

Bacterial Reproductive Pathogens of Cats and Dogs

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KEYWORDS

- Feline • Canine • Infectious disease • Theriogenology
- Bacteria • Urogenital flora

With the notable exception of *Brucella canis* in dogs, exogenous bacterial pathogens are sporadic causes of reproductive disease in cats and dogs. Most commonly, bacterial infection of the reproductive tract is endogenous in origin; many of the bacteria etiologically involved in reproductive disease form part of the urogenital microflora (**Table 1**). Bacterial reproductive disease is therefore frequently opportunistic, and predisposing factors must be present for disease to develop (**Table 2**).

Culture, polymerase chain reaction (PCR), and serology are commonly used to diagnose bacterial reproductive disease, but the limitations of each method must be understood. The presence of a urogenital microflora must be considered when interpreting culture results from vaginal and seminal secretions; furthermore, age, antimicrobial treatment, and stage of the estrus cycle will influence the quantity and quality of bacterial isolates.^{1–4} Isolation of pure profuse cultures, absence of other pathogens, supportive cytological assessment, and response to antimicrobial treatment may all be helpful in establishing a causal role for any recovered organisms.⁵ Isolation of bacteria from sites that are normally sterile, such as blood or parenchymatous organs, together with supportive histopathology, provides a definitive diagnosis. For exogenous infections, PCR presents a rapid and sensitive alternative to culture. However, false-positive results can arise from laboratory contamination, particularly with “open tube” techniques. Serologic evidence of exposure can be useful in diagnosing exogenous infection; assay specificity and timing of sampling are critical.

The authors have nothing to disclose.

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Table 1
Bacteria isolated from the prepuce and vagina of clinically healthy dogs and cats

Prepuce		Vagina	
Male Dog ³⁷	Tom ^{37,103}	Bitch ^{3,37,104,105}	Queen ^{1,37,103}
<i>Staphylococcus</i> spp Coagulase-neg. staphylococci Coagulase-pos. staphylococci	<i>Staphylococcus</i> spp Coagulase-neg. staphylococci	<i>Staphylococcus</i> spp Coagulase-neg. staphylococci Coagulase-pos. staphylococci	<i>Staphylococcus</i> spp Coagulase-neg. staphylococci Coagulase-pos. staphylococci
<i>Streptococcus</i> spp β -hemolytic streptococci α -hemolytic streptococci Nonhemolytic streptococci	<i>Streptococcus</i> spp β -hemolytic streptococci α -hemolytic streptococci Nonhemolytic streptococci	<i>Streptococcus</i> spp β -hemolytic streptococci α -hemolytic streptococci Nonhemolytic streptococci	<i>Streptococcus</i> spp β -hemolytic streptococci α -hemolytic streptococci Nonhemolytic streptococci
<i>Corynebacterium</i> spp	<i>Corynebacterium</i> spp	<i>Corynebacterium</i> spp	<i>Corynebacterium</i> spp
<i>Escherichia coli</i>	<i>Escherichia coli</i>	<i>Escherichia coli</i>	<i>Escherichia coli</i>
<i>Pasteurella</i> spp	<i>Pasteurella</i> spp	<i>Pasteurella</i> spp	<i>Pasteurella</i> spp
<i>Mycoplasma</i> spp	<i>Mycoplasma</i> spp	<i>Mycoplasma</i> spp	<i>Mycoplasma</i> spp
<i>Haemophilus</i> spp	<i>Moraxella/Brahamella</i> spp	<i>Haemophilus</i> spp	<i>Haemophilus</i> spp
<i>Klebsiella pneumoniae</i>	<i>Bacteroides</i> spp	<i>Klebsiella</i> spp	<i>Klebsiella</i> spp
<i>Acinetobacter</i> spp	<i>Fusobacterium</i> spp	<i>Acinetobacter</i> spp	<i>Acinetobacter</i> spp
<i>Moraxella</i> spp	<i>Simonsiella</i> spp	<i>Moraxella</i> spp	<i>Bacteroides</i> spp
<i>Proteus</i> spp		<i>Bacteroides</i> spp	<i>Peptococcus</i> spp

<i>Bacillus</i> spp	<i>Fusobacterium</i> spp	<i>Arcanobacterium pyogenes</i>
<i>Pseudomonas</i> spp	<i>Peptostreptococcus</i> spp	<i>Lactobacillus</i> spp
<i>Enterococcus</i> spp	<i>Proteus</i> spp	
<i>Ureaplasma</i> spp	<i>Bacillus</i> spp	
<i>Flavobacterium</i> spp	<i>Pseudomonas</i> spp	
	<i>Enterococcus</i> spp	
	<i>Ureaplasma</i> spp	
	<i>Flavobacterium</i> spp	
	<i>Citrobacter</i> spp	
	<i>Clostridium</i> spp	
	<i>Neisseria</i> spp	
	<i>Enterobacter</i> spp	
	<i>Micrococcus</i> spp	
	<i>Alcaligenes faecalis</i>	
	<i>Prevotella</i> spp	

Adapted from Barsanti JA. Genitourinary infections. In: Greene CE, editor. Infectious diseases of the dog and cat. 3rd edition. Philadelphia: Saunders Elsevier; 1996. p. 935–61; with permission.

