

Canine and feline abortion diagnostics

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Abstract

Knowledge of the causes of canine or feline pregnancy loss is limited and the success rate for making a definitive diagnosis is disappointingly low. Although these facts are discouraging, there are some things that can be done to improve success rates. This paper will address limitations and explore ways for improvement. For abortions caused by microbial infections, there are many reasons why it may not be possible to identify the agents. “Non-infectious” causes are much more difficult to diagnose, and their relative importance is unknown. These include endocrine failure, underlying endometrial disease, genetic abnormalities, nutritional deficiencies, and toxicosis from drugs or environmental sources. Genetic abnormalities are a major cause of human pregnancy loss, yet we have little specific information about genetic diseases leading to abortion in animals. This paper addresses ways clinicians and diagnosticians can work together to improve diagnostic success. Necropsy techniques for fetal and placental examination and sampling are briefly reviewed. It is hoped that this series of papers will stimulate discussion on the causes and pathogenesis of pregnancy failure, and focus attention on areas where abortion diagnostics can be improved.

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1. Pregnancy losses in bitches and queens

Causes of pregnancy failure are classically divided into infectious and non-infectious etiologies. From a diagnostic perspective, infectious causes of abortion have received the greatest attention because: (1) they may manifest clinically as abortions “storms” in breeding kennels, catteries, or research colonies; (2) abortions caused by infectious diseases may produce grossly visible or microscopically detectable lesions that are diagnostic; (3) infectious diseases that can cause abortion have been studied under controlled experimental conditions and we, therefore, know much more about them; and (4) our diagnostic laboratories have culture or test systems that enable detection of causative

agents, visualization of the lesions they produce, or determination of serological evidence of infection.

Embryonic resorption, fetal death resulting in mummification or abortion, and stillbirth of term fetuses each represent failure of some critical aspect of the structural/functional systems of pregnancy. From a diagnostic perspective, a working hypothesis can be put forward that each and every reproductive failure has a specific cause. This may seem painfully self-evident, but serves the purpose of forcing one to ask why and how things went wrong that led to any given pregnancy “wastage”. Even though we commonly refer to “background” rates of pregnancy loss, each of these losses is the result of some specific failure.

Relatively little firm data are available on resorption, mummification, abortion or stillbirth rates in cats and dogs. In general terms, these represent a continuum that extends from the early embryo to the sick or dead neonate that suffers from events *in utero*. The literature

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on canine pregnancy wastage was reviewed in 2001 [1]. When compared to what is known about abortion in cattle, horses, swine, sheep or goats, there are relatively few hard data available regarding rates of pregnancy loss in cats and dogs. The review reported that one study found 11% of fetuses in 22 bitches, approximately from 3 to 8 wks of gestation, were undergoing resorption and a second study of 12 bitches conducted at ~7 wks gestation found 13% resorptions [1]. Rates of abortion were not given, but the incidence of stillbirths was reported to be in the range of 2.2–4.6% [1]. These are high losses.

Owners of individual bitches or queens that abort are unlikely to pursue diagnostic workups unless encouraged to do so by their veterinarians. Given the realities of cost, common lack of material to work with, and low rates of identification of specific causes, it is no wonder that the relative number of abortion submissions is low and consequently our depth of knowledge so thin. In kennel or commercial production units, diagnostic “action” may not even be initiated in the face of substantial abortion “storms”.

There are many infectious agents shown to be capable of causing canine abortions, e.g. neospora, canine herpesvirus, and minute virus of canines. Unexpected circumstances are occasionally encountered that can challenge any diagnostic system. In the mid 1990’s, pregnant bitches in several areas of the US aborted, and many died following vaccination with a modified live viral vaccine that was contaminated with bluetongue virus [2–4]. From a diagnostic perspective, it was interesting to note that examination of fetal tissues were unremarkable and viral antigen was detected in placental tissue only through use of molecular techniques. Although, the bluetongue infections had little direct effect on conceptuses, they caused devastating systemic and respiratory infections of the dams.

