

Uterine Microbiology of Healthy Cows and Cows That Develop Uterine Disease¹

Klibs N. Galvão²

The dairy cow is unique because virtually all cows are infected with bacteria right after calving (Sheldon and Dobson 2004; Figure 1).

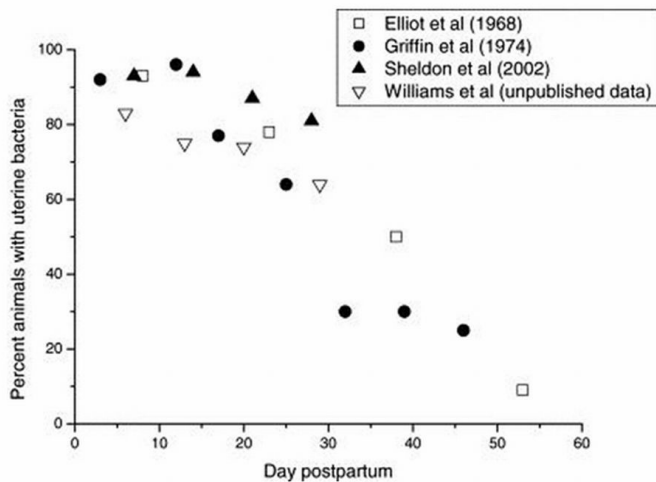


Figure 1. Percent of cows with positive bacterial culture by days postpartum.

Credits: Sheldon and Dobson (2004).

Bacterial culture of the postpartum uterus yields a wide range of isolates (Elliot et al. 1968; Griffin et al. 1974; Sheldon et al. 2002; Galvão et al. 2009). A complete list of isolates can be found in Williams et al. (2005) (see Table 1). Mainly, *Streptococcus* spp., *Staphylococcus* spp., and *Bacillus* spp. were isolated from healthy cows in the first 10 days in milk (DIM), while *Arcanobacterium pyogenes* (*A. pyogenes*),

Escherichia coli (*E. coli*), *Fusobacterium necrophorum* (*F. necrophorum*), and *Prevotella melaninogenica* (*P. melaninogenica*) were primarily isolated from cows with metritis (Bonnett et al. 1991; Bondurant 1999; Huszenicza et al. 1999; Gilbert et al. 2007).

A recent study that used metagenomic analysis to characterize the uterine flora in healthy and metritic cows observed that most clone sequences from the metritic cows were from the phylum Fusobacteria (58%–77%), which included *F. necrophorum* and *F. necrophorum funduliforme*, and Bacteroidetes (9%–16%), which included *Porphyromonas* and *Bacteroides* spp. Interestingly, *P. melaninogenica* was not identified in the phylum Bacteroidetes and neither was *A. pyogenes* (from the phyla Actinobacteria) or *E. coli* (from the phyla Proteobacteria). In healthy cows, the clones were mainly from the phylum Proteobacteria, classis Gammaproteobacteria (100% in one farm and 42% in another), and the phylum Tenericutes (46% in one farm). The classis Gammaproteobacteria included *Mannheimia varigena* and *Pasteurella hemolytica*, and the phylum Tenericutes included *Ureaplasma* and *Mycoplasma*. Using regular culturing methods, the following have been cultured in cows with metritis: *A. pyogenes* (from 33% to 83%), *E. coli* (from 67% to 85%), and Gram negative anaerobes (*F. necrophorum* and *Bacteroides* spp.) (from 49% to 67%) (Bonnett et al. 1991; Huszenicza et al. 1999; Dohmen et al. 2000; Mateus et al. 2002; Földi et al. 2006). Nonetheless, *E. coli* is believed to give way to *A. pyogenes* later in lactation

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2. Klibs N. Galvão, assistant professor, Large Animal Clinical Sciences Department, College of Veterinary Medicine; Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611.

Table 1. Categorization of bacteria isolated by aerobic and anaerobic culture of 328 uterine swabs, according to their expected pathogenic potential in the uterus.

Pathogenic	Potentially Pathogenic	Nonpathogenic
<i>A. pyogenes</i> (137)	<i>Bacillus licheniformis</i> (82)	<i>Clostridium perfringens</i> (6)
<i>P. melaninogenicus</i> (23)	<i>Enterococcus faecalis</i> (40)	<i>Klebsiella pneumoniae</i> (5)
<i>E. coli</i> (104)	<i>M. haemolytica</i> (3)	Micrococcus species (11)
<i>F. necrophorum</i> (18)	<i>Pasteurella multocida</i> (3)	<i>Providencia stuartii</i> (4)
	<i>Peptostreptococcus</i> species (35)	Proteus species (16)
	<i>Staphylococcus aureus</i> (17)	Staphylococcus species, coagulase negative (25)
	Non-haemolytic Streptococci (11)	α-Haemolytic Streptococci (113)
		<i>Streptococcus acidominimus</i> (4)
		Aspergillus species (3)

Adapted from Williams et al. (2005).

in cows with endometritis or pyometra (Olson et al. 1984; Gilbert et al. 2007).