Table 2	
Predisposing factors for infection with endogenous reproductive bacterial pathogens	
Clinical Disease	Predisposing Factors^{37,45,106}
Vaginitis	<ul style="list-style-type: none"> • Congenital anatomic abnormalities (eg, stenosis) • Vaginal atrophy following ovariectomy • Immaturity • Neoplasia • Trauma • Foreign body • Drugs • UTI • Pyometra • Systemic disease (eg, diabetes) • Primary viral infection
Metritis	<ul style="list-style-type: none"> • Trauma (eg, dystocia, obstetric manipulation) • Abortion • Retention of fetal or placental tissue
Pyometra	<ul style="list-style-type: none"> • Middle-aged intact bitch • Unbred intact queen >3-yr-old • Unsuccessful matings (queen) • Exogenous progestins
Mastitis	<ul style="list-style-type: none"> • Trauma to teat and overlying skin • Poor hygiene • Retention of secretions
Epididymitis-Orchitis	<ul style="list-style-type: none"> • Trauma • Concurrent cystitis or prostatitis
Prostatitis	<ul style="list-style-type: none"> • Intact male • Concurrent cystitis or pyelonephritis • Disease of prostatic urethra (eg, urethral urolithiasis) • Disease that interferes with prostatic fluid formation and secretion (eg, prostatic neoplasia)
Neonatal disease	<ul style="list-style-type: none"> • Dystocia • Hypothermia • Poor hygiene • Overcrowding • Insufficient passive immunity • Low body weight • Prematurity • Juvenile queen where GGS endemic • Immature immune system • Mastitis or metritis in dam • Antimicrobial treatment suppressing colonization resistance

BRUCELLA CANIS

Brucella spp are small, aerobic gram-negative coccobacilli, which stain red using the modified Ziehl-Neelsen (MZN) technique. In addition to *B canis*, dogs can be infected with *B abortus*, *B melitensis*, and *B suis*.^{6–8} *Brucella* is not an important cause of reproductive disease in the cat.⁹ However, productive infections can occur; a cat infected with *B suis* was identified as the source of an outbreak of brucellosis in 6 human contacts.¹⁰

Seroprevalence studies indicate that canine brucellosis is widespread in the southern US, Central and Southern America, and Asia.¹¹ Sporadic cases have been reported in Europe. *Brucella* is under statutory control in the UK; the only confirmed case of *B canis* infection was diagnosed post quarantine in a dog imported from Spain.¹² *Brucella* has a predilection for male and female reproductive tracts in sexually mature animals. Infection is acquired by inhalation, ingestion, and insemination; significantly, infection can also be transmitted in utero. Invading bacteria survive phagocytosis¹³ and are transported to the uterus, epididymides, and prostate via a cell-associated bacteremia 2 weeks post infection (PI). Bacteremia persists for at least 6 months PI and can be detected for up to 64 months.¹⁴

Vaginal and seminal secretions from infected animals contain the highest bacterial loads and are therefore the most significant sources of infection.¹⁴ Bacteriuria persists for at least 3 months PI, facilitating horizontal transmission between male dogs. Although bacteria are shed in feces, milk, saliva, and nasal and ocular secretions, these are not regarded as major sources of infection. Infection can also be acquired indirectly; *B abortus* can survive in water and damp soil for up to 4 months, and *B canis* remains viable in semen and mouse cryoprotective agent for up to 48 hours.^{15,16}

Clinical Signs

The clinical signs associated with *Brucella* infection are not pathognomonic. Infected animals are rarely systemically ill and fever is very uncommon, perhaps because this organism lacks the lipopolysaccharide (LPS) antigen associated with endotoxemia.¹⁷ Clinical signs can also reflect localization of the bacteria in extrareproductive tract sites such as the eye, intervertebral disc spaces, and reticuloendothelial system.^{18,19}

Brucellosis causes spontaneous late abortion in an otherwise healthy bitch. This most commonly occurs from days 30 to 57, peaking between days 45 and 55.²⁰ Abortion is usually accompanied by a vaginal discharge lasting up to 6 weeks. Earlier abortions can occur but may be incorrectly reported as conception failure since the bitch typically ingests aborted fetuses. Early embryonic death and fetal resorption can occur 10 to 20 days post-mating. Many bitches that abort will subsequently have normal litters, although some may experience intermittent reproductive failures.²¹ Some litters born to infected bitches contain both live and dead pups, although most live pups die shortly thereafter. Those that survive suffer generalized lymphadenopathy and persistent hyperglobulinemia, and they develop clinical disease on reaching sexual maturity.¹⁴

Clinical signs of epididymitis become apparent from week 5 PI. In acute infection, scrotal distention caused by enlargement of the tail of the epididymis and accumulation of serosanguineous fluid is clinically evident. Primary orchitis is not common but can occur.^{22,23} Infected dogs may also present with scrotal dermatitis caused by constant licking of the scrotal skin and secondary bacterial infection.²¹ Unilateral or bilateral testicular atrophy develops in chronic infection. Sperm abnormalities are detectable with the onset of clinical signs, with over 90% abnormal by week 20. Concurrent prostatitis is also common.²²

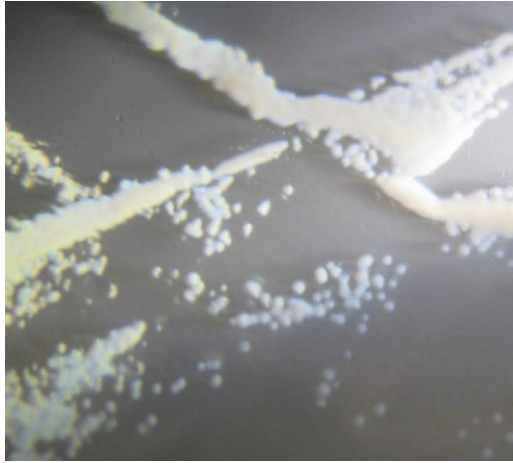


Fig. 1. A 48-hour culture of *B canis* on serum dextrose agar (SDA) incubated in air under 10% CO₂ at 37°C. *B canis* is slow-growing and the characteristic “rough” colonies do not appear before 48 hours on solid media. (Image courtesy of Lorraine Perrett, Animal Health and Veterinary Laboratories Agency [AHVLA], Weybridge, Surrey, UK.)

