

Kirkbride's
Diagnosis of Abortion and
Neonatal Loss in Animals

Fourth Edition

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Kirkbride's Diagnosis of Abortion and Neonatal Loss in Animals

Fourth Edition

Edited by
Bradley L. Njaa



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Clyde A. Kirkbride, 1924-2011

Dr. Clyde A. Kirkbride served the AAVLD and the discipline of veterinary diagnostic medicine for approximately three decades. He did so with distinction, humility and excellence. His public service record goes back to WW II where he served as B-26 bomber pilot, flying 21 missions over Europe. He received his DVM from Oklahoma State University in 1953, was engaged in private practice for 10 years, held a faculty appointment at KSU, and finally served 22 years as a faculty member and diagnostician

at South Dakota State University. He rapidly became the national authority on the diagnostic investigation of the causes of food animal abortion. He taught undergraduate students an animal disease course at SDSU for 20 years, and for which he was awarded teacher of the year in 1982 - the course "Animal Diseases and their Control".

In cooperation with AAVLD he published *Laboratory Diagnosis of Bovine Abortion* in 1974; that book morphed into a second AAVLD publication *Laboratory Diagnosis of Abortion in Food Animals* in 1984; and finally the third edition published in 1989 became *Laboratory Diagnosis of Livestock Abortions*. All of these books were a huge success, drawing upon AAVLD diagnostic experts/ collaborators from across the country. The books are still found at the diagnostic benches of veterinary diagnostic labs around the world. Dr. Kirkbride was delighted and honored when he learned that a 4th edition would be published and would acknowledge him in the title.

Dr. Kirkbride wrote numerous publications and reviews on the subject of reproductive failure in livestock, and conducted research projects that clarified many disease syndromes. In 1985 he described a new infectious agent causing abortion in sheep, the spirillum *Flexispira*. In 1989 he received the SDSU Gamma Sigma Delta Award for excellence in research.

He retired to emeritus status in 1989. In retirement he continued to write, and published a series of JVDI manuscripts that summarized his diagnostic findings while a veterinary diagnostician at the SDSU Animal Disease Research and Diagnostic Laboratory - that series is still frequently referenced by researchers working in the field of livestock reproductive wastage.

David Zeman

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Preface

The first edition of this text was the result of an American Association of Veterinary Laboratory Diagnosticians (AAVLD) committee that formed nearly 40 years ago. Dr. Clyde Kirkbride was the committee chairperson for more than 17 years guiding the first and two subsequent editions of this work. What initially began as a 1974 book covering bovine abortions morphed into a food animal text in 1983 with the addition of sheep and swine. The third edition was the most complete, published in 1990, including cattle, small ruminants, pigs, and horses.

Similarly, this new edition came out of discussions in the AAVLD Pathology Committee. Initially, authors were selected to write about abortion diagnostics based on interest or expertise of a particular species, and their manuscripts were to be placed on a Web site linked to AAVLD. It was later decided that a published manuscript would be a better option.

The previous edition had 43 chapters, each devoted to a particular aspect of abortion diagnostics or a particular pathogen. It was roughly organized into species sections. This new edition has fewer chapters with each one devoted to one or two species. It covers conditions affecting cattle, sheep, goats, pigs, and horses, plus newly added chapters covering general approaches to abortion diagnostics and conditions affecting dogs and cats, and, finally, a chapter devoted to the broad topic of abortion and neonatal loss in nondomestic mammalian species.

The intended audience of this new edition includes veterinary diagnosticians around the world, veterinary pathologists, veterinary practitioners, veterinary educators, animal and public health workers, veterinary students, and veterinary technicians. The format for each chapter begins with an introductory section outlining the pathogenesis of abortions and neonatal loss, a section of special diagnostic techniques, a section of fetal and placental anatomy and physiology, and then detailed descriptions of the various causes of abortion or neonatal loss. In the diseases section, the format is as follows: viruses, bacteria and fungal, nutritional and toxic etiologies, and, finally, miscellaneous causes. The focus for each chapter is disease recognition as well as proper sampling necessary to make a definitive diagnosis.

It is my sincere hope that this publication will enhance our understanding of reproductive disease in animals, will build upon the reputation of previous editions, and will become an invaluable reference that helps decipher abortion and neonatal loss around the world.

Editor's Acknowledgements

Firstly, I would like to thank members of the AAVLD Pathology Committee for entrusting this project to me. In particular, thank you to Matti Kiupel and Donal O'Toole for continually believing in me throughout the process.

Secondly, I would like to express my deepest gratitude to each of the authors and contributors. Each of you contributed greatly to this effort and have built upon the foundation laid by Dr. Kirkbride and the previous authors.

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Fourthly, I wish to thank all of you who have been a professional mentor to me. In particular, I wish to thank Edward G. ("Ted") Clark, Craig Riddell, Greg Stevenson, Evan Janovitz, Sandy de Lahunta ("Dr. D"), Edward Dubovi, Tony Confer, and Roger Panciera for your encouragement, advice, and constructive criticism through the years.

Finally, thank you Leanne, Brynne, Layne, and Brooke Anna, for all of your patience and continued support. I love you all!

Sincerely,
Bradley L. Njaa

Chapter 1

General Approach to Fetal and Neonatal Loss

R. Flint Taylor and Bradley L. Njaa

Introduction

Determining a definitive diagnosis for abortion or neonatal death is frequently a frustrating and unrewarding exercise. Aborted fetuses are often autolyzed at the time of submission limiting the ability to isolate significant pathogens or identify diagnostically relevant gross or histologic lesions. Even if the fetus and associated membranes are fresh, a diagnosis with a specific etiology is rarely achieved. Diagnostician and diagnostic laboratories spend an enormous amount of resources and time on these cases, with the frequent result of the ever-frustrating final diagnosis of “idiopathic abortion” or abortion of unknown cause. Despite such poor odds, a consistent, systematic approach must always be employed in order to arrive at the oft-elusive definitive diagnosis. Submitting veterinarians and owners must be made aware that cases that yield “negative findings” do not equate with a diagnostic failure. Rather, the test negative results for the most common causes of abortion and neonatal loss indicate that the diagnostic focus should be directed toward other possible causes such as genetic abnormalities, nutritional deficiencies or excesses, environmental factors, and management practices.

Effective, improved vaccines and vaccination protocols, a better understanding of infectious diseases, and proven management procedures have greatly reduced the frequency and severity of contagious abortion outbreaks. In livestock, the majority of abortion and neonatal losses are often attributable to deficiencies in management. The diagnostician needs to keep in mind that reproduction is just one specific phase of “production,” and when the animal’s overall state of health is compromised, infertility, fetal death and subsequent abortion, or losses due to neonatal death are a natural outcome. Such submissions will often be unrewarding but may represent a harbinger of further losses and the possible need for management alternatives. Thus, the

diagnostician must be well aware of livestock management procedures, as well as regionally distinct variations, in order to ask the correct questions and be able to effectively work with the owner and referring veterinarian in identification of the genesis of the problem. Although an answer may be forthcoming from a single submission, more often, losses of several offspring with negative findings prompts laboratory staff, along with the referring veterinarian and owner, to pursue less common causes.

Fetal and neonatal losses involving companion animals are equally challenging. In addition to the same pressures of confirming a diagnosis, owners of this group of animals are much more emotionally attached. The spectrum of ownership ranges from "accidental" breeders to professional breeders and breed fanciers, and with this variance comes a broad range of background knowledge. Coupled with myriad Web sites that chronicle anecdotal and rare instances along with factual, science-based accounts of causes, the diagnostician must confront these situations with an added measure of patience. Yet, a systematic and methodical approach is the only way that a specific etiology can be discovered and confirmed.

Background prediagnostics

This general diagnostic approach to fetal and neonatal loss represents a general introduction to the topic to ensure that veterinarians and diagnosticians approach these diagnostic dilemmas in a prototypic, ordered, and organized manner. Specific etiologies will be addressed in the following chapters as they pertain to the spectrum of animal species covered in this book. At the end of this chapter, a section compares and contrasts key concepts specific to abortion diagnostics.

History

A detailed and comprehensive history is paramount for diagnostic success. Over the years, many if not most diagnostic laboratories have borrowed or created questionnaires that provide key prompts when tackling fetal or neonatal losses. Without one, specific questions may be overlooked or forgotten, which can delay or impede the investigation. The outline below is suggested to be used as a starting point. In addition, many North American diagnostic labs have questionnaires posted on their Web sites.

Animal information

- Age of affected dams: Older animals? First pregnancy animals? Mixtures of ages?
- Stage of pregnancy abortion is occurring: first, second, or third trimester? Term? Immediately at or after birth?
- Percentage of animals pregnant that have aborted?

- Are any of aborting animals otherwise clinically ill (beyond the abortion event)?
- Are the abortions clustered by time of year or age group of dams?
- Are the abortions sporadic (that is, a relatively rare event) or are there many in rapid succession (that is, is it an “abortion storm”).
- Is the herd/kennel/flock “closed” (that is, no new introductions) or is it maintained “open” with continuous new introductions?
- If new animals were introduced, when?
- Is there other evidence of disease, besides fetal or neonatal losses?
- Is there history of similar problems in previous years? If so, what were the findings and were management changes made?
- Have congenital defects been reported before with this breeding pair?
- In purebred animals, are there known genetic anomalies? Are any animals tested by the breed registry?

Nutrition

- Are animals fed a balanced ration? Home grown or prepared? Who is the nutritionist?
- What was the quality of the previous years' harvest?
- What additional supplements are provided?
- Have you had any of the feed tested for nutrients? Toxins?
- Is the plane of nutrition increased during periods of extreme weather? As pregnancy progresses?
- Do any of the hay bales have visible or evident mold? Spoilage?
- What is the water source? Has the water been tested for possible toxins? Mineral excesses?

Preventative and treatment therapies

- What therapies have been used without success?
- What vaccines have been used and what is the protocol? Modified live versus killed? A combination of the two?
- What are the serial number and expiration date of any vaccines used?
- How were vaccines handled at time of vaccination (storage, refrigeration, etc. prior to, and at actual time of usage)?
- Is there any modification of the vaccination protocol depending on age of animals in the herd/kennel?
- What is the deworming history? Which products were used?

Environmental influences

- Has the herd or kennel been exposed to any unauthorized visitors? People? Wildlife or feral animals?
- What are the biosecurity measures employed?

- Is there any possibility of exposure to abortifacient plants? Abortifacient or teratogenic pharmaceuticals?
- Is the pasture or area deficient in particular micronutrients (such as, vitamin E or selenium deficient areas)?
- How severe has the weather been? Do abortions or neonatal losses correspond with recent surges in severe weather (that is, typically very cold weather)?

Site visit

With the more vague and difficult abortion cases, an invited formal visit may be a productive exercise that provides a useful addendum to the history. Another "set of eyes" will often pick up some important detail that may not have been clarified by the original information from the referring veterinarian or animal owner. If a visit is not yielding much useful information, sometimes it may be helpful to speak with a hired hand, spouse, or older children in the absence of the owner. Important details sometimes are forgotten or overlooked and may surface when this strategy is employed.

Diagnostic investigation

Necropsy and tissue collection procedures

Necropsy technique and procedures are similar whether the carcass is a fetus, neonate, adolescent, or mature animal, with few exceptions. Prior to beginning the necropsy procedure, a crown-to-rump length of fetus is measured in addition to its weight to approximate or verify stage of gestation (Refer to Appendix A for established species specific gestational age estimates). Overall condition of the fetus is noted (degree of autolysis, etc.). The animal is opened, with ample exposure of all three cavities (that is, pericardial, thoracic, and abdominal cavities) in order to thoroughly examine the major organs and tissues. The calvarium is removed to expose the brain within the cranial vault. Some notable exceptions include a general lack of tinctorial variation between the various tissues, higher water content in many of the tissues, and in the case of fetuses, lack of aerated lungs. All tissues tend to be reddish-pink-gray, whether looking at the lung, heart, or liver, and this lack of distinction worsens as the tissues become autolyzed. Fetal and early neonatal brains tend to lack tissue integrity due to very high water content. As a result, brain tissue is often poured out of the skull into the collection containers.

Gross lesions are rarely seen in aborted fetal tissues. When present, the types of lesions seen are usually small grayish-pink or yellow pinpoint foci on the surface of the liver, lung, or kidney or a combination. These will often translate into systemic infection of viral or bacterial origin. Plaques found on the skin, fetal membranes, or both may be an indication of mycotic infection. Other lesions may be seen such as anatomical anomalies associated with a



Figure 1.1 Contaminated bovine placenta. Fetal membranes are commonly contaminated at the time of collection for submission. (Courtesy of Dr. BL Njaa, Oklahoma State University.)

variety of genetic disorders or exposure to poisonous plants. Finally, lesions may be observed in the fetal membranes that may be an indication of fetomaternal interface disease, either the result of systemic maternal disease or ascending disease processes.

Fetal membranes

Fetal membranes, commonly referred to as the placenta, should always be submitted. They are handled separately from other fetal tissues and reflect the maternal environment. As it is almost always contaminated with straw, manure, shavings, or dirt (Figure 1.1), it should be washed off, then spread out and examined grossly. When there is an obvious surface exudate, remove coarse debris and then sample for microbiological and histological evaluation. Depending on the type of placenta, gravid and non-gravid horns (equine) should be examined; cotyledenal and noncotyledenal (ruminant) portions of fetal membranes should be examined and sampled; and in cats and dogs, zonary and nonzonary placenta should be examined, including the marginal hematoma. Those that lack experience examining fetal membranes should seek advice from more experienced colleagues, include digital images of the purported lesions at the time of submission, or include most or all of the fetal membranes as part of the submission to the diagnostic laboratory. Placentitis is often somewhat focal or “spotty” in its distribution, therefore, multiple sections should be collected and submitted for histologic evaluation. Since different animal species are represented in this text, we will leave the specifics of this part of the examination to the experts in the chapters that follow.

Table 1.1 Fresh specimens collected for fetal and neonatal diagnostics.

Test	Storage	Specimens	Special Collection Notes
Bacteriology/ mycology (via culture, PCR)	Keep refrigerated, NOT frozen	Stomach contents, liver, lung, brain, placenta	• Collect stomach contents in a syringe with a large gauge needle • Package each specimen separately in sterile containers
Virology (via VI, PCR)	May be frozen at –70°C, if necessary	Lung, liver, kidney, heart blood, placenta	• Package each specimen separately in sterile containers
Nutrition, toxicology	May be frozen	Liver, kidney, ocular fluid	
Fetal/ maternal fluids	Keep refrigerated	Dam serum/ fetal fluids	• Paired (acute and convalescent), if possible

Proper collection and handling of fresh tissues

Collection of a comprehensive set of tissues and material is paramount for effective abortion diagnostics. An outline is provided above that may be used as a guideline for any species (Table 1.1). Species-specific and pathogen-specific sampling is further described in later chapters. Tissues samples should be refrigerated for short-term storage when pursuing bacterial or fungal etiologies if the sample cannot be processed immediately. Even if a toxic or nutritional problem is not initially suspected, it is advisable to place liver, kidney, and fetal fluids in frozen storage for possible future testing. If samples are collected for future virological testing, it is a much better idea to freeze the samples at –70°C rather than –20°C. Fetal and maternal fluids can be stored frozen. With blood samples, however, to minimize effects of lysed erythrocytes, serum should be separated before freezing the sample.

A good submission requires the use of proper primary and secondary containers. Fetal fluids and maternal blood samples should be collected in sterile red-top glass vials and then placed into padded, protective containers for shipping. Fresh samples should be collected into plastic, sealable, freezer bags; properly sealed bags are those with a locking seal mechanism as opposed to Whirlpak® plastic bags that are more prone to leakage. When applicable, feed samples should be included in sealable containers. Fixed samples, discussed in more detail later, should be included in jars that have properly sealing lids. Tape can be used to provide an additionally seal between the lid and jar. Alternatively, once the tissues have fixed for at least 24 hours, formalin-fixed tissues can be placed in sealable plastic bags for shipment as long as the excess air is removed

from the bags, thus preventing the tissues from drying out. As a precaution for accidental leakage or breakage during the shipping process, include absorbent materials in the package in order to minimize contamination of other packages. Absorbent materials can be purchased specifically for this purpose. Alternatives to specific packaging absorbent material are disposable diapers, which are made of similar material and designed to absorb large quantities of fluid.

Fixed specimens for histologic evaluation

A full spectrum of specimens should be collected and preserved or fixed in neutral-buffered 10% formalin. Formalin can be made in clinics from raw materials or purchased from commercial companies or from your local diagnostic laboratory. Formalin solutions prepared in clinics are typically inferior due to a lack of proper buffering resulting in excess amounts of artifactual pigmentation of tissue sections, especially fetal and heavily congested tissues. When fixing collected specimens, ensure a proper ratio of formalin to tissues of 10:1. This ratio has proven the test to time, and filling a container with tissues without the proper amount of formalin fixative results in incomplete tissue fixation, continued autolysis, and, overall, a poor quality submission. Increasing the concentration of formalin, such as 30% may hasten fixation of the tissues but can lead to artifactual changes in the fixed tissues (i.e., darkened neurons).

A complete set of tissues should be collected. This would include fetal membranes, brain, lung, heart, liver, kidney, pancreas, adrenal gland, thyroid gland, thymus, lymph node, spleen, and skeletal muscle. In general, alimentary samples are of limited utility for diagnosis of most causes of abortion, stillbirth, or neonatal death, but to ensure that proper tissues are collected, regardless of age, multiple sections of the alimentary tract can be collected and saved. As is the case in non-neonatal or fetal animals, the prosector should pay particular attention to any tissues that appear to have lesions. It is advisable to collect at the margin of normal and abnormal for large lesions. For smaller lesions, collect sections with enough surface area so that several lesions are available for examination. Record any observed lesions in the history in order to alert the pathologists and laboratory staff of your findings. This will ensure proper sampling at the time tissues are processed for histologic evaluation and hasten the return of meaningful results.

As previously mentioned, fetal brain samples often appear as undesirable samples for histologic evaluation due to the high water content and “soupy” consistency. However, for most species, it is advisable to examine multiple sections histologically in order to look for evidence of fetal septicemia or protozoal infections (Figure 2.20).

Feed and water samples

Because compromised nutrition may lead to abortion as a means of survival by the dam, it is always prudent to evaluate both feed and water for quality. A comprehensive feed and water analysis may be useful information and offer insights into deficiencies or excesses that may play a role in fetal or neonatal loss as well as impaired fertility and ongoing herd losses.

Fetal and maternal serology

Fetal fluids, such as heart blood, thoracic fluid, and abdominal fluid, should be routinely collected at the time a fetus or neonate is necropsied for the purpose of serologic testing. It is advisable to collect serum from the aborting dam at the same time in order to compare serology results between dam and offspring. In addition, blood should be collected from herd mates that are unaffected and from those that have aborted during the same time period in order to provide a baseline for the group and evidence for a possible cause, respectively. Many references describe the importance of collecting and analyzing paired serum samples from females in the herd, at the time of abortion and 2 to 3 weeks later. In reality, the dam has typically seroconverted by the time she has aborted and therefore, the convalescent sample may show evidence of declining titers. That is why comparing mean antibody titers of affected with unaffected herd or kennel mates is a much better alternative.

A single sample is of limited diagnostic utility. It cannot differentiate if the antibody levels are due to recent vaccination, an endemic condition in the group, or recent exposure and thus the cause of the abortion. Each laboratory and each individual test method has a broad range. Therefore, determining the significance of a titers magnitude can be very difficult. Certain viruses, such as Parvovirus in swine and BVD in cattle herds, tend to be endemic, and the presence of antibodies to these viruses is more of a reflection of the herd than a cause of neonatal or fetal loss.

Samples reflective of the maternal environment

Fetal membranes represent the actual interface between dam and fetus. When carefully collected directly from the dam during a premature delivery prior to becoming contaminated, it is probably the best sample to assess the maternal environment. However, this is extremely unrealistic and impractical. Instead, stomach contents and portions of fetal lung are specimens that are collected to assess the maternal environment. In a fresh or autolyzed, intact fetus, the lung and stomach contents represent uncontaminated samples reflective of the amniotic environment. Infectious agents that either ascend through the cervix or are transmitted through maternal blood will infect the developing fetus. Swallowed or "inhaled" amniotic fluid during development represents a portal of entry. Thus, bacteria or viruses isolated from these samples should be considered to be significant and likely causative.

Topics of special consideration

Sporadic abortions versus abortion storms

The number of animals that abort due to a particular cause is one way of classifying abortigenic disease. When few animals in a group are affected, they are classified as sporadic abortions. Either a small number of animals abort in

a short, defined period of time or the abortions occur throughout the entire reproductive cycle of the group. They can be caused by infectious agents that primarily target unprotected dams in the group or may be due to feed contaminants, such as *Aspergillus* spp. overgrowth in hay.

Abortions storms are more difficult to define. Although it should represent a certain percentage of animals, such as 1% of a group, affected in a short period of time, in reality, an abortion outbreak becomes defined as a “storm” when three or more animals abort! Three animals in a group of 10 is very different from 3 in a group of 1,000, yet most owners and veterinarians sound the alarm at 3, regardless of herd size. Many infectious agents can cause severe outbreaks of fetal losses, especially when the herd immunity is poor or inapparent for a particular pathogen (i.e., BVD virus outbreak in an unprotected group of pregnant cattle). Although determining the cause of an abortion storm becomes paramount during the event, many times, producers and veterinarians are helpless to prevent it. For some pathogens, such as Equine Herpes Virus, vaccinating in the midst of a storm may be effective. Some pathogens have the potential to cause abortions that occur sporadically or as an outbreak, dependent on the dose and herd immunity.

Acute versus progressive fetal loss

Typically, the ideal submission for diagnostic purposes is an animal that is examined immediately after death or a morbid animal that is euthanatized specifically for diagnostic purposes. When dealing with fetal carcasses, the time and manner of death has even greater implications in terms of quality of the submission. Regardless of cause, various hormonal stimuli in the dam are required to induce physical changes necessary for the aborted fetus to be delivered. If the inciting cause first leads to illness of the dam, fetus, or both, most often these same causes lead to induction of parturition. Typically, so long as the abortus is identified promptly, the fetus and fetal membranes tend to be fresher. However, if the cause leads to abrupt cessation of fetal viability, induction of parturition occurs as a secondary event and may take many hours to a few days. In these instances, the abortus and fetal membranes can be extremely autolyzed and often have limited utility for determining a definitive diagnosis. However, the condition of the fetus and its approximate age can help rule in possible causative categories (i.e., Brucellosis in second trimester bovine abortions). Thus, in cases of death *in utero*, peracute to acute death typically results in fetal retention for many hours leading to severe autolysis of the fetus and associated membranes.

Periparturient fetal viability and stress

During parturition, fetuses are forcibly extricated from a highly controlled, protective womb into a more varied and potentially hostile ambient environment by a combination of powerful, coordinated uterine and abdominal muscular contractions. Delivery of a live neonate is dependent on the fetus entering the pelvic canal in a normal position and in a timely manner so that the newborn can transition to breathing on its own. Prolonged parturition due to fetal malposition,

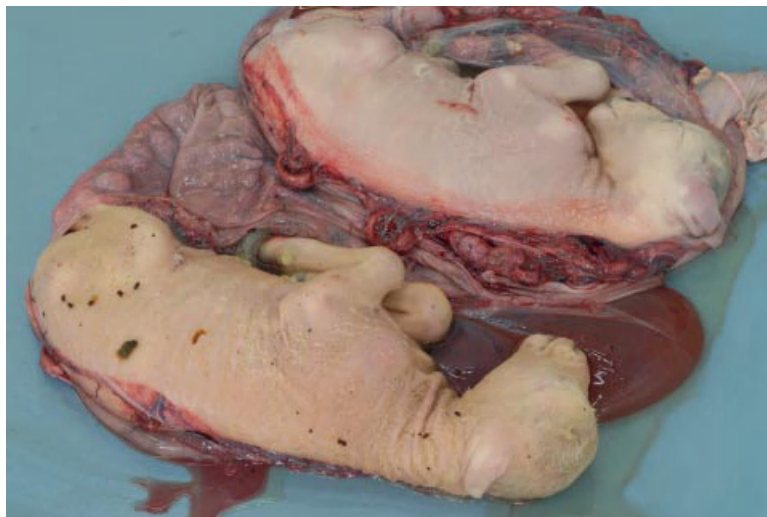


Figure 1.2 Meconium-stained ovine fetus. Two fetuses are present in the image. The fetus in the lower left is stained yellow-brown with flecks of brown meconium over its hair coat. This is an indication of *in utero* stress, likely the result of fetal hypoxia. The twin lamb has a whiter hair coat, with no evidence of meconium staining. (Courtesy of R Irvine, University of Glasgow.)

small pelvic diameter, or possible nutritional deficiencies can result in fetal stress or eventually death. Presumably as a response to fetal hypoxia, the best indication of periparturient fetal stress is relaxation of the anus with release of meconium. This results in orange-brown to orange-yellow discoloration to the fetus (Figure 1.2). In fetuses that die, similar material can be found in the lungs, histologically, indicating aspiration of the meconium during the periparturient period.

Differentiating a stillborn fetus, defined as born dead, from a neonate that was born alive but died in the postparturient period requires necropsy evaluation. Lung inflation is the best method for differentiation. Dark, red-purple lungs in fresh fetuses indicate congenital atelectasis (Figure 1.3); however, this can be a little more difficult in autolyzed lungs. Inability of a section of fetal lung to float in water is another test to confirm lack of antemortem lung aeration.

A second method of determining periparturient fetal viability involves examination of the intra-abdominal umbilical arteries. At the time of parturition, the umbilical connection between dam and fetus is severed. In the live fetus, severed umbilical vessels trigger a combination of vascular smooth muscle spasms and contractions, localized release of mediators of inflammation, as well as continuous delivery of platelets and clotting factors. The result is vascular luminal narrowing, periarteriolar hemorrhages, and thrombosis of these umbilical arteries (Figure 1.4). In stillborn fetuses, severing of the umbilical cord does not result in thrombosis or hemorrhages due, at least in part, to the lack of fetal blood flow.

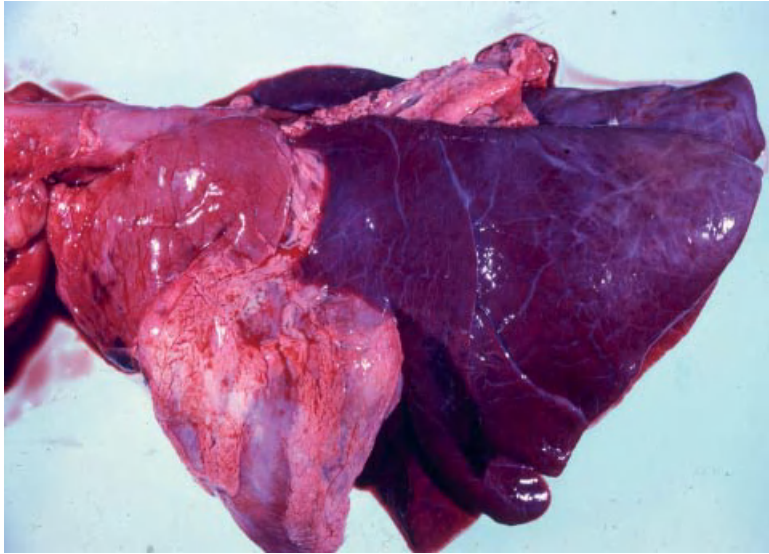


Figure 1.3 Fetal bovine pluck. The lungs from this fetus are dark red to purple indicative of congenital atelectasis. The heart and thymus are visible in this image. (Courtesy of Dr. RJ Panciera, Oklahoma State University.)



Figure 1.4 Neonatal bovine umbilical arteries. Flanking the urinary bladder are two thrombosed umbilical arteries with peripheral areas of hemorrhage within the abdomen of early neonatal foal. In this case, the foal also had urachal inflammation, with pus evident in the region of an opened urachus. (Courtesy of Dr. SD Cramer, Oklahoma State University.)

Summary

In summary, abortion diagnostics are often challenging and frustrating. Even with continued improved techniques, the broad range of possible causes for fetal and neonatal death prevents diagnosticians and diagnostic labs from confirming definitive diagnoses most of the time. One or two abortions likely represent isolated events that do not warrant further investigation. When the number of dead fetuses or neonates begins to increase, more effort should be put into determining a cause. In order to maximize the return on effort, labs try to minimize the cost and effort by pooling tissues and search for a select number of known, more common pathogens during initial screens. If these tests return negative results, other less common causes are explored. Coupled with a thorough, comprehensive, and systematic approach that may include obtaining a more in-depth history, scheduling a site visit, and gaining a broader set of samples that may include maternal serum, water and feed samples, or special genetic analytical tests, a diagnostician has the best chance of arriving at a definitive diagnosis now than ever before.

Chapter 2

Disorders of Cattle

Mark L. Anderson

Introduction

Pathogenesis of abortion and perinatal loss

Surveys of bovine abortion submissions to veterinary diagnostic laboratories reveal that an etiology is identified for less than half of abortion cases and most of these are attributed to infectious causes. A hematogenous route is thought to be the most common way in which many bacterial, viral, fungal, and protozoal infections reach and cause disease in the pregnant uterus of cattle. Ascending infections through the cervix during pregnancy are uncommon mechanisms for abortion in cattle. Other routes of infection include venereal infections, contaminated semen or transferred embryos and pre-existing mucosal infections of the reproductive tract.

The susceptibility of the pregnant uterus to a variety of microbes is likely related to various factors, including the following: possible suppression of the innate or adaptive immune response and local, microenvironmental conditions that may favor colonization by some agents. Depending on the agent and stage of gestation, a placental infection can induce inflammation leading to abortion due to primary placentitis or secondary to fetal infection.

The distribution of fetal lesions may reflect the route of infection from the placenta. Many bacterial and fungal agents proliferate in the placenta and subsequently gain entry into the amniotic fluids surrounding the fetus. This allows the pathogenic agent to enter the fetus through the alimentary tract, respiratory tract, mucous membranes, or the integument. The fetal lesions associated with this route of infection include bronchopneumonia, gastroenterocolitis, and dermatitis. Alternatively, there are other pathogens, such as *Listeria monocytogenes*, *Salmonella* spp., bovine herpesvirus-1 (BHV-1), and *Neospora caninum*, that proliferate within the fetal membranes and

then enter the fetus via the umbilical vasculature. This route leads to hepatic and systemic lesions based on the anatomy of the umbilical vessels and the fact that these pathogens are now blood-borne.

Identifying the cause of an abortion usually requires a variety of procedures, including pathology, microbiology, and serology. Results generated by the diagnostic laboratory should be interpreted by the submitting veterinarian in order to evaluate whether the results provide a sufficient answer when compared with the historical and clinical features. On occasion, an aborted fetus may have an incidental infection, have multiple infections, or may not be representative of the herd problem. When laboratory testing is completed, the clinical situation and laboratory diagnosis can be compared in order to assess whether the submission is a representative sample and if the etiology identified is a significant factor in the herd problem. Information about the herd abortion problem such as the estimated abortion rate, duration, and gestational age of aborted fetuses may help identify potential causes and exclude others. Other information that would be useful to include with the submissions is whether the aborted fetuses are fresh or autolyzed; if the affected dams are heifers, cows, or both; if affected females retain their placentas; if they are clinically ill; if the affected herd breeds naturally or by artificial insemination; and the vaccines or treatments that have been used.

Specialized techniques

Most diagnostic laboratories use a standardized diagnostic protocol that includes a spectrum of diagnostic tests in bovine abortion submissions because the gross examination is not a dependable method by which to select specific diagnostic tests and exclude others. Pathognomonic gross lesions are uncommon, and any lesions present may be obscured by autolysis. The procedures selected by a laboratory to be part of their bovine abortion protocol may vary due to differences in cattle production and the previously identified causes of abortion in the region. The following bovine abortion protocol can be modified depending on the condition and type of samples submitted, the cost associated with testing, and regional variation of abortifacient causes.

Specimens

The entire fetus and placenta along with dam serum samples are the optimal specimens. Inclusion of the placenta may be critical in the diagnosis of some mycotic and bacterial abortions as it may be the primary tissue affected. If the entire fetus cannot be submitted, the preferred samples would include a complete selection of formalin-fixed tissues along with fresh lung, liver, kidney, placenta, fetal fluids (thoracic, abdominal fluid, or heart blood), and abomasal fluid. The fresh samples should be submitted chilled in separate sterile containers with the fluids in sterile tubes, such as red top vacutainer tubes. It is not advisable to submit in syringes because the contents may become accidentally released or react with the rubber or plastic.

Microbiology

The routine microbiological diagnostic protocol on either a complete fetus or the fresh tissues can include an aerobic bacterial culture and *Brucella* spp. culture on the lung, an aerobic culture and *Campylobacter* spp. culture on the liver, and an aerobic culture, *Brucella* spp. culture, and *Campylobacter* spp. culture on abomasal fluid. The abomasal fluid can also be examined with a Gram stain and a dark field examination for *Campylobacter* spp. and Trichomonads. A direct smear on the kidney can be examined for Leptospire by fluorescent antibody staining. Bacterial cultures of the placenta are dependent on its condition and the presence of gross lesions. Fungal cultures can be performed if there are placental or fetal skin lesions suggestive of fungal involvement.

Virology

Routine virology procedures vary among laboratories. Routine virus isolation in tissue culture on an organ pool of fetal tissues, usually including lung, liver, spleen, kidney, adrenal, and placenta, is performed by some diagnostic laboratories. Other protocols may limit routine virology testing to BHV-1 and Bovine Viral Diarrhea Virus (BVDV) by fluorescent antibody (FA) stains on frozen sections of fetal lung (or liver) and kidney. BHV-1 and BVDV immunohistochemistry procedures are relatively specific and sensitive with the advantage that the pathologist can select these as confirmatory tests based on characteristic lesions observed in the fetal tissues.

Histopathology

Examination of a complete selection of tissues is recommended whenever possible. Autolytic changes are commonly present, but unless the fetus is mummified, a complete histopathologic examination of the fetal tissues often will provide useful information. Recommended tissues for routine collection include brain, at least two sections of lung, heart ventricle, one or more sections of liver, kidney, adrenal gland, spleen, thymus with inclusion of adjacent esophagus and trachea, lymph node, skeletal muscle from several sites, including tongue, diaphragm, and limb, abomasum, small intestine, colon, eyelid, and placenta. Ensure that several sections are included in the submission from both intercotyledonary and cotyledonary areas of the placenta. Although the high water content and autolysis may render the fetal brain as liquefied fragments, it is strongly recommended to transfer this brain material into the formalin container as the histologic detail is surprisingly preserved. In some situations with severe placentitis, no significant fetal lesions may be present. A list of differential diagnoses based on gross or microscopic lesions is summarized in Table 2.1.

Table 2.1 Possible causes for gross and microscopic lesions in bovine fetuses.

