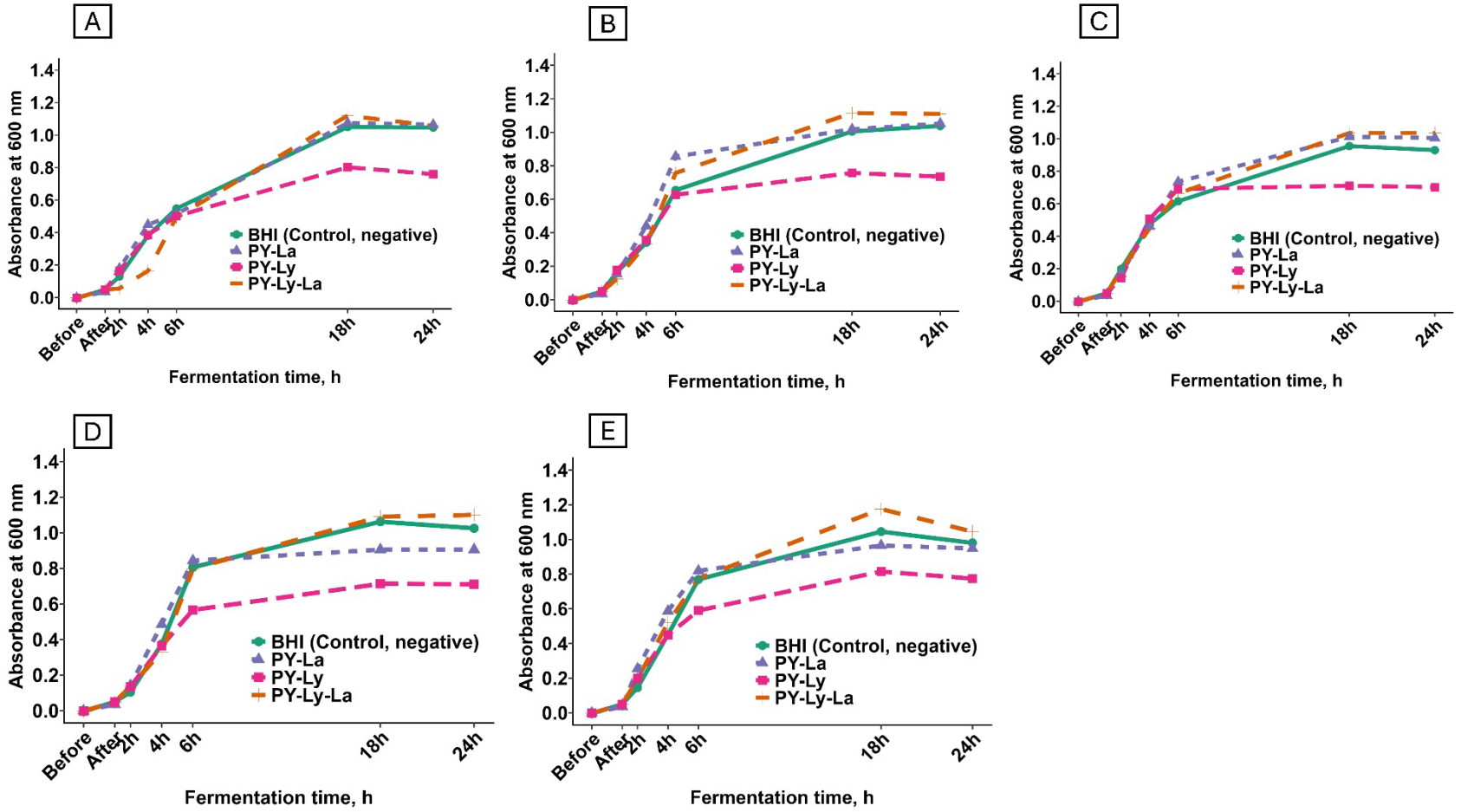

Figure 2. Absorbance of *Fusobacterium necrophorum* subsp. *funduliforme* in different enrichment media BHI, PY-La, PY-Ly, and PY-Ly-La

Absorbance (OD_{600}) was measured at 0 (uninoculated control) and after inoculation at 2, 4, 6, 18, and 24 h to evaluate the growth of *Fusobacterium necrophorum* subsp. *funduliforme* strains in different enrichment media.
Brain heart infusion (BHI) served as the control, while peptone–yeast (PY) enrichment media supplemented with lactate (La), lysine (Ly), or a combination of both substrates (Ly-La) were represented as PY-La, PY-Ly, and PY-Ly-La, respectively. The x-axis represents fermentation time (hours), and the y-axis indicates

optical density at 600 nm (OD_{600}). Panels show results for individual strains: (A) strain 2024-3-926, (B) 2024-3-936, (C) 2024-3-1374, (D) 2024-3-1074, and (E) 2024-3-921.

