INFECTIOUS CAUSES OF PREGNANCY LOSS IN DOGS

Wenche Farstad, PhD, MDNV, Diplomate, ECAR
Department of Production Animal Clinical Sciences
Norwegian School of Veterinary Science
P.O.Box 8146 Dep, N-0033
Oslo, Norway

Introduction

Occurrence and type (re-absorption, abortion, mummification or stillbirth) of pregnancy loss in the dog depend on the cause of the loss and the stage of gestation at which they occur. Pregnancy loss may or may not be associated with vaginal discharge. Vaginal discharge occurs normally in pregnant bitches and is clear to mucoid or pink-tinged and odorless. If the discharge contains blood or pus, is greenish or black-tinged or has an odor, this could imply serious pregnancy complications. Frequently, however, embryonic loss in the bitch occurs without being noticed by the owner.

Embryonic death may occur very early, such as during the passage of the embryo through the oviduct, or the early pre-implantation or free-floating phase of embryonic life. Infections may interfere with the embryos at a very early stage (as early as during fertilization or early cleavage), or when the mother's oviducts or uterus have an inflammatory reaction due to an infection. The early embryo may die either because of the infection, which directly influences its developing cells, or because of a hostile environment in the genital tract, and embryonic deaths occur before day 15-17 when implantation occurs.

The mechanisms for establishing and maintaining pregnancy are not as well known in the dog as in many other species, since the regular time-dependent regression of the corpora lutea, which leads to recurring estrus in non pregnant females of the polyestrous species, is not present in the mono-estrous bitch. Maintaining pregnancy at the critical stage of implantation involves many biological interactions between the mother and the fetuses. If disturbed by dysfunction due to, for example, hormonal imbalance or defects in fetus-to-mother communications through pregnancy specific proteins (known from sheep and cattle) or substances produced by the fetal placenta (relaxin, dog), pregnancy cannot be maintained, and the fetus dies. In the former species, lack of signals from the embryo at critical times during the luteal phase triggers prostaglandin (PF2α) release that induces regression of the corpora lutea, and the pregnancy cannot be maintained due to progesterone deficit, and the polyestrous female returns to estrus. In the dog, pregnancy specific fetal signal proteins have not been identified, but the canine pregnancy is still dependent on normal luteal function throughout pregnancy. Prostaglandins are also luteolytic in the dog, and prostaglandin release may interfere with luteal function and reduce progesterone levels, and anti-progesterones are able to induce abortion. Some species of bacteria, such as *E. coli*, may produce toxins that induce release of prostaglandins, thus causing premature luteal regression.

Embryonic death goes unnoticed when the embryos are re-absorbed before pregnancy is detected, and the bitch is labeled infertile without a specific diagnosis. Most cases of embryonic loss occur during this stage, i.e., in the bitch prior to day 22-24 of pregnancy, when pregnancy diagnosis may be made using ultrasonography. Ultrasonographic imaging is widely used in small animal practice for the diagnosis of pregnancy and the determination of

fetal number (Farstad, 1985). Ultrasonography can be used to monitor abnormal pregnancies, for example, conceptuses that are poorly developed for their gestational age (and therefore are likely to fail), and pregnancies in which there is embryonic re-absorption or fetal abortion (England, 1998). In the following, a short review will be given of known causes of infectious pregnancy loss in the dog.

Pregnancy loss in the dog

Abortion

Abortion is defined as the expulsion of fetuses before full term pregnancy, i.e., before the conceptus is capable of independent life. Abortion is uncommon in the bitch; fetal re-absorption or mummification are more common.

Fetal re-absorption

Fetuses will normally only be reabsorbed if they die during the first half of pregnancy. Incomplete re-absorption is possible as well as re-absorption of a few fetuses, while others develop to term. The most common causes are inadequate hormonal support, endometrial or placental disorders or intoxications, and infections, such as canine herpes virus or brucellosis.

Mummification

The death of fetuses after the calcification of the skeleton, i.e., by day 50 in the dog, may often lead to the mummification of fetuses. The fetuses are maintained within the uterus, and degradation occurs to a certain extent, but the skin dries around the fetus forming a membrane that may delay complete fetal decay and re-absorption for prolonged periods of time. The skull and skeletal parts are also intact, and the fetus remains more or less recognizable in the uterus by ultrasonography and radiography.