Gross Lesion	Causes
Fetal mummification	BVDV, <i>Neospora</i>
Ascites/Anasarca	Congenital heart defect, BVDV, <i>Neospora</i>
Arthrogryposis, other musculoskeletal deformities	Akabane virus, Cache Valley virus, reduced <i>in utero</i> fetal mobility
Fibrinous peritonitis/pleuritis	Many bacteria (<i>Arcanobacterium</i> , <i>Campylobacter</i> , <i>Bacillus</i> , <i>Brucella</i>) and fungi
Fibrinous pericarditis	<i>Bacillus</i> sp., <i>Campylobacter</i> sp.
Icterus	Leptospirosis
Cerebellar Hypoplasia	BVDV
Hydrocephalus Hydrancephaly, porencephaly	BTV, BVDV
Microphthalmia	BVDV
Pulmonary/renal hypoplasia	BVDV
Dermatitis/hyperkeratosis	Fungal, EBA
Multifocal liver necrosis	<i>Listeria</i> , BHV-1, <i>Yersinia</i> , <i>Salmonella</i>
Splenomegaly/Lymphadenopathy	EBA
Placentitis, infarction	Fungal
Microscopic Lesion	Causes
Encephalitis	<i>Neospora</i> , BHV-1, BVDV
Meningitis	EBA, <i>Leptospira</i> , <i>Brucella</i> , other bacteria
Myocarditis	<i>Neospora</i> , BVDV
Suppurative bronchopneumonia	Bacteria
Bronchointerstitial pneumonia	<i>Ureaplasma</i> , <i>Brucella</i>
Multifocal hepatic necrosis	IBR, <i>Listeria</i> , <i>Salmonella</i> , <i>Yersinia</i>
Interstitial nephritis	<i>Neospora</i> , <i>Leptospira</i>
Abomasitis/enterocolitis	Bacteria, Fungi
Conjunctivitis	Bacteria, <i>Ureaplasma</i> , fungi
Placentitis	Bacteria, fungi

Molecular diagnostic procedures

Polymerase Chain Reaction (PCR) procedures are available and used by some diagnostic laboratories for the detection of *Leptospira* spp., BHV-1, BVDV, *Neospora caninum*, and other infectious agents in fetal tissues.

Serology

The use of acute and convalescent serum samples to identify a significantly rising titer to a particular pathogen is standard practice in abortion and neonatal diagnostic approaches. However, in abortion diagnostics, the maternal seroconversion to the common abortifacient agents for which there are serologic tests available usually will have preceded the abortion so that a set of paired serum samples collected at the time of, and several weeks following abortion, may not demonstrate an increasing titer. An option for some situations would be to collect and store serum samples from specific pregnant animals to be used as the acute sample to pair with the abortion serum sample in the event of abortion in that animal. A single serum sample from an aborting cow can help determine if there had been possible exposure to an agent, but this technique usually cannot differentiate between vaccination and natural exposure, or between recent and previous exposure. Recommended routine abortion serologic testing on the dam includes the following agents: BHV-1, BVDV, *Leptospira* (serovars *Canicola*, *Grippotyphosa*, *Hardjo*, *Icterohaemorrhagiae*, and *Pomona*), *Neospora caninum*, and *Brucella abortus*.

Serological evaluation of the fetus depends on its gestational age and condition at the time of the postmortem exam. A quantitative immunoglobulin assay for bovine IgG can be performed on heart blood or thoracic fluid. If the fetal IgG is elevated (>20 mg/dL), this is an indication of an active immune response by the fetus to a foreign antigen, which is most often an infectious agent. If there is evidence of elevated IgG, serology for BHV-1, BVDV, *Leptospira* spp., *Neospora caninum*, *Brucella abortus*, Bluetongue Virus, and Parainfluenza 3 virus can be performed. When specific fetal titers are elevated, they suggest fetal exposure to that agent. On occasion, low titers to one or more of these agents may be present in a fetus without other evidence of infection warranting cautious interpretation. The source of antibody in the fetal fluids, particularly when antibody to multiple agents is present, is not certain but possibly represents maternal antibodies that may have crossed into the fetus as a result of placental lesions.

Table B.1 in Appendix B summarizes what tissues to collect, which tests should be requested, and lists additional ancillary tests that can be performed to maximize the diagnostic effort of attempting to confirm a diagnosis from a list of selected pathogens.

Gross examination

Fetus

A complete necropsy examination is performed on the fetus to identify any gross lesions and to estimate the fetal age and degree of autolysis. An estimation of the gestational age of the fetus can be made from the crown to

rump length, fetal weight, and the extent and distribution of hair development. (See Table A.1 in Appendix A.) Potentially useful external changes include the following: evidence of fetal distress with yellow-orange to orange-brown discoloration of the carcass due to premature release of meconium from the fetal rectum, or round, raised, hyperkeratotic skin plaques that may indicate evidence of a mycotic infection. With the fetus opened, an estimation of the degree of autolysis can be made. Evidence that the fetus may have been alive at the time of parturition includes pink lungs suggestive of antemortem inflation, hemorrhage surrounding the umbilical vessels, or thrombosis of the umbilical arteries. A freshly aborted fetus will usually have clear amber fluid in body cavities, but within 1 to 2 days following death, serosanguinous fluid collects in the body cavities and subcutis. This process is followed by gradual dehydration of the tissues so that by a week following fetal death, the fetus is dehydrated with no abomasal content.

Pathognomonic gross lesions are uncommon, but some abnormalities might be encountered. Icterus of the subcutaneous tissues and internal organs may be observed in Leptospiral infections. Fibrinous exudation in the pleural and/or peritoneal cavities may be observed in a variety of infections including *Campylobacter* spp., *Arcanobacterium pyogenes*, and some mycotic infections. Fibrinous pericarditis can be associated with bacterial infections especially *Bacillus cereus*, *B. licheniformis*, and *Campylobacter* spp. A dilated rounded heart with ascites can be associated with ventricular septal defects, great vessel abnormalities, myocarditis, and myocardial necrosis. Mucosal petechiae in a late term fresh fetus with generalized lymphadenopathy and splenomegaly is commonly observed with Epizootic Bovine Abortion. Scattered white foci in the liver are sometimes observed in abortions due to *Listeria monocytogenes*, *Salmonella* spp., and rarely, BHV-1. White indistinct streaks or foci in the heart and skeletal muscles may be observed in *Neospora caninum* abortions.

Fetal membranes

The placenta in the bovine is epitheliochorial, adeciduate, and cotyledonary. According to Roberts, a normal bovine placenta should weigh 4 to 8 kgs and be 14% of the calf's weight. The maternal portion of the placenta is formed by multifocal points of interface called caruncles. Typically, caruncles are arranged in 4 rows, half dorsal and half ventral along the full length of each uterine horn and totaling 75 to 120 per cow. The fetal, chorioallantoic portion of this interface is called cotyledons. In the pregnant uterus, the caruncle bulges convexly from the endometrial surface as primary and secondary villi, and the cotyledonary villi interdigitate concavely to form a placentome. The intervening portions of the placenta are referred to as intercotyledonary where there is normally no distinct maternal-fetal interface. Where there are areas of interdigitation, these interfaces are called adventitial placentation.

When available, the entire placenta should be examined, because lesions of placentitis may be regional. Reliance on increased placental weight as evidence of inflammation is compromised because placental edema is a common,

relatively nonspecific change. In a fresh placenta, red cotyledons and a clear, translucent intercotyledonary placenta would be considered normal, but with autolysis, the cotyledons develop a dull brown color, and the intercotyledonary placenta is less translucent. Intercotyledonary opacity can be associated with edema, inflammation, or fibrosis. Surface exudate or thickening of the surfaces of the chorioallantoic membranes is evidence of inflammation. Cotyledons whose surface is depressed relative to the surrounding intercotyledonary placenta or cupped can be an indication of inflammation in the surrounding stroma. Gross abnormalities in the cotyledons include adherent caruncular tissue, hemorrhage, necrosis, and exudation. Sections through a cotyledon may reveal infarcts that may suggest a mycotic infection.

Viral causes of abortion and perinatal loss

Bovine herpesvirus type I

Bovine herpesvirus (BHV) type I infection has a worldwide distribution and is associated with abortion, vulvovaginitis, balanoposthitis, respiratory disease, conjunctivitis, encephalomyelitis, and fatal systemic infections in neonatal cattle. The virus may persist as a latent infection following acute infection. Infection occurs through contact with infected cattle shedding virus from respiratory, ocular, and reproductive secretions. Infection at breeding can cause infertility and early embryonic death. Exposure of previously unexposed, nonvaccinated pregnant cattle can result in abortion storms with 25-60% of cows aborting. Experimentally, abortion occurs at any stage of gestation, but in field conditions, abortions are usually seen in the second half of gestation. Most abortions occur several weeks (20 to 52 days) following initial infection of the dam. Cows may exhibit a range of signs reflecting the various clinical disease forms, including subclinical infection but because of the delay from maternal infection to abortion, aborting cows usually do not appear ill.

Aborted fetuses are usually 5 months to term. The fetus is autolyzed with red-tinged fluid in body cavities and fascia. There are usually no gross lesions other than possibly placental edema. Rarely, indistinct, pinpoint white foci may be appreciated in the liver. A presumptive diagnosis can be made from histopathology. Although there is often considerable autolysis, the liver usually has striking multifocal necrosis, best appreciated at low magnification (Figure 2.1). Focal necrotizing lesions are usually present in other fetal tissues, especially lung, adrenal gland, spleen and lymph nodes. Herpes viral intranuclear inclusion bodies are often difficult to identify but may be identified in the adrenal cortical lesions (Figure 2.2). Placentitis with necrosis and vasculitis in placental villi is usually present and associated with abundant viral antigen as detected with immunohistochemistry (IHC) (Figure 2.3). Confirmation of the diagnosis is by viral isolation, detection of viral antigen in fetal tissues by FA on frozen sections or by IHC on formalin fixed tissues using monoclonal antibodies. The best tissues to submit for FA testing are kidney and adrenal

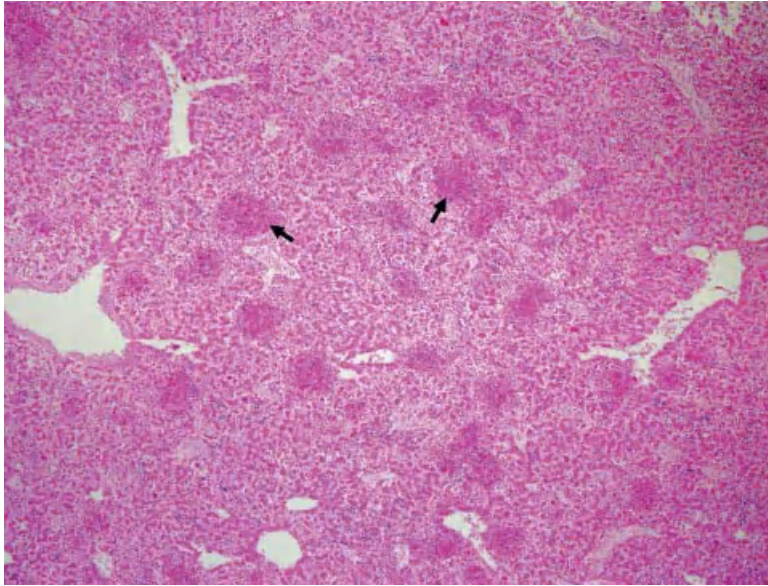


Figure 2.1 Bovine herpesviral hepatitis. Randomly scattered throughout the section of liver are areas of necrosis (*arrows*) associated with BHV-1 infection. Typically, as in this example, affected fetuses are autolyzed by the time they abort. H&E stained section. (Courtesy of Dr. ML Anderson, University of California.)

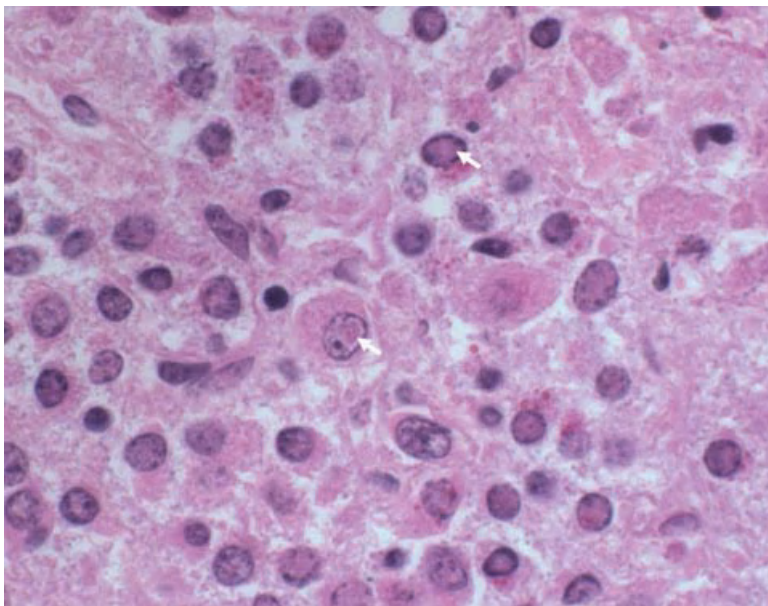


Figure 2.2 Herpesvirus infection of bovine fetal adrenal cortex. Intracellular inclusion bodies are present within numerous infected adrenal cortical cells (*arrows*) due to BHV-1 infection. H&E stained section. (Courtesy of Dr. ML Anderson, University of California.)

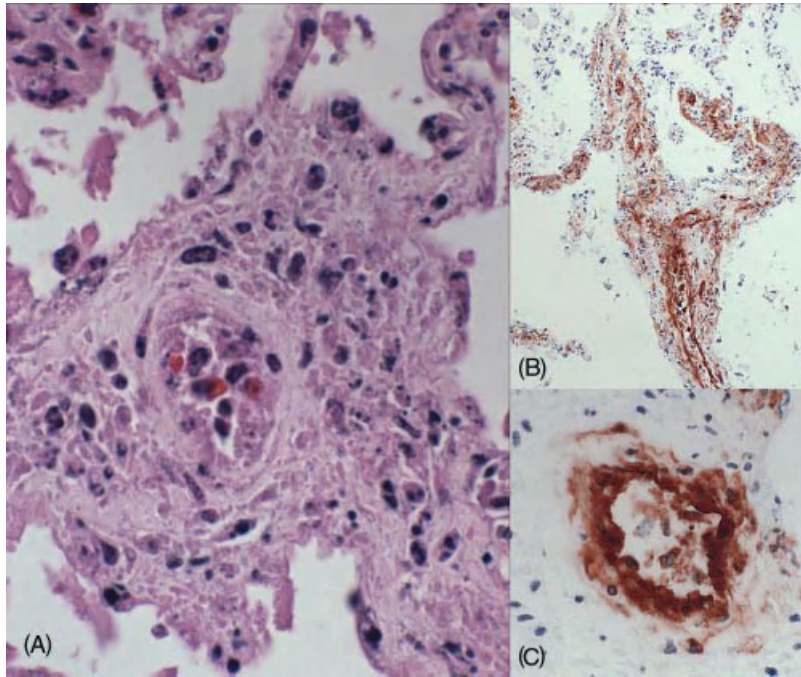


Figure 2.3 Histologic section of herpesviral placentitis in a bovine fetus. (A) Chorioallantois. Degenerate neutrophils infiltrate the vessel wall and surrounding chorioallantoic tissue, diagnosed as vasculitis and perivasculitis. H&E stained section. (B) Immunohistochemical (IHC) stained section of chorioallantois in A. Positively stained viral antigen is detected throughout the chorioallantois. IHC stained section. (C) Higher magnification of affected blood vessel. The chorioallantoic vessel wall is intensely stained because of IHC detection of viral antigen. IHC stained section. (Courtesy of Dr. ML Anderson, University of California.)

gland whereas the best tissues for IHC staining include any tissue with lesions, typically liver, lung, kidney, adrenal gland, and placenta.

Bovine Viral Diarrhea Virus (BVDV)

Fetal BVDV infection is associated with a range of disease manifestations, persistent infections or fetal infection without abortion. Contact with infected animals shedding the virus and contaminated biologics are sources of the virus. There are often no obvious clinical signs seen in herds with fetal losses due to BVDV, and abortions occur a few days or several weeks following maternal infection. Fetal BVDV infection can have variable outcomes depending on the gestational age of the fetus infected and other factors. First trimester infections can cause infertility, embryonic death, fetal resorption, mummification, or abortion. Infection with noncytopathic BVD virus between 2 to 4 months gestation may result in persistently infected live calves that represent a major source of infection for the remainder of the herd. Fetal infections

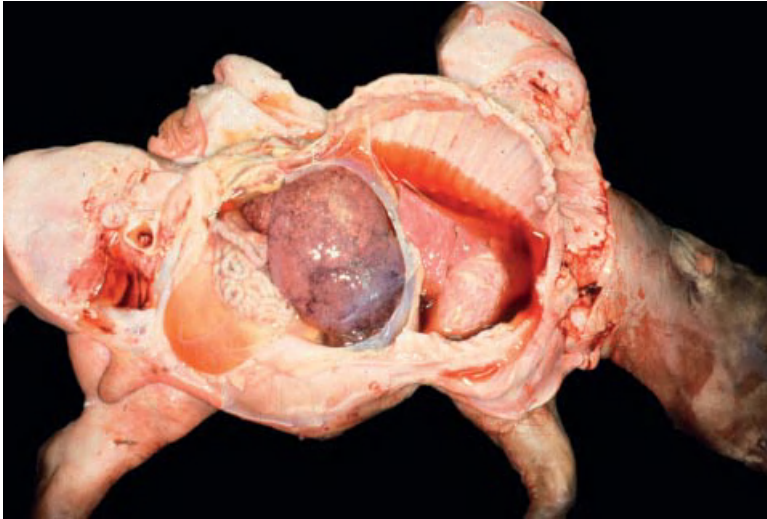


Figure 2.4 Heart failure due to BVD virus infection in a bovine fetus. Abdominal, thoracic, and pericardial sacs are opened. The liver is markedly enlarged with rounded edges. The thoracic and abdominal cavities contain increased volumes of fetal fluids. This fetus had confirmed myocarditis with congestive heart failure due to BVD viral infection. (Courtesy of Dr. ML Anderson, University of California.)

beyond approximately 4 months gestation often result in transient fetal infections, with the development of a fetal immune response, specific fetal antibody production, and elimination of the virus. However, abortions can also occur during later gestational infections. Midgestational infections (from approximately 107 to 183 days of gestation), can also result in the birth of term calves with congenital anomalies. One survey identified BVDV in 33% of abortions that had other bacterial or mycotic infections implying that an initial BVDV infection may contribute to abortion caused by other pathogens perhaps by affecting the dam's immune response during pregnancy.

Fetal lesions attributed to BVDV infection are quite variable. Fetuses can be fresh or autolyzed, and mummification can occur. Aborted fetuses may be small for their gestational age, or calves may be smaller and born premature. Gross lesions suggestive of BVDV infection in the nervous system include microencephaly, cerebellar hypoplasia, hydranencephaly, and hydrocephalus. Ocular lesions including microphthalmia, cataracts, retinal dysplasia, and optic neuritis have been described. Alopecia may be present but is a less specific lesion. Thymic hypoplasia with histologic evidence of thymic cortical atrophy is associated with infection. Pulmonary and renal hypoplasia or dysplasia has also been reported. BVDV infection has been associated with a necrotizing myocarditis with nonsuppurative vasculitis in the heart and other organs. Affected fetuses may exhibit marked ascites with a round dilated heart and chronic passive congestion of the liver (Figure 2.4).

In order to attribute BVDV infection as the cause of abortion, evidence of infection needs to be combined with compatible fetal pathology and/or herd history. Fetal infection can be determined by detection of the virus in fetal tissues by various methods. Virus isolation on a pool of fetal tissues, such as lung, liver, kidney, and lymphoid tissues, is widely used in diagnostic laboratories although it is relatively expensive and time consuming. Virus isolation is specific, but the sensitivity in diagnostic submissions is estimated to be reduced in comparison to other methods presumably due to autolysis and other factors. FA staining for BVDV on frozen sections of either lung or liver and kidney is often used because it is a convenient, rapid, and inexpensive procedure. However, there are significant false positives and false negative results with this procedure compared to either virus isolation or IHC. BVDV IHC using monoclonal antibodies appears to be a sensitive and specific procedure capable of detecting a variety of BVDV isolates. Tissues that are more useful and routinely collected for BVDV IHC staining include kidney, lung, and placenta. In fetuses with vasculitis and necrotizing myocarditis, immunohistochemistry may demonstrate the presence of BVD viral antigens in the vessel wall and/or myocardium. Antigen-capture enzyme-linked immunosorbent assay (ELISA) tests and a number of reverse transcription polymerase chain reaction procedures have been evaluated that appear to be highly sensitive in detecting BVDV.

After 4 months' gestation, the fetus may respond immunologically to infection, so a positive BVDV titer in fetal fluids is an indication of fetal infection. However, if earlier infection by noncytopathic BVDV induces persistent infection, the fetus may be infected but unable to respond immunologically so a fetus that serologically tests negative for BVDV does not rule out infection. Serologically testing the dam for BVDV is of limited diagnostic value, particularly if animals are vaccinated. A fourfold rising titer from paired sera indicates recent exposure to BVDV. BVD virus serologic screening procedures on selected groups of animals in the herd have been used to detect the presence of persistently infected animals. Skin ear notch biopsy procedures using BVD virus IHC or antigen capture ELISA are widely used to identify individual persistently infected animals.

Bluetongue virus (BTV)

Bluetongue virus is an Orbivirus of worldwide distribution. It has multiple serotypes with variable virulence and is transmitted to and between animals by *Culicoides* spp. arthropods. The ability of the virus to cross the placenta and cause fetal infection is enhanced in egg or cell culture adapted vaccine strains. Cattle are frequently infected with BTV and can maintain a prolonged viremia, but reproductive disease associated with BTV has historically been uncommon and sporadic apparently limited to certain cell-adapted strains of BTV. Recently, BTV serotype 8 emerged in Europe as a significant cause of clinical disease in cattle and has an enhanced ability to cause fetal infection. Reproductive disease is influenced by the stage of fetal

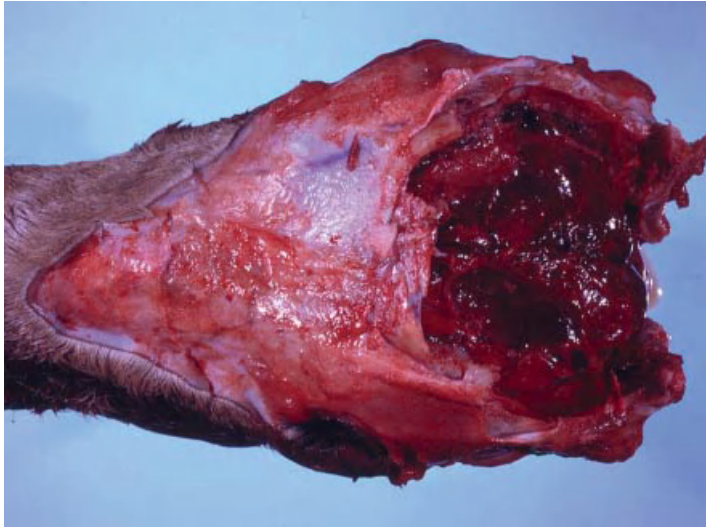


Figure 2.5 Bovine fetal hydrancephaly. The brain of this bovine fetus is dark red and severely malformed as a result of hydrancephaly due to infection with Blue Tongue virus. (Courtesy of Dr. RJ Panciera, Oklahoma State University.)

development at the time of infection. In early gestation under 70 days, death with absorption or abortion can occur. Between 70 and 130 days of gestation, BTV targets neuronal and glial cells. Infection of progenitor cells in the subependymal region of the cerebrum is the pathogenesis for the development of hydranencephaly (Figure 2.5). Slightly later infections but prior to 150 days, approximate time of fetal immunocompetence, can result in porencephaly and hydrocephalus. Later infections may result in encephalitis without brain malformations.

Attempts at cultivating BTV by virus isolation in an immunocompetent fetus may be compromised by the presence of fetal-neutralizing antibodies. Serologic detection of BTV antibodies from fetal fluids or from precolostral serum may provide evidence of *in utero* exposure. BTV PCR assays are available in many diagnostic laboratories.

Bunya virus

A number of bunya virus infections are potentially teratogenic in cattle, and of these, Akabane virus in the Simu serogroup is recognized as a significant cause of congenital arthrogryposis and hydranencephaly in cattle in Israel, Japan, Korea, and Australia. Dependent on the stage of fetal development, Akabane virus, a mosquito-transmitted infection, can lead to abortion or mummification as well as a variety of fetal deformities including hydranencephaly, porencephaly, hydrocephalus, and arthrogryposis. Clinical disease is not observed in the dam. Fetal serology can be useful in detecting exposure. Cache Valley virus, another teratogenic bunya virus, is endemic in North America. Congenital deformities are recognized in small ruminants but not reported in cattle.

Miscellaneous fetal viral infections

Fetal Parainfluenza 3 virus (PI-3) infection has been diagnosed occasionally based on positive virus isolation or detection of fetal antibodies. Experimental fetal inoculation can produce abortion with necrotizing bronchiolitis and interstitial pneumonia with the development of bronchus associated lymphoid tissue (BALT). Even low levels of maternal antibody are protective to fetal infection, and PI-3 appears to be a rare cause of abortion.

The role of Bovine Herpesvirus-4 in bovine abortion is not clear. It is a common reproductive infection and has been associated with endometritis. There is an association between BHV-4 seropositivity and an increased risk of abortion.

Bovine parvovirus infection is widespread in the population and occasionally has been identified in aborted fetuses but its role in abortion is not resolved.

Bacterial causes of abortion and perinatal loss

Opportunistic bacterial infections associated with bovine abortion

A diverse group of bacteria may cause isolated sporadic abortions in cattle. These bacteria are not contagious reproductive pathogens but are commonly found in the environment or on mucous membranes. A maternal bacteremia is the presumed means by which they reach the gravid uterus and subsequently infect the placenta and fetus. Among the bacteria in this group, *Arcanobacterium pyogenes* and *Bacillus* species are most commonly reported, but many other bacteria including *E. coli*, *Histophilus somni*, *Pasteurella* spp., *Pseudomonas* spp., *Serratia marcescens*, *Staphylococcus* spp., and *Streptococcus* spp. can be involved.

Abortions may occur at any stage of gestation, but most are identified in the second half of gestation. There are no specific signs in the dam although the placenta may be retained. The degree of fetal autolysis is variable. The placenta may be thickened and opaque with yellow to brown surface exudation (Figure 2.6). Gross fetal lesions may infrequently include fibrin exudation in body cavities. Histologic lesions include a suppurative placentitis, which is often diffuse and may orient toward either the allantoic or chorionic surfaces. A neutrophilic fetal bronchopneumonia of varying severity is usually present (Figure 2.7). In *Arcanobacterium pyogenes* infection, large clusters of bacterial colonies may be present in the lung with minimal inflammation.

The bacteria involved in these sporadic abortions are common in the environment, therefore, their presence in fetal tissues might reflect incidental postmortem contamination. To establish an etiologic diagnosis, the bacteria should be isolated in nearly pure culture from abomasal contents or tissues, and lesions consistent with a bacterial infection should be identified in the fetus or placenta.

Brucellosis

The incidence of *Brucella abortus* infection and abortion has been reduced in many countries due to government-sponsored eradication programs mostly related to its zoonotic potential. Infection is primarily through ingestion of

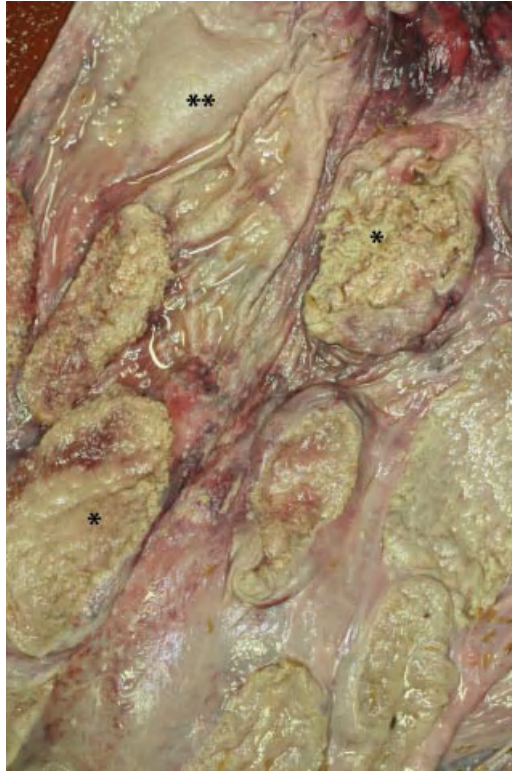


Figure 2.6 Bovine suppurative placentitis. Placental cotyledons are discolored yellow due to an overlying suppurative exudate (asterisk). The intercotyledonary placenta (double asterisk) is marked thickened and opaque due to a similar suppurative exudative process. *Arcanobacterium pyogenes* infection was confirmed. (Courtesy of Dr. ML Anderson, University of California.)

products of abortion. Mature animals are most susceptible to develop a chronic infection. Bacteria multiply in regional lymph nodes nearest the site of entry and then spread via the blood stream to other organs, most importantly, the mammary gland, mammary lymph nodes, and gravid uterus. Uterine infection occurs during the second trimester. Bacteria invade the placental trophoblasts and cause chronic placentitis and fetal infection. Fetuses abort 24 to 72 hours after *in utero* death. Abortion rate is variable but can be high in susceptible herds.

Abortions usually occur after the fifth month of pregnancy. Metritis and retained placentas are common. A severe placentitis with edema, focal necrosis of cotyledons, and thickened intercotyledonary areas with adherent yellowish exudate may be present. The fetus is frequently autolyzed without gross lesions. Histologically, there is a severe placentitis with numerous bacteria visible in chorionic epithelial cells. Fetal lungs are often inflamed varying in severity and character from acute neutrophilic bronchopneumonia to more chronic pleocellular bronchointerstitial pneumonia with periairway infiltration by mononuclear cells. Bacterial isolation is necessary to confirm

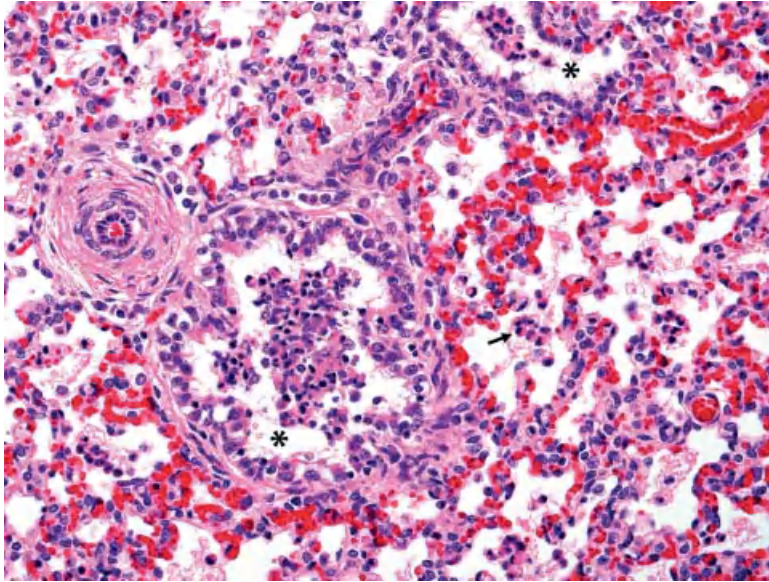


Figure 2.7 Fetal bronchopneumonia. Abundant neutrophils are present within the lumens of two fetal bronchioles (asterisk). Mixtures of fewer neutrophils and strands of fibrin are present within affected alveolar lumens (arrow). *Arcanobacterium pyogenes* was cultured from this case. H&E stained section. (Courtesy of Dr. ML Anderson, University of California.)

the diagnosis. *Brucella abortus* can be isolated from various sources including fetal abomasal fluid, lung, placenta, uterine fluid, and milk. A number of different serologic tests have been developed for governmental surveillance and detection of cattle exposed to *B. abortus*.

Listeriosis

Listeria species are widespread in the environment and abortion due to *Listeria monocytogenes* and *L. ivanovii* infections are encountered throughout the United States. Most abortions are sporadic although abortion storms may occur, some associated with ingestion of poorly fermented silage promoting the growth of *Listeria*. In some instances, there may be illness in aborting cows with fever and anorexia due to metritis. Listeriosis in adult cattle can occasionally cause encephalitis, but this is rarely seen in association with abortion.

The placenta is usually retained at the time of abortion. Fetuses usually abort in the third trimester and are often markedly autolyzed. Gross lesions may be absent or obscured by autolysis, but scattered white to yellow foci may be present throughout the liver (Figure 2.8). Additional findings include small pale foci in placental cotyledons, and fibrin in body cavities. Histopathology changes include suppurative placentitis and multifocal hepatic necrosis with variable suppurative hepatitis (Figure 2.9). Meningitis may also be seen.

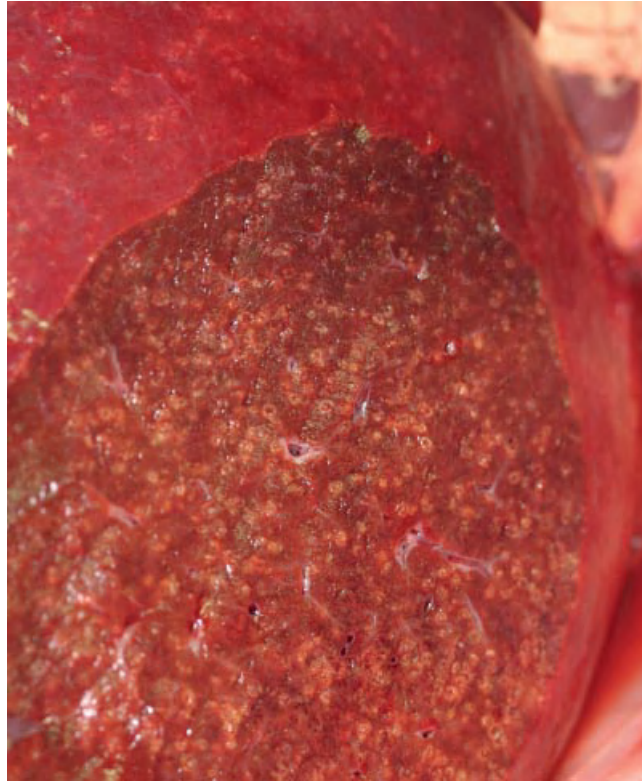


Figure 2.8 Bovine fetal hepatitis. Visible through the liver capsule as well as in the cut surface are myriad, yellow-pink foci representing areas of necrosuppurative hepatitis. In this case, *Listeria ivanovii* was cultured from fetal tissues. (Courtesy of Dr. ML Anderson, University of California.)

Intravascular bacterial colonization in many organs with or without associated inflammatory lesions is sometimes encountered. Gram stains of fresh or fixed tissues are helpful in confirming the diagnosis due to the presence of Gram positive, small rods. *Listeria* spp. are often isolated from multiple tissues. Successful isolation of *Listeria* spp. in abortion cases usually does not require cold enhancement. IHC stains using commercially available antibodies can assist the diagnosis when a positive culture result is unavailable. Some diagnostic laboratories employ *Listeria* PCR procedures.