The relative importance of maternal reproductive endocrinopathies as causes of abortion remains unclear [5–8] and is difficult to document. This is another example of a type of abortion that cannot be diagnosed from examination of abortus alone. Genetic abnormalities are also considered by most veterinarians to be responsible for pregnancy losses, but there are no firm data regarding incidence. Pregnancy failure in the queen and bitch probably involve genetic abnormalities and maternal factors to a greater degree than we appreciate [1,9]. Toxic effects of drugs and environmental toxins on reproduction are receiving a great deal of attention in all species, but toxicology is rarely used in current abortion diagnostics. A search of the literature did

reveal one interesting paper published last year that reported on a study in which pregnant bitches, “were dressed in a garment made of either pure polyester, cotton or wool or of a 50/50% polyester-cotton mix” [10]. Two of seven bitches wearing pure polyester aborted.

Because careful examination of an aborted fetus and/or placenta is an important responsibility of any veterinarian dealing with abortion, stillbirth or neonatal disease, gross pathology examination of fetal and placental tissues will be briefly reviewed.

2. Examination of aborted fetuses and placentas

Like all good mysteries, the crime scene usually contains clues and evidence that can be used to deduce what happened there, and who the culprit is. Understanding how individual microbial perpetrators go about committing their “crimes” (the disease pathogenesis) and what telltale lesions may be left at the crime scene (gross and histopathology) are key diagnostic elements for clinicians and diagnosticians. The absence of a crime trail that can be followed by examination of aborted material alone may make it difficult or impossible to diagnose some types of abortions, e.g. reproductive endocrinopathies, maternal systemic illness, or genetic diseases.

A major limitation to diagnostic success is the time interval between collection, processing, and shipping samples to diagnostic centers. As the time interval increases, tissues autolyze and microbial agents become harder or impossible to culture. However, if practitioners perform thorough necropsy examinations, collect and process samples in a rapid manner and use special transport media, some of these problems could be overcome.

2.1. The fetal necropsy and collection of samples

Costs for full necropsy examination and ancillary testing in most commercial or state veterinary diagnostic laboratories have risen to a point where clinicians are more commonly performing necropsies and collecting appropriate tissues for histopathology, bacteriology, virology, serology, etc., and submitting tissue and serum samples, rather than the entire fetus/neonate.

A key principle of necropsy pathology technique is standardization of the way it is done so that nothing is overlooked. Before starting the necropsy, the history and weights of the fetus and placenta should be recorded. The carcass should be carefully examined for

evidence of congenital defects. Collection of impression smears is indicated if lesions in the placenta or fetus are identified or suspected. A standard set of tissues should always be collected for histopathology; even if gross lesions are missed, they will likely be detected during histopathology.

When possible, fresh tissues, refrigerated on wet ice, should be delivered to a diagnostic laboratory as soon as possible. Tissues that should be submitted (both fixed and on ice) include: liver, kidney, adrenal, small and large intestine, lung, heart, thymus, brain, and fetal membranes. If no fetal fluids can be recovered from the membranes, then stomach contents should be collected. If fetal tissues are not available, swabs of fluids from the bitch's vagina should be placed in transport media and submitted. Even in cases that will be submitted for formal pathology workups, the likelihood of identifying causative bacteria is greatly increased if transport media is used (BBL Port-A-Cult Tubes; Becton Dickson and Co., Sparks, MD, USA). Fetal blood should be included, since older fetuses are capable of responding to infectious agents and if they survive long enough, a specific antibody response may be detected. A serum sample from the dam should be collected as well.

The drawing (Fig. 1) demonstrates how a fetus can be efficiently opened for examination and tissue collection. Larger fetuses are positioned on their left side. Carcasses of smaller canine or feline fetuses can be stabilized to make the necropsy dissection easier by positioning the fetus in a "spread-eagle" position and securing each of the limbs to a board. Once positioned, the dissection is begun by first removing the skin from the ventral abdomen and thorax, followed by incision and removal of the ventral abdominal muscles and clipping ribs on each side to remove both chest walls. Fetal bones can easily be cut with heavy scissors.