Bacterial infections

Normal vaginal flora

Bacterial vaginitis may be a cause of infertility; however, the bacterial species found in the vagina in bitches with reproductive problems do not differ significantly from those of fertile bitches (Farstad, 1982a). Vaginal cultures are almost always positive, and it has been well established that normal flora exist in the vagina, whereas the uterus is normally sterile. The species found from vaginal swabs may vary with the stage of the cycle, with the location of the swab specimen (anterior or posterior vagina) as well as the age of the bitch (juvenile or mature). The normal flora, i.e., bacteria isolated from clinically healthy bitches, includes species such as Staphylococci, streptococci (Alfa or beta hemolytic and non-hemolytic), *Escherichia coli*, *Pasteurella spp*, *Proteus spp*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Moraxella spp* and *Haemophilus spp*., all of which may be opportunistic pathogens. However, some studies indicate that pasturella- and haemophilus- like bacteria may be associated with reproductive disorders (Farstad, 1982b). *E. coli* is most certainly involved as an ascending infection and a sequel to hormonal disturbances in pyometra, urinary tract infections and endometritis in bitches. Endotoxin production by certain strains of *E.coli* may also cause more systemic effects such as septicemia, as well as abortion and

perinatal puppy death in bitches and queens (Pohl et al, 1993; Beutin, 1999). *Pseudomonas spp* and *Proteus spp* and certain streptococci may also be implicated as causes of infertility (Bjurstrøm and Linde-Forsberg, 1992). Bacteria may enter the uterus as an ascending infection during proestrus and estrus when the cervix is open, and may cause local effects such as production of spermicidal toxins and metabolic products that may alter the vaginal microenvironment. However, due to estrogenic influence, the local immune defense mechanisms are enhanced during estrus, but adhesive bacteria may remain in the tract after estrus and cause infection after closure of the cervix during the period of progesterone dominance.

Bacteriological examination of the vaginal mucus of the bitch should be carried out if the bitch has a history of repeated infertility despite normal clinical and hormonal progression of the estrus cycle if the vaginal discharge smells foul or has a deviant color, or if the bitch has had discharge during anoestrus, and the dog has been eliminated as the direct cause of the infertility due to sperm defects. Mixed cultures of bacteria are more commonly found from vaginal isolates, but more pure cultures are found in diestrus and anoestrus than in estrus (Farstad, 1982a). Although pure cultures may be found in normally healthy bitches, the tendency to treat such infections is larger if the bacterial growth is abundant. Appropriate antibiotic therapy may be given after a sensitivity determination of the species in question, but the widespread use of antimicrobial therapy may induce vaginal colonization of other antibiotic-resistant species, such as *Pseudomonas aeruginosa* or *Mycoplasma spp* (Strøm and Linde Forsberg, 1993). Mycoplasma spp have also been isolated from 30-88% of normal bitches, so the interpretation is that they may also be opportunistic pathogens. Thus, their presence does not necessarily implicate reproductive disease.

In the following, some reports of bacterial infections causing pregnancy loss are cited.

Mixed cultures

An 18-month-old Pekingese bitch aborted 7 fetuses at Day 55 of pregnancy. Necrosis and/or abscess formation in all 7 placental sites was found following a panhysterectomy performed 4 days later. *Escherichia coli*, *Proteus mirabilis* and a beta-hemolytic *Streptococcus sp*. were cultured from the vaginal discharge and from a uterine abscess (Penzhorn, 1985).

Salmonella

More than 50 salmonella types have been isolated from dogs, of which *S. typhimurium* and *S. anatum* were the most prevalent in one study. Non-clinical salmonellosis occurs in most cases, but a more severe form of the disease may result in fever, vomiting, depression abortion and even death. Dogs may remain carriers and fecal shedders, and may thus represent a reservoir for infection in both humans and other dogs. Aborted fetuses, fetal membranes and meconium should be considered potential health hazards (Morse and Duncan, 1975). *Salmonella montevideo* is a recognized cause of ovine abortion and can cause disease in other domestic animals and humans. The organism was isolated from the aborted fetuses of a bitch from a pack of foxhounds in Scotland, UK (Caldow and Graham, 1998). *Salmonella panama*: isolation from aborted and newborn canine fetuses (Redwood and Bell, 1983).