Salmonellosis

In North America, *Salmonella* spp. infections usually present as sporadic abortions, but in some regions of the world, *Salmonella* abortion is a significant cause of both sporadic and epizootic abortion. Most bovine abortions due to *Salmonella* infection are associated with *Salmonella enterica*, serovar *Dublin*, but other serotypes can be involved. The infection is presumed to originate

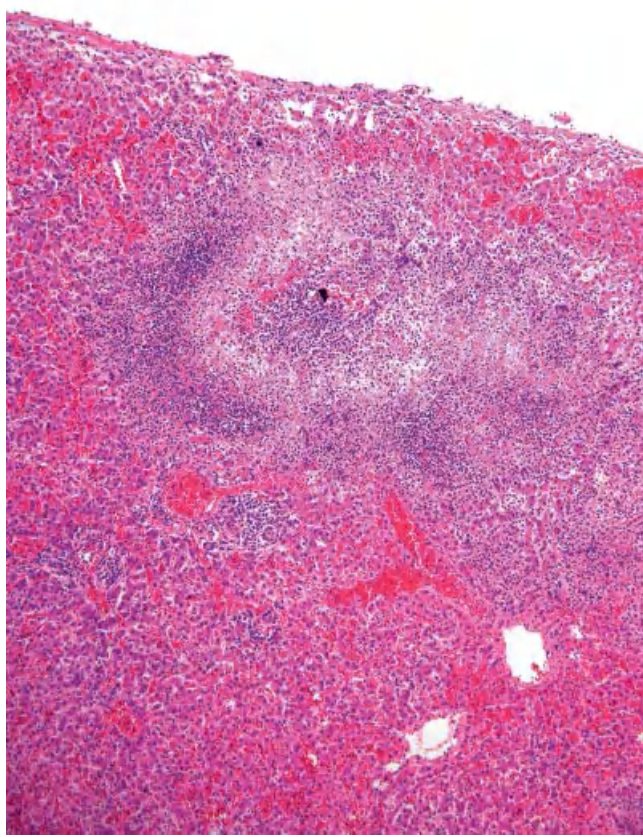


Figure 2.9 Histology of necrosuppurative hepatitis in a calf. In this histologic section of Figure 2.8, a focal area of hepatic necrosis is heavily infiltrated by degenerate neutrophils due to *Listeria ivanovii* infection. Surrounding sinusoids and blood vessels are markedly congested. H&E stained section. (Courtesy of Dr. ML Anderson, University of California.)

from the intestinal tract. Bacteremic episodes can lead to the localization and proliferation of the infection in the placentome causing destruction of fetal villi that may lead to abortion without bacterial invasion of the fetus.

The abortions are usually in the second half of gestation, and the placenta is often retained. The chorioallantois is thickened by fibrinous fluid with a diffusely grey to red chorionic surface. Portions of caruncular tissue may be adherent to the cotyledons. The fetus is usually quite autolyzed and may be emphysematous. Usually there are no remarkable gross lesions. Rarely, indistinct pale foci may be present in the liver. Histologic changes include neutrophilic placentitis and mineralization with bacterial proliferation in cotyledonary villi and when present a multifocal suppurative hepatitis. Lung lesions may be minimal consisting of neutrophilic bronchial exudate. *Salmonella* and *Listeria* abortions have some similarities in that the bacteria

proliferate in the placenta followed by massive infection of the fetal liver, septicemia, and death, often without development of bronchopneumonia typical of some other bacterial infections of the placenta.

Yersinia pseudotuberculosis

Sporadic abortion due to *Yersinia pseudotuberculosis* infection occurs in cattle. The bacterium, a frequent inhabitant of the intestinal tract, is presumed to hematogenously spread to the placenta.

Placentitis is characterized as thickened cotyledons with pericotyledonary fibrosis. The aborted fetuses are usually in the second half of gestation and not autolyzed. Gross fetal changes consist of excess fluid with varying amounts of fibrin in body cavities and occasionally multifocal hepatic necrosis. Histologic changes include pleocellular cotyledonary placentitis with attached necrotic caruncular tissue containing thrombosed vessels. Fetal lesions include focal hepatic necrosis with a pleocellular infiltrate, conjunctivitis, and enterocolitis.

Leptospirosis

The most significant *Leptospira interrogans* serovars associated with reproductive problems in cattle are *L. interrogans* serovars Hardjo and Pomona although serovars Icterohaemorrhagiae and Grippotyphosa can affect the reproductive tract. Genetic analysis indicates that *L. interrogans* serovar Hardjo has undergone genomic reduction and has been placed in a separate genus, *L. borgpetersenii* serovar Hardjo, which is host dependent and adapted to cattle. Affected cattle can become persistently infected. Other serovars of *Leptospira* (including *L. Pomona*) are maintained within domestic or wildlife species. Leptospire can be shed in urine, and nonhost-dependent serovars may survive in wet environments. The organisms can penetrate intact mucous membranes or abraded skin. Abortion is a manifestation of chronic leptospirosis in adult cattle, and is frequently the only clinical sign observed in a herd. Signs of acute leptospirosis including fever, hemolytic anemia, hemoglobinuria, icterus, and high mortality rates are reported in calves. In lactating cattle, agalactia and mastitis can develop with flaccid udders and thick yellow to occasionally blood-tinged secretions.

Abortion can occur 1 to 3 months following initial infection with *L. borgpetersenii* serovar Hardjo and 1 to 6 weeks following infection with *L. interrogans* serovar pomona. Hardjo infections cause infertility, abortion of fetuses 4 months to term, and weak calves at birth. Abortion due to Pomona usually occurs in the last trimester. The herd abortion rate seldom exceeds 10% with Hardjo infections but can approach 50% in herds infected with Pomona. The aborted fetus is usually autolyzed. Icterus may be seen in late gestation fetuses infected with Pomona. Histologic lesions may not be observed, but in some cases, renal tubular necrosis and interstitial nephritis is present (Figure 2.10). Bile retention within canaliculi may be present. Nonsuppurative meningitis has also been reported.

Because leptospire are labile and difficult to culture, bacterial isolation is impractical for routine diagnostics. Identification of leptospire by darkfield

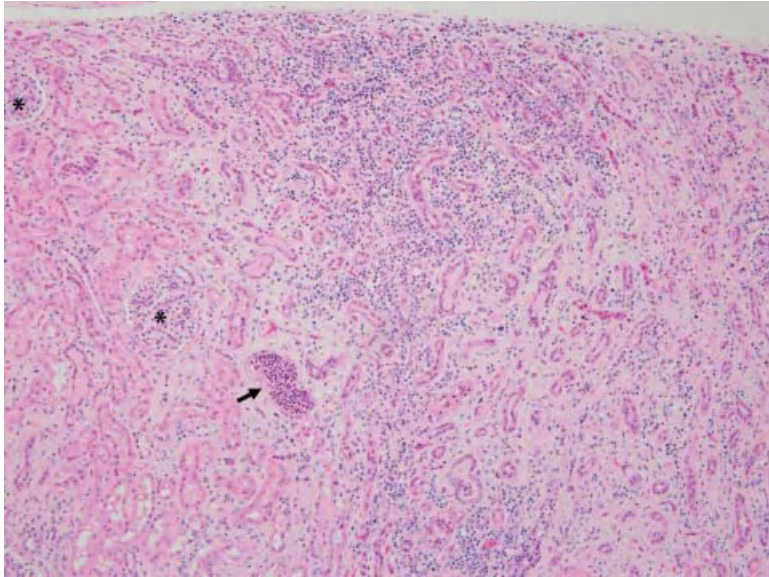


Figure 2.10 Histology of interstitial nephritis due to *Leptospira* infection in a bovine fetus. Within the interstitium between renal tubules are large numbers of mononuclear cells. One renal tubule is infiltrated by large numbers of inflammatory cells (arrow). Renal glomeruli (asterisks) are unaffected in this case of fetal *Leptospira* infection. H&E stained section. (Courtesy of Dr. ML Anderson, University of California.)

microscopy of fetal fluids or silver stains of fetal tissues is rarely successful. FA examination of fetal kidney smears using multivalent antisera is a convenient, rapid, and inexpensive procedure although not highly sensitive and specific. Typically, FA tests will not determine the specific *Leptospira* serovar involved. IHC staining is sometimes useful in identifying leptospires in bovine fetal tissues, but autolytic fragmentation of the spirochetes can make interpretation difficult. PCR techniques may be useful for identifying *Leptospira* organisms shed in urine. Some laboratories employ this PCR test to identify leptospires in fetal tissues and placenta.

Maternal serology using the modified agglutination microtiter test may be useful in the diagnosis of leptospirosis, though caution must be used to distinguish between vaccination, previous exposure, and recent infection. Serology for Hardjo is especially difficult to interpret since infected animals often have a low titer rarely exceeding 1:1,600, and a proportion of infected cattle can have a negative titer at the time of abortion. Serologic titers to Pomona may be higher at the time of abortion and can exceed 1:12,800. Since abortion occurs weeks to months following maternal infection, seroconversion usually precedes abortion, and the titer may be declining at the time of abortion. In addition, it can be difficult to distinguish between vaccination and natural exposure. Vaccination usually results in lower titers compared to recent natural exposures (e.g., Pomona titers in vaccinated animals seldom exceed

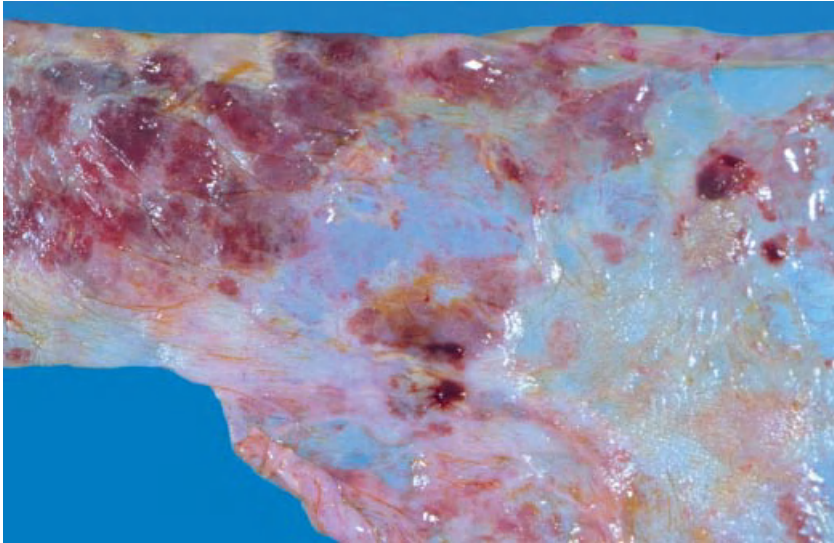


Figure 2.11 Hemorrhagic amnionitis in a cow. Scattered throughout the amnion are areas of thickening and hemorrhage due to *Ureaplasma diversum* infection. (From Dr. R Miller, 4th Edition of McGavin and Zachary's Pathologic Basis of Veterinary Disease, pg. 1,297.)

1:3,200), and multivalent bacterins usually result in elevated titers to multiple serovars compared to elevations of a single serovar following field exposure. However, a newer single serovar vaccine for *Leptospira borgpetersenii* serovar Hardjo (formerly *L. interrogans* serovar *hardjo-bovis*) might induce high titers to *Leptospira* serovar Hardjo. In the fetus, titers greater than 1:40 would be supportive of a diagnosis, but infected fetuses may have no detectable titer.

Ureaplasma diversum

Ureaplasma diversum is a common inhabitant of the upper respiratory tract and lower reproductive tract of cattle. The diagnosis of *U. diversum* abortion in cattle is variably reported. In North America, although in Canada, a substantial portion of abortions has been attributed to this infection. It is not clear whether this regionally higher incidence reflects an actual difference in infection rate or is due to differences in microbiologic procedures used by diagnostic laboratories to identify this fastidious organism.

Fetuses are usually aborted the last third of gestation, and stillbirths or weak calves may occur. Aborted fetuses are often in fresh condition. Placenta retention is frequently reported. A placentitis develops in which the amnion is often the most affected portion of the fetal membranes. The amnion is thick and opaque with multifocal to extensive areas of hemorrhage, fibrin exudation, necrosis, and fibrosis (Figure 2.11). Similar changes may be present in the chorioallantois with the allantoic surface affected more severely than the chorionic surface. Cotyledons may be tan to dark red, cupped with adherent caruncular material. Fetal lungs may be swollen and firm.

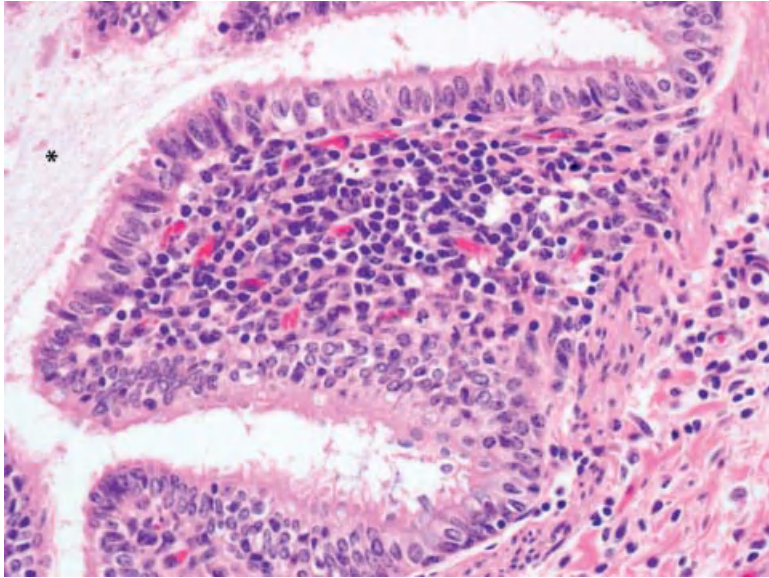


Figure 2.12 Histology of lymphocytic infiltration of a fetal bovine bronchiole. Large numbers of mononuclear cells, mostly lymphocytes, infiltrate the submucosa of a bronchiole. The calf aborted due to an *Ureaplasma diversum* infection. Bronchiolar lumen (asterisk). H&E stained section. (Courtesy of Dr. ML Anderson, University of California.)

Histologic changes in the stroma of the chorioallantois and amnion include fibrosis, necrosis, and mineralization with macrophage and plasma cell infiltration and mononuclear vasculitis. The inflammation in the chorioallantois is often oriented toward the allantoic surface. Lesions in the lung consist of a nonsuppurative alveolitis and periairway mononuclear infiltrate often with the formation lymphofollicular nodules (Figure 2.12). This prominent, periairway lymphoid infiltrate and marked BALF stimulation may also be observed with other fetal infections including BVDV, PI-3, and other chronic bacterial infections. Erosive lymphoplasmacytic conjunctivitis is also associated with *Ureaplasma* sp. infections.

Since *Ureaplasma* is a commensal organism of the reproductive tract of cattle, contamination of placental and fetal tissues is a potential confounding factor in confirming a diagnosis. Diagnosing *Ureaplasma diversum* as the cause of abortion should be based on isolation of the organism from the fetal lung, its aseptically collected stomach contents, or placenta coupled with the presence of compatible lesions in the fetus and placenta.

***Campylobacter* species**

Campylobacter fetus subspecies *venerealis* is a widespread, venereally transmitted cause of infertility due to early embryonic death and occasional abortions. Most abortions are noted at 4 to 6 months gestation. Following initial infection in cows, bacteria are cleared from the uterus but may persist for longer periods in vaginal mucus. Reinfection can occur, though some

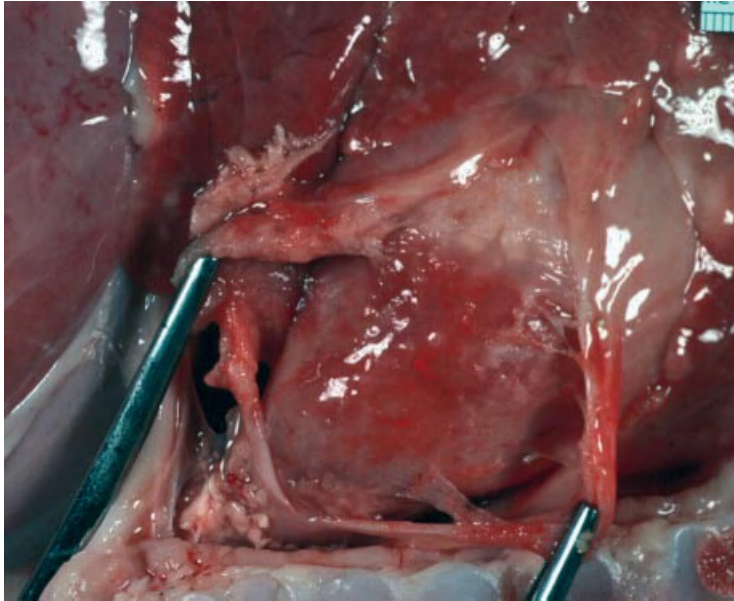


Figure 2.13 Fetal pericarditis in a calf. The pericardial sac is thickened. Strands of fibrin extend between the pericardium and epicardium indicating pericarditis. This calf aborted due to *Campylobacter fetus* subsp *venerealis*. (Courtesy of Dr. ML Anderson, University of California.)

immunity exists following infection in the cow. This immunity explains why there is a higher incidence of infertility among heifers within chronically infected herds. As with trichomoniasis, bulls with campylobacteriosis are sub-clinical carriers, and when a herd infection is established, it is more prevalent in older bulls. The disease can also be transmitted by contaminated semen during artificial insemination and with contaminated semen collection equipment.

Aborted fetuses may be fresh but can be in variable postmortem condition. In the placenta, a fibrinous intercotyledonary placentitis may be present. The fetus may have gross evidence of fibrinous exudation in the pleural cavity, peritoneal cavity, and especially within the pericardial sac (Figure 2.13). Splenomegaly is a variable feature. Fetal serum immunoglobulin levels may be moderately elevated. Histologic changes include fibrinous neutrophilic placentitis, neutrophilic bronchopneumonia, fibrinous neutrophilic serositis and occasionally abomasitis.

Silver stains of fetal tissues that are inflamed may assist in the diagnosis by identifying curved bacteria. IHC has been used to detect *Campylobacter fetus* in fetal tissues and differentiate it from other *Campylobacter* species. *Campylobacter* may be identified in the abomasal fluid using direct darkfield microscopy. *Campylobacter fetus* ss. *venerealis* is fastidious, requiring careful sample collection and proper transport. The bacteria can be cultured from lung, placenta, and abomasal fluid. The bacteria can also be identified in preputial samples and vaginal secretions. *C. fetus* ss. *venerealis* must be differentiated from *Campylobacter fetus* ss. *fetus* and *Campylobacter jejuni*,

which are not venereally transmitted infections but instead occur in cattle as sporadic, hematogenous infections from the intestinal tract with similar fetal pathology. Isolation requires special media and oxygen tension. Phenotypic characterization and more recently molecular techniques have been used to differentiate *Campylobacter* species.

Epizootic bovine abortion

Epizootic bovine abortion (EBA), also known as Foothill Abortion, is a cause of abortion and premature calving in cattle grazing oak woodlands and sagebrush-juniper-piñon pine rangelands in California, Nevada, and Oregon. The infection is transmitted to susceptible pregnant cattle by an argasid tick (*Ornithodoros coriaceus*) that feeds on deer and cattle. The disease is seen in heifers or cows exposed to endemic areas for the first time while in the first trimester of pregnancy. Abortions, either sporadic or as an outbreak, usually occur in the last trimester, and premature weak calves may also occur. Following an abortion, affected cattle are resistant to repeat abortion.

The etiology of EBA remains under investigation, but an unnamed delta-proteobacteria in the Myxobacteria family has been implicated based on molecular techniques on extracted 16S bacterial ribosomal DNA fragments in infected fetal tissues and ticks. The bacterium is a plump 2- to 3-micron-long Gram-negative rod observed in smears from infected tissues. The bacterium has not been successfully grown on artificial media. Histochemical identification in tissues has been successful only using a modified Steiner silver stain procedure as adapted for staining *Lawsonia intracellularis*. An IHC procedure using sera from infected fetuses can stain the bacteria in thymus, spleen, lymph node, and in other tissues in the presence of inflammatory lesions.

The diagnosis of EBA is based on the identification of characteristic gross and histologic lesions that are chronic, having developed over a period of 3 months or longer. The affected fetus is usually fresh and may be born alive. Petechial hemorrhages are common in the mucosa of the conjunctiva and oral cavity and are especially prominent in the ventral lingual mucosa (Figure 2.14). There is marked enlargement of peripheral lymph nodes that are easily palpated through the skin. Abdominal distension due to ascites and liver enlargement is often present. Splenic and lymph node enlargement is very common. The thymus may be reduced in size with interlobular or widespread hemorrhage and edema in the cranial portion.

Histologic examination of fetal tissues, particularly the lymphoid organs, is required to confirm a diagnosis of EBA. Thymic lesions, which are unique in EBA, develop late in the course of the disease and consist of a loss of cortical thymocytes and infiltration of the medullary region with macrophages (Figure 2.15). The thymic interlobular septa are distended with edema, fibrin, hemorrhage, and cellular infiltrates consisting of macrophages and other mixed inflammatory cells. The gross enlargement of the lymph nodes is associated with lymphoid hyperplasia and widespread macrophage infiltration in the sinuses and medulla. There is lymphoid hyperplasia and histiocytic infiltration in the spleen. Late in



Figure 2.14 Sublingual hemorrhages in a bovine fetus. Multifocal hemorrhages scattered throughout the ventrum of the tongue is a lesion that is prominent in cases of enzootic bovine abortions (EBA). (Courtesy of Dr. ML Anderson, University of California.)

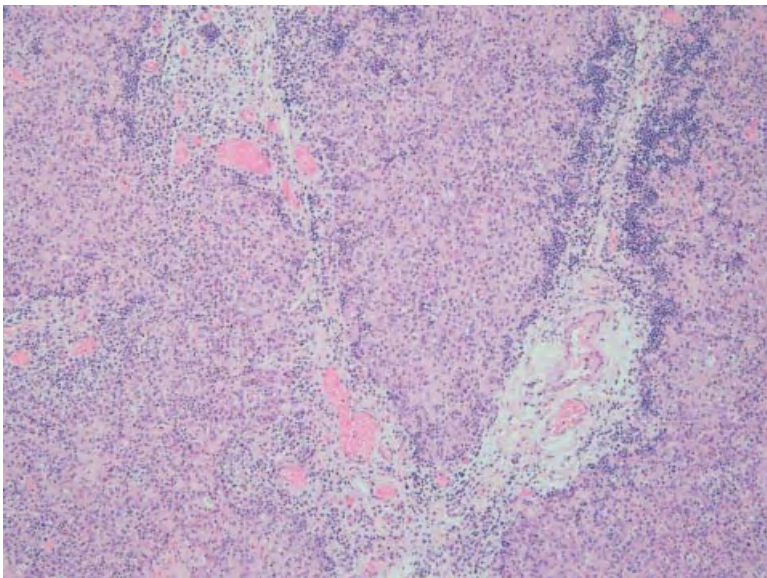


Figure 2.15 Histology of thymus from a case of EBA. There is marked infiltration and replacement of cortical thymocytes by macrophages. This is a characteristic feature observed in cases of enzootic bovine abortion (EBA). H&E stained section. (Courtesy of Dr. ML Anderson, University of California.)

the course of disease, following the proliferative response, acute necrotic foci develop in lymphoid organs. There are widespread inflammatory lesions with a vascular orientation in most organs, including the brain, lung, heart, liver, kidney, skeletal muscle, and other organs. Fetal serum immunoglobulin levels are usually markedly elevated and range from 100 to 1,500 milligrams per deciliter (mg/dL).

***Chlamydia*-related organisms**

Chlamydophila abortus, *Parachlamydia* species, and *Waddlia chondrophila* are members of the order *Chlamydiales*. *Chlamydophila abortus*, a common cause of abortion in small ruminants, is a rarely diagnosed cause of cattle abortion. Recent evidence primarily through the use of PCR methods on placental samples suggests the involvement by related *Parachlamydia* species and *Waddlia chondrophila* infections in placentitis and late-term bovine abortions. Suppurative to necrotizing placentitis often with vasculitis in the stromal vessels is the primary lesion reported. Diagnosis is based on PCR and immunohistochemistry.

Coxiella burnetii

Coxiella burnetii infection is an uncommonly diagnosed cause of bovine abortion although the placental infection is more common with zoonotic implications. A significant association between placentitis and *C. burnetii* placental infection in cases of bovine abortion has been reported suggestive that it has a role in bovine abortion. Lesions attributed to *C. burnetii* infection include mononuclear cell infiltration in the placental stroma with enlarged trophoblasts containing intracytoplasmic organisms, chorionic trophoblast necrosis and fibrinosuppurative exudation. Fetal bronchopneumonia may be present.

Diagnosis is on the basis of the histologic changes in the placenta with identifiable intracytoplasmic organism in trophoblast with histochemical stains such as modified acid-fast, Macchiavello's, or Gimenez stains. Further confirmation of the presence of *Coxiella burnetii* is achieved by IHC or PCR techniques.

Mycotic infections

The percentage of mycotic abortions diagnosed varies depending on the geographic location. The majority of mycotic abortions in cattle are caused by *Aspergillus fumigatus*. Infections by other *Aspergillus* spp., *Absidia* spp., *Mucor* spp., *Rhizopus* spp. and *Candida* spp. occur less commonly. The fungi responsible are ubiquitous saprophytes in the environment. The concentration of fungal conidia in the environment may increase the risk for fetal infection. Injury to the respiratory or digestive tract of the dam may also enhance the entry of fungi into the bloodstream. Localization in the uterus probably occurs by hematogenous spread from these sites of entry. Experimentally, intravenous



Figure 2.16 Mycotic placentitis from a cow. Both the cotyledonary (arrow) and intercotyledonary (asterisk) placenta is thickened and leathery due to *in utero* fungal infection. In this particular case from New Zealand, *Mortierella wolfii* was recovered. This pathogen is rarely recovered from North American cases. (Courtesy of Dr. KG Thompson, Massey University.)

injection of *A. fumigatus* causes abortion 23–35 days post-infection. *Mortierella wolfii*, a common abortifacient agent in the southern hemisphere, is rare in North America, and fetal infection by *M. wolfii* is associated with a primary mycotic pneumonia in the dam.

Mycotic abortions usually occur as sporadic third trimester abortions. Clinical signs in the dam are infrequent aside from retained placentas. Since placentitis is the primary lesion, submission of the placenta is critical for confirming the diagnosis. Grossly there is often a severe placentitis involving both the cotyledons and intercotyledonary placenta producing a diffusely thickened, leathery placenta (Figure 2.16). Cotyledons may have necrotic, hemorrhagic infarcts with adherent caruncular tissue. Histologically, the placenta may have suppurative inflammation, necrosis, and vasculitis with thrombosis associated with fungal invasion. Fetal autolysis may be minimal, especially with *Aspergillus fumigatus* infections. Fetal lesions are variable and may be absent. Fetal lesions associated with mycotic abortion include bronchopneumonia, occasionally focal digestive tract inflammation associated with fungal mucosal invasion, and focal to locally extensive circular skin lesions (Figure 2.17). The diagnosis is based on identifying gross lesions and demonstration of fungus by culture of the placenta, abomasal fluid, and lung, and direct identification of the fungi with potassium hydroxide (KOH) wet-mount exam of scrapings from skin lesions and in histologic sections.



Figure 2.17 Mycotic dermatitis in a bovine fetus. Scattered throughout the skin are numerous multifocal to coalescing, circular, raised epidermal plaques. These lesions are characteristic of *in utero* infections with fungal organisms. *Aspergillus* spp. was recovered from this case. (Courtesy of Dr. KS Palyada, Cornell University.)

Protozoal infections

Tritrichomonas foetus

Tritrichomonas foetus is a widespread venereally transmitted protozoal pathogen that is a major cause of infertility similar to *C. fetus* ss. *Venerealis*. This infection is usually associated with early embryonic loss and only occasional abortion and pyometra. The abortion rate is usually moderate from 5 to 30%, and most fetal loss is in the first 5 months of gestation although later-term abortion may occur. Infection in the cow usually is transient, and bulls, especially older bulls, are inapparent carriers.

The clinical history of infertility in bull-bred cows leaves suspicion for trichomoniasis or campylobacteriosis. The parasites are most efficiently obtained and identified by aspirating smegma from the preputial fornix of infected bulls using a dry insemination pipette. The diagnosis may also be made from the aspirated cervical or vaginal mucous of infected cows. Uterine fluid from infected cows with pyometra can be an excellent sample. The protozoa are very fastidious, so special care should be taken to properly collect, maintain, and transport collected fluid according to the needs of your diagnostic laboratory.

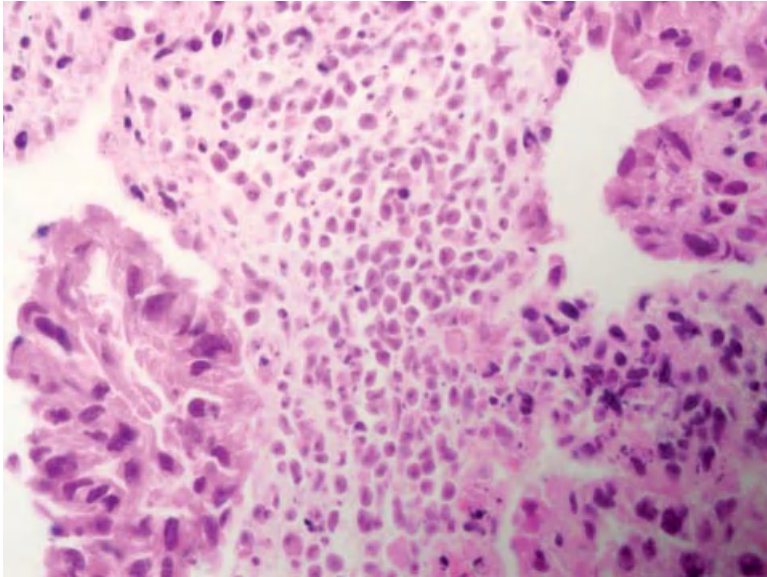


Figure 2.18 Histology of *Tritrichomonas* placentitis. Scattered throughout the stroma of a chorionic villus of a bovine placenta are myriad protozoa identified as *Tritrichomonas foetus*. H&E stained section. (Courtesy of Dr. ML Anderson, University of California.)

Gestational age of aborted fetuses may range from 2 months to late gestation with variable autolysis. In most cases, there are no gross lesions other than placental edema. Histologic placental lesions consist of stromal edema with pleocellular infiltrates and chorionic epithelial necrosis. In non-autolyzed placentas, trichomonads can be identified invading the placental stroma in hematoxylin and eosin (H&E) stained sections (Figure 2.18). Inflammation in association with the presence of the protozoa is variable. Fetal lung lesions consist of a bronchopneumonia with neutrophilic and macrophage exudation. Occasionally, trichomonads can be visualized within alveoli and airways in histologic sections. Langhans and/or foreign body giant cells in pulmonary airways are associated with trichomoniasis although multinucleated giant cells in fetal pneumonia are not unique to *T. foetus* infection (Figure 2.19). Trichomonads may be identified in abomasal fluid samples with darkfield microscopy. Giemsa and Bodian's silver protargol histochemical stains are useful aids in identifying trichomonads in tissue sections. IHC using polyclonal and monoclonal antibodies are also an effective method to detect *T. foetus* in fetal and placental sections.

Neospora caninum

Neosporosis is a common protozoal infection of cattle and a major cause of abortion with a worldwide distribution. An important feature of the disease is that the parasite can be maintained in the cow as a chronic infection and is

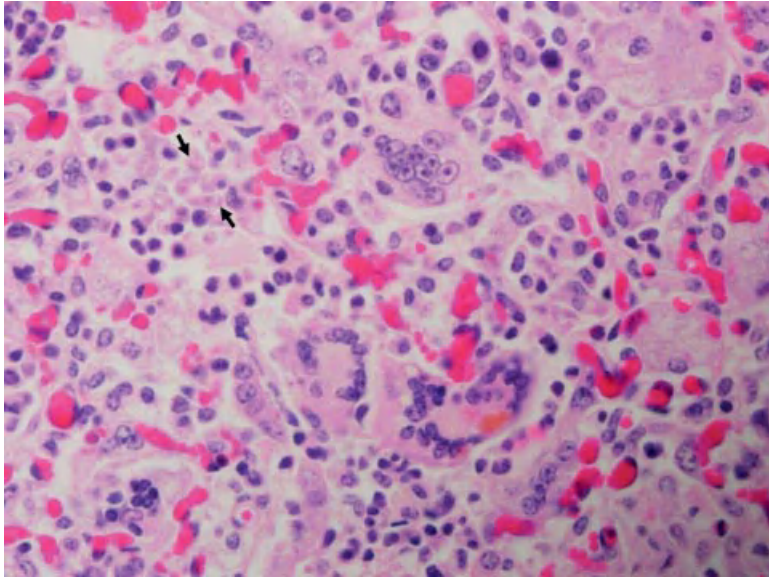


Figure 2.19 Histology of fetal bovine pneumonia due to *T. foetus*. A mixture of neutrophils, mononuclear cells, and multinucleated giant cells are present within alveoli mixed with protozoal organisms (arrows). This is a characteristic of *T. foetus* infection but not unique to this pathogen. H&E stained sections. (Courtesy of Dr. ML Anderson, University of California.)

very efficiently transmitted to the fetus during pregnancy. Some infected pregnancies result in abortion, but the majority of fetal infections result in an asymptomatic, congenitally infected calf. A congenitally infected heifer calf is capable of transmitting the infection onto the next generation when she becomes pregnant, thus maintaining the infection in the herd. A high percentage (as high as 80 to 90%) of calves born to seropositive cows are congenitally infected based on serology, and these clinically normal, congenitally infected calves are important in maintaining the infection in the herd. Cattle may also acquire infection by horizontal or postnatal infection through ingestion of oocytes shed by the definitive hosts for *N. caninum*, currently identified as dogs, coyotes, and dingoes.

The serologic prevalence of *N. caninum* infection is highly variable, ranging from 14 to 60% in U.S. cattle herds. Seropositive cattle have a threefold to thirteen-fold increased risk of abortion compared to seronegative cattle. Both endemic and epidemic abortion patterns may occur. Endemic abortions of greater than 5% may persist for years. The epidemic pattern of abortion is less common. In subsequent pregnancies, cows that abort may have repeat abortions or produce infected calves. The clinical outcome of these subsequent pregnancies is variable, but a seropositive cow that has an abortion has a greater risk of abortion in subsequent pregnancies.