2.2. Examination of placental tissues and sample collection

As most infectious agents establish infection in placental tissue when they enter the gravid uterus, inclusion of placental tissue is very important. The gross anatomy of the canine placenta is demonstrated (Fig. 2). The membranes should be clear and fresh placental tissues are surprisingly brightly colored.

Placental examination begins by removing any bedding material by briefly rinsing under a rapidly flowing stream of cold water and laying the fetal membrane on a clean, flat surface in an area that can be subsequently disinfected. If fetal fluid remains trapped within the membranes, it should be collected for culture

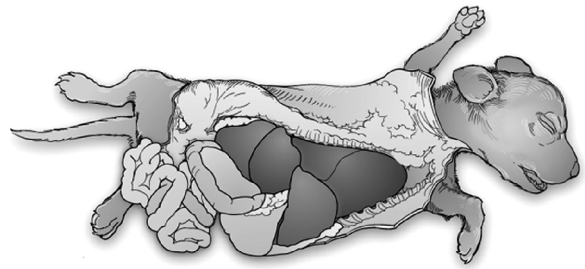


Fig. 1. Necropsy examination of fetal puppies or kittens begins with complete exposure of both the abdominal and thoracic cavities, as well as the pericardial sac. This is most easily done in large fetuses by initially making a cut through the skin along the ventral midline, extending from the tip of the mandible to the anus. The carcass is then placed in left lateral recumbency and the skin is reflected dorsally. The right lateral abdominal muscles are removed. The diaphragm is cut free from the rib cage and, using a heavy pair of scissors, proximal and distal cuts are made through the ribs on the right side, the chest wall is removed, and the pericardial sac is opened. All abdominal and thoracic organs can be easily examined and samples taken. Smaller fetuses can be necropsied using ventral exposure with the carcass on its back, with all four limbs secured to a board. Removal of the brain can be done by hemi-sectioning the head, or by removing the calvaria with heavy scissors. In smaller fetuses, once cultures have been taken and organs opened to ensure adequate fixation, the entire carcass can be submerged in 10% buffered formalin for fixation and submission for histopathology. Submission of digital photographs of the fetal internal organs is recommended, along with a written description of the gross necropsy findings.

before rinsing the membranes. Fetal and placental tissues should always be handled with gloves, and attention must be paid to personal hygiene.

Each placenta should be carefully spread out on a clean flat surface for examination. The slightly thickened band of tissue that extends circumferentially around the uterus and is intimately interdigitated with the surface of the endometrium is the labyrinth. Running on each side of the labyrinth are the marginal hematomas that contain partially processed maternal blood that has accumulated between the chorioallantoic membrane and the endometrium. When the chorioallantoic membranes are torn free from the endometrium, a characteristic greenish-colored fluid from within the marginal hematomas spills onto the surface of the endometrium and partially covers the deep (endometrial) surface of the fetal membranes. It is this fluid that is responsible for the greenish color of uterine lochial fluids.

In normal placentas, the membranes are thin and clear. Both the allantoic and deep (endometrial) surfaces should be examined and cytologic samples collected for examination if placentitis is suspected. Bacterial placentitis frequently has a slightly fetid odor. Several slab sections taken from different parts of the placental labyrinth should be fixed and other fresh