Diagnosis

Isolation and identification of *B canis* is the gold standard (**Fig. 1**). Placenta, lymph nodes, prostate, and spleen are suitable samples for culture, whereas semen, vaginal secretions, and urine (unless collected by cystocentesis) are frequently contaminated with other organisms. Blood submitted in an aerobic blood culture bottle is the sample of choice because of the lack of contaminating organisms and prolonged bacteremia. Culture is time-consuming and presents a potential biohazard to laboratory personnel, necessitating Containment Level 3 (CL3) facilities (**Table 3**).

PCR can be a rapid, highly sensitive and specific assay and presents a useful alternative to culture for the direct detection of *Brucella*. Most PCR assays are designed to detect gene sequences conserved across all *Brucella* species and biovars, and therefore detect *Brucella* to genus level only. A multiplex PCR assay that can differentiate all known species, including *B canis*, has recently been published.²⁴ In dogs, *Brucella* DNA has been detected in whole blood, serum, semen, vaginal swabs, inguinal lymph nodes, and aqueous humor.^{16,18,25–27} Compared to blood culture, the diagnostic sensitivity of whole blood PCR was 100% in naturally infected dogs.²⁵ However, some dogs were PCR positive and blood culture negative, which likely reflects the lower sensitivity of blood culture.²⁸

Serology is widely used to diagnose canine brucellosis. An understanding of assay sensitivity and specificity, the chronology of antibody development, and the antigen used are all required to successfully interpret test results. Antibodies are not detectable for 3 to 4 weeks PI, and occasionally up to 12 weeks PI.¹⁴ False-positive reactions can be problematic since epitopes within the LPS antigens are frequently shared with other bacterial species. The most commonly used antigens are *B ovis* or *B canis* LPS antigens; assays based on the *B canis* nonpathogenic (M–) strains are more specific.²⁹

The rapid slide agglutination test (RSAT) is a simple, rapid, and sensitive test designed to detect antibodies to *Brucella* LPS antigen. This is most accurate as a screening test from 8 to 12 weeks PI. The 2ME-RSAT is considered more specific

Table 3 Diagnosis of major bacterial reproductive pathogens by culture						
Pathogen	Optimal Samples	Direct Microscopy	Culture Time	Additional Tests	Comments	Alternative Tests
<i>Brucella</i>	<ul style="list-style-type: none"> • Blood • Placenta 	<ul style="list-style-type: none"> • MZN-positive clusters of cells 	<ul style="list-style-type: none"> • Min. 48 hr 	<ul style="list-style-type: none"> • Multiplex PCR • Serotyping 	<ul style="list-style-type: none"> • Exogenous • CL3 Laboratory • Selective media • Reportable in UK 	<ul style="list-style-type: none"> • AGID (serum) • PCR (whole blood, tissues, secretions)
<i>Escherichia coli</i>	<ul style="list-style-type: none"> • Genital swabs • Neonatal tissues • Milk 	<ul style="list-style-type: none"> • Gram-negative rods 	<ul style="list-style-type: none"> • Min. 24–48 hr 	<ul style="list-style-type: none"> • API 20E 	<ul style="list-style-type: none"> • Endogenous 	<ul style="list-style-type: none"> • N/A
<i>Streptococcus</i>	<ul style="list-style-type: none"> • Genital swabs • Placenta • Fetal tissues • Neonatal tissues 	<ul style="list-style-type: none"> • Gram-positive cocci in chains or pairs 	<ul style="list-style-type: none"> • Min. 24–48 hr 	<ul style="list-style-type: none"> • API 20 Strep • Lancefield Grouping 	<ul style="list-style-type: none"> • Endogenous 	<ul style="list-style-type: none"> • N/A
<i>Leptospira</i>	<ul style="list-style-type: none"> • Urine • Fetal tissues 	<ul style="list-style-type: none"> • Motile helical bacteria using dark ground microscopy 	<ul style="list-style-type: none"> • Min. 2–6 wk 	<ul style="list-style-type: none"> • Serotyping • DNA profiling 	<ul style="list-style-type: none"> • Exogenous • CL3 Laboratory • Selective media • Fragile 	<ul style="list-style-type: none"> • MAT (serum) • PCR (tissues, urine) • FA (tissues)
<i>Salmonella</i>	<ul style="list-style-type: none"> • Placenta • Fetal tissues • Genital swabs 	<ul style="list-style-type: none"> • Gram-negative rods 	<ul style="list-style-type: none"> • Min. 48 hr 	<ul style="list-style-type: none"> • API 20E • Serotyping • Phage typing 	<ul style="list-style-type: none"> • Exogenous • Selective media 	<ul style="list-style-type: none"> • PCR (feces, tissues)
<i>Campylobacter</i>	<ul style="list-style-type: none"> • Placenta • Fetal tissues • Genital swabs 	<ul style="list-style-type: none"> • Slender, curved Gram-negative rods 	<ul style="list-style-type: none"> • Min. 48 hr 	<ul style="list-style-type: none"> • API Campy 	<ul style="list-style-type: none"> • Exogenous • Selective media • Microaerobic 	<ul style="list-style-type: none"> • PCR (feces, tissues)
<i>Staphylococcus</i>	<ul style="list-style-type: none"> • Genital swabs • Neonatal tissues • Milk 	<ul style="list-style-type: none"> • Gram-positive cocci in bunches of grapes 	<ul style="list-style-type: none"> • Min. 24–48 hr 	<ul style="list-style-type: none"> • ID32<i>Staphylococcus</i> • DNase test • Coagulase test 	<ul style="list-style-type: none"> • Endogenous 	<ul style="list-style-type: none"> • N/A

than the RSAT, as 2-mercaptoethanol (2-ME) destroys cross-reacting IgM antibodies. Agar gel immunodiffusion (AGID) detects precipitating antibodies against *Brucella* cell wall or cytoplasmic antigen. The assay is highly specific for *Brucella* spp, particularly if based on the *B canis* cytoplasmic antigens (CPag). CPag antibodies are detectable from 8 to 12 weeks PI and can persist for up to 36 months after bacteremia has resolved, making the test useful for detecting chronic infections.¹⁴ The AGID assay is frequently used as a confirmatory test for other serologic assays.³⁰ Enzyme-linked immunosorbent assays (ELISAs) have been developed to detect antibodies to *B canis* cell wall, cytoplasmic, and recombinant antigens and may prove a simpler and more rapid alternative to agglutination assays. A recently described ELISA based on heat-soluble *B canis* antigen was highly sensitive (91.1%) and specific (100%) compared to AGID.³⁰ Samples with positive or equivocal results on serology should be submitted for a direct confirmatory method given the propensity for inaccurate results. Preliminary PCR data suggest that the 2ME-RSAT and AGID may be less sensitive but more specific than previously reported.³¹