N. caninum abortions occur throughout the year and have been reported in both dairy and beef cattle. There are no signs of clinical illness in cows that

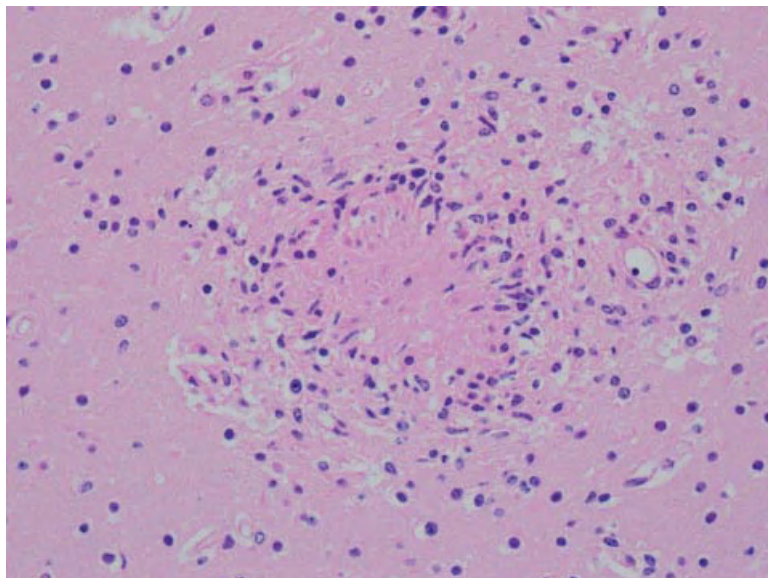


Figure 2.20 Histology of neuropil necrosis in a bovine fetus. A focal area of necrosis within the fetal neuropil is surrounded by mononuclear inflammatory cells and hyperplastic glial cells. The cause in this case was *Neospora caninum*. H&E stained sections. (Courtesy of Dr. ML Anderson, University of California.)

abort due to *N. caninum* infection and the placentas are not retained. Abortions have been diagnosed in both heifers and cows from 3 months gestation to term, but the majority occur in the second trimester of pregnancy, a distinctive feature of this agent. Fetal mummification is reported. A rare outcome of fetal *N. caninum* infection is the birth of a clinically affected full-term calf with signs ranging from mild proprioceptive defects to complete paralysis associated with a multifocal encephalomyelitis, which may be localized to the spinal cord. The majority of calves that acquire infection during gestation are born clinically normal and will have high precolostral antibody titers, which are useful in confirming the diagnosis.

The aborted fetus is usually 4 to 6 months in gestation age and autolyzed with serosanguinous fluid accumulation in body cavities. Rarely there are subtle gross lesions, consisting of pale white foci or streaks affecting skeletal muscles or the myocardium. The most diagnostically significant histologic lesions are found in the brain consisting of scattered foci of nonsuppurative cellular infiltrates and/or foci of necrosis with a surrounding nonsuppurative cellular rim (Figure 2.20). Protozoa are not usually seen in H&E stained sections although occasional tissue cysts may be encountered in the brain which are usually not directly associated with inflammation. In the heart, a nonsuppurative epicarditis and/or myocarditis are present. Focal mononuclear and occasionally necrotizing myositis are scattered throughout skeletal muscle. Hepatic portal areas are infiltrated with mononuclear cells, and there are often indistinct foci of paracentral hepatic necrosis. Scattered mononu-

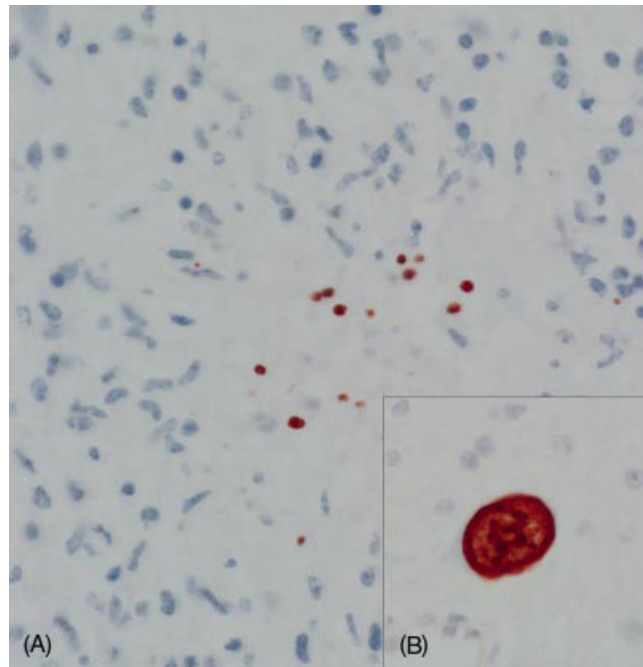


Figure 2.21 Immunohistochemically stained section of a bovine fetal brain for *Neospora caninum*. (A) IHC stained section of brain. Scattered within the center of an area of neuropil necrosis are positively stained *Neospora caninum* organisms. IHC stained section. (B) Higher magnification of A. A strongly positively stained tissue cyst is presented in this image. IHC stained section. (A and B, courtesy of Dr. ML Anderson, University of California.)

clear interstitial infiltrates with or without necrosis are seen in sections of lung. The interstitium of the kidney may have focal mononuclear infiltrates. Placental lesions can vary considerably in severity and on occasion appear to be the primary cause of fetal death and abortion. Multifocal chorionic epithelial necrosis with inflammatory cellular infiltration and edema in the subjacent stroma is commonly present.

IHC testing using antibodies raised against *N. caninum* antigens is an effective method to identify the tachyzoite and tissue cyst stages of the parasite in fetal tissues (Figure 2.21). Tissues that are most useful for IHC testing include brain, lung, kidney, skeletal muscle, and placenta. In aggregate, lesions and IHC identification of protozoan in aborted fetuses to establish a diagnosis has come into question because the fetus can be infected and not abort due to the infection. The presumption is that in early gestation there is little resistance to disseminated infection resulting in abortion or mummification while later in gestation, the fetus can survive the infection. Confirmation of *N. caninum* infection as the cause of abortion should consider the gestational age and condition of the fetus, the presence of compatible disseminated lesions, detectable parasites, and the absence of other causes. An association between

N. caninum abortion and concurrent BVDV infection has been reported. A fetus with mild lesions, often limited to focal encephalitis in late-term fetuses, may have an incidental *N. caninum* infection so other causes for the abortion should be investigated.

PCR techniques for detection of *N. caninum* have been used to assist in identification of fetal infection and are reported to be more sensitive than IHC in identifying fetal infections. Establishing *N. caninum* as the cause of abortion on the basis of a positive PCR test should be viewed with similar caution as is employed with positive IHC.

Serologic tests are available to assist in the diagnosis and include the indirect immunofluorescent antibody (IFA) test, the modified agglutination test, and a number of ELISAs. The assays use *N. caninum* tachyzoites or specific derived antigens. The specificity and sensitivity of the various serologic tests are comparable depending on the minimum antibody titer that has been established as the cutoff for a positive result. Laboratories using any of the serologic tests for *N. caninum* should establish appropriate cut-off titers using sera from known infected and noninfected cattle. In some tests, the positive cut-off titer has been selected based on the antibody titer in a cow that has aborted an infected fetus so this cutoff may not be the most appropriate for the serologic diagnosis of a chronic infection in cattle that vary in age and pregnancy status. A single serum sample from an individual cow may not accurately reflect her infection status since titers in known positive cattle fluctuate and may fall below the cut-off value for some period of time. In rare instances, cows that abort a *N. caninum* infected fetus may not have a significantly elevated titer. Also, previously elevated titers at abortion may decline over several months following abortion.

Serology is effective in detecting elevated *N. caninum* antibodies in the serum of congenitally infected calves, and a proportion of aborted fetuses have elevated antibody titers. A negative fetal titer does not rule out infection, and a positive titer does not prove that this infection caused the abortion. In the individual aborting cow, a positive serology result does not prove that the abortion was due to neosporosis, but it can assist the diagnosis. Serology can be used on a herd basis to investigate the association between seropositivity and abortion by comparing results among aborting and non-aborting cattle to estimate the extent that the abortions can be attributed to *N. caninum* infection.

Sarcocystosis

Sarcocystis spp. infections are extremely common in cattle although Sarcocystosis is an uncommon cause of abortion. This parasite has a two-host life cycle in which cattle are intermediate hosts infected by ingestion of infective parasite stages from the feces of the carnivorous definitive host. *Sarcocystis cruzi*, *S. hirsute*, and *S. hominis* are infective to cattle, but *S. cruzi* is most pathogenic.

A definitive diagnosis of sarcocystis-induced abortion must rely on postmortem examination of aborted fetuses. Lesions in aborted fetuses are similar,

although usually less severe, to those associated with *N. caninum* infections. Parasites may be found in virtually all organs but are most common in the brain. Recognition of immature schizonts or mature schizonts in a rosette form within endothelial cells is diagnostic of *Sarcocystis* spp.. *N. caninum* will not form these structures. However, histologic detection of these parasite stages may be difficult. *Sarcocystis* spp. infections should be differentiated from *N. caninum* infections by the schizont morphology in endothelial cells or with IHC. However, standardized *Sarcocystis* spp. antiserum is not readily available, so an approach to parasite differentiation could be to test for reactivity with *N. caninum* IHC. Molecular diagnostic assays potentially could provide other methods for detection and differentiation of these protozoal parasites. A genomic probe for *S. cruzi* and ribosomal RNA-based assays for the molecular differentiation of *Sarcocystis* spp. and other coccidia have been developed, but their utility for diagnostic purposes has not been established.

Abortifacient plants

A number of plants have been implicated as causes of embryonic death, teratogenesis, and abortion in cattle. Confirmation that a plant toxin is involved is difficult because there are likely multiple factors involved, including the stage of gestation and amount of toxicant ingested. In addition, the exposure may have long preceded the observed problem, and it is difficult to rule out genetic defects or other non-infectious developmental abnormalities. Some of the better-recognized plant causes of reproductive failure are included here.

Needles for the Ponderosa pine tree (*Pinus ponderosa*), lodgepole pine (*P. contorta*), and common juniper (*Juniperus communis*) cause late-term abortion and placental retention within a few days to several weeks of ingestion, which may be precipitated by range or weather conditions. The toxin, isocupressic acid, is produced in the rumen from metabolites contained in both the green and dry needles and the bark and stems of the trees. The mechanism for the abortion or premature delivery is contraction of the caruncular vessels. No specific diagnostic lesions are present in the aborted fetus, so the presumptive diagnosis is dependent on clinical history and elimination of other causes.

Chronic ingestion of certain species of locoweed (*Oxytropis* and *Astragalus* spp.) containing swainsonine can induce a storage disease that can affect both the dam and the fetus inducing abortion and teratogenic effects. Limb and joint deformities have been reported. The toxin is presumed to induce increased vascular resistance in the fetus resulting in cardiac hypertrophy and dilation with fluid accumulation and abortion.

Ingestion of poison hemlock (*Conium maculatum*) during 50 to 75 days of gestation is associated with limb contractures, arthrogryposis, spinal curvatures, and cleft palate. Coniine, the toxin, is presumed to cause suppression of fetal movement. Similar teratogenic effects of limb and spinal deformities including lateral rotation of the forelimbs can be observed

with ingestion of tree tobacco (*Nicotiana glauca*) during 50 to 75 days of gestation. Anabasine is thought to be the teratogenic compound. Ingestion of certain lupine species by pregnant cattle between about 40 to 100 days of gestation is associated with fetal teratogenic effects including limb and spinal deformities and cleft palate similar to those observed with either poison hemlock or tree tobacco. The teratogenic compound in *Lupinus sericeus* and *L. caudatus* is anagryne, whereas, it is ammodendrine in *Lupinus formosus*.

Broomweed (*Gutierrezia microcephala* and *G. sarothrae*) ingestion over a broad period of time during pregnancy is associated with abortion, premature weak calves, and retained placenta.

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Chapter 3

Disorders of Sheep and Goats

Robert B. Moeller Jr.

Introduction

It is often easier to find an etiologic agent for the cause of abortions in sheep and goats than in many of our other domestic animals. The reason is likely due to the presentation of both the fetus and placenta for examination. A thorough gross and histologic evaluation of fetal and placental tissues and routine microbiological evaluation often aid in the identification of a causative agent for the abortion. However, two retrospective studies involving sheep and goat abortions presented to two diagnostic laboratories in the United States for evaluation demonstrated that a cause was not identified in 56% of the ovine abortions and 53% of the caprine abortions.

Sample collection and ancillary testing

Lung, liver, abomasal content, and placenta should be cultured for bacterial pathogens. Standard aerobic cultures (chocolate blood agar, blood agar, and MacConkey agar) of the lungs, liver, abomasal fluid, and placenta as well as *Campylobacter* cultures of the lungs, placenta, and abomasal content should be performed. If *Mycoplasma* spp. is a suspected cause, special *Mycoplasma* cultures of the lungs and abomasal fluid are warranted. If fetal fluids are available, they should be saved and evaluated for *Toxoplasma* titers. Kidney impressions on glass slides should be made for *Leptospira* evaluation. Glass slide impressions of cotyledons and/or frozen molds of cotyledons should be prepared for *Chlamydophila* spp. evaluations. In both instances, fluorescent antibodies specific to *Leptospira* serovars and *Chlamydophila* spp. are applied for identification.

Table B.2 in Appendix B summarizes what tissues to collect, which tests should be requested, and lists additional ancillary tests that can be performed to maximize the diagnostic effort of attempting to confirm a diagnosis from a list of selected pathogens.

Kirkbride's Diagnosis of Abortion and Neonatal Loss in Animals, Fourth Edition.
Edited by Bradley L. Njaa.
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For histopathology, a complete set of tissues should be placed in 10% neutral buffered formalin. The following tissue samples should be collected from the fetus: brain, heart, lung, liver, kidney, spleen, adrenal gland, thymus, rumen, abomasum, intestines, skeletal muscle from the leg and diaphragm, skin lesions (if present), and multiple sections of the placenta (cotyledons and noncotyledonary regions).

If nutritional deficiencies are suspected, liver samples may be submitted for evaluation for copper, selenium, and other selected metal levels. In the author's experience, fetal levels of selenium can be variable in the aborted fetus and very difficult to interpret. If fetal lesions of copper or selenium deficiency are not confirmed histologically in fetal or stillborn tissues, then the possibility of a deficiency causing the problem should be questioned. Since trace element levels in fetal livers are highly variable, trace elements in dam sera and blood should be assayed to determine if a true deficiency exists in the dam.

Under most situations, evaluation of dam serology to determine the cause of the abortion is unrewarding due to the endemic nature of many of the bacterial and viral agents that cause abortions in sheep and goats. Consequently, elevated titers to these agents without significant fetal pathology may suggest only exposure to the organisms but not the cause of the abortion. Acute and convalescent serum samples may be helpful for the evaluation of certain diseases. Serology for *Coxiella burnetii* and *Toxoplasma gondii* may be of little use since most adult animals are exposed to these agents. Paired serum samples collected 2 to 3 weeks apart, which demonstrate rising titers to a particular agent, may suggest recent exposure and may possibly be responsible for the abortion. The collection of fetal serum or fluids or serum from precolostral newborns may also assist in identifying exposure. Under some circumstances, the infected fetus may develop antibodies to the infectious agent before dying and aborting.

Examination of the aborted lamb/kid fetus

Fetal examinations should consist of an external examination looking for easily identifiable fetal malformations or cutaneous lesions consistent with an infectious agent.

Fetal gestational age can be determined by measuring the crown to rump length of the fetus (Table A.2 in Appendix A) and recording fetal weights. Examination of the animal's skin and hair covering may assist in identifying certain disease entities. Lambs that are excessively hairy may have been exposed to the Border disease agent. Alopecic areas on the skin and eyelids may suggest possible mycotic infections. The identification of fetal malformations may suggest a toxic or viral infection.

The gross examination of internal organs may also aid in rendering a diagnosis. Fibrin adhered to serosal surfaces may suggest a bacterial infection (*Brucella*, *Campylobacter*, *Flexispira*, and *Listeria*). Large areas of hepatic necrosis suggest a *Campylobacter* or *Flexispira* infection. Fetal pneumonias

Table 3.1 Possible causes for gross and microscopic lesions in sheep and goat fetuses.

Placental lesions (Gross)	Causes
Cotyledonary suppuration and intercotyledonary thickening and suppuration	<i>Brucella</i> , <i>Campylobacter</i> , <i>Coxiella</i> , <i>Chlamydophila</i> , and other bacteria
Cotyledonary necrosis, intercotyledonary areas normal or only edematous	<i>Toxoplasma gondii</i> , <i>Sarcocystis</i> spp.
Infarction of cotyledons and intercotyledonary thickening and suppuration	Mycotic
Placental lesions (Microscopic)	Causes
Vasculitis	<i>Chlamydophila</i> , <i>Campylobacter</i> , <i>Coxiella</i> , <i>Brucella</i>
Bacteria easily seen in trophoblasts	<i>Coxiella</i> , <i>Campylobacter</i> , <i>Brucella</i>
Zoites present in placental tissues	<i>Toxoplasma gondii</i> , <i>Sarcocystis</i> spp.
Fetal lesions (Gross)	Causes
Hyperkeratosis of skin and eyelids	Mycotic
Calcified plaques on walls and soles of feet (sheep)	<i>Brucella ovis</i>
Aberrant growth of hair/excessive hair coat	Border disease virus
Mummified fetuses	Border disease virus, Bluetongue virus, Cash Valley virus, <i>Toxoplasma gondii</i>
Cyclops/craniofacial malformations	<i>Veratrum californicum</i>
Musculoskeletal lesions (Arthrogryposis, scoliosis, torticollis, contracted tendons, hypoplasia of limbs)	Cache Valley virus, Border disease virus, Akabane virus, Wesselsbron disease virus, <i>Lathyrus</i> spp. plants, Locoweed, <i>Veratrum californicum</i> , Lupine, Tree tobacco
Blind newborns/retinal dysplasia	Bluetongue virus
Fibrin over serosal surfaces	<i>Brucella</i> , <i>Campylobacter</i> , <i>Flexispira</i> , <i>Listeria</i> , other bacteria
Large multifocal areas of necrosis of liver (targetoid pattern)	<i>Campylobacter</i> , <i>Flexispira</i>
Small pinpoint foci of necrosis in liver, adrenal glands, kidney, and/or lungs	<i>Chlamydophila</i> , <i>Listeria</i> , <i>Francisella tularensis</i> , Caprine herpesvirus
Massive liver necrosis	Rift Valley fever virus, Wesselsbron disease virus
Cerebellar hypoplasia	Border disease virus, Cache Valley virus, Akabane virus and other <i>Bunyaviruses</i> , Wesselsbron disease virus

(Continued)

Table 3.1 (Continued)

Fetal lesions (Gross)	Causes
Hydranencephaly, porencephaly, hydrocephalus	Bluetongue virus, Border disease virus, Akabane virus, Cache Valley virus and other <i>Bunyaviruses</i> , Wesselsbron disease virus, <i>Sarcocystis</i> spp., <i>N. caninum</i>
Ruminal epithelial hyperplasia/plaques	Mycotic
Icterus	<i>Leptospira</i> spp., Rift Valley Fever virus
Goiter	<i>Brassica</i> spp. plants
Fetal lesions (Microscopic)	Causes
Neutrophilic to pleocellular pneumonia	Bacterial
Large necrotic areas in the liver	<i>Campylobacter</i> , <i>Flexispira</i>
Central to midzonal to massive necrosis of liver	Rift Valley Fever virus, Wesselsbron disease virus, <i>N. caninum</i>
Smaller foci of necrosis in liver	<i>Chlamydophila</i> , <i>Listeria</i> , <i>Fransicella tularensis</i> , <i>Salmonella</i> , <i>Herpesvirus</i>
Portal lymphocytic cuffing	<i>Coxiella burnetii</i>
Intranuclear inclusions in multiple foci of necrosis in the kidney, lungs, liver, adrenal gland	Caprine herpesvirus
Lymphocytic abomasal infiltrates	Bacteria
Demyelination	Border disease virus

are often difficult to identify grossly. Small, pinpoint areas of necrosis in the liver, kidneys, and adrenal gland are often difficult to see grossly, but, when identified, may suggest a bacterial septicemia (*Listeria* or *Chlamydophila*). Examination of the rumen for hyperplastic plaques may also be helpful suggesting a bacterial or fungal infection (See Table 3.1.).

Examination of fetal membranes

Gross examination of the placenta can be very helpful in interpreting the problems associated with the fetus. Sheep and goats, like most ruminants, have a cotyledonary-type placentation. In these animals, the chorionic surface of the chorioallantoic membrane is apposed to the fetomaternal epithelial layer in the placentome, which replaces the epithelial layer of the caruncle. This leads to development of synepitheliochorial placentation. Placental attachment to the caruncles occurs at approximately 17 days gestation with development of the placentome occurring at about 90 days gestation. At this time, the placentome has reached its maximum size to ensure proper nourishment to the developing fetus.

As the fetal cotyledons develop in the maternal caruncle to form the placentome, the fetal chorioallantoic blood vessels enter the placentome at the hilus. This region is noted as a centrally located depression in the chorioallantois consisting of thick-walled vessels in a sparingly cellular matrix. On the surface of the hilus are primary chorioallantoic villi that come in contact with the maternal caruncular tissues. Primary villi develop branching villi that interdigitate in the branching caruncular septa. By day 58 of gestation at the junction of the primary villi and hilus of the cotyledon, the maternal tissues form small hemorrhagic foci (hematophagous areas or arcade hematmata) of maternally derived blood and golden-brown granular pigment in fetal trophoblasts. These foci are believed to be responsible for the transfer of iron from the dam to the fetus. Since these sites are areas of blood pooling, they may also be sites for blood-borne pathogens to collect during maternal septicemia and cause placentitis.

Examination of cotyledons, as well as intercotyledonary areas, is extremely important. Many bacterial diseases affect both the cotyledonary and intercotyledonary regions. Examination of the cotyledons for necrosis, infarction, and suppuration often suggest a bacterial infection. The intercotyledonary areas should also be examined for areas of thickening, increased opacity, proliferative regions, and necrosis. Examination of the umbilical vessels may also be useful in determining fetal problems.

Common agents associated with cotyledonary separation, suppuration, and necrosis with intercotyledonary thickening, opacity, and necrosis are *Chlamydomphila* (*Chlamydia*), *Campylobacter*, *Coxiella*, and *Brucella*. Multifocal, small, necrotic areas on the cotyledons with little involvement of the intercotyledonary areas may suggest *Toxoplasma* or *Sarcocystis* infections. Infarctions of the cotyledons and intercotyledonary areas may suggest a possible mycotic placentitis. Viral infections often have minimal or no placental lesions. Although lesions seen in the placenta may suggest a particular agent, thorough gross and histological examination of the placenta and fetus, along with bacterial cultures, are important in determining the cause of the abortion. For a list of placental and fetal lesions and possible differential diagnosis for these lesions, see Table 3.1.

Viral abortions

Bluetongue virus

Bluetongue virus is an arthropod-borne viral disease that affects many domestic and wild ruminants. Infections are most severe in sheep with the severity of infection depending on the breed of sheep (Merino and British breeds most susceptible) and strain of virus. The virus is an Orbivirus (double-stranded RNA virus) of the Reoviridae family. Twenty-five different serotypes of the virus are present worldwide. Small midges or *Culicoides* sp. are the primary vector for transmission of the virus. Since this disease is arthropod

borne, it seems to be primarily a seasonal disease in the temperate climates with most cases occurring in summer and early fall.

Clinically affected sheep develop a high persistent fever, 41-42°C, facial edema, excessive salivation, and nasal discharge. Swelling and hyperemia of the lips, gums, and feet often occur with ulceration of the tongue, oral mucosa, and esophagus. Intense swelling of the head may occur with drooping of the ears. Reluctance to move is often due to coronitis and laminitis. Affected animals may progress to pulmonary edema, due to heart failure, and death. Recovery can be prolonged, with partial or complete loss of the fleece. Although severe lesions are often hallmark for bluetongue outbreaks, many animals have less severe clinical disease or inapparent infections, particularly in enzootic regions.

Mild subclinical infections in pregnant ewes are a concern since the virus can cross the placenta and infect the fetus. Infection of ewes during the first 40-60 days of gestation can result in fetal death and resorption. Infections in fetal lambs later in gestation may result in abortions but more often result in stillbirths or the birth of weak, nonviable neonates with severe neural and ocular defects.

Gross examinations of affected stillborn or neonatal lambs often demonstrate hydranencephaly or porencephaly. Near-term aborted fetuses may also demonstrate similar lesions in the brain or show no gross lesions. The placenta will often appear normal.

Histologically, lamb fetuses exposed between 40-60 days of gestation often develop a necrotizing encephalitis and retinitis, which, if the fetus survives to birth, will develop into hydranencephaly and retinal dysplasia. Fetuses exposed between 60 and 100 days of gestation may develop necrotizing encephalitis, which results in porencephalic cysts in the brain. Fetuses exposed after 100 days of gestation often develop mild multifocal meningoencephalitis with gliosis. The diagnosis of bluetongue in the fetus can be difficult since most fetuses that were exposed before 100 days of gestation have cleared the virus. Infected late-term fetuses may still be viremic at birth possibly enabling recovery of the virus through virus isolation or PCR techniques. Sera from aborted fetuses and precolostral serum from newborn lambs can be tested for antibodies to bluetongue virus. The presence of bluetongue antibodies in precolostral or fetal serum is good evidence of fetal infection.

Border disease

Border disease is a viral disease in sheep and goats. The virus is a pestivirus and is closely related to bovine viral diarrhea virus (BVDV) and classical swine fever virus. Nearly all isolates of border disease virus (BDV-1 through BDV-6) are noncytopathogenic in cell culture. Infections most often occur by the introduction of a persistently infected carrier into to a flock. Susceptible animals become infected by oral ingestion of virally contaminated feeds, contact with infected animal's oral or nasal secretions, or semen from persistently infected males. Healthy young lambs and adult sheep when exposed to the virus usually experience only mild, often inapparent, infections.

Animals infected with the virus may have a mild febrile response and leukopenia lasting 4–11 days. During that time, the animals are viremic. Consequently, there are rarely clinical signs of infection in the ewes, and infections become only apparent with the presentation of the exposed fetus or newborn. Animals infected with the virus early in gestation (less than 60 days), often result in fetal death and resorption. Infected sheep often develop a protective immune response to further infections and should be retained for next year's breeding. Pregnant ewes infected later in gestation (60 days or greater) can result in fetal death with abortion and mummification of fetuses, fetal abnormalities, stillbirths, and birth of premature small weak or normal young.

It is often the gross appearance of affected lambs that suggests Border disease. Affected lambs are short, stocky, and often have severe neurological deficits that may regress over time. Neurological problems, also known as "hairy shakers," usually consist of rhythmic contractions of the hind limbs to fine tremors of the head, ear, and tail. Some animals are ataxic and cannot stand. The hair coat is often thick and protruding on the neck and back. The hair may be abnormally pigmented. Affected lambs often remain weak and develop poorly. Gross examination of the brain may identify cerebellar hypoplasia, hydrocephalus, hydranencephaly, porencephaly, or leukoencephalomalacia.

Histologically, the primary lesions in the CNS are hypomyelinogenesis of the brain and spinal cord. Gliosis of the cerebral white matter with destruction and swelling of nerve fibers is a common finding. Gitter cells are rarely observed. The poor myelination of axons throughout the CNS is due to viral predilections for oligodendrocytes. Arteries and arterioles of the white matter may have a lymphohistiocytic periarteritis involving primarily the adventitia. Aberrant hair growth is related to abnormally enlarged primary hair follicles and fewer secondary hair follicles. In the placenta, the chorionic villi may be necrotic.

Fetal infections of border disease in goats are slightly different from that seen in sheep. In goats, there is a higher incidence of fetal death and mummification. Infected neonatal kids may develop ataxia with tremors similar to neonatal lambs. Histologically, there is cerebral white matter necrosis and gliosis with perivascular lymphocytic cuffing. Cerebellar dysplasia is often a feature of the disease in infected kids. The fetal infections prior to the development of the immune system (60–80 days gestation) may lead to persistent infections. Virus isolation or PCR techniques can confirm infection in fetal or neonatal kids.

Serological testing of precolostral lambs or fetal fluids for border disease may assist in a diagnosis. Antibodies to Border Disease Virus or BVDV can also be useful in fetal and placental tissues for the detection of the virus by immunofluorescent or immunohistochemical techniques.

Caprine herpesvirus type 1

Caprine herpesvirus type 1 (CHV-1) is an alpha herpesvirus that is closely related to bovine herpesvirus type 1. In the adult goat, this virus is associated with vulvovaginitis, balanoposthitis, occasional respiratory disease, and

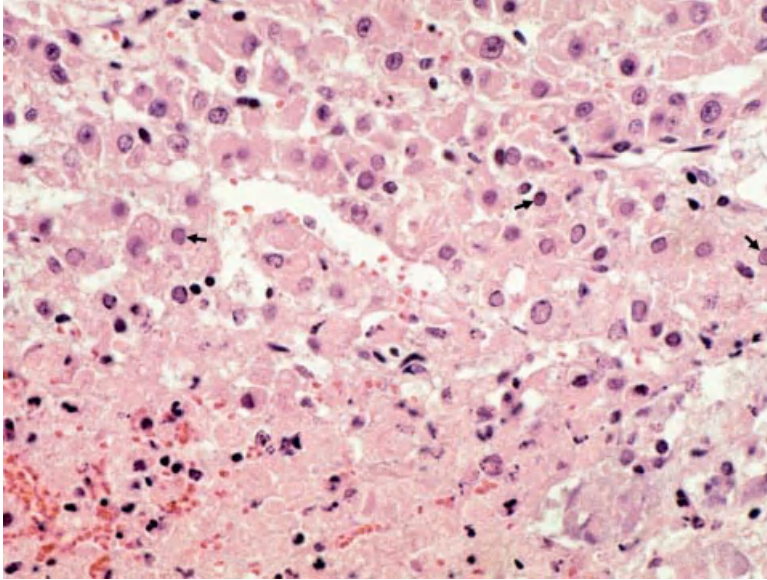


Figure 3.1 Caprine herpes virus infection of fetal adrenal gland. This histologic image demonstrates adrenal cortical necrosis with numerous cortical cells that have nuclei with chromatin clearing and intranuclear viral inclusion bodies (arrows). H&E stained section. (Courtesy of Dr. FA Uzal, California Animal Health and Food Safety Laboratory.)

abortions. In neonatal kids, CHV-1 has been associated with necrotizing enteritis and generalized systemic infections that are often fatal.

Abortions caused by CHV-1 are usually not associated with clinical disease in the herd. Infected does will appear normal with no clinical history of illness prior to the abortion. Occasionally, does may develop mild vulvovaginitis (edema, erythema, and pinpoint ulcers). An ulcerative balanoposthitis may be seen in bucks. Abortions tend to be late term or stillbirths. Weak, nonviable neonates are often associated with abortion storms.

The placenta is usually autolytic but appears normal with no evidence of necrosis or exudation in the cotyledons or intercotyledonary areas. Affected fetuses are often aborted late in the last trimester and are moderately autolyzed. Multifocal, small, 1-2 mm, white necrotic foci may be identified in lungs, liver, kidneys, and occasionally the adrenal cortex.

Microscopic examination of fetal tissues identifies multifocal areas of necrosis in the lungs, liver, adrenal cortex, kidneys, thymus, and spleen. Minimal to mild neutrophilic infiltrates may be associated with the areas of necrosis. Large eosinophilic to amphophilic intranuclear inclusion bodies are often found in necrotic cells or cells adjacent to the areas of necrosis. Intranuclear inclusions are most commonly found in the adrenal glands, liver, lungs, and kidneys (Figure 3.1). Histological lesions are not found in the placenta.

A presumptive diagnosis of caprine herpesvirus-1 abortions is made on the basis of pathological changes in the fetus and the finding of herpetic

eosinophilic intranuclear inclusions. Antibodies to bovine herpesvirus-1 often cross react with this agent on FA or immunohistochemical staining and assist in a presumptive diagnosis. Caprine herpesvirus type-1 DNA can also be detected by PCR techniques, which can aid in confirmation.

Cache Valley virus

Cache Valley Virus (CVV) is an arthropod-borne (mosquito) disease primarily affecting sheep. The virus is a member of the Bunyamwera serogroup of the genus *Bunyavirus*. The disease primarily is seen in some provinces and states of Canada, United States, and Mexico. Multiple mosquito species (*Anopheles*, *Aedes*, *Coquilleltidia*, *Culiseta*, and *Psorophora* spp.) are reported to become infected with the virus. Some of the mosquitoes allow the virus to persist with transovarian infection possible. This allows the virus to survive over winter in temperate and northern climates. Most infections in adult sheep are asymptomatic and subclinical. Experimental CVV infections can produce low-grade fevers with malaise, anorexia, and muscle stiffness. Problems tend to become evident when serious congenital malformations are observed at lambing. Increased early embryonic deaths, a common finding in CVV, may also be observed in ewes when infected early in pregnancy. Congenital malformations of the CNS and musculoskeletal system are the primary gross features of CVV. Malformations of the brain include hydranencephaly, hydrocephalus, porencephaly, microencephaly, cerebellar and cerebral hypoplasia, and micromyelia. Musculoskeletal lesions consist of arthrogryposis of one or multiple limbs, scoliosis, torticollis, and hypoplasia of limb muscle groups.

Mummified and stillborn fetuses are also a feature of infection. Some stillborn or mummified fetuses may not demonstrate skeletal or neural abnormalities. Infections of the fetus earlier than 32 days of gestation often result in early embryonic death. Infections from 32 to 37 days of gestation result in both musculoskeletal and CNS lesions. Infections from 37 to 48 days of gestation result in only musculoskeletal lesions.

The diagnosis of CVV in fetal and newborn tissues is difficult because the virus is often eliminated by the fetal immune system. Precolostral serum from affected lambs should be submitted for antibody testing for CVV.

Three other *bunyaviruses*, La Crosse virus, San Angelo Virus, and Main drain virus, are also known to cause fetal death, arthrogryposis, hydrocephalus, axial skeletal deviations, and anasarca.

Rift Valley fever

Rift Valley fever (RVF) is caused by a phlebovirus (Bunyaviridae family). The virus is an arbovirus that is transmitted by *Culex* and *Aedes* spp. mosquitoes. The disease primarily affects young newborn sheep and goats but is also known to cause severe abortion storms. The virus is primarily found in subSahara Africa, particularly in the Rift Valley region running from Kenya to South Africa. Outbreaks in Western Africa, as well as along the Nile River,

have occurred periodically. Outbreaks have also occurred in the Middle East (particularly the Arabian Peninsula), probably from the importation of infected sheep and goats to the region. Most outbreaks occur during times of increased mosquito populations brought on by rainy weather. The virus can cause a serious disease in humans primarily from contact with infected mosquitoes. However, the handling of virally infected tissues and blood from infected animals is also a major source of infection.

Sheep and goats are very susceptible to the virus. In endemic areas, infection of adult animals is often mild and inapparent. Naïve animals may develop severe illness with high fevers of 40–41°C, anorexia, weakness, excessive salivation, vomiting, abdominal pain, mucopurulent nasal discharge, and diarrhea that may become bloody. Death may occur within 24 hours. Sheep are very sensitive to the virus with death losses up to 50% in naïve animals. Newborn lambs as well as newborn goats may have peracute disease with no clinical symptoms prior to death. Pregnant animals usually abort during illness or during convalescents.

Gross evaluation in the fetus often identifies a fairly fresh fetus. The placenta usually appears normal. The fetus may be icteric with hemorrhage over serosal surfaces. The liver is often enlarged and swollen with the surface being yellow to grayish/red. Multiple white or gray/white foci of hepatic necrosis may be seen grossly.