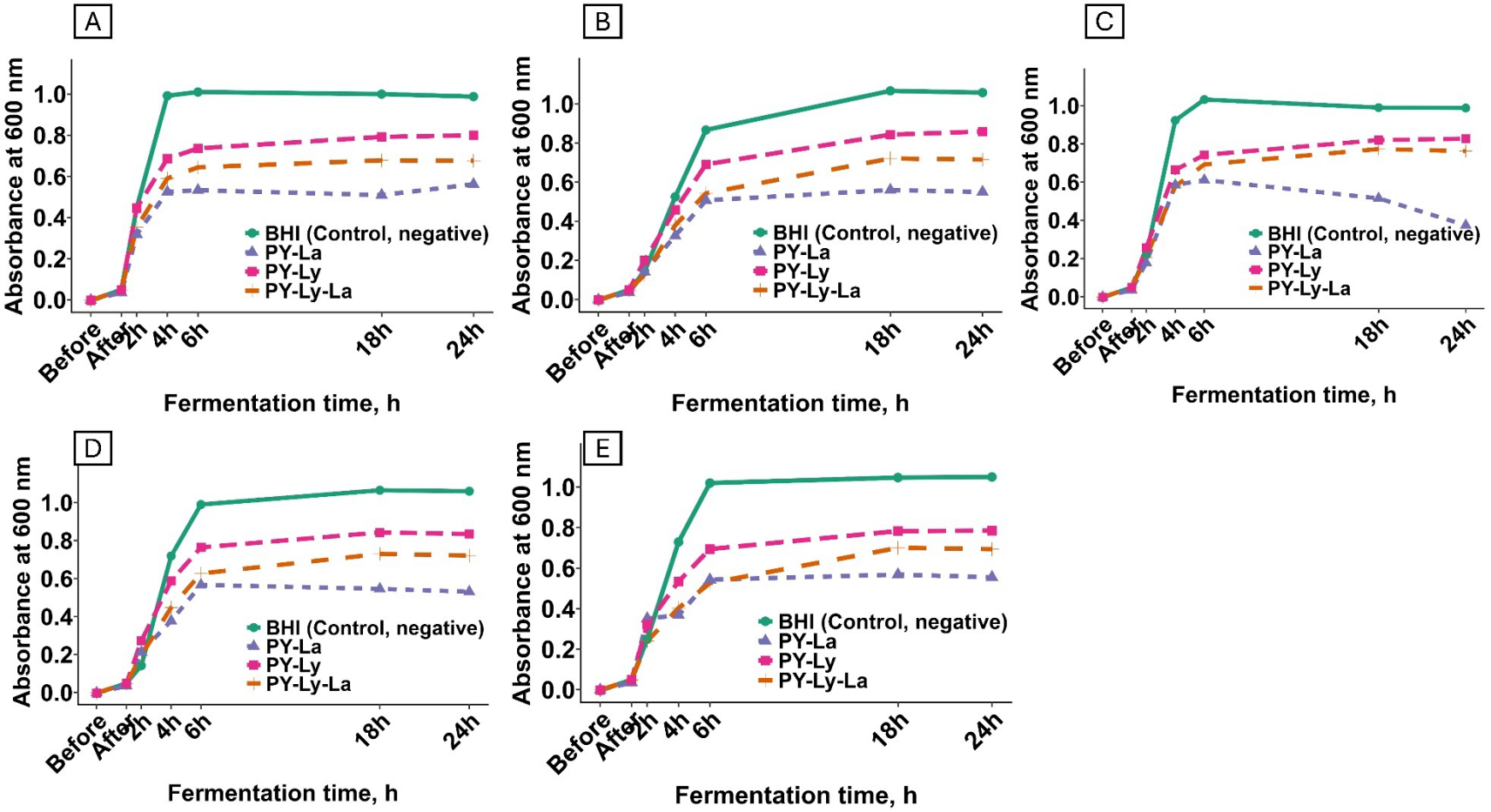

Figure 3. Absorbance of *Fusobacterium. varium* in different enrichment media BHI, PY-La, PY-Ly, and PY-Ly-La
Absorbance (OD_{600}) was measured at 0 (uninoculated control) and after inoculation at 2, 4, 6, 18, and 24 h to evaluate the growth of *Fusobacterium varium* strains in different enrichment media.
Brain heart infusion (BHI) served as the control, while peptone–yeast (PY) enrichment media supplemented with lactate (La), lysine (Ly), or a combination of

both substrates (Ly-La) were represented as PY-La, PY-Ly, and PY-Ly-La, respectively.
The x-axis represents fermentation time (hours), and the y-axis indicates optical density at 600 nm (OD_{600}).
Panels show results for individual strains: (A) strain 2024-3-775, (B) 2024-3-1236, (C) 2024-3-774, (D) 2024-3-1093, and (E) 2024-3-776.