Antimicrobial Therapy and Control

Brucellosis is problematic to treat, given the inability of many antimicrobials to attain adequate concentrations at intracellular level. No antimicrobial protocol has been shown to consistently achieve a long-term cure. Combination therapy is frequently recommended; the most efficacious is reported to be a combination of tetracyclines and aminoglycosides, or fluoroquinolones and aminoglycosides.^{11,18,32} Where *Brucella* is identified within kennels, a test and elimination strategy can be instigated. To prevent new infections, all incoming animals should be isolated until 2 seronegative tests are returned 30 days apart.¹⁴ Prebreeding tests should also be carried out.¹¹

B canis is not considered a significant zoonosis under normal circumstances, with infections reported to be mild and uncommon. However, serious illness can occur in immunocompromised patients.³³ Infection is usually transmitted by direct contact with infected animals or through occupational aerosol exposure. The true incidence of infection is unknown as clinical signs are usually nonspecific, and diagnosis is challenging.

ESCHERICHIA COLI

Escherichia coli is a facultatively anaerobic, gram-negative member of the Enterobacteriaceae family. *E coli* forms part of the intestinal microflora in all mammals and can also be isolated from healthy epithelial tissues. *E coli* is one of the most frequently isolated bacteria from the lower urogenital tract in clinically healthy cats and dogs.

E coli infections of the reproductive tract are opportunistic infections caused by strains within the gastrointestinal (GI) microflora. In humans, 2 distinct groups of *E coli* reside in the healthy GI tract: commensal strains that rarely cause disease and pathogenic strains with potential to cause disease at any site external to the GI tract. The latter are called extraintestinal pathogenic *E coli* (ExPEC). These strains possess a toolkit of virulence genes that encode resistance to host defenses (eg, capsular K antigen), iron-acquisition systems (eg, aerobactin), adhesins (eg, P fimbriae), and exotoxins (eg, hemolysins).³⁴ It is likely that equivalent groups of *E coli* reside in the canine and feline GI tracts and that many *E coli* infections of the reproductive tract are caused by ExPEC strains. It is known that *E coli* isolates recovered from bitches with pyometra are derived from the GI microflora³⁵ and carry a wide range of virulence genes, including uropathogenic specific protein (*usp*), cytotoxin necrotizing factor (*cnf-1*), β -hemolysin (*hlyA*), and P fimbriae (*papC*) genes.³⁶ Additional research is

required to ascertain whether a particular combination of virulence genes can accurately define ExPEC strains.

While only virulent *E coli* isolates secrete exotoxins, all *E coli* strains release endotoxin on cell lysis. Endotoxin can activate the host systemic inflammatory response syndrome (SIRS) on entering the systemic circulation. Uncontrolled SIRS can trigger sepsis and multiorgan failure, and endotoxemia is a potentially fatal sequel to pyometra, metritis, neonatal bacteremia, and mastitis caused by *E coli*.

Clinical Signs

E coli is a common cause of vaginitis, metritis, mastitis, and pyometra in bitches and queens and is an uncommon cause of abortion.³⁷ A wide range of vaginal microflora organisms have been recovered from pyometra cases, with *E coli* isolated from 70%.^{2,38,39} Peak binding of *E coli* to the endometrium occurs in the early luteal phase,⁴⁰ when bitches are most vulnerable to the development of pyometra.⁴¹ *E coli* can be isolated from the healthy uterus during proestrus and estrus,^{1,3} which may provide a residual source of infection. Concurrent urinary tract infection (UTI) with identical strains of *E coli* is common,^{35,40,42} but it is unclear whether UTI predisposes to, or is a consequence of, pyometra. Endotoxin is abortifacient in other species and has been cited as a possible cause of partial abortion in a bitch.⁴³ However, *E coli* does not appear to be a common cause of abortion in cats and dogs.⁴⁴

Most cases of epididymitis, orchitis, and prostatitis in companion animals are bacterial in origin, with *E coli* most frequently isolated.³⁷ Infection can affect 1 or all 3 organs. In most cases, infection ascends from the distal urethra with concurrent UTI commonly reported. None of these conditions are common in companion animals, particularly the cat.

Disease and death are common in neonatal pups or kittens (days 0–14). Infectious diseases of bacterial origin are the second most common cause of death after dystocia, with *E coli* and β -hemolytic (β H) *Streptococcus* spp the most significant bacterial pathogens. Puppies and kittens are colonized with *E coli* in their first 24 hours, most commonly from maternal vaginal discharges and from the environment; over 60% of *E coli* strains isolated from pups were identical to strains isolated from dams and other dogs in the kennel.⁴⁵ Under normal circumstances, colonization should be asymptomatic or cause mild self-limiting disease. However, the presence of predisposing factors can trigger bacteremia and endotoxemia (**Table 2**). It is not yet clear whether particular virulent strains of *E coli* are consistently involved.

Diagnosis

E coli is readily cultured from clinical specimens using routine diagnostic media (**Fig. 2**). PCR can be used to detect virulence genes present in *E coli* isolates,³⁶ but additional research is needed to identify the combination of genes that define virulent strains before such assays can provide useful information to veterinary practitioners.