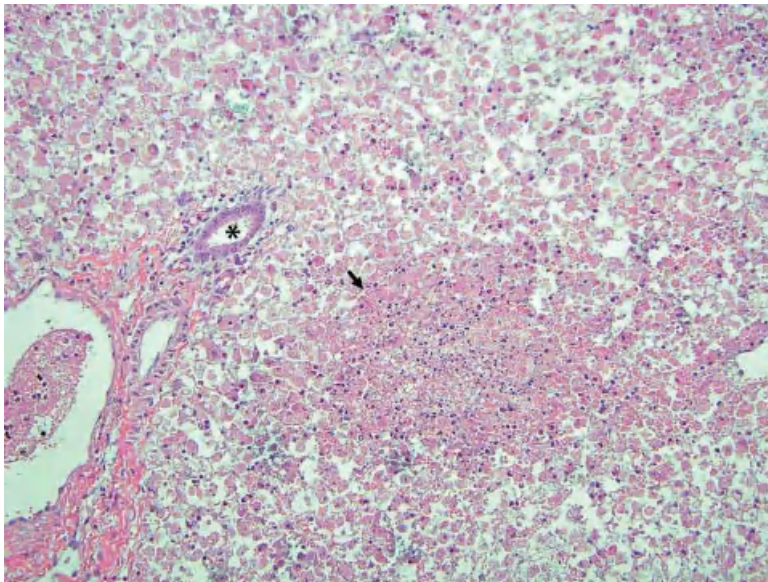


Figure 3.2 Rift Valley Fever associated fetal hepatic necrosis. This is a histologic section of liver from a term ovine fetus. Hepatic necrosis (arrow) is a feature of *in utero* Rift Valley Fever virus infection. Bile ductule (asterisk). H&E stained section. (Courtesy Dr. R Moeller, California Animal Health and Food Safety Laboratory.)

Histologically, the placenta appears normal with lesions seen only in the fetus. The liver is the primary target organ with pronounced necrosis of hepatocytes. Necrosis primarily begins around the central vein but often will become massive and involve the entire hepatic lobule. Minimal to no inflammatory changes are seen (Figure 3.2). Intranuclear inclusion bodies in hepatocytes have been described with RVF but are rare. Necrosis of lymphocytes in the splenic white pulp may also be observed along with hemorrhage.

RVF is a reportable disease. Consequently, a diagnosis is confirmed by federal laboratories. RVF should be suspected in outbreaks of a highly fatal disease in newborns and/or an extensive abortion storm. Liver, spleen, lymph node, brain, heparinized blood, and serum should be saved and submitted for further laboratory testing. Liver, spleen, and blood samples are excellent tissues for virus isolation. Impression smears of frozen sections of liver and spleen can be used for fluorescent antibody testing. Electron microscopic evaluation of the liver often identifies viral particles consistent with phlebovirus. PCR techniques are currently available for rapid confirmation. Enzyme-linked immunosorbent assay (ELISA) testing is also available.

Additional viral agents

Many viral infections of sheep and goats have the potential of causing abortions. Infections tend to primarily involve the dam with minor problems in the aborted fetus. Three additional viruses are discussed for their known ability to cause fetal death and malformation.

Akabane virus

Akabane virus (Simbu subgroup of the family *Bunyaviridae*) is a major cause of fetal malformations in cattle but also affects sheep and goats. This virus is a major cause of arthrogryposis and hydranencephaly in ruminants from Australia, Japan, Israel, and Korea. Akabane virus is a mosquito-borne disease involving primarily *Aedes* and *Culex* spp. Adult animals rarely demonstrate clinical disease. However, at lambing, stillbirths, mummified fetuses, and newborns with congenital malformations are often observed. Major abnormalities include arthrogryposis, hydranencephaly, porencephaly, hydrocephalus, and cerebellar hypoplasia. Since the virus is often cleared from the fetus, diagnosis is made by identifying antibodies to Akabane virus in fetal sera or in the precolostral sera from affected neonates.

Nairobi sheep disease virus

Nairobi Sheep Disease Virus (Nairovirus of the family *Bunyaviridae*) is known to cause abortions in infected ewes and does in East Africa. Transmission is tick-borne by the bite of the brown tick (*Rhipicephalus appendiculatus*). Affected sheep develop a high fever up to 41°C, depression, anorexia, hemorrhagic diarrhea, hemorrhagic mucopurulent nasal discharge, and conjunctivitis.

Naïve animals introduced into endemic regions often die. Abortions are common during active clinical disease or convalescence. Gross examination of the fetus often reveals a fresh fetus with petechial and ecchymotic hemorrhages on serosal surfaces of many organs along with a placenta that is edematous and hemorrhagic.

Wesselsbron disease virus

Wesselsbron disease is an acute mosquito-borne flavivirus infection in sheep and goats, in southern Africa. The disease causes mortality in newborn lambs and kids. In adults, the disease is often inapparent, however, in pregnant ewes, prolonged gestation and *hydrops amnii* are common features of the disease. Abortions, stillbirths, and fetal malformations are common in infected dams. The virus is very neurotropic resulting in necrosis of the fetal CNS. Affected fetuses or newborns may develop segmental aplasia of the spinal cord, hydranencephaly, hydrocephalus, porencephaly, or cerebellar hypoplasia. Brachygnathia and arthrogryposis are also seen in infected lambs. Necropsy findings in young animals include icterus and yellow/brown megalic livers. Multifocal areas of hepatocellular necrosis are seen histologically.

Bacterial agents of abortion

***Brucella* (*Brucella ovis* and *Brucella melitensis*)**

The principal species of *Brucella* infecting sheep and goats are *Brucella ovis* (sheep) and *Brucella melitensis* (goats and sheep). *Brucella ovis* tends to be a disease of the ram. Orchitis and epididymitis are the primary clinical manifestations of these bacteria in infected rams. Infected rams shed the bacteria in their semen, which can result in early embryonic death and repeat breeding in the infected ewe. Third trimester abortions or the birth of weak, unthrifty neonatal lambs are occasionally seen with *Brucella ovis* infections.

Both sheep and goats are highly susceptible to infections with *Brucella melitensis*. This organism is also known to affect cattle and swine. Goats tend to be universally susceptible to infections while the susceptibility of sheep varies with breed. It has been reported that Maltese sheep show considerable resistance to *Brucella melitensis* infections. The source of *Brucella melitensis* infections is often the introduction of infected animals into a naïve herd or flock resulting in severe abortion storms. Following the abortion storm, most animals become immune, and further abortions are only seen in young, naïve animals or newly introduced animals. Some carrier animals exist with carrier does or ewes excreting the organism from the reproductive tract and in the milk after normal delivery. This results in animals becoming infected by ingestion of contaminated feed or placental tissues. Infected does shed large numbers of organisms in the uterine discharge and placenta. Organisms can be present in the uterine discharge for up to 2 months following parturition. In goats, the bacteria can be

found in the milk and often will be shed in the milk for all future lactations due to a persistent infection. Sheep tend to shed fewer bacteria in uterine secretions. Like goats, bacteria persist in the sheep's milk throughout the lactation.

The source of *Brucella ovis* infections tends to be from an infected ram. Rams excrete the bacteria in the semen. Once a ram is infected, it is felt that it is persistently infected and will shed the bacteria indefinitely in the semen. Infection of ewes is through breeding. Ewes are fairly resistant to the bacteria and usually will only maintain infections and secrete bacteria in the vaginal secretions only for several heat cycles. The major problem with *Brucella ovis* in ewes is early embryonic death and repeat breeding. Third trimester abortions or the birth of weak, nonviable lambs are not a common finding in infections. However, in aborting animals, the bacteria are easily identified in the placenta, uterine discharge, and milk.

Clinically, ewes rarely show signs of *Brucella ovis* infection. However, as stated earlier, some animals have third trimester abortions or the birth of weak and stillborn lambs. In infections with *Brucella melitensis*, sheep and goats rarely are sick prior to the abortion. Some goats rarely become ill with fever, depression, anorexia with weight loss, and diarrhea prior to aborting. Some animals may die during this acute bacteremic phase. Lameness and mastitis have been reported rarely in some aborting goats. Goats with mastitis may have palpable nodules in the mammary gland and secrete watery milk that contains clots. Some sheep infrequently develop arthritis and encephalitis.

The gross examination of the placentas infected with either *Brucella ovis* or *Brucella melitensis* is similar in late-term abortions. Cotyledons are necrotic, granular, and often have a gray/white to brownish/red exudate. Intercotyledonary areas are opaque, thickened with edema, and leathery or plaque-like. Variable amounts of debris and brownish/red to yellow or gray/white thick, sticky exudate are often present. A prolonged suppurative metritis may be present in infected dams with retention of the placenta. The aborted fetuses are often fresh but can be somewhat autolyzed. Often, there are no gross lesions in the fetus. Less often, fetuses are edematous with extensive fetal fluids present in the thoracic and abdominal cavity and have hyperplastic lymph nodes. Occasionally, fibrinous pleuritis and peritonitis are present. In *Brucella ovis* infections, calcified plaques on the walls and soles of the hooves and accessory digits are considered a characteristic lesion (Figure 3.3). However, this may represent a more basic and nonspecific fetal lesion.

Microscopically, *Brucella* infections result in placental necrosis and inflammation involving both cotyledons and intercotyledonary regions. The inflammatory reaction is primarily composed of neutrophils and macrophages. The chorioallantoic connective tissue is often edematous and infiltrated by variable numbers of neutrophils and macrophages. Many placental vessels have mural and perivascular infiltrates of neutrophils and macrophages. Both the chorionic villi and chorionic surface of the intercotyledonary regions have necrosis of the surface epithelium with variable amounts of debris and suppurative inflammation. Many trophoblastic epithelial cells are filled with small intracytoplasmic Gram-negative coccobacilli.



Figure 3.3 Hoof calcification in an ovine fetus. A very rare gross manifestation of *in utero* infections by bacteria as well as many other less specific causes. In this case, *Brucella ovis* was cultured. (Courtesy of Dr. A Julian, Gribbles Veterinary Pathology.)

The infected fetus often has a bronchopneumonia. Infiltrates will vary from neutrophilic to mixed pleocellular infiltrates composed predominately of neutrophils and macrophages. Occasionally, multinucleated giant cells may be present. In the kidneys, a mild neutrophilic and histiocytic interstitial nephritis involving the corticomedullary regions are occasionally observed. A mild inflammatory infiltrate in the portal regions of the liver is occasionally seen.

A diagnosis of brucella abortion is confirmed through the identification of the organism using standard bacteriological techniques. Antibodies to this organism are available for immunohistochemical staining techniques. PCR techniques are available for identification of the organisms. Various serological tests are available for detecting exposure. Complement fixation test and ELISA testing are used for *Brucella ovis*. Serum agglutination tests, complement fixation, rose bengal tests, and ELISA techniques are used for the detection of antibodies to *Brucella melitensis*.

Campylobacter infections of sheep and goats

Campylobacter fetus subsp. *fetus*, *Campylobacter jejuni* subsp. *jejuni*, and *Campylobacter lari* can infect and cause abortions in both sheep and goats. Although both species are susceptible to *Campylobacter* infection, sheep tend to be the more susceptible to abortions.

Campylobacter infections often occur as late-term abortions, stillbirths, or the birth of weak, nonviable lambs. Infected dams rarely demonstrate any clinical signs prior to aborting. The susceptible dam is infected through

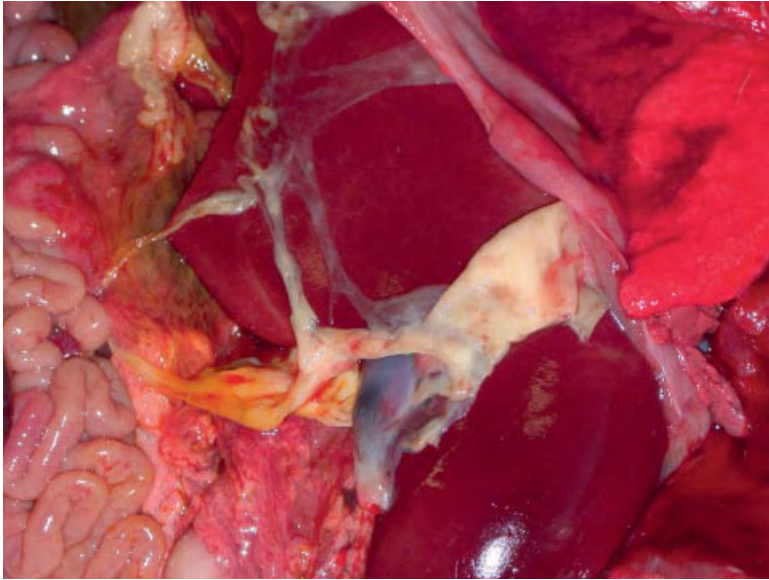


Figure 3.4 *Campylobacter* fetal septicemia. The presence of fibrinous peritonitis in this ovine fetus is an indication of a fetal septicemia. In this case, the fibrinous lesion is due to *in utero* *Campylobacter* infection. (Courtesy of Dr. KG Thompson, Massey University.)

ingestion of contaminated feces on feed from infected carrier animals. The organism colonizes the intestinal tract of the adult animal usually without clinical signs of diarrhea. A bacteremia may occur in susceptible pregnant animals leading to infection of the uterus and placenta. Placental infections often lead to fetal septicemia prior to abortions. Many abortion storms occur when susceptible does or ewes are brought into a herd with *Campylobacter* infected herdmates. These organisms are highly contagious resulting in most of the flock becoming infected by the time the abortion storm begins. Ewes that abort are often immune to reinfection and usually will not abort again when re-exposed to these organisms. Some ewes that abort will develop a suppurative metritis and die from complications of the uterine infection.

Cotyledons are enlarged, yellowish, and covered with a brownish/red suppurative exudate. Intercotyledonary areas are often edematous and hyperemic but usually lack any exudate. Aborted fetuses may be fresh and well preserved or autolytic. Meconium staining of the fetus and placental tissues may occur. Variable amounts of serosanguinous fluid are present in both the thoracic and peritoneal cavities. Fibrin may be present in the thoracic and abdominal cavities (Figure 3.4). The liver may have multifocal areas of necrosis up to 2-4 cm diameter. These areas of necrosis are often white to yellow brown, well demarcated, and often have depressed centers (Figure 3.5).

Histologically, chorionic villi are necrotic characterized by abundant necrotic debris, mixed with variable numbers of neutrophils, lymphocytes, and mac-

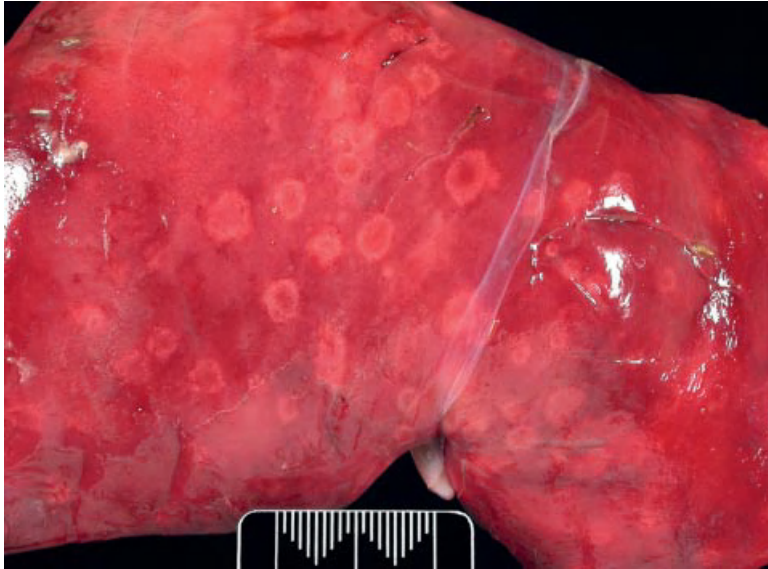


Figure 3.5 *Campylobacter* hepatic necrosis. Scattered throughout the liver of a newborn lamb are multifocal, circular areas of yellow-tan discoloration. These lesions have a peripheral rim of pallor with a darker, central area of depression. These lesions represent multifocal hepatic necrosis and are virtually pathognomonic for *Campylobacter* infection in newborn or fetal sheep. (Courtesy of Dr. KG Thompson, Massey University.)

rophages. Numerous colonies of small, Gram-negative, rod-shaped bacteria are present in trophoblasts and the placental stroma. A mild vasculitis is often present with mononuclear inflammatory cells present in the vessel wall and associated perivascular stroma. The chorioallantoic connective tissue is often mildly edematous with leukocytic infiltrates. A suppurative bronchopneumonia occurs frequently with or without meconium. The liver has multifocal areas of coagulative necrosis with minimal mononuclear and neutrophilic infiltrates. Lymphocytic infiltrates are often seen in the abomasal mucosa.

Bacterial cultures of the placenta, lungs, liver, and abomasum are essential in the confirmation of the diagnosis. Currently, immunohistochemical staining and fluorescent antibody testing are also available for a rapid diagnosis. Darkfield and phase contrast microscopy may also be used on wet mounts from abomasal contents or affected cotyledons to see the characteristic darting motility of the bacteria.

***Chlamydophila (Chlamydia) abortus* (ovine enzootic abortion)**

Chlamydophila abortus was previously known as *Chlamydia psittaci* immunotype 1. Recent work has identified the agent as being a separate organism from *Chlamydia psittaci*. This organism is a major cause of bacterial abortions in both sheep and goats. Abortion occurs most often during the last

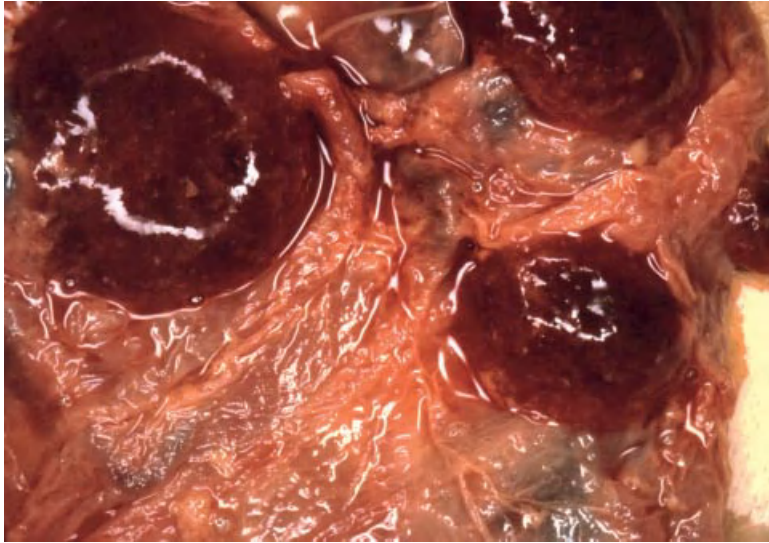


Figure 3.6 Chlamydophila placentitis in a goat. A combination of multifocal necrosis affecting the cotyledons as well as thickening and opacity of the intercotyledonary portions of the placenta are the features most often attributed to placentitis and abortion due to Chlamydophila infections. Often there is an accompanying overlying exudate. (Courtesy of Dr. P Blanchard, California Animal Health and Food Safety Laboratory.)

trimester of gestation. Alternatively, affected sheep and goats may give birth to weak or stillborn young. Infected females rarely demonstrate clinical illness or a febrile response before aborting. Naïve yearling ewes and does that have recently been introduced into an infected herd are usually the animals that abort. Animals become infected by oral exposure to contaminated feces or discarded abortion products. After ingestion, the bacteria colonize the intestinal epithelium. Animals infected with the intestinal organisms rarely present with clinical illness. However, once the organisms have become established in the intestinal mucosa, the bacteria may spread systemically via the blood stream to the uterus and placenta of pregnant animals. Placental lesions often take at least 2 weeks to develop. Once the placenta is infected, the organisms may cause a localized placentitis and then spread to the fetus causing generalized disease. Animals that become infected and abort often maintain a protective immunity. Rarely, immune animals may shed organisms in uterine discharges or placental tissues on subsequent pregnancies.

Gross examination of fetal and placental tissues often identifies lesions. Cotyledons are necrotic and have a gray to dark brownish/red exudate. The intercotyledonary regions often have localized areas that are thickened, opaque, and leathery. Often the intercotyledonary region is covered by a flakey, gray/white to brownish/red exudate (Figure 3.6). The abortus is often well preserved. Examination of the fetus often fails to identify visible gross lesions. However, some fetuses develop petechial hemorrhages in the subcutis,

thymus, and lymph nodes. The peritoneal cavity may contain abundant ascites. Internal organs are usually well preserved. The liver may appear congested and mildly swollen. Pinpoint white areas of necrosis may be scattered throughout the liver. Lymph nodes are often enlarged.

Placental changes are characterized by necrosis of the chorion with abundant debris, neutrophils, and lymphocytes present. Many trophoblasts contain intracytoplasmic organisms that fill the trophoblastic epithelium. Chorioallantoic connective tissue is thickened by edema, lymphocytes, macrophages, and neutrophils. Vasculitis is evident in many placental vessels with fibrinoid necrosis and variable mural accumulations of neutrophils and mononuclear cells and as well as in the surrounding connective tissue. In the fetus, multifocal areas of coagulative hepatocellular necrosis are often mixed with scant neutrophilic and mononuclear cell infiltrates. Increased mononuclear cells infiltrate portal areas. Similarly, multifocal areas of necrosis mixed with scant neutrophils and mononuclear cells affect the spleen and lymph nodes. A mild, nonsuppurative meningoencephalitis with vasculitis may be present in the brain. In the lungs, mild interstitial infiltrates of mononuclear inflammatory cells may result in thickening of the alveolar septa and pulmonary interstitium.

Confirmation of *Chlamydophila abortus* abortions can be accomplished by impression smears of the cotyledons and evaluating the smears for aggregates of small elementary bodies. Using the Gimenez stain, elementary bodies are red whereas when using Giemsa stains, elementary bodies are blue. Antibodies to *Chlamydophila abortus* are used for fluorescent antibody or immunohistochemical staining techniques for the detection of the organisms in placental trophoblast and splenic or hepatic tissues. PCR techniques are also available.

Since *Chlamydophila abortus* is often ubiquitous in the sheep and goat environment, antibody testing of dam sera is of little use in identifying this agent as a cause of the abortion. Since this is often a chronic late-term infection of the fetus, antibodies to the bacteria may be detected in fetal fluids.

Coxiella burnetii

Coxiella burnetii is a common bacterial agent that causes abortion in sheep, goats, and occasionally in cattle. This organism is an intracellular, pleomorphic, Gram-negative coccobacilli. The organism is placed in the gamma division of the *Proteobacteria* and is classified in the *Rickettsiaceae* family and *Rickettsiae* tribe. Although *Coxiella burnetii* is considered to be a member of the *Rickettsiae* tribe, there are major differences between this organism and other members of this tribe of bacteria, including the following: (1) it is resistant to dehydration and destruction by physical heat and chemical agents; (2) it contains endospores found in the phagosomes of infected eukaryotic cells; and (3) it undergoes phase variation in the phagosome of the host cell.

Abortions tend to occur primarily in young, naïve animals. Exposure is primarily via the ingestion or inhalation of contaminated placental tissues or uterine discharges. Ticks are able to transfer the bacteria between animals but are believed to be a minor source of infection. *Coxiella burnetii* remains viable

in the phagosome of free-living amoeba. The ability of this organism to survive in protozoa, along with the organism's resistance to desiccation, may play a role in maintaining the organism in the environment. Once infected, rare animals can become carriers with re-activation of the bacteria during pregnancy and the shedding of large numbers of bacteria in feces, uterine discharges, and milk at the time of parturition. Consequently, intense agricultural practices that place large numbers of naïve animals in small, compact areas can result in exposure and re-infection of pregnant animals. This can result in large abortion storms with up to 50% or greater abortion rates in the flock or herd.

The primary clinical presentation of *Coxiella burnetii* infections in sheep and goats are late-term abortions, stillbirths, and weak neonates that do poorly during the first several days of life and often die. Infected dams tend to be young adult, primiparous animals. Infected animals rarely become ill before the abortion occurs. Sheep and goats often quickly recover with no evidence of disease. Vaginal or uterine discharges from clinically normal animals, as well as animals with a severe metritis, may contain organisms which may infect more animals. Ewes tend to shed more organisms than does in the uterine discharge. Goats shed the organism in the feces for up to 20 days before and after parturition while sheep shed organisms for up to eight days after lambing. Ewes and does can shed the bacteria in milk. Goats are known to shed the bacteria for prolonged periods in milk. Sheep tend to shed *Coxiella* in milk intermittently and for a short period of time. In one study, monitoring by PCR for the presence of *Coxiella burnetii* for 30 days after abortion or normal delivery showed that the organism is shed into vaginal mucus, feces, and milk of 44%, 21%, and 38%, respectively, of goats that aborted and 27%, 20%, and 31%, respectively, of goats that delivered normally.

Aborted fetuses are often fresh with little evidence of *in utero* autolysis. Aborted animals often are normal size or appear slightly thin. Major internal organs are without lesion. The placenta is often the only tissue affected and is extremely useful in confirming a diagnosis. Cotyledons are often diffusely thickened with multiple areas of necrosis, and covered with gray/white to brownish/red exudate. Intercotyledonary areas are thickened, leathery, and covered with gray/white to brownish/red exudate (Figure 3.7). Weak, nonviable neonates often have no lesions; however, some animals may have a mild bronchopneumonia.

Histologically, the placental chorioallantoic connective tissue is thickened by edema and fibrosis with variable neutrophilic and lymphocytic inflammation. The chorionic trophoblastic epithelium is often necrotic with abundant surface mixtures of neutrophils and debris. A mild vasculitis may be present in placental vessels but often is not a major feature. Similar changes are observed in the chorionic villi. Many of the chorionic trophoblasts in both the cotyledonary and intercotyledonary regions are filled with intracytoplasmic small coccobacilli (Figure 3.8). These organisms are readily visible with routine hematoxylin and eosin staining but are most pronounced with Gimenez or Wolbach's Giemsa stains.

The infected fetus usually does not demonstrate histopathologic lesions other than the placentitis. Approximately 10% of goat fetuses may have mild

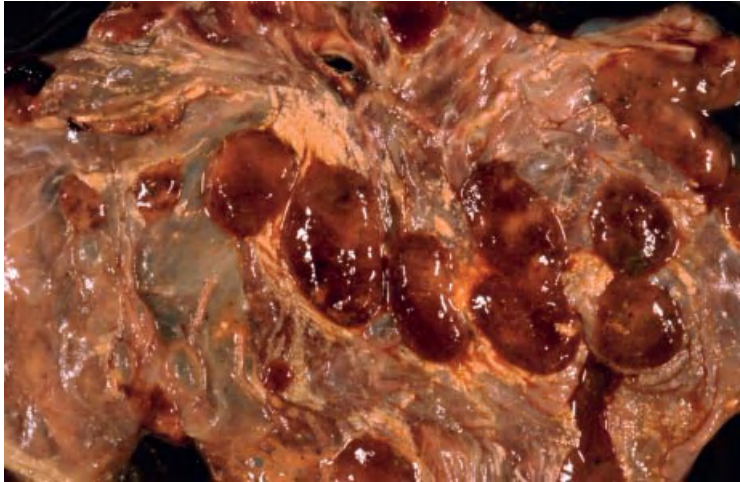


Figure 3.7 *Coxiella* placentitis in a goat. Placental lesions attributed to *Coxiella burnetii* infections include cotyledon thickening with areas of multifocal necrosis and intercotyledonal thickening and opacity with an almost leathery appearance. Commonly, there is an associated brownish exudate. (Courtesy of Dr. RB Moeller, California Animal Health and Food Safety Laboratory.)

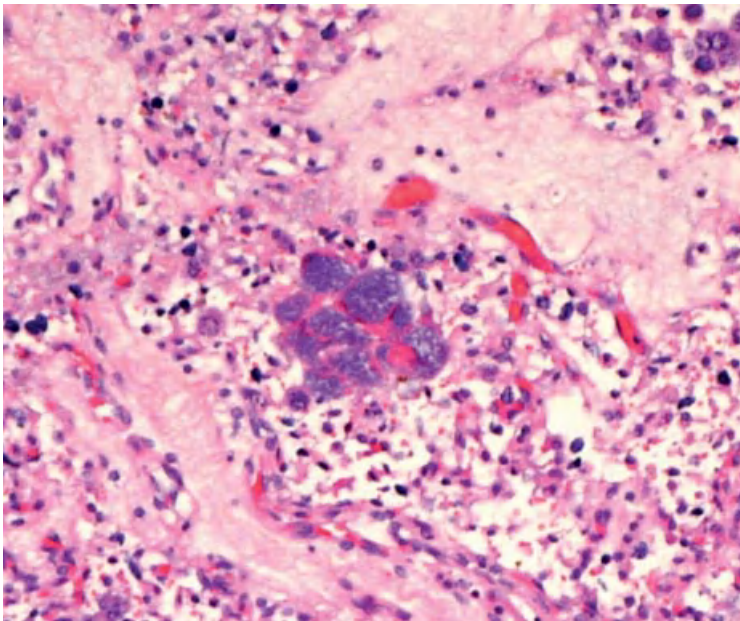


Figure 3.8 *Coxiella* placentitis in a goat. Numerous, dark blue organisms are present within the cytoplasm of infected placental epithelial cells. H&E stained section. (Courtesy of Dr. RB Moeller, California Animal Health and Food Safety Laboratory.)

lymphocytic hepatitis, pleocellular bronchopneumonia, and/or lymphocytic nephritis. In one report, an aborted goat fetus had granulomatous hepatitis. The identification of a mild lymphocytic portal hepatitis, granulomatous hepatitis, pleocellular bronchopneumonia, and/or lymphocytic nephritis in a fetus without placental tissues or the inability to culture other bacterial pathogens from standard bacterial medias is strongly suggestive of a *Coxiella* abortion. In infected newborns with a bronchopneumonia, the lung changes are primarily a pleocellular bronchopneumonia with or without meconium present in the lungs. As in the fetus, the organisms are difficult to identify in the infected organs.

Organisms seen in the placental trophoblasts must be differentiated from *Brucella*, *Chlamydophila*, and *Campylobacter* species. *Brucella* and *Campylobacter* species are easily isolated from the placenta and fetal tissues using standard bacteriological techniques; *Chlamydophila abortus* and *Coxiella burnetii* are not. *Coxiella burnetii* is often identified in the placental tissues using antibody-based detection methods such as fluorescent antibody or immunohistochemical staining techniques. PCR testing procedures are also available for identifying organisms in fetal tissues. Serological testing for *Coxiella burnetii* is of little use in rendering a rapid diagnosis of infection. Acute and convalescent sera routinely give a retrospective study of the flock's problem. Indirect immunofluorescent antibody (IFA) assays are considered the standard for diagnosing *Coxiella burnetii* and is often used in the diagnosis in people. These assays can differentiate antibody to both Phase I and Phase II variants in the IgG, IgM, or IgA fractions. ELISA testing has shown some promise in identification of *Coxiella* abortions in animals. The ELISA detects both Phase I and Phase II antibodies. High response levels from both the ELISA and IFA testing would strongly suggest a *Coxiella* infection. It is recommended that serum samples be taken on aborting animals around day 15 postabortion rather than waiting later to draw sera for testing. Complement fixation testing, microagglutination testing, and radioimmunoassays are also available.

Flexispira rappini

Flexispira rappini is a microaerophilic, faintly Gram-negative bacteria with bipolar tufts of sheathed flagella and spiral periplasmic fibers. Although the taxonomic classification of *Flexispira rappini* is not clear, 16SrRNA sequencing indicates that this organism belongs to the genus *Helicobacter*. This organism has been identified as an occasional etiologic agent for abortions in sheep. It has not been associated with abortions in goats.

Flexispira rappini infection in pregnant ewes usually results in abortion (in the last trimester of pregnancy), stillbirths, or the birth of weak nonviable lambs. Lesions seen in the fetus or newborn are very similar to *Campylobacter* sp. and must be differentiated from this organism.

Grossly, fetuses usually do not show any significant postmortem autolysis. A fibrinous peritonitis and pleuritis may be present with fibrin adhered to the liver surface. The liver may be congested and swollen with multifocal, well-demarcated oval to round gray/white areas of necrosis, up to several

centimeters in diameter. The intercotyledonary regions of the placenta are thickened with a brownish/red to grayish/white exudate. Cotyledons often are necrotic with a similar brownish/red to grey/white exudate.

Microscopically, the liver has multifocal areas of coagulative necrosis infiltrated by variable numbers of neutrophils, lymphocytes, and macrophages. In adjacent areas, there is dissociation of hepatocytes from the hepatic cords along with dilation and congestion of sinusoids. Placental lesions consist of multifocal necrosis of the chorionic epithelium in both the cotyledons and intercotyledonary areas infiltrated by numerous neutrophils. The chorioallantoic connective tissue is often edematous with variable numbers of neutrophils and macrophages. Vasculitis of placental vessels is prominent with numerous transmural and perivascular neutrophils. Capillaries in the chorionic villi often contain myriad bacteria.

This bacterium should be suspected when lesions are present and *Campylobacter* spp. are not cultured. Special stains identify weakly stained Gram-negative bacteria in capillaries of the chorionic villi. However, Giemsa and crystal violet stains readily identify these bacteria. Darkfield or phase-microscopic examination of the abomasal fluid can identify highly motile organisms. Bacterial culturing is difficult and requires special conditions to allow the organism to survive and grow.

***Francisella tularensis* (Tularemia)**

Francisella tularensis is a Gram-negative coccobacillus. In sheep, this bacterium causes late-term abortions and death in young lambs. Tularemia is a problem in the Western United States and Canadian provinces. The bacteria appear to be transmitted to sheep by ticks (*Dermacentor andersoni*). Water does not appear to be a significant means of spread.

Aborted fetuses are often autolytic. Multifocal, pinpoint white foci affect fetal lungs, liver, and/or spleen. Often gross lesions are not presented in the fetus or placenta. Histologic lesions include multifocal areas of parenchymal necrosis infiltrated by macrophages and neutrophils. Since the organisms are very small and the tissues are usually autolytic, Gram stains often fail to detect the bacteria. Immunohistochemical stains for *Francisella tularensis* often demonstrate both intracellular and extracellular organism in areas of necrosis and in blood vessels.

Bacterial culturing of the organism is difficult. It is felt that fetal autolysis and specific growth requirements by the bacteria often hinder efforts to isolate the bacteria. Current methods for identification of the organism are to use specific bacterial media for growth, immunohistochemistry, and PCR techniques.

Leptospirosis

Leptospirosis has been thought to be a minor cause of abortions in sheep and goats. These animals have been considered fairly resistant to *Leptospira interrogans* infections. However, as intense agricultural practices place these

animals in more crowded conditions, more cases of leptospirosis are being seen. Serological studies of *Leptospira* titers in sheep and goats have identified 12.13% of sheep in Italy, 3.3% of sheep and 5% of goats in Portugal, and 5.7% of sheep in Chile to be seropositive. In one study in Ireland, 17% of the aborted lambs were positive for leptospirosis by FA. In Spain, 1.7% of the sheep abortions were attributed to leptospirosis. The primary serovars of leptospirosis considered infectious to sheep and goats are *L. hardjo*, *L. pomona*, *L. icterohaemorrhagiae*, *L. autumnalis*, *L. canicola*, *L. grippityphosa*, *L. ballum*, *L. bratislava*, *L. pyogenes*, and *L. castellanis*. It is unclear if all of these serovars cause abortions in seropositive sheep and goats; however, severe abortion storms have been attributed to *L. hardjo*, *L. pomona*, *L. castellanis*, and *L. icterohaemorrhagiae*.