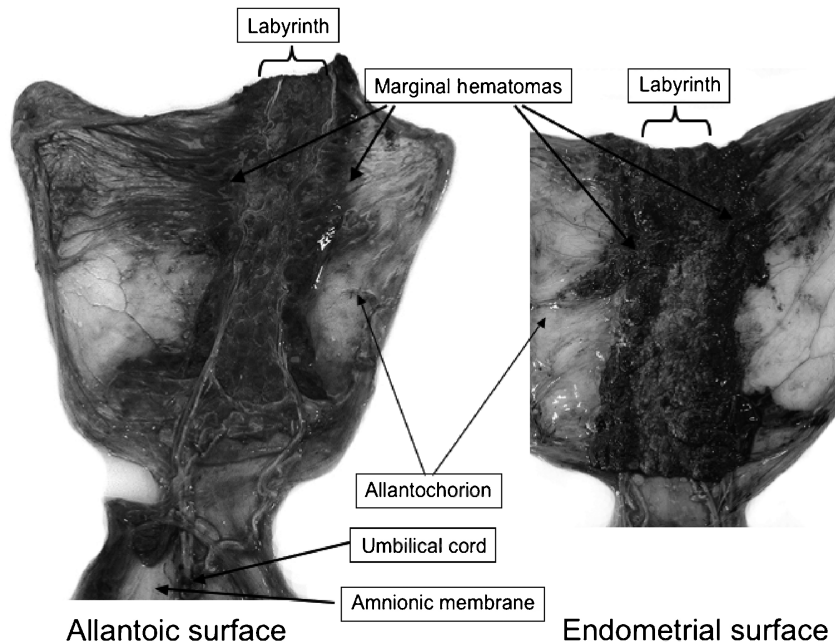


Fig. 2. Normal placenta (fetal membranes) from a term canine pregnancy. The placenta has been laid out to demonstrate major features. Each placenta should be carefully spread out on a clean, flat surface for examination. The fetus would have been attached to the umbilical vessels that form a broad umbilical cord and are contained within a thin amniotic membrane. If fetal fluid remains trapped within the membranes, it should be collected for culture. The slightly thickened band of tissue that extends circumferentially around the uterus and is intimately interdigitated with the surface of the endometrium is the labyrinth. Running on each side of the labyrinth are the marginal hematomas that contain partially processed maternal blood that has accumulated between the chorioallantoic membrane and the endometrium. The characteristic greenish-colored fluid spills onto the surface of the endometrium and partially covers the deep (endometrial) surface of the fetal membranes and is responsible for the greenish color of uterine lochial fluids. In normal placentas, the membranes should be thin and clear. Both the allantoic and deep (endometrial) surfaces should be examined and cytologic samples collected for examination, if placentitis is suspected. Then slab sections cut perpendicularly through the labyrinth should be fixed and fresh samples collected for submission, as should any areas thought to contain lesions. Submission of digital images of each placenta is advised, along with a written description of the postmortem findings.

samples collected for submission. Any areas with hemorrhage, discoloration, uneven surface features, or prominent vascular supply, should also be sampled and submitted with an appropriate gross description. Submission of digital images of each placenta and the internal organs of necropsied fetuses with samples that have been collected is advised. A written description of the postmortem findings should be filed in the medical record and a copy submitted with the samples to a diagnostic lab as soon as possible.

3. Microbiology

Limitations exist in even the largest laboratories in their ability to identify microbial agents from abortions. Why are some infections missed? Tenable explanations include: (1) testing error: samples submitted are rarely very fresh and fragile microbes have died (this can be partially overcome by using transport media and taking samples as soon as possible after an abortion); (2) samples were not included from critical tissues (i.e.

placenta); (3) some bacteria, viruses, protozoa, etc., are difficult or impossible to grow; (4) some microbes are extremely fragile and do not survive in tissues, even for very short intervals after fetal death; (5) the infectious agents may affect maternal systems with no or little direct involvement of the fetus or placenta; (6) individual labs may not have the expertise or experience to conduct appropriate tests; and (7) special testing is not available or too expensive.

4. Discussion

The author believes that there are a number of ways to improve abortion diagnostic rates. Set reasonable expectations with clients (discussion of costs, outcomes, etc.). Maintain a close working relationship with your colleagues in the diagnostic labs. Learn how to conduct a thorough fetal necropsy and placental examination. Submit samples to your diagnostic lab as soon as possible. Use special transport media to increase the likelihood of success in recovery of causative microbial

agents. Make your submissions as complete as possible. Provide complete histories and full tissue sets of both fixed and unfixed specimens. In situations where more than one animal is involved, acknowledge that identification of the cause may require more than one submission.

The health of the bitch or queen must also be monitored. As we look to the future, we can anticipate more attention will be paid to the role of endocrinopathies and genetic diseases in the pathogenesis of pregnancy losses in the cat and dog. There will undoubtedly be more extensive use of molecular diagnostic techniques to confirm the presence of infectious agents; however, it seems very unlikely that the need for thorough gross necropsy examinations will be replaced.

Acknowledgement

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