Antimicrobial Treatment and Control

Sensitivity testing should be carried out if antimicrobial therapy is indicated, as antimicrobial resistant isolates are increasingly prevalent.⁴⁶ A recent study monitoring *E coli* isolates from healthy dogs in the United Kingdom (N = 183), found that 29% of healthy dogs were shedding isolates resistant to at least one antimicrobial, with 15% carrying multidrug-resistant (MDR) isolates. Resistance to ampicillin, tetracycline, and trimethoprim was most prevalent.⁴⁷ To control disease, predisposing causes must be identified and minimized.

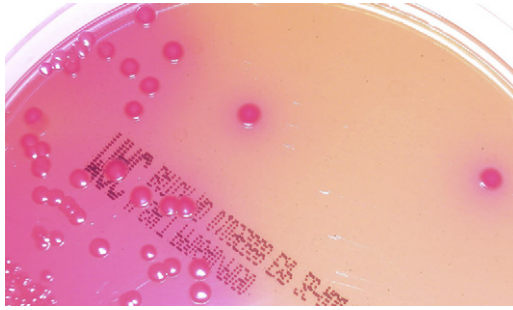


Fig. 2. *E coli* is a strong lactose fermenter, producing bright pink colonies on MacConkey agar within 24 hours. Identification based on biochemical properties can be made using the Analytical Profile Index (API) numerical system (API 20E; bioMérieux, Marcy l’Etoile, France).

Animal handlers and veterinarians are potentially at risk from ExPEC strains. Virulence factors detected in strains carried by healthy dogs are known to be significant in human infections.³⁶ Furthermore, MDR organisms and resistance plasmids could readily be transferred between species and organisms, respectively. MDR ExPEC strains (ST131) have been detected in both clinical and healthy companion animal samples.⁴⁸

STREPTOCOCCUS

Streptococcus spp are small, nonmotile, facultatively anaerobic gram-positive cocci, which frequently appear in chains in clinical specimens (**Fig. 3**). Many species form part of the canine and feline microflora populating skin and mucous membranes.

Streptococci are common opportunistic pathogens in animals and humans, and predisposing factors for infection are listed in **Table 2**. Streptococci can be categorized in several ways—first, by their effect on erythrocytes in culture medium; most pathogenic streptococci are β H, whereas α -hemolytic and non-hemolytic organisms are less likely to be clinically significant. Streptococcal species are also categorized by antigenic differences in their cell wall C-substance (Lancefield groups). In both cats and dogs, clinical



Fig. 3. *S canis* manifests as gram-positive cocci in chains or pairs in smears made from clinical specimens or isolates.

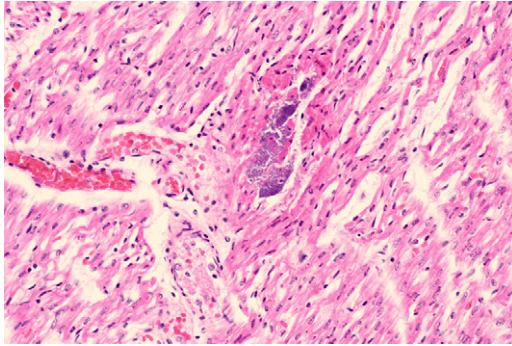


Fig. 4. Gram-positive cocci within cardiac tissue from a case of neonatal septicemia. *β H Streptococcus* sp was subsequently isolated from this sample. (Image courtesy of Dr Catherine Lamm, University of Glasgow.)

disease is most commonly associated with Lancefield group G streptococci (GGS), predominantly *S canis*. However, some organisms within Lancefield groups B (GBS), C (GCS), L (GLS), and M (GMS) have been etiologically associated with neonatal septicemia, abortion, and endometritis.^{49–53}

Clinical Signs

In the past, β H streptococci have been linked to multiple diseases of the reproductive tract, including infertility. However, many early reports need to be interpreted carefully, given the frequency with which β H streptococci can be isolated from the urogenital tracts of clinically healthy animals. Currently, there is little causal evidence to link β H streptococci with infertility in the bitch or the queen.⁵⁴ Nonetheless, β H streptococci are a recognized cause of metritis, pyometra, placentitis, and abortion,^{38,50,52,54} usually as a result of ascending infection. β H streptococci have also been isolated from sporadic cases of mastitis and vaginitis.^{55,56}

β H streptococci are a major cause of neonatal death. Typically, neonates become infected from the maternal birth canal via the umbilicus or, less commonly, from mastitic milk.^{57,58} GGS bacteria predominate, with *S canis* the most frequently identified species.⁵³ However, many isolates are identified only to genus level. *S canis* septicemia is a particularly well-described problem in breeding catteries.⁵⁴ Several kittens in a litter can be infected at once, most commonly the first litter of a young queen. Young queens have higher bacterial loads in the vagina, which persist throughout pregnancy, whereas older queens can eliminate infection by mid-gestation.

β H streptococci were causally associated with all 24 canine fetal or neonatal deaths investigated over a 33-month period in a regional diagnostic laboratory.⁵⁹ Many isolates were not speciated; of those that were, *S canis* predominated (**Fig. 4**). GBS and GCS are rare causes of reproductive disease in cats and dogs. *S agalactiae* (GBS) caused neonatal septicemia in 2 litters of pups in a research colony. One bitch presented systemically ill with a purulent vaginal discharge; the other bitch remained well. The organism was isolated from vaginal swabs and pup tissues.⁵¹ *S dysgalactiae* subsp *dysgalactiae* (GCS) was isolated in pure culture from neonatal puppies that died of septicemia within 72 hours of birth.⁴⁹



Fig. 5. *S canis* is β H on sheep blood agar, producing a wide zone of clear hemolysis. Lancefield group testing can be carried out on isolates using commercial agglutination kits or the ring precipitation test. Principal species can be distinguished biochemically using the API 20S numerical system.