These four main bacteria — *A. pyogenes*, *E. coli*, *F. necrophorum*, and *P. melaninogenicus* — are believed to work synergistically to cause uterine disease (Griffin et al. 1974; Ruder et al. 1981; Bonnett et al. 1991). In fact, *E. coli* might increase the susceptibility of the endometrium to subsequent infection with *A. pyogenes* (Olson et al. 1984; Gilbert et al. 2007; Williams et al. 2007), while *A. pyogenes* acts synergistically with *F. necrophorum* and *P. melaninogenicus* to enhance the severity of uterine disease (Griffin et al. 1974; Ruder et al. 1981; Bonnett et al. 1991). Among their effects, *E. coli* releases bacterial-wall lipopolysaccharides (LPS) (Williams et al. 2008); *A. pyogenes* produces the cholesterol-dependent cytotoxin pyolysin (Miller et al. 2007) and a growth factor for *F. necrophorum* (Sheldon and Dobson 2004); *F. necrophorum* produces a leukotoxin; and *P. melaninogenicus* produces a substance that inhibits phagocytosis (Sheldon and Dobson 2004).

E. coli and *A. pyogenes* have been more extensively studied than the other bacteria. Recently, it was observed that a specific *E. coli* causes uterine disease, which is different from known diarrhoeic or extra-intestinal pathogenic *E. coli* (Sheldon et al. 2010). This specific *E. coli* was named endometrial pathogenic *E. coli* or EnPEC. EnPEC was found to be more adherent and invasive to endometrial cells and also to stimulate greater production of prostaglandin E2 and interleukin 8 (Sheldon et al. 2010). Interleukin 8 is the main neutrophil chemokine. In another study, six virulence factors were found to be associated with metritis and endometritis: fimbriae components (fim) fimH, hemolysin A (hlyA), cytolethal distending toxin (cdt), group II capsule (kpsMII), invasion of brain endothelium (ibeA), and arginine succinyltransferase (astA). However, fimH

was the most prevalent and significant factor (Bicalho et al. 2010). The authors concluded that *E. coli* carrying fimH and at least one of the other factors were pathogenic to dairy cows.

Arcanobacterium pyogenes has been highlighted in several studies as the main causative agent of endometrial damage and infertility (Ruder et al. 1991; Bonnett et al. 1991; Dohmen et al. 2000; Bondurant 1999). A recent study also tried to find specific virulent factors associated with uterine disease (Silva et al. 2008). They evaluated a series of virulence factors including pyolysin (plo), neuraminidases (nan) nanP, nanH, collagen-binding protein A (cbpA), fimA, fimC, fimE, and fimG, but were unable to find any association with incidence of metritis. They concluded that synergism between *A. pyogenes* and other bacteria and differential gene expression of virulence factors might be more important for establishment of infection. In another study, only fimA was found to be overrepresented in cows with metritis, while the other virulence factors were similarly found in both healthy and metritic cows (Santos et al. 2010).

Besides the four main bacteria involved in the pathogenesis of uterine disease, only one virus, bovine herpesvirus IV (BoHV-4), has been linked to uterine disease (Donofrio et al. 2007; Donofrio et al. 2008; Donofrio et al. 2009; Donofrio et al. 2010). Donofrio et al. (2007) observed that BoHV-4 had a tropism for endometrial cells and rapidly infected, replicated, and killed endometrial cells. BoHV-4 is usually isolated concurrently with bacteria that cause disease (Frazier et al. 2001; Monge et al. 2006); however, BoHV-4 itself can also stimulate an immune response by stimulating interleukin-8 production by endometrial cells. Interestingly, viral replication was stimulated by *E. coli* or its

LPS (Donofrio et al. 2008), indicating synergism between *E. coli* infection and BoHV-4 replication.

In summary, pathogenic bacteria associated with metritis and endometritis are *E. coli*, *A. pyogenes*, *F. necrophorum*, and *P. melaninogenicus*. *E. coli* increases the susceptibility of the endometrium to subsequent infection with *A. pyogenes*, and *A. pyogenes* acts synergistically with *F. necrophorum* and *P. melaninogenicus* to enhance the severity of uterine disease. Among their effects, *E. coli* releases bacterial-wall LPS; *A. pyogenes* produces the cholesterol-dependent cytotoxin pyolysin (Miller et al. 2007) and a growth factor for *F. necrophorum*; *F. necrophorum* produces a leukotoxin; and *P. melaninogenicus* produces a substance that inhibits phagocytosis. A specific *E. coli*, called EnPEC, causes uterine disease, and the virulence factor fimH was mostly associated with disease. For *A. pyogenes*, fimA was the only virulence factor associated with uterine disease. BoHV-4 seems to be involved in the pathogenesis of uterine disease.

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