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# *Fusobacterium necrophorum* infections in animals: Pathogenesis and pathogenic mechanisms ☆

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**Abstract**

*Fusobacterium necrophorum*, a Gram-negative, non-spore-forming anaerobe, is a normal inhabitant of the alimentary tract of animals and humans. Two subspecies of *F. necrophorum,* subsp. *necrophorum* (biotype A) and subsp. *funduliforme* (biotype B), have been recognized, that differ morphologically, biochemically, and biologically. The subsp. *necrophorum* is more virulent and is isolated more frequently from infections than the subsp. *funduliforme*. The organism is an opportunistic pathogen that causes numerous necrotic conditions (necrobacillosis), either specific or non-specific infections, in a variety of animals. Of these, bovine liver abscesses and foot rot are of significant concern to the cattle industry. Liver abscesses arise with the organisms that inhabit the rumen gaining entry into the portal circulation, and are often secondary to ruminal acidosis and rumenitis complex in grain-fed cattle. Foot rot is the major cause of lameness in dairy and beef cattle. The pathogenic mechanism of *F. necrophorum* is complex and not well defined. Several toxins or secreted products, such as leukotoxin, endotoxin, hemolysin, hemagglutinin, proteases, and adhesin, etc., have been implicated as virulence factors. The major virulence factor appears to be leukotoxin, a secreted protein of high molecular weight, active specifically against leukocytes from ruminants. The complete nucleotide sequence of the leukotoxin operon of *F. necrophorum* has been determined. The operon consists of three genes (*lktBAC*) of which the second gene (*lktA*) is the leukotoxin structural gene. The leukotoxin appears to be a novel protein and does not share sequence similarity with any other leukotoxin. *F. necrophorum* is also a human pathogen and the human strains appear to be different from the strains involved in animal infections.



*Keywords:* *Fusobacterium necrophorum*; Virulence factors; Leukotoxin; Liver abscesses; Foot rot; Calf diphtheria

## 1. Introduction

Fusobacteria are Gram-negative, non-spore-forming, rod-shaped anaerobes that produce butyric acid as a major product of fermentation. The genus name comes from the Latin word 'fusus' meaning spindle. However, not all species of *Fusobacterium* have the characteristic spindle shaped cells. Recently, the taxonomy of the genus *Fusobacterium* has been updated and it currently includes 13 species [1]. The species included are: *F. nucleatum, F. simiae, F. periodonticum, F. naviforme, F. russi, F. necrophorum, F. equinum, F. gonidiaformans, F. mortiferum, F. ulcerans, F. varium, F. necrogenes*, and *F. perfoetans*. Of these *F. nucleatum* and *F. necrophorum* are the two most prevalent species of *Fusobacterium* in clinical samples. Some species previously included in the genus *Fusobacterium* have been assigned to different genera based generally on 16S rRNA gene sequences. *Fusobacterium sulci* and *F. alocis* isolated from the human gingival sulcus [2] have been reclassified as *Eubacterium sulci* and *Filifactor alocis*, respectively [3]. Similarly, *F. prausnitzii*, reportedly one of the 10 most

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predominant members of the human fecal flora, is really a Gram-positive organism and is now under a new genus, *Faecalibacterium* [4,5].

Among the 13 species, *Fusobacterium mortiferum*, *F. naviforme*, *F. nucleatum*, *F. periodonticum*, *F. ulcerans*, and *F. varium* have been isolated mainly from human clinical samples [1,6–9]. *Fusobacterium naviforme*, *F. peridonticum*, and *F. simiae* are typically members of the human oral flora, and with the exception of *F. nucleatum*, are rarely associated with human infections [1]. *Fusobacterium nucleatum* is one of the predominant organisms involved in gingivitis and periodontal diseases, particularly in children and young adults [9]. *Fusobacterium gonidiaformans* has been isolated from normal urogenital and intestinal tracts and occasionally from infections [6]. *Fusobacterium ulcerans* has been associated with tropical ulcers in humans [10]. *Fusobacterium russi* is a member of the canine and feline flora and has been isolated from infected dog and cat bite wounds in humans [11]. *Fusobacterium simiae* was isolated from monkey mouth and differs phenotypically from other oral species of *Fusobacterium* [12]. *Fusobacterium varium* has been implicated in ulcerative colitis in humans and the main virulence factor is believed to be butyric acid [13]