Diagnosis

Samples should be submitted in transport medium as streptococci are susceptible to desiccation.⁶⁰ Streptococci are readily cultured from clinical specimens using routine diagnostic media. β H species produce a clear zone of hemolysis on blood agar within 24 to 48 hours (**Fig. 5**).

Antimicrobial Therapy and Control

Generally, streptococci are sensitive to penicillin and its derivatives, erythromycin, clindamycin, and cephalexin; however, sensitivity testing should be carried out prior to antimicrobial treatment. In endemically affected catteries, vulnerable kittens can be prophylactically treated and the umbilicus dipped in 2% tincture of iodine.⁵⁴ Predisposing factors for disease must be minimized.

GGs can be transferred to humans via direct contact and bite wounds. However, the public health risk from *S canis* is low and infection is uncommon, representing 1% of all human streptococcal infections in France over a 5-year period.⁶¹ Serious illness is mainly confined to elderly or immunocompromised patients.

LEPTOSPIRA

Leptospira are fine spiral bacteria (spirochetes), with a central body hooked at each end and surrounded by an envelope. Within this lies a single flagellum arising from each end and overlapping centrally. Species (eg, *L interrogans*) are divided into serovars on the basis of shared envelope antigens.

Leptospirosis occurs in dogs worldwide. The range of serovars reported from each country varies. Reporting depends on (1) those actually present and (2) those sought (which can vary according to the strains used as antigens and the ability of laboratories to isolate and identify them). The dog is a maintenance host of *L interrogans* serovar Canicola, and perhaps of serovar Bratislava.⁶² Infection with other serovars is usually sporadic and depends on contact with sources of infection. Serovars present worldwide include Icterohaemorrhagiae and Canicola. Serovars Pomona, Grippityphosa, and Bratislava are among the most common serovars in North America,⁶³ with Grippityphosa and Bratislava the most common in continental Europe.⁶²

Infection is transmitted by direct or indirect contact with leptospirae, via ingestion, entry through abrasions, transplacental, and possibly venereal routes. The organisms multiply rapidly to produce a bacteremia, which may cause clinical signs. Leptospirae may cause damage to the liver, kidneys, or other organs; however, the relationship between infecting serovar and organ system involvement is not as well defined as previously thought.⁶⁴ Circulating antibody normally limits leptospiremia within 7 to 10 days PI. However, organisms can enter, replicate, and persist in sites protected from circulating antibody, such as renal tissue.⁶⁵ Localization may also occur in the pregnant uterus, causing abortion or birth of stillborn or weak progeny.^{66,67} Leptospirae may enter the male genital tract from systemic infection.

Clinical Signs

Clinical leptospirosis is rarely recorded in cats,⁶⁷ although infection may be common.⁶⁸ In dogs, disease of the reproductive tract is uncommon and is associated with systemic infection in most cases (serovars Pomona, Grippityphosa, and Canicola), and may result from carrier infections (Canicola and Bratislava). Fever and icterus may accompany or precede abortion, or the birth of weak or stillborn progeny. Reproductive disease has been described most consistently in breeding colonies, often associated with serovar Bratislava.⁶⁶

Diagnosis

Diagnosis is confirmed by demonstration of the organism or by the presence of specific antibody. The organisms may be demonstrated by dark ground microscopy of the urine from aborting bitches or in urine, aborted fetuses, or stillborn pups by culture. Dark ground microscopy is relatively insensitive and nonspecific and is no longer recommended.⁶⁵ The fragile and fastidious leptospirae may be grown aerobically in complex media, and must be carried out in CL3 facilities to protect laboratory personnel. Primary isolation from tissue or urine takes at least 2 to 6 weeks and is most reliable when the tissue concerned is fresh, uncontaminated, and submitted in a suitable transport medium. Isolation remains the gold standard⁶³ but is not routinely carried out given that the assay is time-consuming, requires specialist facilities, and may be lacking in sensitivity. The organism, its antigens, or products may also be demonstrated in tissue by silver staining of fixed tissue, by immunofluorescence or immunoperoxidase, and by DNA probes. PCR tests based on sequences from the 16S rRNA gene can also be used to identify the presence of pathogenic leptospirae.⁶⁹

Serologic tests are widely used. Antibodies to leptospirae appear in the serum within 1 to 2 weeks of infection and reach titers of 1:10,000 to 1:30,000 (microscopic agglutination lysis test [MAT]), which may persist for some weeks. The MAT, using live organisms, is the most sensitive test and detects rising titers that follow infection particularly well. ELISAs based on whole cells, axial filaments, and, most specifically, lipoprotein LIPL32 have been described. A competitive ELISA has been produced for serovar Bratislava.⁷⁰ Serum antibody can be used to confirm recent infections, but aborting animals or those with reproductive disease may be seronegative. Antibody detected in thoracic exudates from stillborn fetuses is diagnostic. Cross-reaction between serogroups and serovars may occur and accurate serology is best carried out using local serovars.

Antimicrobial Therapy and Control

The parenteral administration of a number of antibiotics such as penicillin, semisynthetic penicillins, streptomycin, and doxycycline is of value in acutely ill animals.

Abortions may be prevented and renal carriers eliminated by doxycycline treatment.⁷¹ Vaccination of uninfected dogs with killed vaccines containing the appropriate serovars prevents reproductive disease. In North America, serovars Pomona, Gripotyphosa, Canicola, and Icterohaemorrhagiae are available in combination. Only serovars Icterohaemorrhagiae and Canicola are available in commercial European vaccines.⁶² Infection may be prevented by vaccination, disinfection, elimination of rodents, and restriction of access to rodent-contaminated areas. Leptospirae can survive in uncooked offal and survive freezing, so feeding raw animal byproducts should be avoided.