In adults, leptospirosis is usually subclinical; however, some animals, during the bacteremic phase, may have a fever and/or develop agalactiae. Some animals may develop severe infections leading to renal disease, hemoglobinuria, and rarely anemia. Abortions tend to primarily affect primiparous or naïve replacement stock. Does may manifest metritis after aborting. Caution should be used in handling the uterine discharge since this and the urine may harbor organisms. The aborting fetus is often late gestation or near term. Fetal tissues may be mildly autolytic or fresh. Affected animals are often icteric. Fetal organs may be hemosiderin stained due to autolysis but otherwise normal in appearance. Placental examination is often unrewarding but may have some edema of the intercotyledonary regions. The cotyledons are rarely necrotic.

Microscopic lesions may include mild lymphocytic or plasmacytic interstitial nephritis with minimal renal tubular necrosis. In many cases, kidney lesions are absent. Bile canalicular stasis is occasionally seen. The chorioallantoic connective tissue may be mildly edematous with lymphocytic infiltrates.

Use Warthin-Starry or antibody-based immunohistochemical stains to identify *Leptospira* organisms in renal tubules, chorionic trophoblastic epithelium, lungs, and liver. A diagnosis of leptospirosis is often made by identifying spirochetes by fluorescent antibody techniques in kidney impression smears. PCR techniques are available for the identification of *Leptospira* in urine, but there are no reports of this technique in sheep or goats. Serological evaluation of dam sera or fetal fluids for various serovars may assist in the identification of the serovar responsible for the abortion.

***Listeria* spp.**

Listeria infections in sheep or goats are caused by *Listeria monocytogenes* and *Listeria ivanovii*. These organisms are small Gram-positive coccobacilli. Of the 16 serovars of *Listeria monocytogenes*, 4b is the most common isolate from abortions in sheep. Listerial infections are primarily a problem in temperate and cold climates where seasonal feeding of silage is associated with the disease. Most cases in North America and Northern Europe occur from December to May. The ingestion of poor quality or spoiled silage (pH >5.0) has been incriminated in many of the cases. However, infections in pastured animals do occur.

Infected sheep and goats usually develop a septicemia prior to abortion and may or may not demonstrate clinical signs of infection. Infected does and ewes may develop a fever with depression and anorexia prior to or during the abortion. Some aborting animals may die from the septicemia. Aborting animals rarely develop signs suggestive of neural involvement. Invasion of the placenta and fetus occurs usually within 24 hours of the onset of the bacteremia. Abortions usually occur 6 to 13 days later. Most abortions occur after 12 weeks of gestation during the second and third trimester. Stillbirths of term fetuses and birth of weak nonviable offspring is common. Aborting animals may retain a portion of their placenta resulting in a purulent metritis for up to 3 weeks after abortion.

Grossly, fetuses are usually slightly to markedly autolyzed with occasional mummified fetuses present. Fetal fluids are abundant and reddened. A fibrinous pleuritis, pericarditis, and peritonitis may be seen. The liver often has multifocal, small gray/white, necrotic foci 0.5-2mm in diameter. Necrotic gray/white foci can also be noted in the spleen, kidneys, lung, myocardium, and adrenal glands. Hemorrhages may be noted on the serosal surfaces in fresh fetuses. The abomasum may have small erosions, 1-2mm in diameter, on the mucosa. The intercotyledonary regions of the placenta are often thickened, congested, and edematous. Necrosis, debris, and brownish/red exudate are often seen on the surface of cotyledons.

Microscopically, the necrotic foci in the liver and other organs (particularly the spleen, heart, kidneys, and adrenal glands) are composed of necrotic debris and variable numbers of coalescing aggregates of neutrophils and some macrophages. A congenital neutrophilic pneumonia may be seen. Gram-positive coccobacilli are often present in necrotic foci. The cotyledons and intercotyledonary regions have multifocal areas of necrosis of the chorionic epithelium and superficial connective tissue. Variable numbers of nondegenerate and degenerate neutrophils are present in the necrotic debris. Chorioallantoic connective tissue is edematous and infiltrated by variable numbers of neutrophils and macrophages. Neutrophilic vasculitis with thrombosis commonly affects chorioallantoic vessels.

Bacterial cultures of the placenta, lungs, liver, and abomasal fluid confirm the diagnosis. Gram-positive organisms are identified in placental impressions, abomasal fluid, and in histologic tissue sections. Antibodies specific for *Listeria* can be used for immunohistochemical staining on formalin-fixed tissue.

Salmonella

Salmonella abortions are considered rare events in sheep and have not been reported in goats. *Salmonella* Abortus-ovis and *S. Brandenburg* have been noted in Europe and New Zealand (*S. Brandenburg*) to cause severe abortion storms in heavily concentrated sheep flocks. Other *Salmonella* infections that have been identified in sheep abortions and are considered to be sporadic infections are *S. Typhimurium*, *S. Hindmarsh*, *S. Dublin*, *S. Arizona*, *S. Oranienburg*, and *S. Montevideo*.

Salmonella infections occur most often in large flocks that are tightly concentrated leading to overcrowding, poor sanitary conditions, and stress. Contaminated feeds are the most common source of infectious organisms. Most infected dams do not demonstrate clinical illness; however, some animals will develop fever and diarrhea prior to aborting. Abortion storms tend to occur 6 to 8 weeks prior to lambing and can affect up to 10-20% of the flock. Earlier abortions can occur if the ewe is infected earlier in gestation. Infected ewes may develop a septic metritis with a fetid, dark vaginal discharge and peritonitis, which can be fatal. Weak newborn lambs or lambs up to 2 weeks of age may develop a bronchopneumonia.

Placental infections produce swollen, pale, or hemorrhagic cotyledons mixed with areas of necrosis. Variable amounts of brownish/red suppurative exudate may be present on the cotyledonary surface. Intercotyledonary areas are often edematous and opaque covered by variable amounts of gray/white to brownish/red exudate. The fetus often dies *in utero* and is usually autolytic. Some edema of the subcutaneous tissues, hemosiderin staining of organs, and abundant serosanguinous fluid present in the thoracic and abdominal cavities may be seen. Other organs, though autolytic, appear normal.

Histologically, the placenta is edematous, and variable numbers of neutrophils, lymphocytes, and histiocytes infiltrate the chorioallantoic connective tissue. The chorionic villi are often necrotic and attract variable numbers of neutrophils. Rarely, fetuses may develop a mild neutrophilic bronchopneumonia.

Standard bacterial cultures of the placenta and fetus (lung and abomasum), and vaginal discharges often identify these bacteria.

Minor bacterial causes

Many other bacteria have been isolated from aborting sheep and goats. These bacteria cause sporadic, individual animal problems and do not represent a serious flock problem. Mechanistically, most infections are associated with an initial septicemia in the dam followed by localization of the bacteria to the uterine caruncle and infection of the placental cotyledons. Bacteria in this category include *Escherichia coli*, *Arcanobacterium pyogenes*, *Aeromonas* species, *Fusobacterium* species, *Staphylococcus* species, *Streptococcus* species, *Pseudomonas* species, *Bacillus* species, *Pasteurella multocida*, *Mannheimia haemolytica*, *Yersinia pseudotuberculosis*, *Mycoplasma mycoides*, subspecies *mycoides large colony type*, and *Mycoplasma agalactiae*.

Infections in the dam often go unnoticed; however, if the dam is closely observed, they may develop fever, depression, and anorexia prior to the abortion. Diarrhea may affect some animals (*Yersinia pseudotuberculosis*). These bacterial abortions usually occur during the second and last trimester. Most abortions are usually noticed around 12 to 20 weeks of gestation. Stillbirths and the birth of weak, nonviable neonates may occur. Infected dams may develop postpartum metritis. Since the bacteria tend to localize at the interface of the uterine caruncle and cotyledon, the primary lesion tends to involve the cotyledon. Multifocal areas of necrosis with red/brown or gray/

white exudate may affect the cotyledons. Infarction and reddening of the chorionic villi may be seen. The intercotyledonary regions are often spared, but they may be slightly edematous, transparent, and/or have superficial fibrinous exudation. Fetuses may undergo various stages of autolysis.

Rarely, fibrin may be on peritoneal or pleural surfaces. Excessive fluid may be found in the thoracic and abdominal cavities. Most fetal tissues appear normal. Histologically, chorionic villi are necrotic with variable numbers of neutrophils present in the debris. Macrophages and lymphocytes may infiltrate the chorioallantoic connective tissue. Thrombosis and vasculitis of small vessels in the chorionic villi can occur. However, the large placental vessels are rarely diseased. The affected fetus often has a neutrophilic or pleocellular bronchopneumonia. Necrosis of the liver with pleocellular infiltrates is rare. Often no lesions are present, but a pure growth of bacteria from the infected fetal tissues suggests a bacteremia at the time of death.

Diagnosis of these infections is often accomplished using routine bacterial culture of fetal tissues (placenta, lung, liver, and abomasal contents).

Protozoal abortions

***Sarcocystis* and *Neospora* abortions**

Sarcocystis infections in most adult sheep and goats are usually asymptomatic and rare. *Sarcocystis tenella* (sheep) and *Sarcocystis capracanis* (goats) are the most pathogenic species of *Sarcocystis* that infect these animals. Some animals infected with these organisms develop disseminated disease characterized by fever, anorexia, weakness, anemia, muscle twitching, prostration, and death. If a pregnant animal becomes infected, fetal death can occur. Fetal infections early in gestation can result in fetal death and reabsorption, and infections later in gestation can result in abortions and stillbirths. Most abortions usually occur after 10 to 14 weeks of gestation. Most infections result in sporadic abortions in a herd. Rarely are major abortion storms seen.

Fetuses are often autolytic with internal organs lacking any abnormalities. Placental lesions, when observed, include pinpoint, white necrotic foci of cotyledons. Fetal lesions are rare. However, hydrocephalus has been reported, particularly in full-term fetuses. Under most circumstances, the brain and liver are the most common organs affected. The brain usually develops multifocal areas of necrosis and gliosis with mild lymphocytic and histiocytic perivascular cuffing. The liver may have multifocal areas of necrosis with minimal inflammation. Often numerous rosette-forming schizonts are present in endothelial cells.

Diagnosis of *Sarcocystis* infections often involves ultrastructural evaluation of the organisms (zoites) present in the endothelial cells. Immunohistochemical detection is also employed.

N. caninum infections in sheep and goats are rare. Infections in adult animals are usually asymptomatic. However, the outcome of the pregnancy is often dependent on when the doe or ewe is infected. Infections early in preg-

nancy can result in fetal death and re-absorption. Infections later in pregnancy can lead to fetal death and abortion or the birth of weak neonates.

Rarely are gross lesions seen in the fetus or placenta. If infected over 12 weeks gestational age, aborted fetuses tend to be severely autolyzed. Hydrocephalus and cerebellar hypoplasia have been reported in aborted fetuses and newborns. Some animals may be born alive with severe CNS disturbances characterized by ataxia, opisthotonos, difficulty rising, and inability to nurse.

Histologically, CNS lesions include necrotizing encephalitis with gliosis, lymphocytic perivascular cuffing, and nonsuppurative meningitis. Dystrophic mineralization may involve the neuropil. Necrosis of the liver, mild interstitial lymphocytic pneumonia, and lymphocytic myocarditis can also be seen in fetal infections. Involvement of other organs is rare. In newborn animals, lesions tend to only involve the brain. When present, a placentitis is often non-suppurative and necrotizing.

Neosporosis is confirmed by finding the organism or cysts in infected tissues using immunohistochemical staining. PCR techniques are also available for the detection of organisms in fetal tissues. Serologic testing of fetal fluids or precolostral sera from affected neonates can also be useful in confirmation of *Neospora* infections.

Toxoplasma gondii

In some areas of the world, *Toxoplasma gondii* is a major cause of abortions in sheep and goats. Based on serological data, this organism infects many animals and is ubiquitous throughout the world. *T. gondii* has a two-host life cycle with the cat as the definitive host and most warm-blooded animals (birds and mammals) as intermediate host. Most sheep and goats acquire infections after birth usually through the ingestion of forage contaminated with cat feces containing sporulated coccidian oocysts. Less than 4% of persistently infected animals transmit the parasite vertically through transplacental transmission. Fetuses are infected through transplacental transmission.

Under most circumstances, infection of naïve immunocompetent dams rarely causes clinical disease. Most animals are asymptomatic before and after aborting. Experimental studies have shown that infected does and ewes may develop mild transient lethargy, diarrhea, and respiratory distress after exposure to the organism. Exposure of susceptible pregnant animals to *T. gondii* will result in a wide range of abortions from the involvement of several animals in the herd to a major herd wide abortion storm. The outcome of fetal infections depends on when the naïve dam is infected. If the dam is infected during early gestation, fetal death and resorption usually occur. Infections later in gestation result in mummification, stillbirths, and birth of weak neonates. Not all fetuses presented from the infected dam may demonstrate organisms.

Aborted fetuses usually have no significant gross lesions. If fetuses are presented from multiple dams, there is often a marked variation in fetal

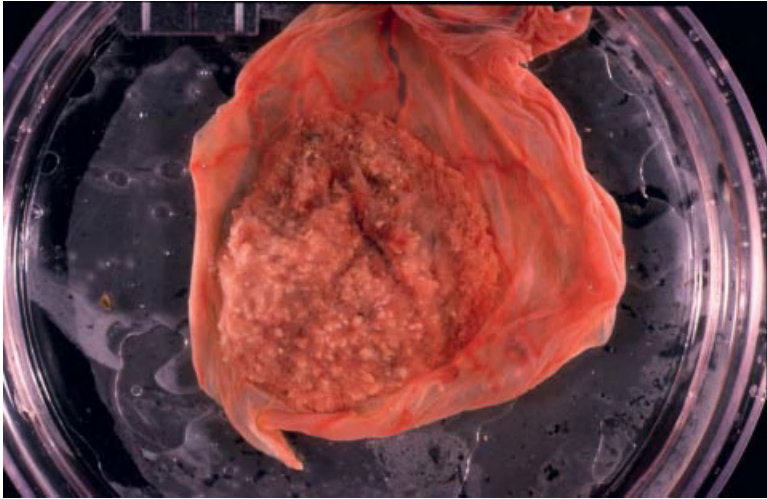


Figure 3.9 *Toxoplasma* placentitis in a ewe. Scattered throughout the surface of this placental cotyledon are multifocal areas of necrosis due to *Toxoplasma gondii* infection. (Courtesy of Dr. ML Anderson, California Animal Health and Food Safety Laboratory.)

ages and autolytic state (often varies from mummification to fresh term fetuses). Presentation of twin and triplet abortions often reveals fetuses in variable postmortem conditions (one mummified and one fresh fetus). Usually a thorough history of the flock or herd will identify that the abortions are primarily in young ewes and does or recently purchased animals. Placental changes are often the only gross lesion observed in the aborted animals. Placental lesions consist of small, pale, white foci of necrosis in the cotyledons (Figure 3.9). Often the necrotic foci are very small and difficult to discern.

Histologically, the lesions vary with duration of fetal infection. The placental lesions consist of necrosis and mineralization of the cotyledonary villi with a variable, nonsuppurative inflammatory change (Figure 3.10). Mild nonsuppurative inflammation is also seen in the chorioallantoic connective tissue. The brain is the primary target tissue. Lesions consist of multifocal areas of necrosis and gliosis, primarily in the periventricular cerebral white matter. Mild perivascular cuffing composed of lymphocytes and macrophages are often associated with the necrotic regions. Other lesions in the fetus are rare, but mild mononuclear cell infiltrates may be identified in the heart, lung, and liver. Tachyzoites or tissue cysts are rarely identified. However, if tissue cysts are found, they are usually in cotyledonary villi or in the brain.

The diagnosis of *Toxoplasma gondii* abortions involves identification of tissue cysts and zoites using *T. gondii* antibodies for immunochemical staining. Also the detection of fetal titers to *T. gondii* in fetal fluids also indicates exposure. However, fetal exposure must be of significant duration for the fetus

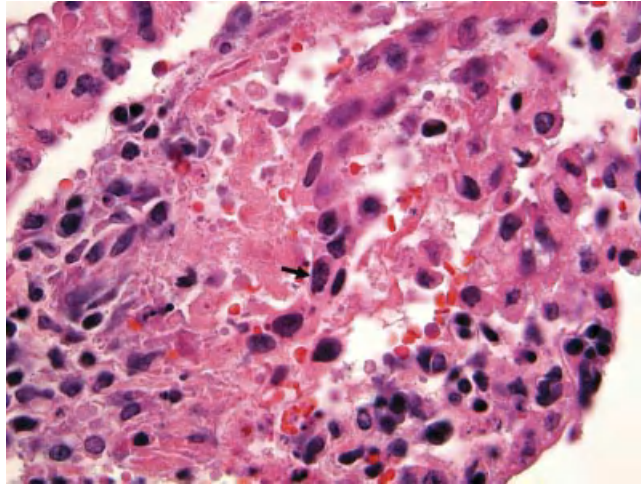


Figure 3.10 Histology of *Toxoplasma placentitis*. Within this section of chorioallantois are scattered free tachyzoites as well as one, intratrophoblastic *Toxoplasma* cyst (arrow). H&E stained section. (Courtesy of Dr. J Ortega, California Animal Health and Food Safety Laboratory.)

to develop fetal antibodies. Precolostral serology of weak lambs or kids may also be useful. Maternal serology often is unrewarding since exposure is common and titers often are prevalent throughout the herd, even in non-aborting animals.

Toxic plants

Many poisonous plants are capable of causing abortions in sheep and goats. If the plant is capable of causing injury or stress in the pregnant animal, there is always the potential for an abortion. Consequently, the diagnostician should not rule out any particular poisonous plant or compound as a potential abortifacient. This review will deal with toxicoses that have been demonstrated to cause abortions and/or deformities in the fetus or newborn.

Annual ryegrass

Annual ryegrass (*Lolium rigidum*) is known to be toxic to sheep and cattle in Australia and South Africa. This toxicosis can cause abortions. The disease is caused by infection of annual ryegrass with the plant-parasitic nematode, *Anguina* spp. *Anguina* spp. larva damages the seed heads forming seed galls. These seed galls are infected with toxin-producing bacteria (*Clavibacter rathayi*). The toxin is known as corynetoxin. Affected animals become excitable with ataxia and convulsions. Pulmonary edema may develop. Pregnant ewes may abort, however, the pathogenesis is not completely understood. Fetal lesions are not described.

***Gutierrezia microcephala* and *G. sarothrae* (Broomweed, Snakeweed, Stinkweed, and Turpentine weed)**

Gutierrezia microcephala and *G. sarothrae* (Broomweed, Snakeweed, Stinkweed, and Turpentine weed) cause abortions and death in sheep and goats. Acute poisoning in adult animals may result in nasal discharge, listlessness, anorexia, and diarrhea or constipation. Excessive mucous productions in the feces, as well as excessive vaginal discharges, are reported in some animals. Hemoglobinuria has been detected. Abortions may occur several days to weeks after ingestion. Fetal lesions are not seen. The toxic and abortifacient principles are not completely characterized but believed to be due to flavonol methyl esters, diterpenoid acid, and saponins.

***Lathyrus* spp. (singletary pea, wild pea, everlasting pea, sweet pea)**

Ingestion of *Lathyrus* sp. plants (primarily the seeds) causes abortion and the birth of weak lambs and kids with deformities. Affected does and ewes usually do not have visible problems during pregnancy. The primary concern includes fetal and neonatal limb and axial skeletal deformities. Fetal lesions consist primarily of contraction or overextension of the pastern and carpal joints. Scoliosis, kyphosis, and torticollis are often seen. Osteoporosis is a common finding. The primary cause of the structural defects is due to aminonitriles that interfere with inter- and intra-molecular cross-linkage of collagen during collagen synthesis. This results in an abnormal collagen matrix formation and skeletal deformities.

Locoweed

Prolonged consumption of locoweed (*Astragalus* and *Oxytropis* sp.) by pregnant ewes can lead to abortions and skeletal deformities in the newborn. Both the dam and fetus are affected by the ingestion of these plants. Dams often become emaciated and ataxic, with proprioceptive defects and abnormal behavioral changes. Fetuses may abort and show vacuolization of neurons in the brain, convoluted renal tubules, placentome trophoblasts, and macrophages in lymph nodes. Fetal skeletal abnormalities include brachygnathism, contraction or overextension of joints, limb rotation, osteoporosis, and bone fragility. The pathogenesis of these deformities is unknown. Delayed placentation also occurs in ewes grazing locoweed early in gestation (less than 50 days). Placentomes are often pale and anemic and fail to attach to the uterine wall.

Subterranean clover

Subterranean clover (*Trifolium subterraneum*) is known to cause early embryonic death and abortions in sheep. Phytoestrogens, genistein, formononetin and biochanin A, are believed to be the primary isoflavone estrogens



Figure 3.11 Cyclopic ovine fetus. Fetal cyclopia is a virtually pathognomonic lesion attributed to the ingestion of *Veratrum californicum* by the pregnant ewe on day 14 or 15 of gestation. (Courtesy of Dr. B Stegelmeier, USDA ARS, Poisonous Plant Research Laboratory, Logan, Utah.)

responsible for the problems in sheep. Formononetin is low in estrogenic activity and is converted to equol, an estrogenic metabolite. Although some ewes may abort, the primary problem seems to be infertility. The estrogenic compounds induce the development of cystic glandular hyperplasia of the cervical and uterine mucosa. Changes to cervical mucous production impair sperm transport and embryonal implantation. If affected animals are removed from the pasture, fertility often returns. However, if the animal remains on the pasture for a prolonged time, infertility may persist and become permanent. In addition to infertility, pregnant animals may develop uterine inertia and failure of the cervix to dilate at parturition. Affected ewes also have an increased incidence of cervicitis and metritis.

***Veratrum californicum* (skunk cabbage, western or false hellebore, or wild corn)**

Skunk cabbage causes numerous fetal abnormalities in sheep and goats. The ingestion of the plant at different stages of fetal development will result in different fetal abnormalities. The ingestion of the plant at day 14 or 15 of pregnancy can result in cyclopean and cebocephalic malformation (Figure 3.11). Ingestion of the plant at this time, as well as earlier, may also result in embryonic death. Ingestion of the plant at day 29 to 31 results in pronounced limb shortening of the tibiae, radii, metacarpals, and metatarsals. Fusion of metacarpals may result in bowing of the legs and arthrogryposis with fusion of

the joints. Cleft palate can also occur in the affected fetus. Tracheal and laryngeal stenosis occurs in offspring of ewes ingesting the plant on day 30 to 31. The toxic compounds responsible for these teratogenic events are the alkaloids cyclopamine, veratramine, cycloposine, and jervine. Cyclopamine is believed to be the most important toxic teratogenic agent due to being the alkaloid in the greatest concentration in the plant.

Other minor toxic plants

Other plants that are known to cause skeletal malformation and cleft palate in sheep and goat fetuses and newborns are poison hemlock (*Conium maculatum*), tree tobacco (*Nicotiana glauca*), and lunara lupine (*Lupinus formosus*). All of these plants produce congenital lesions when the does are exposed to these piperidine alkaloid-containing plants during days 30 to 60 of gestation.

Mineral deficiencies and abortions

Copper

Copper is required to ensure good reproductive performance in both male and female animals. Deficient adult animals may have difficulty becoming pregnant or fail to have proper embryo implantation, resulting in a decrease in the numbers of animal births. Ewes may have depigmented and uncrimped wool. Fetal copper deficiencies have been shown to appear in the last half of pregnancy with histological evidence of demyelination of the cerebellum and motor tracts of the spinal cord. Though this can happen during fetal development, it is a very rare occurrence. Instead, copper deficiency is more common in lambs and kids that are newborn or up to 3 months of age. Deficient neonates demonstrate posterior limb weakness, ataxia, and progressive paralysis.

Iodine

Iodine deficiencies occur worldwide. Deficiencies can cause stillbirths (premature or term) and weak live lambs or kids. An enlarged thyroid gland is most often observed. The enlarged thyroid glands often weigh 10-50 gm (normal is 2 gm). The iodine content in affected glands is often very low. Some animals may have partial or complete alopecia. Although true iodine deficiencies can occur, goitrogenic plants (*Brassica* sp., kale, rape, cabbage, and turnips) more commonly induce iodine deficiency. The goitrogenic properties of these plants are due to goitrin (L-5-vinylyl-2-thioexazolidone), which is a breakdown product of glucosinolate. Goitrin inhibits the incorporation of iodine into precursors of thyroxine and interferes with the secretion of iodine.

Selenium

Selenium and vitamin E, like copper, are important in enhancing the reproductive performance of both male and female animals. Deficiencies in the dam may result in poor lambing due to early embryonic death or failure of the embryo to implant in the uterus. Rarely are abortions conclusively linked to selenium/vitamin E deficiencies. Very low selenium levels in fetal livers may be suggestive of selenium deficiency in the fetus, but confirmation of these trace elements as the cause of the abortion is speculative. Consequently, if the fetal liver has very low selenium levels, dam blood selenium levels should be evaluated to see if values correlate with selenium deficiency. Rarely, if ever, are skeletal or cardiac lesions suggestive of white muscle disease seen in an aborted fetus. Most cases of selenium deficiency affect kids and lambs over 2 weeks of age. Affected neonates often develop necrosis of skeletal and/or cardiac muscle leading to acute death or stiff animals reluctant to get up.

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Chapter 4

Disorders of Pigs

Michael J. Yaeger

Introduction

Mechanisms of abortion and neonatal loss

Embryonic and fetal death are a diagnostic challenge due, in part, to the broad array of processes that have been associated with *in utero* mortality, including genetic, hormonal, nutritional, toxic, traumatic, metabolic, hypoxic, and infectious. Laboratory diagnosticians have traditionally focused on infectious and toxic causes of reproductive wastage because of the potential to detect these agents through routine testing of fetal and maternal samples and the possibility of ameliorating losses with intervention strategies. Similar to other domestic species, this approach successfully leads to a diagnosis in only one-third of abortion cases whereas approximately two-thirds of porcine abortions are classified as idiopathic (Table 4.1).

A brief review of a generalized representation of the pathogenesis of abortion will help to illustrate several key issues regarding mechanisms of infectious abortion (Figure 4.1). In swine, the principal mechanisms whereby infectious agents cause abortion include, most commonly, infection of the fetoplacental unit, and, less commonly, the systemic effects of maternal illness. The diagnostic approach differs depending on the mechanism of abortion. Abortion due to maternal illness generally occurs during the acute phase of the disease process, at which time: (1) sows typically exhibit signs of systemic illness; (2) sows will not have seroconverted; (3) the causative agent may be detected in dam sera or tissues; and (4) there has generally been insufficient time to develop detectable fetal infection. Since abortion is related to maternal causes, examination of fetuses is generally unrewarding. Diagnostic testing should focus on samples collected from the sow based on her clinical signs at the time of abortion.

Conversely, when abortion is due to fetal infection, the following may occur: (1) the dam will typically have seroconverted at the time of abortion;

Table 4.1 Diagnostic results on 1,396 porcine abortion cases submitted to the Iowa State University Veterinary Diagnostic Laboratory from 1/2003 to 1/2010.

	Number of cases (N = 1,396)	Percent total abortions
Idiopathic	942	67
Infectious	409	30
Viral	318	23
Bacterial	89	6.4
Fetoplacental inflammation, cause not determined	40	3

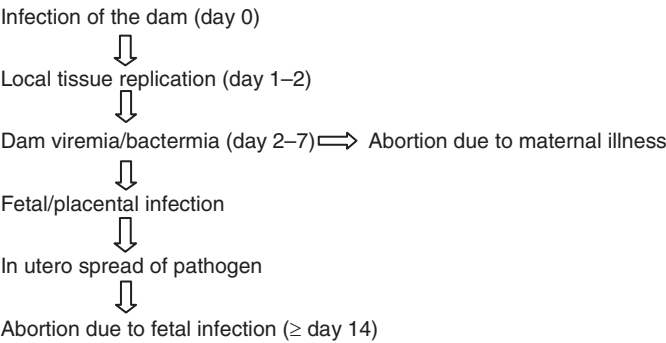


Figure 4.1 Generalized representation of the pathogenesis of infectious abortion in swine.

(2) the dam is generally no longer viremic/bacteremic; and (3) the organism can typically be detected in the fetus and/or placenta. For several agents (for example, Porcine Reproductive and Respiratory Syndrome Virus [PRRSV], pseudorabies [PRV], *Erysipelothrix rhusiopathiae*), abortion may result from either maternal illness or fetal infection. When investigating an abortion outbreak, the first step is to assess whether aborting sows are exhibiting signs of systemic illness at or near the time of abortion. If aborting sows are systemically ill, abortion may be due to maternal illness, and specimens from both the dam and fetus should be evaluated. When abortion results from fetal infection, the agent can generally be detected in fetal tissues and/or placental.

Tissue sampling

Specialized techniques

There has been a progressive shift toward the use of molecular techniques, specifically PCR, for porcine abortion diagnostics. Studies have demonstrated that PCR is the most sensitive method for detecting a number of agents in

aborted or mummified porcine fetuses. The astounding sensitivity of this assay helps to overcome the detrimental effects of fetal autolysis and the potential for agent dilution by pooling samples from infected and non-infected fetuses. PRRSV is currently the most common infectious cause of abortion in swine, and PCR is considered to be the only reliable technique for detecting this virus in fetal tissues.

The increasing use of PCR to detect infectious agents in postmortem specimens requires more stringent procedures for tissue collection and processing, especially in facilities that process large numbers of porcine cases. The reliability of PCR depends on avoiding cross contamination among specimens. Whenever possible, samples should be collected with disposable instruments and on disposable surfaces, and the use of instruments that may be contaminated from previous use should be avoided.

In the past, mummified fetuses from a number of domestic species were routinely discarded due to the perception of limited diagnostic value. However, mummification is a common feature of viral-induced reproductive disease in swine. Thus, practitioners should be encouraged to submit mummified fetuses when an elevated mummy rate is an important clinical manifestation of the reproductive problem. Tissues from mummified fetuses are typically appropriate for fluorescent antibody (FA) or PCR.

Porcine abortion serology

The mechanism of abortion dictates the approach to serologic diagnosis. The majority of infectious abortions are the result of fetal infection. Although abortion appears to be an acute process, abortion resulting from fetal infection typically occurs ≥ 14 days after the dam became infected, maternal seroconversion can generally be demonstrated at the time of abortion, and paired serum samples are of limited diagnostic value because significant titer changes will not be observed post-abortion. When abortion results from maternal illness, the sow aborts during the acute phase of infection. The sow will not have seroconverted at the time of abortion, and acute and convalescent samples are needed to establish a serologic diagnosis and will demonstrate a significant rise in titer. As mentioned above, an assessment of maternal health at the time of abortion is vital for appropriate sampling strategy.

Whenever sows are systemically ill at or near the time of abortion, maternal illness should be considered as a potential mechanism, and both acute and convalescent serum samples should be evaluated. In cases of abortion due to fetal infection, negative post-abortion serology would tend to eliminate a specific pathogen from consideration. Positive titers demonstrate exposure without a time reference provided by seroconversion in relation to abortion. If titers to an agent are unexpected (for example, positive titers in a PRRSV-free herd, or high titers to *Leptospira* serovars in animals not recently vaccinated), a single, post-abortion serum sample can provide valuable diagnostic information. Titers to endemic agents (such as PPV, PCV2) or in vaccinated animals are difficult to interpret.

Pooling of samples

Swine typically have the largest litters of our domestic species. This would be a moot point if all agents infected all fetuses in all litters. Unfortunately, this is not the case. Studies have demonstrated that it can be a relatively uncommon occurrence for a bacterial or viral pathogen to pass from the dam to an individual fetus. This uncommon event is typically followed by *in utero* spread from fetus to fetus. The ramifications of these processes include the following: (1) all fetuses in a litter are typically not infected at the time of abortion; (2) fetuses may be infected and die at different stages of gestation; and (3) the diagnosis can be missed if a limited number of fetuses are evaluated. PRRS is an excellent example of this phenomenon. In two large studies, PRRSV was detected in approximately 50% of fetuses in infected litters. If one fetus per litter were randomly evaluated, a diagnosis would be missed in half of the cases.

The ideal number of fetuses to sample varies depending on the pathogen, strain of the agent, and stage of gestation. However, a good general recommendation is to submit or sample four to six fetuses per litter. This is based on a calculation to achieve 90-95% confidence that at least one infected fetus is represented in a submission, assuming a fetal infection rate of 50% and a litter size of 12. Fortunately, pooling of samples from several fetuses generally has limited impact on diagnostics, especially when highly sensitive tests, such as PCR, are used.

Table B.3 in Appendix B summarizes what tissues to collect, which tests should be requested, and lists additional ancillary tests that can be performed to maximize the diagnostic effort of attempting to confirm a diagnosis from a list of selected pathogens.

Gross examination

Fetus

An attempt should be made to assess fetal viability at birth. Partially inflated lungs, lungs that float in formalin, and air or milk in the stomach are indicators that the fetus had been born alive. If the lung and other tissues float in formalin, this may be due to gas-producing bacteria, either as a cause of abortion or contributing to putrefaction. Crown-to-rump length should be measured to obtain an estimate of fetal age at the time of *in utero* death or abortion. (See Table A.3 in Appendix A.) This is especially true with mummified fetuses, since *in utero* death has occurred well before fetal expulsion. Unfortunately, gross lesions in aborted swine are uncommon, rarely diagnostic, and lack of gross lesions does not exclude the possibility that abortion was due to an infectious agent. Table 4.2 lists differential diagnoses for common gross lesions identified in porcine fetuses.

Fetal membranes

Swine have diffuse, epitheliochorial placentation. The allantochorion has three, relatively distinct zones. The bulk of the allantochorion consists of a large central zone that has transverse folds, numerous areolae (shallow cups in the chorion opposite the openings of uterine endometrial glands), and a velvety surface

Table 4.2 Possible causes of gross and microscopic lesions in swine fetuses.