Brucella

Brucella canis is a gram-negative rod that causes late abortion in otherwise clinically healthy bitches, and infertility, epidimyditis and testicular atrophy in dogs. This is the only bacteria that is known to be a specific cause of infertility in dogs. This *Brucella* species as a rule only infects canines, but human infections have been reported. Brucellosis in dogs is endemic in South America and parts of North America, has been reported once during later years in France, but is not endemic in Europe. It has never been detected in the Nordic countries. Import regulations in many European countries contain specific requirements to serum Brucella titres when live animals or semen is imported from countries in which Brucella is endemic. Diagnosis is made through isolation of the causative agent and serology. Outbreak in kennels will invariably result in stamping out of all animals in the kennel, which makes this disease a very serious threat to dog breeders. Antibiotic therapy may be attempted, followed by monthly testing for free status, but elimination is not easy due to *Brucella canis* being an intracellular organism. Vaccine is not available.

Infection with other *Brucella* species in dogs is rare. However, oral inoculation of pregnant female beagles with *Brucella abortus* strain SRB51 caused infection of the placenta, with resulting placentitis and fetal infection, but abortion was not apparent. Intravenous inoculation resulted in infection of maternal spleen, liver, and placenta; however, fetal infection and abortion were not observed. Infected canine placental membranes or fluids may be a source of infection for other animals and human beings (Palmer and Cheville, 1997).

Campylobacter

Campylobacter jejuni was isolated in pure culture from the vaginal discharge from three German shepherd bitches after late-pregnancy abortions in South Africa. The main clinical sign occurring in the bitches was a profuse and odorless hemorrhagic vaginal discharge (Odendaal et al, 1994). *Campylobacter jejuni* was isolated from a Wheaten Terrier bitch which had aborted (in southern Idaho). A Campylobacter of undetermined species also was isolated from aborted Poodle pups (in central Washington). Pathologic evaluation of cases involving premature and aborted pups should include bacteriologic tests, using appropriate media and atmospheric conditions necessary for the cultivation of these organisms (Bulgin et al, 1984).

Listeria

Abortion caused by *Listeria monocytogenes* has been reported in a bitch (Sturgess, 1989). The bitch was in late pregnancy (7 weeks) and was presented depressed and lethargic with a brown vulvar discharge. Digital palpation revealed a partially opened cervix, and a Caesarean section was performed due to incomplete dilation of the cervix. The bacteriological swab from the uterine contents and vaginal discharge showed pure culture of *Listeria monocytogenes.*

Escherichia coli

A five-and-a-half weeks pregnant Airedale terrier with a history of estrous irregularities presented with genital hemorrhage. It was found to be anemic, and *Escherichia coli* was isolated from vaginal swabs. Estradiol and progesterone levels were normal. Two fetuses were aborted on day 41 of gestation. Antibiotic treatment was given. The hemorrhages

ceased but on day 61 an abnormal vaginal discharge was seen. A caesarean was performed, from which resulted three dead and two live fetuses, which died within two days. *E. coli* was isolated from the uterine contents, although the bitch was still receiving antibiotics; it was also still anemic. It is probable that the hemorrhages were caused by an endotoxin produced by *E. coli (* Linde, 1983).

Parasitic infections

The protozoan *Toxoplasma* gondii has been shown to infect puppies following experimental intravenous or intraperitoneal inoculation of pregnant bitches with tachyzoites. Infected dams showed systemic illness 3-5 days after infection, and infected puppies were delivered 4-6 days after inoculation of the dams (Chamberlain et al, 1953). Toxoplasmosis does not seem to be an important cause of abortion in dogs compared with other domestic animals since few reports are available in the veterinary literature that implicate this organism as a cause of canine abortion.

Neospora caninum, which is responsible for abortion storms in cattle, is a known pathogen in dogs (Bjerkås et al, 1984). Abortion following infection with this organism is not common in dogs. However, when transmitted to canine fetuses during pregnancy or to puppies via the milk, this organism can produce weak puppies; however, experimental infection of newborn pups produced variable results with regard to establishing infection (Cole et al, 1995).