*Fusobacterium necrogenes*, *F. equinum* and *F. simiae* are isolated mainly from animal infections [7]. In addition, *F. russi* and *F. nucleatum* are commonly isolated from abscesses in cats [14]. *Fusobacterium equinum* is a new species that is phenotypically similar to *F. necrophroum* and has been isolated from the normal oral cavity and oral-associated disease of horses [15]. *Fusobacterium necrophorum* is a major human and animal pathogen and is isolated from the mouths, gastrointestinal tracts, and genitourinary tracts of animals and humans. It is one of the most common anaerobes isolated from abscesses and respiratory tract infections in animals [16].

## 2. Characteristics of *Fusobacterium necrophorum*

*F. necrophorum* is a pleomorphic, rod-shaped bacterium and one of the important characteristics is its ability to produce propionic acid from lactic acid. Traditionally, *F. necrophorum* has been classified into four biotypes or biovars: A, B, AB, and C [17–19]. Biotype AB was isolated from ovine abscesses and had similar characteristics to both biotypes A and B (20). Based on 16S ribosomal RNA sequence it is closely related to both biotypes A and B [21]. Currently the taxonomic status of biotype AB is unresolved. Biotype C is non-pathogenic, was initially named *F. pseudonecrophorum* [22], and based on DNA-DNA hybridization analysis [23] and comparison of the 16S-23S intergenic spacer region sequence analysis [24], it is currently considered as identical to *F. varium*. Biotype A is called *F. necrophorum* subspecies *necrophorum* and biotype B is called *F. necrophorum* subspecies *funduliforme* [25]. These two subspecies differ in cell morphology, colony characteristics, growth patterns in broth, extracellular enzymes, hemagglutination properties, hemolytic activities, leukotoxin activities, chemical composition of LPS, and virulence in mice [17–19,26]. However, subspecies identification with conventional tests is sometimes misleading and even conflicting. Phylogenetic methods such as DNA restriction fragment length polymorphism, 16S rRNA sequences, 16S-23S intergenic spacer region sequences , or DNA gyrase B subunit (*gyr*B) sequences have been used for the differentiation of the subspecies [4,27–31].

Subspecies *necrophorum* is more frequently encountered in infections than subsp. *funduliforme* and the latter tends to occur more frequently in mixed infections [17,18,32]. In humans, *F. necrophorum* is associated with Lemierre's syndrome, a condition that primarily affects young and healthy persons. The condition starts out as an acute sore throat with purulent exudate, high fever, cervical and submandibular lymphadenopathy and leads rapidly to disseminated metastatic abscesses, frequently involving septic thrombophlebitis of the internal jugular vein [33]. Strains of *F. necrophorum* causing human infections appear to be distinct from the subspecies *necrophorum* of animal infections and seem to resemble subsp. *funduliforme* [34,35]. However, this requires confirmatory DNA homology or 16S rRNA sequence studies.

## 3. Virulence factors

Virulence factors implicated in the pathogenesis of *F. necrophorum* include leukotoxin; endotoxic lipopolysaccharide (LPS); hemolysin; hemagglutinin; capsule; adhesins or pili; platelet aggregation factor; dermonecrotic toxin; and several extracellular enzymes, including proteases and deoxyribonucleases (Table 1). All these factors contribute to entry, colonization, proliferation, establishment of the organism and to the development of lesions, however, leukotoxin is considered to be the major virulence factor involved in fusobacterial infections in animals [36].

## 4. Leukotoxin

*F. necrophorum* leukotoxin is a secreted protein that is cytotoxic to neutrophils, macrophages, hepatocytes, and possibly to ruminal epithelial cells [37]. The cytotoxicity appears to be specific to ruminant (cattle and sheep) and human neutrophils, but not those from pigs or rabbits and only moderately toxic to neutrophils of horses [37]. The toxin induces apoptosis at low concentrations and lyses the cells at higher concentrations and is more active against PMNs than against lymphocytes [38]. The