Gross Lesions	Causes
Fetal mummification	PPV, PCV2, PRV, TEV/PEV, EMCV, CSFV, PCMV, JEV, LPMV, Menangle virus, <i>Leptospira</i>
Cerebellar Hypoplasia	CSFV, JEV, BVDV-BDV, Menangle virus
Multifocal hepatic necrosis	PRV, JEV, <i>Leptospira</i>
Myocardial lesions	EMCV, PCV2
Perirenal edema	PRRSV, PCV2
Mesocolonic edema	PRRSV, PCV2
Pulmonary hypoplasia	SIV*, CSFV
Arthrogryposis	CSFV, Menangle virus
Jaundice	<i>Leptospira</i>
Umbilical cord hemorrhage and edema	PRRSV
Enlarged congested liver	PCV2
Pulmonary edema	PCMV
Microscopic Lesions	Causes
Nonsuppurative encephalitis	PRRSV, EMCV, PRV, PEV/PTV, CSFV, BVDV-BDV, JEV, Menangle virus, PPV*
Hepatitis	PPV*, TEV/PEV, Menangle virus, <i>Leptospira</i> , <i>Chlamydia</i> , fetal bacterial septicemia, <i>B. suis</i>
Placentitis	PPV*, PRV, <i>Leptospira</i> , <i>B. suis</i> , <i>Chlamydia</i> , bacterial abortion
Myocarditis	PRRSV, EMCV, PCV2, Menangle virus, PPV*
Intranuclear inclusions	PCMV, PRV, PCV2, Menangle virus
Interstitial pneumonia	PRRSV, PCV2, PRV
Suppurative bronchopneumonia	Bacterial abortion, <i>Leptospira</i> , <i>B. suis</i>
Interstitial nephritis	<i>Leptospira</i> , PPV*, CSFV
Multifocal hepatic necrosis	PRV, JEV, <i>Leptospira</i>
Arteritis	PRRSV

Key:

PRRSV: Porcine Reproductive and Respiratory Syndrome Virus

PCV2: Porcine Circovirus type 2

PPV: Porcine Parvovirus

PRV: Pseudorabies

EMCV: Encephalomyocarditis virus

PEV: Porcine enteroviruses

PVT: Porcine teschoviruses

CSFV: Classical Swine Fever Virus

BVDV-BDV: Bovine Viral Diarrhea Virus and Border Disease Virus

LPMV: La Piedad-Michoacan Virus

JEV: Japanese Encephalitis Virus

PCMV: Porcine Cytomegalovirus

SIV: Swine Influenza

*Rarely observed in field cases

composed of numerous, villus-like projections which interdigitate with the endometrium. This central zone is bordered on each side by paraplacental zones characterized by a smooth glistening surface, distinctive longitudinal blood vessels, and almost complete lack of areolae. The extremities of the placental sac (necrotic tips) are sharply demarcated from the paraplacental zones by constrictions. These extremities are the ischemic zones, which are often dry, shriveled, and caked with dark brown, sticky material. The necrotic tips of neighboring embryos normally form a 3- to 8-cm-wide avascular region between one conceptus and the next. It has been speculated that the ischemic zones prevent end-to-end fusion of adjacent placentas, discouraging the formation of vascular anastomoses and preventing the development of freemartins, which appear to be rare in swine. Though the necrotic tips do not prevent fusion of adjacent placentae in the latter half of gestation, they tend to inhibit interembryonic vascular anastomosis between days 29 and 50 of gestation, which may be the critical period for preventing freemartin development.

Evaluation of the placenta is essential for establishing an etiologic diagnosis for a number of abortion-causing diseases, particularly bacterial (including *Chlamydia* spp. and *Coxiella burnetii*) and fungal. For many domestic species, diagnostic success plummets when placenta is not available for examination. Swine are largely an exception to this general rule. Mycotic abortion is rare in swine, placentitis and abortion due to *Chlamydia* spp. is not well documented, and bacteria account for less than one-fourth of all infectious abortions (Table 4.1). Compared to other species, placental lesions are less common and of less diagnostic significance in swine. Nonetheless, evaluation of the placenta is recommended. The presence or absence of placental lesions can be of value in categorizing an abortion as infectious or noninfectious and aid in interpreting the significance of bacteria isolated from fetal tissues.

Placental mineralization, manifest as gritty, white plaques in the amnion and/or allantois, is common in swine and of no apparent clinical significance.

Viral causes of abortion

When compared to other domestic species (small ruminants, bovine, equine) where bacteria and/or protozoa (small ruminants, cattle) account for the majority of infectious abortions, swine are relatively unique in that viral infection of the fetoplacental unit is clearly the most common infectious cause of reproductive wastage, accounting for 78% (318/409) of infectious abortions and 23% (318/1,396) of total abortions (Table 4.1). PRRSV alone accounts for nearly 60% of all infectious abortions (Table 4.3). Viral-induced reproductive disease is typically the result of infection of the fetoplacental unit, though maternal illness can be the primary cause (for example, Swine Influenza [SIV], Transmissible Gastroenteritis [TGE]) or a contributing cause (for example, PRV, PRRSV) with a number of viruses.

A large and diverse assortment of viruses have been associated with porcine abortion, including viruses indigenous to the United States (PRRSV, PCV2,

Table 4.3 Common agents detected in 409 porcine cases classified as infectious abortion at Iowa State University Veterinary Diagnostic Laboratory from 1/2003 to 1/2010.

Agents	Number of diagnoses (N = 409)	Percent infectious abortions
PRRSV	232	57
PCV2	52	13
PPV (mummified fetuses)	34	8.3
Leptosira ¹	24	5.9
<i>Strep sp.</i> (<i>S. suis</i> most common)	12	2.9
<i>E. coli</i>	11	2.7
<i>A. pyo</i>	6	1.5
<i>Staph sp.</i> (<i>S. hyicus</i> most common)	5	1.2
<i>Salmonella sp.</i>	2	0.5

¹The number of *Leptospira* abortions is artificially elevated. 71% of the cases (17/24) were from a single large outbreak.

PPV, PRV, SIV, enteroviruses, teschoviruses, Bovine Viral Diarrhea Virus [BVDV]-Border Disease Virus [BDV], swine vesicular disease, TGE, PRNS, Porcine Cytomegalovirus [PCMV]) as well as a variety of foreign animal diseases (Japanese encephalitis virus, classical swine fever, African Swine Fever, Menangle virus and La Piedad-Michoacan virus).

Many laboratories have largely discontinued the use of conventional techniques, such as virus isolation, and currently detect viral causes of abortion by specific methodologies, such as PCR, that provide rapid results, are sensitive, specific, and less impacted by autolysis. This approach has benefits and significant drawbacks. The sensitivity of PCR improves the diagnostician's ability to detect specific agents. However, test specificity precludes the possibility of detecting other agents. The preference for use of specific diagnostic methods is partially responsible for the current dearth of information on the diagnostic frequency of less common viral causes of abortion. When faced with an ongoing abortion problem where routine testing for viruses has been negative, virus isolation should be considered, particularly if fetal anomalies, mummification, interstitial pneumonia, myocarditis, encephalitis, or necrotic lesions have been identified and freshly dead fetuses are available.

Porcine reproductive and respiratory syndrome virus (PRRSV)

PRRSV is an enveloped, positive-sense, single-stranded RNA virus in the family Arteriviridae. PRRS is one of the most economically significant diseases of swine. The total annual economic impact of PRRSV infection was estimated to be \$66.75 million in breeding herds and \$493.57 million in growing-pig

populations in 2004. As illustrated in Table 4.3, PRRSV is unrivalled as the most important infectious cause of abortion in pigs in the Midwestern United States, accounting for nearly 60% of infectious abortions from 2003 to 2010. Two characteristics of this virus make it particularly difficult to control: (1) genome plasticity and (2) persistent shedding.

Single-stranded RNA viruses typically have a high error rate during replication leading to the development of multiple strains that may differ in virulence and antigenicity. The existence of multiple strains of PRRSV has been confirmed by sequencing, monoclonal antibody analysis, and virulence studies. The antigenic differences associated with strain variation result in a failure of complete cross protection between strains, including vaccine strains, and clinical disease can occur in previously seropositive or vaccinated herds. Genetic and antigenic variation can also interfere with diagnostic tests. There is sufficient antigenic variation that a single monoclonal antibody will not recognize all strains of the virus and differences at primer binding sites have the potential to lead to false negative PCR results.

Studies have demonstrated that 90% of PRRSV-infected pigs are carriers after 105 days and that PRRSV may be isolated from oropharyngeal samples from pigs for up to 157 days. This extended period of virus shedding favors continued cycling of the virus and greatly complicates control.

Clinical signs in the breeding herd are variable and depend on genetics, the stage of gestation, strain virulence, and the overall immune status of the sow herd. Despite this potential complexity, certain clinical features are relatively consistent in the breeding herd. Sows will generally exhibit a mild, febrile illness when initially exposed to the virus. They will be slightly depressed, off feed, and have a mild fever of 39.5–39.7°C for 1–2 days. Sows occasionally abort during the initial febrile phase of the illness, but typically abort 2–4 weeks following infection.

PRRSV classically causes late gestation reproductive disease manifest as late gestation abortions, stillbirths, premature farrowings, and the birth of weak (“squeaker”) piglets. Exposure of naïve sows to a virulent strain early in gestation (<45 days) often fails to result in fetal infection, and exposure late in gestation (<70 days) leads to abortion.

The immune status of the breeding herd influences clinical signs. If a virulent strain is introduced into a naïve sow herd, a massive outbreak of abortions and weak piglets may result. If herd turnover results in an ever-increasing number of females that lack immunity to the herd strain of the virus, the presence of pockets of nonimmune animals may result in abortion in scattered groups of females.

Gross and microscopic lesions are not consistently observed in cases of PRRSV abortion, and lack of lesions does not preclude a diagnosis of PRRSV abortion. Gross changes (late gestation autolyzed fetuses, umbilical cord hemorrhage, and edema) were observed in 12% of PRRSV abortion cases, and microscopic lesions were only identified in 4% of PCR-positive litters (ISU VDL data, 2006–2009).

A typical PRRSV litter will consist of 1 to 2, dark, autolyzed fetuses with abundant fluid in the body cavities and several fetuses with meconium staining of fetal skin. The remainder of the litter may appear essentially normal.

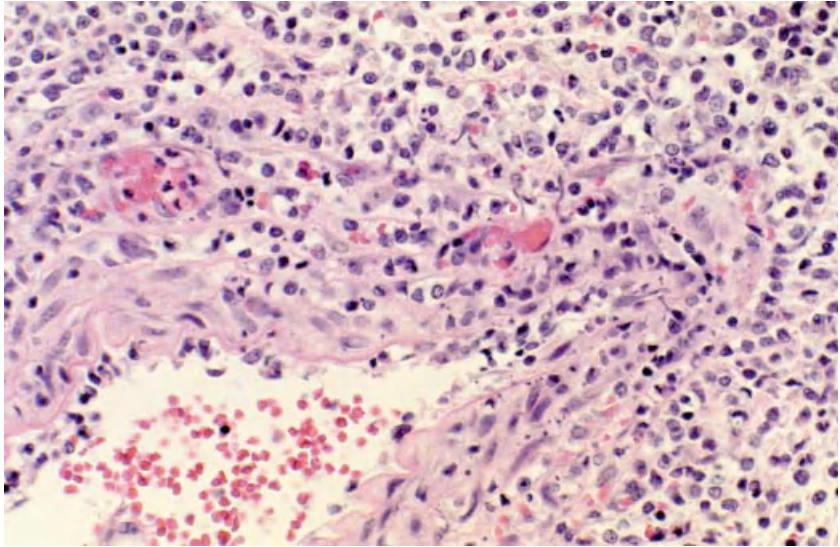


Figure 4.2 Histology of PRRSV-associated vasculitis. Mixtures of degenerate leukocytes infiltrate the vessel wall and surrounding tissue from an aborted porcine fetus. H&E stained section. (Courtesy of Dr. KD Rossow, Minnesota Veterinary Diagnostic Laboratory.)

Additional gross changes include umbilical cord edema with segmental umbilical cord hemorrhage, perirenal edema, mesocolonic edema, and increased amounts of clear to serosanguinous fluid in the body cavities.

Microscopic lesions are uncommon. Edematous umbilical cords with segmental hemorrhage typically have a necrotizing arteritis with periarterial hemorrhage. Arteritis is occasionally observed in a variety of tissues (Figure 4.2). Though uncommon in clinical specimens, reports describe additional microscopic fetal lesions including nonsuppurative encephalitis, myocarditis, and interstitial pneumonia.

Several issues complicate PRRSV abortion diagnostics. Firstly, not all fetuses in the litter are infected. The number of infected fetuses per litter varies with the stage of gestation and viral strain. The percentage of infected fetuses is reported to vary from 18.8 to 73.8%, with an overall rate of approximately 50% reported in two large studies. Assuming a 50% fetal infection rate, material from four to six fetuses should be pooled to achieve 90–95% confidence that at least one infected fetus is represented in a submission.

Secondly, sows can abort due to maternal illness. On an individual animal basis, examination of fetuses alone may be insufficient to establish a diagnosis of PRRSV abortion. Sows exhibiting a mild febrile illness at the time of abortion may abort due to maternal illness. In these cases, sow sera should be submitted for PRRSV Enzyme-linked Immunosorbent Assay (ELISA) and PCR testing. Results that support a diagnosis of PRRSV abortion due to maternal illness include the following: (1) failure to demonstrate fetal infection; (2) negative

PRRSV ELISA (in a sow that has not previously been exposed or vaccinated); and (3) demonstration of PRRSV viremia in the affected sow.

Finally, strain variation can lead to false negative results. Substantial genetic and antigenic differences exist between PRRSV strains. One of the consequences of this variability is that a single monoclonal antibody or single set of PCR primers will not detect all strains. To address this issue, laboratories should use multiple primers, including primers for North American (NA) and European-like (EU) strains. Many laboratories use an antibody cocktail, containing more than one monoclonal antibody, to increase the percentage of strains detected. If there is a disparity between clinical signs, serology, and laboratory results, it may be necessary to retest samples with different reagents because strain variation has the potential to lead to false negative results with both antibody- and nucleotide-based tests.

Reports have documented the superiority of PCR over all other testing methodologies for the diagnosis of PRRSV in aborted fetuses. Material from one positive fetus can be pooled with seven negative fetuses without an apparent loss of signal. Autolysis, which is common in aborted porcine fetuses, has a limited impact on PCR techniques. In one study, 88% of samples autolyzed for 2–4 days at 21° and 37°C remained PCR-positive.

Because of the marked strain variation and the presence of both North American and European-like strains, there is the need for simultaneous detection of multiple strains by PCR. This is accomplished by using several primers in a multiplex PCR.

Studies have demonstrated that tissue pools and fetal thoracic fluid are essentially diagnostically equivalent. Because fetal thoracic fluid is easier to pool and process, and can be collected in individual disposable syringes, minimizing the opportunity for contamination, it is the preferred fetal sample for PRRSV detection. Pooling fetal thoracic fluid from four to six fetuses/litter for PCR testing is recommended. Preferred tissue pools for PCR detection can be inferred from IHC results, and should consist of lymphoid tissue (thymus, tonsil, lymph node, spleen), liver, and lung.

PRRSV can cause abortion as a result of maternal illness (minority of cases) or fetal infection. In either instance, aborting sows will seroconvert. If abortion is due to maternal illness, paired samples will be necessary to demonstrate seroconversion. The sow will have seroconverted at the time of abortion in the vast majority of cases, and a single sample, collected at the time of abortion, will demonstrate high PRRSV titers. Results can be difficult to interpret in vaccinated or known seropositive herds. If sows are seronegative at the time of abortion and remain seronegative, PRRSV can be ruled out with relative certainty.

PRRSV is very stable at refrigeration or freezer temperatures but degrades rapidly at room or body temperature. Autolysis, which is common in aborted fetuses, can lead to virus degradation, loss of viability, and false negative virus isolation (VI) results. In experimentally infected litters, fetuses dead *in utero* were compared to those that were viable at harvest. PRRSV was isolated from only 11.8% of dead fetuses compared to 65.7% of live fetuses. Because of the limited sensitivity, VI should not be the primary testing methodology in cases

Table 4.4 A comparison of two studies evaluating the percentage of tissues from PRRSV-positive fetuses in which viral antigen was detected by IHC.

Tissue	Study A % PRRSV IHC positive	Study B % PRRSV IHC positive
Thymus	48	31
Liver	31	38
Spleen	29	54
Lung	15	38
Umbilical Cord	10	NA

NA: not assessed.

of suspect PRRSV abortion. If an isolate is desired to produce an autogenous vaccine, submission of serum from multiple, precolostral, weakborn pigs will prove more productive than sampling autolyzed fetuses.

IHC suffers from three serious limitations when applied for fetal diagnostics: (1) limited sensitivity; (2) highly variable tissue distribution; and (3) inherent difficulty in pooling samples. Firstly, in one study multiple fetal tissues were evaluated and PRRSV antigen was detected by IHC in only 48.1% of PRRSV-positive fetuses. When the tissues with the highest IHC positivity rate from two studies were compared (Table 4.4), a consistently high-yielding tissue was not identified. If a single tissue from each fetus/litter were pooled and evaluated by IHC, the likelihood of detecting a positive litter would be $\leq 54\%$. Finally, VI or PCR are test methods conducive to sample pooling; however, IHC requires the placement of samples on 1×3 glass slides, and charges are incurred per slide. Thus, IHC has limited utility in PRRS abortion diagnostics.

Fetal serology can be a useful diagnostic tool in late gestation abortions if the fetus is immunocompetent at the time of *in utero* exposure and if there is sufficient time for fetal seroconversion prior to abortion. Fetal serology was critically evaluated for PRRSV abortion diagnostics. Individual animal sensitivity was poor—only 30.8% of PCR positive fetuses had detectable antibodies. Studies evaluating the pattern of fetal infection suggest that it is a relatively rare event for the virus to cross the placenta and cause fetal infection. This is followed by lateral spread *in utero* to adjacent fetuses. The poor sensitivity of fetal serology is likely due to insufficient time for the majority of fetuses to seroconvert prior to abortion.

It is currently possible to generate sequence data from an isolate or PCR product. This information is used primarily as an epidemiologic tool. The value of PRRSV sequencing depends on the availability of baseline data and the client's approach to PRRSV control in the breeding herd. If PRRSV control is based on herd-wide, homologous immunity accomplished by exposing all females to the herd strain of PRRSV prior to entry into the breeding herd, then

baseline sequence information should be gathered. In this situation, if the sequence from the initial outbreak and subsequent outbreaks are the same, then abortions are the result of exposure failure during acclimation. If the sequences are different, a new strain of the virus has been introduced suggesting a lapse in biosecurity.

Porcine circovirus type 2 (PCV2)

Porcine circovirus (PCV) is a small, circular, single-stranded, non-enveloped DNA virus. There are two recognized types of PCV: PCV type 1 (PCV1) and PCV type 2 (PCV2). PCV1 was first discovered as a contaminant of a porcine kidney cell line (PK-15) in the early 1970s and has since been determined to be non-pathogenic in pigs. In contrast, PCV2 was first isolated in Canada in the early 1990s in cases of postweaning multisystemic syndrome (PMWS), is pathogenic, and has been linked to many disease entities in growing swine commonly referred to as PCV associated disease (PCVAD), including PCV2-associated reproductive failure. Five different genotypes of PCV2 are currently recognized: PCV2a to PCV2e. Today, PCV2a and PCV2b are distributed worldwide, PCV2c was identified in archived Danish sera, and PCV2d and PCV2e have only been reported in China.

PCV2 is a ubiquitous virus and global. Most swine herds have evidence of exposure based on the presence of PCV2-specific antibodies. However, clinical signs of PCV2 infection may be absent. A variety of clinical signs may be observed in growing pigs with PCVAD including icterus, pneumonia, wasting, and diarrhea with gross lesions of lymphadenopathy. In mature breeding animals, PCV2-associated reproductive failure can manifest as abortion or be associated with increased rates of mummified, macerated, stillborn, and weak-born piglets. Early embryonic death has also been linked to PCV2 infection.

The first field report of PCV2-associated reproductive failure was described in 1999 in a Canadian breeding facility stocked entirely with first parity gilts. Farrowing rates fell below 60% with 20% of the reduced farrowing rate accounted for by losses after 13 weeks of gestation. Many late-term abortions were characterized by mummified, macerated, autolyzed, and stillborn fetuses. Gilts that farrowed during the peak of the outbreak had litters composed of approximately 75% mummified fetuses or stillborn piglets. The majority of reported field cases of PCV2-associated reproductive failure has been observed predominately in gilts or newly populated gilt farms. It is speculated that affected dams were seronegative prior to being bred.

Multiple investigations in many geographical regions have analyzed the incidence of PCV2-associated reproductive failure. On North American farms that exhibited greater than 10% nonviable fetuses at farrowing, anti-PCV2 antibodies and PCV2 DNA was demonstrated in 16.4% (28/171) and 7.6% (13/171) of serum samples obtained from stillborn piglets, respectively. A Korean retrospective study found PCV2 DNA in 10% of examined fetuses at all stages of gestation with the highest frequency in fetuses aborted in late gestation.

The incidence of PCV2-associated reproductive failure in European countries is variable. A study that evaluated German herds demonstrated PCV2 DNA in 27.1% of stillborn or weakborn piglets. A Spanish study found only 0.3 % of the cases contained PCV2 DNA. In the Czech Republic, PCR results of fetal tissue samples demonstrated a gradual increase in the diagnosis of PCV2 fetal infection from 2005 to 2007 whereas diagnosed cases of porcine parvovirus (PPV) and porcine reproductive and respiratory syndrome virus (PRRSV) had decreased during the same time span.

PCV2 fetal infections occur following exposure of the dam to PCV2 or after insemination with PCV2 spiked semen. Dam viremia may last for multiple weeks during the gestation period, and PCV2 can infect fetuses *in utero* at different stages of gestation resulting in variable sized mummified and stillborn fetuses at parturition. PCV2 can also spread from fetus-to-fetus *in utero*.

Timing of PCV2 fetal infection influences gross lesions and the distribution of PCV2. Myocardium has been identified as the primary site of PCV2 replication in early to mid gestation leading to myocardial inflammation and dysfunction. PCV2 infection occurring during late gestation, results in an increased amount of PCV2 DNA and antigen within lymphoid tissues. Fetuses infected after 70 days of gestation can clear viral infection prior to parturition or be born PCV2 viremic with minimal gross or microscopic lesions.

Clinical signs of PCV2 infection in the dam are generally absent; however, pyrexia and anorexia may be observed in aborting dams, occasionally resulting in abortion due to systemic illness. Abortions are typically seen in a low percentage of females on a given farm with the exception of rare cases of PCV2-infection of naïve populations. Delayed farrowing (after 118 days of gestation) or pseudopregnancy may be additional clinical features. Coughing, diarrhea, or wasting has not been reported in affected dams; however, replacement gilts in development units may have signs of systemic PCVAD. It is important to realize that most gilts and sows have been exposed to PCV2 prior to breeding and are partially protected against disease and PCV2-associated reproductive failure.

PCV2-associated microscopic lesions commonly seen in affected growing pigs (lymphoid depletion; granulomatous inflammation of organ systems) have not been reported in dams with PCV2-associated reproductive failure, and lymphoid tissues are usually normal. Placental sections are normal as well.

Sow serology is difficult to interpret because PCV2 is ubiquitous, and most dams have been previously exposed to PCV2. PCV2 viremia at parturition may indicate fetal infection but is not diagnostic for PCV2-associated reproductive failure.

Affected litters may contain normal appearing live-born piglets, weak-born piglets, macerated fetuses, stillborn fetuses, and mummified fetuses. All fetuses in a single litter can vary greatly in crown to rump lengths (Figure 4.3). Large numbers of variably sized mummified fetuses is the most consistent finding. Increased pre-weaning mortality can also be observed.



Figure 4.3 Variably mummified litter due to PCV2 infection. This litter is composed of variably sized mummified and partially mummified fetuses expelled from a sow experimentally infected with PCV2. The partially mummified fetuses on the far left have a crown-rump length >6 inches (>16 cm). Mummies >6 inches may be observed following PCV2 infection but do not tend to occur with PPV-induced fetal mummification. (Courtesy of Dr. MJ Yaeger, Iowa State University.)

Gross heart lesions are infrequent but when present are seen in stillborn or late gestational mummified fetuses. Individual fetuses may have lesions of heart failure including dilated and hypertrophied ventricles, plural effusion, ascites, and enlarged, congested livers (Figure 4.4). Late gestational fetuses may exhibit generalized anasarca. Perirenal and mesocolonic edema is infrequently seen in stillborn pigs.

Fetal histological lesions, when present, vary depending on the stage of fetal development at the time of infection. The most consistent lesions include myocardial degeneration and necrosis, nonsuppurative myocarditis, fibrosis, and mineralization with occasional amphophilic to basophilic intranuclear inclusion bodies. Liver congestion with hepatocellular atrophy may also be present along with nonsuppurative pneumonia, but both are of minor importance. Lymphoid follicular hyperplasia and lymphocyte depletion with marked macrophage infiltration and rare multinucleated giant cells has been reported.

Independent of the stage of infection, the heart contains the highest amount of viral antigen. Therefore, fetal myocardium should be collected in 10% buffered formalin. Lung, tonsil, thymus, and spleen can also be included for microscopic examination if PCV2-associated reproductive failure is suspected. Precolostral sera from live-born piglets is a useful diagnostic sample. Finding PCV2 DNA or antigen in precolostral serum samples from live-born piglets,

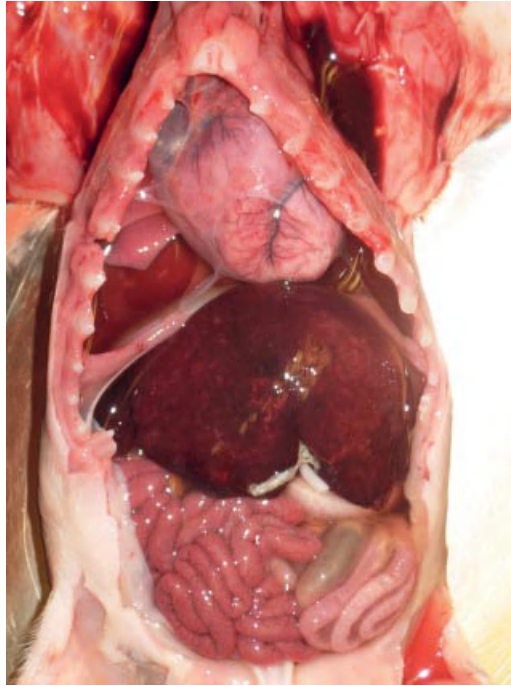


Figure 4.4 PCV2-induced heart failure. Gross lesions in this weakborn piglet experimentally infected with PCV2 include an enlarged and globose heart, hydrothorax, ascites and a markedly enlarged, mottled, congested liver. (Courtesy of Dr. MJ Yaeger, Iowa State University.)

tissues of aborted fetuses, or tissues from litters with increased nonviable piglets at parturition is indicative of intrauterine exposure to PCV2.

Immunohistochemistry (IHC) and *in-situ* hybridization (ISH) are considered the gold standard for detection of PCV2 antigen or PCV2 DNA in formalin-fixed, paraffin-embedded tissues. PCV2 antigen is readily detected in the cytoplasm as well as the nucleus of infected myocardium. PCV2 antigen or PCV2 DNA can also be present in thymus, spleen, tonsil, and lung. Demonstrating the presence of PCV2 DNA or anti-PCV2 antibodies in precolostral live-born piglet sera, aborted, mummified, or stillborn fetuses can also be accomplished by PCR, virus isolation, ELISA, indirect immunofluorescent antibody (IFA), and immunoperoxidase monolayer assay (IPMA).

Clinical abortion or increased numbers of nonviable fetuses at parturition and the detection of PCV2 DNA or PCV2 antigen in fetal tissues together confirm the diagnosis of PCV2-associated reproductive failure. Fetal myocardium and lymphoid tissues are the most appropriate samples to test. Dam serology or viremia should not be used to diagnose PCV2-associated reproductive failure.

Sequencing allows distinguishing between PCV2 strains. Previously, it was documented that DNA sequences from PCV2 isolates associated with

reproductive failure were different compared to sequences from PCV2 isolates recovered from growing pigs; however, PCV2-associated reproductive failure is not limited to infection with these selected isolates. It appears that PCV2-associated reproductive failure can be reproduced with any PCV2 isolate. At this point in time, sequencing results may be a useful epidemiological tool, but it is currently not possible to associate virulence with certain sequences.

Porcine Parvovirus (PPV)

Porcine Parvovirus is a non-enveloped, single-stranded DNA virus. Until the recent discovery of a new antigenic variant, all PPV isolates appeared to have been antigenically similar, if not identical. In nonimmune, pregnant females, PPV causes reproductive failure characterized by embryonic and fetal death, mummification, stillbirths, and delayed return to estrus. Acute infection of postnatal, nonpregnant pigs is usually subclinical.

Keys to understanding PPV-induced reproductive disease include the following: (1) the ubiquitous presence of the virus in breeding herds; (2) the impact of immunity on the dynamics of PPV infection; and (3) fetal gestational age at the time of infection.

PPV is endemic in most swine herds. Serological surveys have demonstrated PPV antibodies in 70-100% of the herds studied and in 73-83.5% of the animals bled in those herds. The virus is shed in secretions for 2 weeks and can survive for 4 months in the environment. Contaminated premises appear to be the major reservoir of the virus.

Immunity plays a key roll in the dynamics of this disease in the breeding herd. Because the virus is ubiquitous and vaccination is common, the majority of females in the breeding herd are immune. The populations at greatest risk for PPV-induced reproductive disease are immunologically naïve gilts or gilts with high passive antibody titers. Passive immunity to PPV is generally undetectable by 3-6 months of age. However, passive protection of gilts with high levels of colostral antibody may last for 7-8 months. Passive antibody in these gilts may interfere with the development of active immunity, and they may not become susceptible to infection until their first pregnancy. When these gilts become pregnant, waning of passive antibody leaves them susceptible to reproductive wastage associated with PPV infection.

PPV infection of the dam does not typically result in maternal illness. Maternal viremia leads to fetal infection, and gestational age at the time of infection determines the clinical outcome. Fetal infection at <30 days of gestation leads to embryonic death with resorption, which can manifest clinically as infertility or decreased litter sizes. Fetal infection between 30 and 70 days of gestation results in fetal mummification, the major clinical manifestation of PPV infection. Studies have demonstrated that as the percentage of litter mummification increases, the likelihood of PPV infection also increases. Porcine fetuses become immunocompetent at approximately 70 days of gestation. If they are exposed after this stage of gestation, infection

is generally without clinical consequence and is manifest as *in utero* seroconversion. PPV has the potential to cause outbreaks of reproductive failure in naïve populations. However, outbreaks are currently uncommon largely because of widespread natural or vaccine-induced immunity.

In natural infections, the primary gross lesion is fetal mummification. Fetuses are dark in color, markedly dehydrated, the eyelids are depressed deep into the orbits, and the skin is dry and leathery. Mummified fetuses typically have crown rump lengths ranging from 3–16 cm. Due to *in utero* spread of the virus, fetuses are often mummified at different stages of gestation resulting in mummies of varying sizes.

Microscopic lesions are rarely reported in field infections because mummified fetuses are generally not appropriate for histologic examination. Microscopic lesions have been described in experimentally infected fetuses from sows that have been euthanized at selected times following infection and from stillborn fetuses. Lesions most commonly consist of perivascular accumulations of mononuclear inflammatory cells in both grey and white matter of the cerebrum and meninges, necrotizing and nonsuppurative hepatitis, interstitial nephritis, placentitis with calcification, and myocarditis.

Mummified fetuses are the preferred sample to establish a diagnosis of PPV-induced reproductive wastage. They should be true mummies and not late gestation, dark, autolyzed, fluid-filled fetuses that are common in PRRSV-infected litters. Quantitative PCR has demonstrated that PPV has a predilection for the heart, lung, spleen, and testis in the fetus, with the highest viral loads in the testis. Fetal mummification due to PPV or PCV2 is indistinguishable. However, in many cases of PCV2-associated fetal death, the mummies were reported to be larger than 16 cm crown-rump length (Figure 4.3).

PPV antigen can be detected by ELISA, FA, IHC, and HA tests. FAs have traditionally been used for the diagnosis of PPV. Mummies are reported to have abundant antigen throughout most tissues. Lung is the tissue of choice, because it is easily identified, generally has abundant antigen, and has minimal autofluorescence. Because it has become increasingly difficult to obtain positive control tissues, some labs currently employ methods other than FAs for routine PPV diagnostics. Mummified tissues are infrequently processed for histologic evaluation, therefore, IHC is rarely used.

Studies in older pigs have demonstrated that PCR is more sensitive than IHC, VI, and ISH for the detection of PPV. PCR has been used to successfully detect PPV in mummified fetuses. Viral nucleic acids are most consistently detected in fetal lung, heart, spleen, and testis.

PPV can be isolated using PK15 cells. PPV infectivity is progressively lost following fetal death, and mummies may have died and been retained *in utero* for 40–70 days following PPV infection. Because of the potential for false negative results due to loss of virus viability, virus isolation is not well suited for routine diagnostics on mummified fetuses.

Hemagglutination inhibition (HI) assays are commonly used to detect an antibody response to PPV. A variety of additional methodologies, including ELISA, complement fixation, and virus neutralization assays have been

employed. Interpretation of antibody titers is difficult for the following reasons: (1) the majority of farms vaccinate against PPV; (2) the virus is ubiquitous, and natural exposure is common prior to introduction into the breeding herd; (3) there is evidence for an anamnestic response in sows that do not develop reproductive disease; and (4) high HI titers can occur in a herd without any PPV-related symptoms.

Interpretation of serology is further complicated by the delay in time between maternal infection and the observation of clinical signs. In cases of PPV-induced early embryonic death and resorption, the insult occurs prior to 30 days of gestation, but the problem may not become manifest until the sow comes back into heat or fails to deliver piglets. With PPV-induced fetal mummification, exposure has occurred between days 30 and 70 of gestation yet the problem will not be evident until >40 days later when an elevated mummy rate is identified at parturition. In both cases, PPV titers will be high, but there will have been no possibility to collect paired serum samples to demonstrate seroconversion in relation to clinical signs. Negative serology would eliminate PPV from consideration.

Serology on stillborn pigs or neonates can provide useful information. The presence of PPV antibodies in the serum of piglets that have not suckled or the thoracic fluid of stillborn pigs indicates *in utero* exposure to the virus.

Pseudorabies virus (PRV)

Pseudorabies virus (PRV) (Aujeszky's disease) has been eradicated from commercial swine operations in the US and throughout many intensive swine-rearing regions across the globe. It is tempting to characterize the disease as primarily of historical significance. This would be a disservice to the animal health industries. Transmission from feral swine has the potential to introduce PRV into commercial populations. PRV is endemic in many European wild boar populations. In the US, feral swine have been identified in 32 states, PRV has been detected in feral swine populations in at least 12 states, and there is evidence for infection of peccaries in the southwest. Rapid detection of incursions into domestic populations by astute diagnosticians will allow for the expeditious control of this disease.

PRV (suid herpesvirus-1) is an alphaherpesvirus. The development of lifelong, latent infection and the failure of vaccination to prevent either infection or transmission complicate control and eradication of this disease. Pigs are the primary host and primary reservoir of PRV. The severity of clinical signs, morbidity, and mortality are dependent on the virulence of the isolate, immune status of the host, and age at the time of infection. Non-immune pregnant females, suckling piglets, and recently weaned pigs born to non-immune females are generally considered to be most susceptible to clinical PRV infection.

PRV-associated maternal illness may result in abortion or fetal mummification without evidence of fetal infection due to the nonspecific effects of maternal pyrexia. The stage of gestation at which the sow is infected determines the



Figure 4.5 Herpesviral hepatic necrosis. Multifocal pinpoint tan/white foci (necrosis) are scattered throughout the liver of this late-term fetus that aborted due to Pseudorabies virus infection. (Courtesy of Dr. GW Stevenson, Iowa State University.)

reproductive signs. Fetal infection in the first trimester results in embryonic death and resorption or the expulsion of embryos. Fetal infection during the later two-thirds of gestation leads to fetal mummification, abortion, stillborn, and weakborn piglets. Piglets born infected with PRV are weak, develop neurologic signs, and die within 1 to 2 days. Suckling piglets will develop a high fever, neurologic signs (trembling, incoordination, ataxia, nystagmus, opisthotonos and seizures), and die within 1 to 2 days of the onset of clinical signs. Prewaning mortality in suckling pigs born to naïve dams has been reported to be 82-95% and may reach 100%.