Viral infections

Canine herpesvirus 1, (alpha-herpesvirus), the minute virus of canines and the bluetongue virus have all been shown to cause abortions or neonatal deaths in dogs. (CHV I) was first described from the USA, the disease later spreading worldwide. Herpes virus causes different clinical symptoms depending on the age and immune status of the infected dog. CHV is in endemic areas a common inhabitant of the upper respiratory tract of adult dogs. If susceptible dogs come in direct contact with dogs harboring the virus, they will experience mild respiratory symptoms, and in the genital mucosa (vestibulum and vagina of the bitch and penis and prepuce of the male), hyperemia and swollen lymphoid nodules are found rather than the more typical vesiculation or ulceration seen in herpes virus infections in other animal species. Infection in pregnant bitches may affect the litter by causing necrosis of the placentae, and if the bitch is infected during the last three weeks of pregnancy or the first three weeks post partum the infection is most likely to result in late fetal or perinatal deaths. Natural infection in bitches is succeeded by latency, and recrudescence and repeated nasal and vaginal shedding may be achieved by prednisolone administration (Smith, 1997). Hence, if a bitch has previously been exposed to the virus, she will remain infected for life and during times of stress the virus may be reactivated. The bitch will pass on antibodies to the newborn puppies through colostrum that will usually protect the litter. In kennels with an outbreak of CHV infection both breeding males and other breeding bitches may be infected through exposure to infected whelping areas, through direct contact with sick or dead puppies and infected placentas. The virus is relatively sensitive to cleaning agents and to high temperatures, and does not survive for a long time in the environment. Recently, a vaccine has been introduced in the United Kingdom by Merial Animal health (Eurican herpes 205®) that may be used for preventive purposes in kennels that have a high incidence of herpes viral infertility or abortion and stillbirth (Cullen, 2003).

Other viruses

Minute virus of canines or MAC (CPV1) was initially believed to be non-pathogenic, but it has been shown that it can cause mild to severe illness in experimentally infected neonatal specific-pathogen-free puppies (respiratory illness) and in spontaneously infected puppies (respiratory and intestinal illness). The symptoms are usually milder than with CPV2 infections. The infection may occur transplacentally in the fetus or via the oronasal route in pups and adult dogs. Infection in utero can cause embryo re-absorption and abortion, especially when the dam is infected prior to day 30 of gestation. In one late abortion in a Yorkshire terrier in Germany the minute virus of canines (MVC) antigen could be demonstrated in fetal tissues by immunofluorescence. The bitch had a high specific anti-MVC serum titer when tested about four weeks after the abortion. Those results implicate an etiological role of MVC in this abortion (Truyen et al, 1996). The disease has been reported to cause problems in the USA, Central Europe and Japan (Carmichael et al, 1991, Carmichael et al, 1994, Truyen et al, 1996, Hashimoto et al, 2001).

Bluetongue virus infection is an International Office of Epizootics List Category A disease described as the century's most economically devastating affliction of sheep. Bluetongue viruses were thought to infect only ruminants, shrews, and some rodents, but inadvertent administration of a virus-contaminated vaccine has resulted in mortality and abortion among domestic dogs (Levings et al, 1996). Natural viral infection among African carnivores (wild or domestic) dramatically widens the spectrum of susceptible hosts. The authors hypothesized that such infection occurred after ingestion of meat and organs from bluetongue virus-infected prey species (Alexander et al, 1994).

Conclusion

Infectious causes of canine pregnancy loss are not as well studied in bitches as in many other domestic animals, and there seem to be few microorganisms that are specifically known to cause infertility in dogs such as *Brucella canis*. Most infections that cause pregnancy loss in dogs are opportunistic pathogens, but viral infections such as canine herpes virus, may present a real problem in infected breeding kennels.

References

Alexander KA, MacLachlan NJ, Kat PW, House C, O'Brien SJ, Lerche NW, Sawyer M, Frank LG, Holekamp K, Smale L, et al. 1994. Evidence of natural bluetongue virus infection among African carnivores. Am J Trop Med Hyg 51, 568-576.

Beutin L. 1999. Escherichia coli as a pathogen in dogs and cats. Vet Res 30, 285-98.

Bjerkås I, Mohn SF Presthus J. 1984. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. Z Parasitenk 70;271-274.

Bjurstrøm L, Linde- Forsberg C.1992. Long term study of aerobic bacterial flora of the genital tract in breeding bitches. Amer J Vet Res 53,665.