Humans are a dead-end host for leptospiral infection; contact with the urine or products of abortion of infected dogs should be avoided. Leptospirae enter susceptible hosts via mucous membranes or damaged skin, and gloves and protective clothing should always be worn when handling infected animals. All known shedders or suspected shedders should be treated with antimicrobials to eliminate the carrier state and minimize environmental infectivity. Contaminated areas can be treated with iodophor disinfectants.^{65,72}

SALMONELLA

Salmonella spp are gram-negative coliform bacteria. Those causing disease in cats and dogs are serotypes of *S enterica*, especially Typhimurium, Panama, and Montevideo. Salmonellosis is an uncommon cause of reproductive tract disease in the cat and dog and usually follows enteric or systemic disease. Fever or direct bacterial invasion of the products of conception may cause abortion, or the birth of stillborn and weak pups or kittens.⁷³ *Salmonella* infection can cause prostatitis, orchitis, and epididymitis in males. Cases of *Salmonella* septicemia in greyhound pups can be associated with raw meat diets and a high prevalence of healthy *Salmonella* shedders within racing kennels.⁷⁴

Successful culture confirms infection or carriage. Isolation of salmonellae from organs aseptically sampled from aborted fetuses or stillborn pups or kittens confirms their involvement in reproductive disease.⁷⁵ Organisms in feces may be detected by simple PCR, but real-time PCR gives more rapid results. Serum antibody can be detected using ELISA tests based on "O" antigens modified for the dog and cat.

Salmonella may be sensitive in vitro to a wide range of suitable antimicrobials, such as ampicillin, clavulanate-potentiated amoxicillin, gentamicin, or fluoroquinolones, but resistance is common in serotypes such as *S enterica* Typhimurium. Supportive treatment should be given for enteric or systemic signs. *Salmonella* causes clinical disease in humans and protective measures should be taken by those exposed to the products of abortion or to weak puppies or kittens.

CAMPYLOBACTER

Campylobacter spp are microaerobic gram-negative curved rods or short spiral organisms. Up to 14 species have been isolated from dog and cat feces, principally *C jejuni*, *C coli*, and *C upsaliensis*. Abortion and infection of the female reproductive tract sometimes follow *Campylobacter enteritis*, but are rarely identified in the dog and cat.⁷⁶ Infection of the reproductive tract may be ascending or blood-borne; the fetus becomes infected in gravid females, causing abortion or the birth of weak or stillborn pups and kittens. Diagnosis is confirmed by demonstrating the organism in aborted fetuses and vaginal swabs by culture or PCR.⁷⁷ Antibody detection is not used routinely in diagnosing infection. Macrolides and fluoroquinolones can be used for treatment. Infection spreads rapidly in multicat or multidog households where

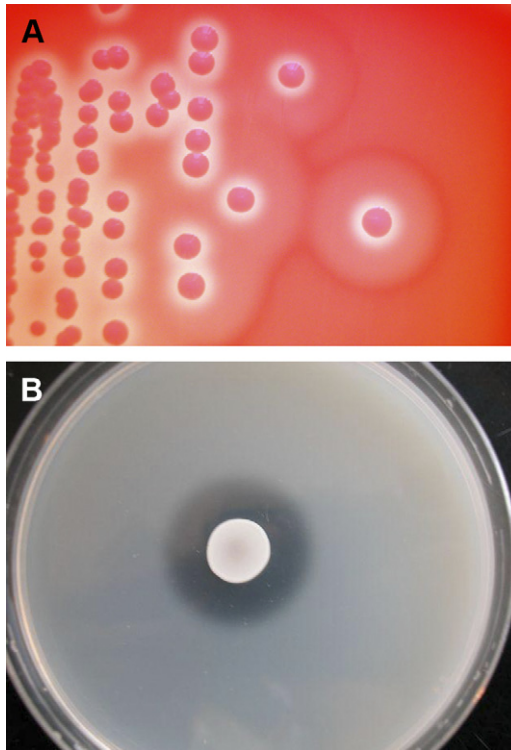


Fig. 6. (A) *S. pseudintermedius* isolates frequently produce target or double hemolysis on sheep blood agar, a narrow clear zone of hemolysis surrounded by a wide zone of incomplete hemolysis. The ID32 *Staphylococcus* API system can be used to identify *S. pseudintermedius*. (B) *S. pseudintermedius* is DNase-positive, producing a wide zone of clearing after 24 hours on a DNase agar plate.

hygiene practice is inadequate. *Campylobacter* spp from cats and dogs can cause disease in humans.

STAPHYLOCOCCUS

Staphylococcus spp contribute to the microflora populating feline and canine skin and mucous membranes. *Staphylococcus pseudintermedius* is the predominant species colonizing canine skin and mucous membranes, and *Staphylococcus felis* is the major species colonizing feline mucous membranes. *S. pseudintermedius* is occasionally isolated from feline skin and mucous membranes.

Opportunistic infection of the reproductive tract with staphylococci occurs in the presence of predisposing factors (Table 2). Staphylococci are the major cause of mastitis in the bitch and are sporadically recovered from cases of neonatal septicemia, vaginitis, and pyometra.^{2,58} A role for *S. felis* in feline reproductive disease has yet to be described, but these are significant UTI pathogens⁷⁸ and are frequently isolated from other clinical specimens.⁷⁹

Direct microscopy on clinical specimens is useful, with the arrangement of gram-positive cocci in “bunches of grapes.” *S. pseudintermedius* causes complete or double hemolysis on blood agar (Fig. 6) and tests positive on coagulase and DNase

tests. *S felis* is weakly hemolytic, coagulase-negative, and weakly DNase-positive⁸⁰ and cannot be identified using the commercial ID32 *Staphylococcus* API system. Given the difficulty in distinguishing *S felis* from other staphylococci and that these are coagulase-negative staphylococci, *S felis* infections may be underdiagnosed. Most staphylococcal isolates are susceptible to β -lactamase-resistant synthetic penicillins, first-generation cephalosporins, aminoglycosides, and fluoroquinolones.