Bulgin MS, Ward AC, Sriranganathan N, Saras P. 1984. Abortion in the dog due to Campylobacter species. Am J Vet Res 45,555-556.

Caldow GL, Graham MM**.** 1998. Abortion in foxhounds and a ewe flock associated with *Salmonella montevideo* infection. Vet Rec 142,138-139.

Carmichael LE, Schlafer DH , Hashimoto A. 1991. Pathogenicity of minute virus of canines (MCV) for the canine fetus. Cornell Vet 81, 151-171.

Carmichael LE, Schlafer DH , Hashimoto A. 1994. Minute virus of canines (MVC, canine parvovirus type-1) pathogenicity for pups and seroprevalence estimate. J Vet Diagn Invest 6, 165-174.

Chamberlain DM, Doctor FL, Cole CR. 1953. Toxoplasmosis II. Intra-uterine infection in dogs, premature birth and presence of organisms in milk. Proc Soc Exp Biol Med 82,198-200.

Cole RA, Lindsay DS Blaghburn BL Sorjonen DC Dubey JP. 1995. Vertical transmission of *Neospra caninum* in dogs. J Parasitol 81, 208-211.

Cullen, C. 2003. Vaccination against canine herpes virus. The III Annual Congress of the European Veterinary Society for Small Animal Reproduction, Dublin 7-8 September, pp.78-79.

England, GC. 1998.Ultrasonographic assessment of abnormal pregnancy. Vet Clin North Am Small Anim Pract 28, 849-68.

Farstad W. 1982a. Bacterial findings in the genital tract of bitches with reproductive disorders. Nord Vet Med 34, 451-56.

Farstad W. 1982b. Haemophilus and Pasturella –like organisms from the canine vagina. Acta vet scand 234, 619-21.

Farstad W. 1985. Bruk av ultralyd til drektighetsdiagnostikk hos hund.(The use of ultrasongraphy for pregnancy diagnois in the dog). Norsk veterinærtidsskrift (N Vet J).

Hashimoto A, Takiguchi M, Hirai K, Kida H, Carmichael LE. 2001. A serological survey of minute virus of canines (MVC; canine parvovirus type 1) in dogs in the Tokai area of Japan. Jpn J Vet Res , 49, 249-253.

Levings RL, Wilbur LA, Evermann JF, Stoll IR, Starling DE, Spillers CA, Gustafson GA, McKeiman AJ, Rhyan JC, Halverson DH, Rosenbusch RF. 1996**.** Abortion and death in pregnant bitches associated with a canine vaccine contaminated with bluetongue virus. Dev Biol Stand 88:219-220.

Linde C. 1983.Partial abortion associated with genital Escherichia coli infection in a bitch. Vet Rec. 1983 May 7;112(19):454-5.

Morse EV, Duncan MA. 1975. Canine salmonellosis: prevalence, epizootology, signs and public health significance. J Amer Vet Med Assoc 167, 817-820.

Odendaal MW, de Cramer KG, van der Walt ML, Botha AD, Pieterson PM. 1994. First isolation of *Campylobacter jejuni* from the vaginal discharge of three bitches after abortion in South Africa. Onderstepoort J Vet Res 6,1193-1195.

Palmer MV, Cheville NF.1997. Effects of oral or intravenous inoculation with Brucella abortus strain RB51 vaccine in beagles. Am J Vet Res 58, 851-856.

Penzhorn BL. 1985. Necrosis and abcessation of placental sites in a Pekingese bitch. J S Afr Vet Assoc 56, 135-136.

Pohl P, Oswald O, van Muylem K, Jaccquemin E, Lintermans P, Mainil J. 1993. Vet Res 24, 311-315.

Redwood DW, Bell DA. 1983.*Salmonella panama*: isolation from aborted and newborn canine fetuses .Vet Rec 112, 362.

Smith KC.1997. Herpesviral abortion in domestic animals. Vet J 153, 239-244.

Sturgess CP. Listerial abortion in the bitch. Vet Rec124, 177.

Strøm B, Linde Forsberg, C. 1993. Amer J Vet Res 54, 891.

Truyen U, Wolf G, Carmichael LE. 1996. The other parvovirus: first description of the minute virus of canines (canine parvovirus type 1 in Germany). Tierarztl Prax 24, 511-513.