MYCOPLASMA

The family Mycoplasmataceae contains 3 genera of veterinary significance: *Mycoplasma* spp, *Ureaplasma* spp, and *Acholeplasma* spp, collectively referred to as *Mycoplasma*. Mycoplasmas colonize the epithelial lining of the lower genital tract in cats and dogs (**Table 1**), and disease is likely to be opportunistic.

Experimentally, reproductive disease can be elicited in male and female dogs, and in queens.^{81–83} Clinical reproductive disease is occasionally reported but may be underdiagnosed given that mycoplasma culture is not routine in many laboratories. *M canis* was the probable cause of chronic prostatitis and concurrent cystitis in 1 dog, and chronic purulent epididymitis in another.⁸⁴ There are few published reports of reproductive disease in cats resulting from natural infection.

Culture is considered the gold standard for diagnosis. However, mycoplasmas have fastidious growth requirements and culture may not be routinely available. Diagnostic laboratories should be consulted prior to sampling to ascertain whether they can carry out the required testing and to request mycoplasma transport medium. The publication of data identifying species-specific sequences has assisted the development of molecular techniques that identify mycoplasmas at both genus and species levels.⁸⁵ Mycoplasmas lack rigid cell walls and are inherently resistant to antimicrobial classes that target the cell wall such as the β -lactams. However, mycoplasmas are susceptible to tetracyclines, fluoroquinolones, macrolides and lincosamides.

CHLAMYDOPHILA FELIS

Chlamydophila felis is an obligate intracellular gram-negative organism, which predominantly causes conjunctivitis in cats. Following ocular infection, organisms may spread systemically and persist in many tissues, including the reproductive tract. Cats experimentally infected after 4 months of age shed *C felis* from the reproductive tract within 1 week of ocular infection.⁸⁶ Reactivation of shedding may occur in late pregnancy, with the likely spread of infection from the birth canal to kittens via the nasolacrimal ducts.⁸⁷ Experimental infection can cause clinical reproductive disease in both queens and toms.^{87,88} However, clinical disease caused by natural infection has not been described, and no significant link between reproductive failure and infection has yet been established.⁸⁹ A PCR assay to detect the organisms in swabs or tissues is the most sensitive diagnostic test.⁹⁰ Prolonged treatment with doxycycline will eliminate conjunctival shedding; persistence in the reproductive tract was not investigated⁹¹ but should be eliminated. Transmission of *C felis* from cats to humans has occasionally been reported.⁹²

COXIELLA BURNETII

Coxiella burnetii is an obligate intracellular gram-negative bacterium, the causative agent of query (Q) fever. The organism can infect virtually all animal kingdoms⁹³ and has a worldwide distribution with the remarkable exception of New Zealand. Infection of cats and dogs may be acquired by tick bites or by ingestion of organisms in

infected tissues. The uterus and mammary glands are sites of chronic infection, with recrudescence of shedding during parturition. *C burnetii* was implicated in several cases of abortion and stillbirth in cats, as well as neonatal death in puppies.^{94–96} Although all animals were seropositive, in some cases the demonstration of *C burnetii* organisms either was not attempted or was unsuccessful.^{95,96} Furthermore, *C burnetii* organisms and DNA have been detected in healthy cat vaginal and uterine tissues^{94,97}; therefore, a clear causal relationship cannot be proven. Nonetheless, most reported cases were investigated only because of subsequent disease in humans, and thus *C burnetii*-associated disease in cats and dogs may be underdiagnosed. Because of the zoonotic risk, most cases are diagnosed using serologic methods,⁹³ although PCR can also be used to demonstrate the organism within tissues. *C burnetii* is sensitive to tetracyclines and fluoroquinolones. Q fever is an important zoonosis worldwide, and seropositive periparturient cats and dogs may provide a source of infection for humans.

LISTERIA MONOCYTOGENES

Listeria monocytogenes is a rare reproductive pathogen in cats and dogs. Clinical disease is usually associated with ingestion of infected meat products. A single report of abortion in a bitch caused by *L monocytogenes* has been published; the bitch presented clinically ill with a brown vaginal discharge 7 weeks into pregnancy, and a pure culture of *L monocytogenes* was isolated from the discharge.⁹⁸ Persistent infection of mammary glands can occur, and *L monocytogenes* transmitted in human breast milk caused neonatal death in pups.⁹⁹ The organism can be cultured using routine diagnostic media. Individual colonies are surrounded by a wide zone of hemolysis and must be differentiated from β H streptococci. *L monocytogenes* can cause local and systemic disease in humans. *Listeria* is susceptible to aminoglycosides and trimethoprim-sulfonamide.

BARTONELLA

Bartonella spp are intracellular hemotropic gram-negative organisms. The cat is the reservoir host for at least 2 species, *B henselae* and *B clarridgeiae*, and transmission is mainly by arthropods. Reproductive failure has been associated with experimental infection in cats although *Bartonella* does not appear to be transmitted transplacentally.^{100,101} Establishing a diagnosis is difficult; serology is not always useful since infection is widespread, but a negative result has a high negative predictive value. Bacterial culture is the most reliable diagnostic test, although bacteremia can be intermittent.¹⁰² No antimicrobial protocol has been shown to achieve a long-term cure and antimicrobials should only be used in cats showing clinical signs.¹⁰¹ Bartonellosis is a significant zoonotic infection.

SUMMARY

Primary exogenous bacterial pathogens, with the exception of *B canis*, are sporadic causes of reproductive disease in the cat and dog. A speculative role for some pathogens such as *C felis*, *C burnetii*, and *Bartonella* spp in reproductive disease has yet to be confirmed. Most bacterial infections of the reproductive tract are caused by endogenous microflora in the presence of predisposing factors, and establishing a definitive diagnosis can be challenging. Bacterial reproductive disease appears to be less significant in the cat, although *Mycoplasma* and *S felis* infections may be underdiagnosed.

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