In nonmummified fetuses, PRV infection may result in the development of pinpoint (1-2 mm in diameter) foci of necrosis in liver (Figure 4.5), spleen, and occasionally lung. This lesion is not pathognomonic for PRV because fetal septicemia may cause similar lesions. Microscopic foci of necrosis are detected more commonly than gross changes, and a lack of gross lesions does not exclude the possibility of PRV.

PRV infection causes multifocal random coagulative necrosis in many fetal tissues including liver, spleen, adrenal gland, lung, lymph node and placenta. Eosinophilic to amphophilic intranuclear inclusions are occasionally observed in cells adjacent to foci of necrosis. Lung changes include multifocal, often peribronchiolar, coagulation necrosis and a diffuse pleocellular interstitial pneumonia. Compared to the severe multifocal random necrotizing hepatitis typical of Infectious Bovine Rhinotracheitis (bovine herpesvirus-1) in cattle, PRV-associated fetal necrosis may be much less spectacular. Often, only one to

two foci of necrosis are identified in each section of tissue. CNS lesions typical of a viral encephalitis may be identified in stillborn and weakborn pigs and include nonsuppurative perivascular cuffs, multiple foci of gliosis and neuronal necrosis. Placental changes are less common than visceral lesions and consist of multifocal necrosis of chorionic fossae, edema of the chorionic mesenchyme, and a nonsuppurative placentitis often accompanied by intranuclear inclusions in degenerating trophoblasts and mesenchymal cells in the chorionic stroma.

In recent years, PRV diagnostics have focused on eradication efforts. To the author's knowledge, comparative studies to determine the most sensitive and specific fetal diagnostic tests have not been undertaken. The most common methods for detecting PRV in aborted fetuses include FA, VI, and PCR. Fetal tissues of choice include lung, liver, spleen, brain, and kidney.

Porcine kidney cell lines (PK-15, SK-6) are commonly used for virus isolation. The virus generally induces cytopathic effect (CPE) within 24-72 hours, and virus identity should be confirmed with immunoperoxidase or immunofluorescence. In the absence of obvious CPE, two blind passages are advisable. Virus isolation from fetal tissues suffers from the same drawbacks that have been mentioned previously.

PRV antigen can be demonstrated by direct FA. Lung, liver, and spleen are reported to be the tissues of choice. Antigen is rarely identified in mummified fetuses.

PCR has been shown to be more sensitive than VI, especially in latently infected animals. Another advantage of PCR is that it can be used to rapidly differentiate wild-type PRV from gene-deleted marker vaccine virus in herds currently using gene-deleted, modified live vaccines.

Virus sequencing is used primarily as an epidemiologic tool to determine the source of an outbreak. Following a recent outbreak in Wisconsin, sequence information demonstrated that feral swine were the likely source of infection. (<http://www.avma.org/onlnews/javma/jun07/070615r.asp>)

ELISA and virus neutralization (VN) are commonly used to detect an antibody response to PRV. Serology has been an invaluable tool in eradicating this disease from commercial populations, and serologic surveillance continues. Because vaccination is generally no longer allowed in PRV-free regions, positive titers, in any phase of production, are highly significant.

There are several issues that should be considered when using maternal serology to document PRV abortion. Because vaccination generally protects against reproductive disease, the demonstration of maternal titers to field virus in a vaccinated animal is not diagnostic for PRV-abortion. Conclusive evidence for a diagnosis of PRV abortion in vaccinated animals requires the identification of the virus in fetal tissues.

Swine influenza (SIV)

Influenza A viruses are enveloped, negative-sense RNA viruses. SIV is most typically associated with acute respiratory disease. Infertility, decreased litter size, abortion, and stillbirths have been reported in association with outbreaks

of SIV. With rare exception, SIV is not thought to directly impact the reproductive tract or fetuses. Abortion is thought to be the result of maternal illness, and examination of fetuses is generally unrewarding. In abortion cases submitted to the ISU VDL in which SIV was isolated from nasal swabs of aborting sows, virus has never been detected in fetal tissues by PCR or VI.

Aborting sows typically have a fever, are depressed and anorexic, and have respiratory signs, including a cough. Reproductive manifestations are reported to include infertility, decreased litter size, abortion, and stillbirths.

Reports of fetal lesions associated with SIV abortion are rare. Direct *in utero* inoculation of the fetus resulted in severe epithelial necrosis in bronchial buds and moderate epithelial necrosis in more fully differentiated major bronchi, leading to the development of pulmonary hypoplasia. This lesion is not typically identified in field cases of abortion, and studies involving natural routes of infection have generally failed to demonstrate fetal infection.

Diagnostics should focus on the sow because abortion is likely the result of maternal illness. Since uncomplicated SIV is rarely fatal in sows, antemortem specimens are generally used to establish a diagnosis, and nasal swabs are the sample of choice. The period of viral shedding can be limited; therefore, swabs should be collected from several acutely ill sows. Samples can be pooled and submitted for PCR, VI, or antigen-capture ELISA.

Sows typically abort during the acute phase of illness, and paired serum samples are necessary to establish a diagnosis. On the initial visit during the acute phase of the outbreak, serum and nasal swabs should be harvested. Convalescent serum collected 2 weeks later will typically exhibit $\geq$ fourfold increase in titer.

Transmissible gastroenteritis (TGE)

TGE is a highly contagious viral enteric disease caused by a coronavirus. Reproductive sequelae are occasionally evident. During outbreaks of TGE, farms report an increased incidence of abortion and stillbirths as well as a decreased conception rate and decreased litter size. There is no evidence of transplacental infection, and abortion is likely the result of maternal illness.

There is an age-related resistance to Transmissible Gastroenteritis Virus (TGEV), and clinical signs in adults are typically mild. It is suggested that severe clinical illness in breeding animals may be the result of massive exposure from infected offspring. Signs in sows may vary from slightly loose stools to severe diarrhea accompanied by fever, inappetence, and occasional vomiting.

Examination of fetal tissues is typically unrewarding because abortion is thought to be the result of maternal illness. TGEV infection can be confirmed by submitting feces from acutely affected sows for PCR.

Sows abort during the acute phase of illness. Evaluation of serum samples collected at the time of abortion and then 2 weeks later will demonstrate seroconversion. Serologic diagnosis of TGEV is complicated by the presence of antibodies that neutralize both TGEV and porcine respiratory coronavirus (PRCV), a respiratory mutant of TGEV. On a herd basis, a differential ELISA can

be used to discriminate the serologic response to TGEV or PRCV. This test requires a matured immune response and is not accurate for recent infections (<28 days). If serology is used to differentiate TGEV and PRCV infection, serum samples should be collected a month after the outbreak.

Encephalomyocarditis virus (EMCV)

Encephalomyocarditis virus (EMCV) is an RNA virus of the genus *Cardiovirus*, family Picornaviridae. Rodents are thought to be the natural host of EMCV where the virus typically persists without causing disease. Rodents can serve as the source of infection for swine. Variants of EMCV may differ considerably in virulence and their ability to cause myocarditis, reproductive disease, or both.

EMCV outbreaks have been reported in swine throughout the world, and the pig is considered to be the most common and severely affected domestic species. Although rodents are believed to be the source of infection and may facilitate spread throughout the facility, pig-to-pig transmission appears to play a role in viral spread during outbreaks. In naïve pregnant sows, transplacental EMCV infection may lead to reproductive failure characterized by early embryonic death, abortion, fetal mummification, and stillbirths. Serologic studies suggest that infection in swine is not uncommon, but clinical disease is infrequent. The significance of EMC as a cause of reproductive disease in swine is currently unclear because many laboratories do not routinely screen for EMCV in aborted fetuses. Studies have demonstrated evidence for *in utero* exposure to EMCV in 18–36.6% of fetal samples.

Infection in postweaning and adult pigs is generally inapparent. In preweaning pigs, EMCV may cause acute myocarditis with sudden death or neurologic signs including trembling, staggering, and paralysis. In pregnant sows, EMCV may cause decreased farrowing rates, abortion, fetal mummification, stillbirths, weakborn piglets, and neonatal deaths often preceded by rapid abdominal breathing. Fetal death has been observed as early as 2 weeks post-inoculation in experimentally infected sows. In an outbreak of reproductive disease in which EMCV was isolated from aborted fetuses, 17% of sows produced mummified fetuses and there was a 10% reduction in farrowing rate attributed to early embryonic death.

Litters aborted due to EMCV infection are reported to contain a mixture of large, partially autolyzed fetuses that are dark and edematous, and fetuses that have become mummified at various stages of gestation. Aborted fetuses occasionally have scattered pale to chalky white foci in the heart and evidence of heart failure (hydrothorax, hydropericardium, and ascities).

As the name implies, histologic lesions may be identified in the brain and heart. Fetuses may have nonsuppurative encephalitis and/or myocardial lesions that vary from myocardial degeneration with mineralization to myocarditis with focal or diffuse mononuclear cell infiltration. However, EMCV-positive aborted fetuses may lack microscopic lesions.

Screening for EMCV may not be part of the routine abortion protocol at many laboratories. EMCV should be considered when fetal encephalitis and/or

myocarditis are identified. However, absence of these microscopic lesions does not exclude EMCV from consideration because reproductive failure has been experimentally induced without evidence of microscopic lesions. Diagnostics have focused on identifying the virus in the tissues of abnormal fetuses by virus isolation or PCR, and by detecting EMCV-specific antibodies in fetal sera or fetal fluids.

Lung, spleen, kidney, heart, and brain should be harvested from fetuses for virus isolation. Appropriate cell lines for isolation include baby hamster kidney (BHK-21), continuous monkey kidney (Vero), and HeLa. Vero cells have been found to be more sensitive than BHK-21 cells and pig cell lines for primary isolation of EMC virus. Daily inspection for cytopathic effect (CPE) is recommended followed by FA or virus neutralization confirmation.

RT-PCR has been used for the rapid and specific diagnosis of EMCV in a variety of clinical samples. Heart has been shown to be the most suitable tissue.

VN and ELISA are the most commonly used serologic methods and are considered to be specific for EMCV. For VN, titers $\geq 1:16$ appear to be significant. Because seroconversion is common in endemic regions in the absence of disease and aborting sows have typically seroconverted at the time of abortion, serology can be difficult to interpret.

Specific antibodies to the virus have been detected in both fetal and neonatal sera and are considered to be diagnostic for *in utero* EMCV infection. Virus neutralization antibody titers up to 1:12,800 have been detected in samples of thoracic or abdominal fluids from aborted fetuses.

Enterovirus and teschovirus abortion

Porcine enteroviruses (PEV) and porcine teschoviruses (PTVs) are small, non-enveloped RNA viruses belonging to the Family *Picornaviridae*. Porcine PEVs and TEVs are ubiquitous, can be isolated from healthy animals, and infection is generally asymptomatic. Swine are the only known natural host for PEVs and PTVs, and pigs of any age are susceptible to infection. These viruses have been associated with polioencephalomyelitis, reproductive disease, pneumonia, pericarditis, myocarditis, and enteric disease. SMEDI, which stands for Stillbirths, Mummified fetuses, Embryonic Death, and Infertility, describes the typical features of clinical enteroviral infection in naïve pregnant females.

The relative importance of PEVs and PTVs as a cause of abortion is currently unknown, in part, because many diagnostic laboratories have discontinued virus isolation as a standard diagnostic technique. In a 6-year study conducted in the 1970s, viral infection of the fetus was detected by inoculating several primary porcine cell lines and passaging for 3 weeks. Enterovirus infection was identified in 10.9% of 824 porcine abortions in the study.

Infected dams generally do not exhibit clinical signs. Intrauterine infection may result in infertility, embryonic death, mummification, stillbirths, and neonatal mortality. PEVs, PTVs, and Porcine Parvovirus should be considered when reproductive problems consist of small litters accompanied by increased numbers of mummies.

Specific gross lesions have not been reported. Microscopic lesions are occasionally described and include mild perivascular cuffing and gliosis in the brain, cerebral vaculitis with malacia, lymphocytic spinal ganglioneuritis and meningitis with neuronal necrosis, lymphoplasmacytic periportal hepatitis, focal hepatic necrosis associated with vascular thrombosis, and necrotic epithelial cells in the airways of fetal lung.

Four serogroups, including PTV-1, PTV-2, PTV-6, and PEV-8, have been associated with reproductive losses. PEVs and PTVs can be isolated on primary pig kidney or PK-15 cell lines and typically cause rapid cytopathic effect. Lung is reported to have the highest concentration of virus. Isolates may be identified by immunofluorescence, immunoperoxidase staining, complement fixation, virus neutralization, or PCR.

Since virus isolation is not suitable for mummies due to a lack of viable virus, FA or PCR on fetal lung are the most appropriate procedures to detect PEVs and PTVs in mummified fetuses. PCR primers should be used that will detect multiple serogroups (PTV-1, PTV-2, PTV-6, and PEV-8).

Sow serology is of little to no diagnostic value. These viruses are ubiquitous, and seroconversion is common. Because the dam has typically seroconverted at the time of abortion, it is generally not possible to demonstrate seroconversion. Fetal serology can be a useful diagnostic tool. The identification of anti-PEV or PTV antibodies in the fetal thoracic fluid of stillborn pigs or serum of precolostral weakborn animals from small litters with increased numbers of mummies can be used to confirm *in utero* exposure to these viruses.

Pestiviral abortion

Swine can be infected by several pestiviruses including Classical Swine Fever (CSF or "hog cholera"), Bovine Viral Diarrhea Virus (BVDV), Border Disease Virus (BDV), and an additional putative pestivirus, Porcine Reproductive and Neurological Syndrome Virus (PRNSV).

Classical swine fever virus (CSFV) is a small, enveloped, predominantly noncytopathogenic, single-stranded RNA virus classified in the genus *Pestivirus*, family *Flaviviridae*. Classical swine fever (CSF) is a highly contagious febrile disease of domestic and wild pigs that has the potential to cause severe losses and significantly hamper international trade in animals and animal products.

The ruminant pestiviruses, Bovine Viral Diarrhea Virus (BVDV) and Border Disease Virus (BDV), are closely related to Classical Swine Fever Virus (CSFV). Both natural and experimental BVDV and BDV infections have been reported in swine. Infection of postnatal swine by the ruminant pestiviruses is generally subclinical, but *in utero* exposure may lead to reproductive consequences. Infection by the ruminant pestiviruses is generally the result of a close association between pigs and small ruminants or cattle, the feeding of bovine offal, whey or milk, or from the administration of pestivirus-contaminated vaccines.

Aside from the limited consequences of *in utero* infection, the primary concern with ruminant pestivirus infection in swine has been the potential for

false positive results during surveillance for CSFV, due to the close antigenic relationship between these viruses. Fortunately, this is becoming less of a concern because the seroprevalence of BVDV-BDV in swine appears to be declining. The incidence of BVD in cattle is steadily decreasing due to ongoing herd elimination efforts and mixed farms, where different species live in close contact potentially exchanging infectious agents, are becoming rare. Additionally, the use of testing methodologies that target CSFV-specific antigens or nucleic acids has greatly diminished false positive CSF test results following ruminant pestivirus infection.

In the early 1900s, CSF was distributed worldwide. Since then, the disease has been eradicated from Australia, Canada, New Zealand, and the United States. It is endemic in Eastern Europe, Asia, Central America, Mexico, and parts of the Caribbean and South America. CSF is a highly contagious disease with variable clinical presentation dependent on host age, viral strain, exposure dose, and the host's immune and nutritional status. The virulence of field strains varies widely, and there are no pathognomonic signs associated with CSFV infection. Wild and domestic pigs are the only known reservoir of CSFV.

Clinical signs in sows infected during pregnancy are variable, and reproductive disease may be the only overt clinical manifestation of CSFV infection. Clinical signs described in experimentally infected gestating females include fever, reduced feed intake, depression, ataxia, conjunctivitis, constipation, cachexia, and cutaneous erythema. CSFV is able to cross the placenta of pregnant animals and infect the fetus during all stages of gestation. The outcome of transplacental fetal infection depends on the stage of gestation and virulence of the virus. Fetal infection may cause embryonic death and resorption, abortion, mummification, stillbirths, fetal malformations, and increased neonatal mortality. Experimentally infected females have been reported to abort between 13 and 49 days post-infection or farrow numerous mummified and stillborn pigs. Piglets from sows infected on day 22 or 43 of gestation exhibited varying degrees of muscular tremors, ataxia, splayleg, inability to suckle, and increased perinatal mortality. Fetal infection between 22 and 70 days of gestation may lead to the development of persistently viremic piglets. These animals may be born stunted, exhibit congenital tremors, or appear essentially normal. Persistently infected piglets do not develop antibodies to CSFV, shed high levels of virus, frequently die in the first few weeks of life, or develop a chronic form of the disease with poor growth, wasting, and death at 3 to 8 weeks of age.

BVDV-BDV infection in postnatal swine is typically subclinical and self-limiting. Ruminant pestivirus infection of pregnant sows can lead to maternal viremia followed by fetal infection; however, transplacental infection appears to be inefficient and does not occur with all strains. Clinical signs depend on viral strain and the stage of gestation at the time of infection. More severe signs occur in fetuses infected during the first trimester. Similar to pestivirus infection in other species, *in utero* infection can lead to the development of an immune tolerant, persistently infected state where piglets continuously

excreted virus in oropharyngeal fluid, urine, feces, and semen. Persistently infected swine appear to be important in pig-to-pig transmission of the ruminant pestiviruses, whereas transmission by postnatally infected swine does not appear to occur. Because BVDV-BDV infection in pigs is typically self-limiting, control measures for these viruses in swine are generally not required.

Sows infected with ruminant pestiviruses develop a viremia, which may be accompanied by a transient febrile illness, and seroconvert. *In utero* infection may be associated with poor conception rates, small litters, occasional abortions, stillbirths, congenital anomalies, and increased neonatal mortality, which may reach 30-70% by 2 days of age. In nearly all naturally occurring cases, the disease has been limited to only one litter in a herd. Fetal infection with ruminant pestiviruses can result in a disorder comparable to congenital CSF in piglets, with signs that included a mild fever, alopecia, anemia, rough hair coat, growth retardation, wasting, congenital tremors, conjunctivitis, diarrhea, and death within the first few weeks to months of life.

In utero CSFV infection has led to the development of fetal lesions that include ascities, widespread petechiation of the skin and internal organs, hepatic nodularity, pulmonary hypoplasia, pulmonary artery malformations, micrognathia, arthrogryposis, and central nervous system abnormalities including cerebellar hypoplasia, microcephaly, and defective myelination of the brain and spinal cord. Histologically, scattered mononuclear cell infiltrates may be identified in fetal brain and kidney, and foci of hemorrhage and necrosis may be observed in the kidney and spleen. Piglets with congenital tremors due to CSFV infection typically have cerebellar hypoplasia and dysplasia accompanied by CNS hypomyelination, which is most severe in the spinal cord. Persistently infected piglets may have severe thymic atrophy, pale swollen lymph nodes, and focal colonic mucosal necrosis. Microscopic lesions in persistently infected piglets may include marked depletion of lymphocytes in the thymus, spleen, and lymph nodes, portal fibrosis, intestinal villus atrophy and ulceration, interstitial pneumonia, neuronal degeneration in the central nervous system, and hydrocephalus. Gross lesions in BVDV-BDV infected aborted or stillborn fetuses are rarely reported. Cerebellar hypoplasia has been described in fetuses inoculated with BDV on day 34 of gestation. Fetal infection with BVDV at days 42 to 46 of gestation resulted in mild, lymphohistiocytic vascular and perivascular cuffs in the leptomeninges and choroid plexus, without apparent clinical consequence. Congenitally infected piglets may exhibit a variety of lesions similar to those observed in animals congenitally infected with CSFV including chronic gastroenteritis with ulceration, hemorrhages in the skin, lymph nodes, epicardium, and kidneys, necrotic tonsillitis, and thymic atrophy.

Several reports describe the comparative sensitivities of various testing methodologies for the diagnosis of CSF. These studies have typically focused on herd diagnostics and compared test sensitivities on antemortem samples or tissues harvested from postnatal pigs. Preferred methodologies and tissues for abortion diagnostics have not been thoroughly described.

In many localities, CSF is considered to be a transboundary animal disease (TAD). The choice of approved diagnostic tests and reagents may vary by country, and diagnostics are often carried out at a central reference laboratory. In the US, certified laboratories participating in the National Animal Health Laboratory Network (NAHLN) can screen for CSFV with a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) developed and validated by USDA's Agricultural Research Service at its Plum Island Animal Disease Center and by FADDL (Foreign Animal Disease Diagnostic Laboratory). All suspect or positive samples are sent to FADDL for confirmatory testing. CSFV shares common antigenic determinants with ruminant pestiviruses that can infect pigs. Infection of swine by ruminant pestiviruses can lead to false positive CSFV results due to cross-reactivity when using polyclonal antibody-based tests (FA, IHC) and serologic assays (virus neutralization). The use of more specific testing methodologies targeting CSFV-specific antigens or nucleic acids has greatly diminished this traditional concern.

Virus isolation is time consuming and less sensitive than other diagnostic methods, such as PCR. Nonetheless, VI is indispensable for building virus collections for use in differentiating CSFV strains based on virulence.

Appropriate fetal tissues for CSFV diagnostics include tonsil, kidney, spleen, lung, and placenta. The most widely used cell lines for virus isolation are porcine kidney cells, specifically PK15 and SK6 cells. Ruminant pestiviruses have been isolated from brain, spleen, lung, and kidney of fetuses on PK15 cells, but the ruminant pestiviruses are reported to grow to higher titers when isolated on ruminant cell lines. Since the vast majority of CSFV, BVDV, and BDV strains do not cause cytopathic effect, cell cultures must be fixed and stained for virus detection using either direct or indirect immunofluorescence or immunoperoxidase assays. Due to the close antigenic relationship between the ruminant pestivirus and CSFV, there is the potential for false positive CSFV results following fetal infection by BVDV or BDV. This can be overcome by using CSFV-specific monoclonal antibodies or a panel of monoclonal antibodies that can differentiate pestiviruses capable of infecting swine. A monoclonal antibody panel can also assist in tracing the likely epidemiological origins of a virus following an outbreak. Due to loss of infectivity, it may not be possible to recover virus from fetuses infected early in gestation.

Antigen detection may be carried out on fixed tissue cryosections using direct or indirect immunofluorescence tests or immunoperoxidase assays with monoclonal or polyclonal antibodies. These tests provide rapid results and are particularly valuable for the initial evaluation of suspect cases. Due to the frequent occurrence of nonspecific staining, low virus levels with some CSFV strains, and cross-reactivity with ruminant pestiviruses when using less specific reagents, FAT results may not be conclusive. When polyclonal or nonspecific reagents are used, positive results should be followed by a panel of monoclonal antibodies to differentiate the ruminant pestiviruses from CSFV. Preferred fetal tissues for CSFV diagnostics, in decreasing order of preference, are pancreas, tonsil, kidney, liver, lung, and brainstem. Tonsil is the

tissue of choice in postnatal swine, followed by spleen, kidney, mandibular lymph node, and the distal portion of the ileum.

Immunohistochemistry has primarily been evaluated in postnatal pigs where the highest level of antigen was detected in tonsil, spleen, and pancreas. Viral antigen has been identified in epithelial cells, macrophages and circulating monocytes, endothelial cells, lymphoid cells, and glial cells.

Commercially available CSFV antigen-capture ELISAs can be used for analyzing blood, plasma, serum, or tissue samples. This is a rapid, fully automated method that can provide results in 4 hours. Unfortunately, test performance is hampered by low sensitivity. Virus isolation and RT-PCR detect infection earlier in the course of disease and identify a greater proportion of infected pigs, when compared to antigen capture ELISA. This test can be used as a herd-screening tool but should not be used to establish a diagnosis of CSF in individual animals.

RT-PCR has been established as the most sensitive method for the detection of CSFV. When compared to virus isolation, viral nucleic acid can be detected earlier after infection, and for a longer period of time, PCR primers can be designed to amplify a broad range of pestivirus strains. BVDV, BDV, and CSFV can be reliably differentiated by restriction fragment length polymorphism (RFLP) analysis of the amplicons or through the use of separate primers specific for CSFV or the ruminant pestiviruses. Real-time procedures have been validated that are specific for CSFV, do not cross-react with other pestiviruses capable of infecting swine, and are applicable for the rapid detection of CSFV in large numbers of samples. RT-PCR is currently used as a surveillance tool to screen for CSFV in the US. Sequencing is a powerful tool for characterizing strains of CSFV. Sequencing of the variable regions of different isolates has made it possible to establish phylogenetic relationships among isolates that aid in tracing the origin and spread of the disease. Sequencing provides greater discrimination between isolates than monoclonal antibody analysis.

The most commonly used methods for antibody detection are virus neutralization and ELISAs. Pestiviruses share common antigenic determinants and serologic tests that detect anti-CSFV antibodies may cross-react with antibodies to ruminant pestiviruses, leading to false-positive results. Cross-reactions with antibodies to other pestiviruses are greatly reduced in the competitive ELISA format using CSFV-specific monoclonal antibodies against glycoprotein E2. These competitive ELISAs are able to distinguish pigs infected with CSFV from those infected by BVDV-BDV virus with a high degree of reliability. Nonetheless, in CSFV-free regions, positive serologic results are typically verified by additional testing. Comparative virus neutralization assays, such as the neutralizing peroxidase-link assay (NPLA) and the fluorescent antibody virus neutralization test, can be used to determine the infecting pestivirus. Antibody titers will be highest against the homologous virus, and in most cases, titers against heterologous pestivirus species will be considerably lower. However, CSFV challenge in swine previously infected with BVDV has resulted in higher titers against BVDV than CSFV, demonstrating that prior BVDV infection could lead to false-negative CSFV results.

CSFV is immunosuppressive, and virus-specific antibodies are usually not detectable until the third week post-infection. Additional shortcomings of CSFV serology include false-negative results in chronically infected animals in which antibodies have disappeared, and the inability to identify persistently viremic, congenitally infected pigs that seldom produce specific antibodies. Because of the above-described limitations, serology is most appropriately used on a herd basis when clinical signs have been present for more than 2 weeks. Maternal serology is of limited value in establishing a diagnosis of CSFV-, BVDV-, or BDV-associated reproductive disease.

Seroconversion will typically have occurred prior to evidence of reproductive disease, and it is generally not possible to determine whether titers resulted from previous exposure or were induced in association with *in utero* pestivirus infection. The utility of fetal serology has not been thoroughly investigated; however, in one study, seroconversion was not demonstrated in any of the CSFV-infected offspring. BVDV-BDV titers have been detected in fetuses, and fetal serology may provide evidence for *in utero* ruminant pestivirus infection.

Porcine reproductive and neurological syndrome (PRNS) and virus X

Since at least 1995, pork producers and swine practitioners have reported disease outbreaks characterized primarily by early infertility and neurological signs. The syndrome was initially termed “sow infertility syndrome” because it was typified by an acute decline in farrowing rate, increased early or delayed returns, and prolonged suboptimal farrowing performance. Affected animals confirmed pregnant at 30 days either aborted or demonstrated irregular returns to estrus. The neurologic manifestations of this disease were characterized by posterior weakness/paralysis, ataxia, bar chewing, and head pressing/banging. Neurologic signs typically occurred in nursery and fattening pigs, were progressive, and generally resulted in death within 2 to 3 days. A cytopathic agent that could pass through a 0.22- μ m membrane filter was consistently isolated from clinically affected animals and was tentatively named “Virus X.”

Sows experimentally infected with “Virus X” became viremic, embryonic, or fetal death was identified, and the virus was recovered from fetal tissues, demonstrating the ability to cross the placental barrier. Experimentally infected 7-week-old CDCD pigs did not develop clinical signs but did have microscopic lesions characterized by nonsuppurative meningoencephalitis, nonsuppurative interstitial pneumonia, and nonsuppurative periportal hepatitis. “Virus X,” currently referred to as PRNSV, is proposed to be a novel swine pestivirus that awaits further characterization.

PRNSV is most frequently isolated from tonsils, spleens, and lymph nodes of pigs with neurologic signs and can be recovered from serum and the brain. The cytopathic virus can be isolated from a variety of fetal tissues including lung, spleen, heart, and kidney. In cell culture or frozen tissue sections, the virus can

be recognized to a degree by the polyclonal antiserum specific for BVDV (polyclonal antiserum for ruminant/caprine pestivirus antibodies 440-BDV 9001) from the National Veterinary Services Laboratory (Ames, IA, USA) followed by application of an anti-bovine conjugate or swine sera collected from naturally or experimentally infected pigs followed by incubation with FITC-labeled anti-porcine IgG.

Japanese encephalitis virus (JEV)

Japanese encephalitis virus (JEV), a member of the genus *Flavivirus*, is an important, zoonotic, mosquito-borne disease that causes encephalitis in humans, horses, and piglets and abortion in sows. JEV is naturally maintained in a mosquito-bird-mosquito cycle. The virus is distributed in temperate and tropical areas throughout Southeast Asia, the Indian subcontinent, Indonesian archipelago, and the Australasian zoogeographical region. JEV is not currently present in the US, and the potential for JEV to spread to the Americas, Europe, or Africa is considered to be limited due to the long distance from endemic areas.

Pigs are the only mammals that are significant in the transmission cycle of JEV. They serve as amplifying hosts and can be important in the pre-epizootic amplification of JEV leading to vector-borne transmission to humans. Because this is a mosquito-borne virus, the occurrence of clinical disease typically corresponds with the mosquito season.

Adult, nonpregnant swine typically do not exhibit overt clinical signs following infection. Reproductive failure occurs in non-immune females that become infected prior to 60-70 days of gestation. Pregnant sows may abort, produce mummified fetuses, or give birth to stillborn or weak piglets. Abortion rates of 5.4-34% have been reported.

Litters from infected sows contain fetuses that are mummified and dark in appearance. Subcutaneous edema, hydrocephalus, cerebellar hypoplasia, bicavitary effusions, serosal petechia, and necrotic foci in the liver and spleen have been described in infected fetuses.

Histological lesions are largely restricted to the central nervous system. Diffuse nonsuppurative encephalitis with neuronal necrosis, neuronophagia, gliosis, and spinal hypomyelination have been described in cases of JEV abortion.

Virus has been isolated from brain, liver, spleen, lung, and placenta of affected fetuses. The virus may be isolated on cultures of porcine or hamster kidney cells, the African green monkey kidney (Vero) cell line, and mosquito cell lines. In cell culture, JEV will cause cytopathic effect, and the identity of the isolate can be confirmed by indirect immunofluorescence using a JEV specific monoclonal antibody against E protein.

Immunohistochemistry (IHC) can be used to confirm JEV involvement in piglets with encephalitis as well as aborted fetuses. In minimally autolyzed fetuses, viral antigen has been identified in the cytoplasm of neurons and neuronal processes in the cerebral hemisphere, thalamus, and olfactory bulb. Viral antigen was detected in relatively intact nerve cells but not in the necrotic lesions.

RT-PCR and nested RT-PCR has been used to detect viral nucleic acid in tissue and blood samples.

Antibodies to Japanese encephalitis virus (JEV) can be detected by hemagglutination inhibition (HI), virus neutralization, or ELISA tests. Serology on dams bearing affected litters is generally not diagnostic. Antibody is common in endemic regions, and it is difficult to demonstrate titer changes in females with affected litters because seroconversion has typically occurred prior to abortion.

Infected fetuses will generally produce a detectable antibody response. The demonstration of antibodies in the serum, thoracic, or abdominal fluid of stillborn piglets is diagnostic.

Porcine cytomegalovirus (PCMV)

Porcine cytomegalovirus (suid herpesvirus 2) is a betaherpesvirus. PCMV is distributed worldwide and is ubiquitous in most swine populations where up to 98% of pigs may be seropositive in infected herds. PCMV is most commonly associated with "inclusion body rhinitis," where infection of the nasal mucosa in young pigs results in sneezing, nasal discharge, coughing, and poor weight gain.

In postnatal swine, the primary site of viral replication is the nasal mucosa. This is followed by a cell-associated viremia, during which time PCMV may cross the placenta and infect the embryo or fetus. Infection of the developing embryo has been demonstrated in sows exposed 48 hours postcoitus. Inoculation of sows between days 31 and 85 of gestation led to fetal infection with fetal death typically occurring 28-42 days post-maternal exposure. The consequences of *in utero* infection include decreased litter size, fetal mummification, stillbirths, and congenitally infected piglets, which tend to die within the first few weeks of life.

Affected sows may exhibit a brief period of afebrile depression and anorexia. The reproductive manifestations include decreased litter size accompanied by increased numbers of mummified fetuses and stillbirths. Litters from sows experimentally infected with PCMV at various stages of gestation contained 30% (18/60) mummies, and 27% (16/60) of liveborn pigs were congenitally infected. Prewaning mortality is generally increased. Congenitally infected piglets may exhibit stunting, pneumonia, rhinitis, and typically die. PCMV infection of the newborn can result in fatal generalized disease.

The majority of infected fetuses are grossly normal. Interlobular pulmonary edema is occasionally observed. Cytomegaly and basophilic intranuclear inclusions may be seen in the liver and/or lung of better-preserved fetuses. Placental lesions have not been identified, and virus has not been isolated from placenta.

In both the fetus and neonate, PCMV targets the capillary endothelium, and endothelial damage may result in hemorrhage and edema. In neonates, gross lesions may include enlarged edematous lymph nodes, subcutaneous edema, interlobular pulmonary edema, pleural and pericardial effusion, and petechial hemorrhages in the kidneys, heart, lymph nodes, and intestines. Histologically,

intranuclear inclusion bodies may be observed in endothelial cells in liver, lung, and less frequently in the spleen, kidney, nasal mucosa, and brain. Inclusions are also present in hepatocytes and pulmonary macrophages. Neonates may occasionally develop hepatic necrosis, renal tubular necrosis, and/or a non-suppurative meningoencephalitis.

PCMV can be isolated on porcine alveolar macrophages and porcine fallopian tube cell lines. Cytopathic effect, typically involving only a small proportion of the cells, appears from 11 to 18 days post-inoculation. Cells become progressively enlarged (cytomegaly), multinucleate cells are common, and intranuclear inclusions can be observed. In the fetus, lung appears to be the tissue of choice for diagnostics. Virus has also been recovered from spleen, and brain. PCMV has been isolated from lung, liver, and kidney of congenitally infected neonates.

The virus has a predilection for fetal lung where it has been identified in monocytes and capillary endothelium by indirect immunofluorescence. In the embryo, the virus is reported to localize in leptomeningeal cells, hepatic sinusoidal cells, peritoneal macrophages, periosteal cells, and occasional alveolar cells.

Though the literature does not describe the use of PCR to assess PCMV infection in fetal tissues, PCR has proven to be a sensitive and specific method for detecting PCMV in a variety of samples from postnatal pigs.

The serologic response to PCMV has been measured by indirect FA and ELISA. The latter method appears to be the more sensitive technique. Due to the ubiquitous presence of PCMV in most swine herds, seroconversion is common, and sow serology may be difficult to interpret in suspect cases of PCMV abortion.

Fetal serology can provide evidence for *in utero* infection by a number of viruses. Fetal serology does not appear to be useful for the diagnosis of PCMV abortion because seroconversion was not demonstrated in congenitally infected fetuses using either IgG or IgM antiglobulin preparations.

Paramyxovirus abortion

Until recently, economically important porcine paramyxovirus infection had not been described. The discovery of La Piedad-Michoacan virus in Mexico and outbreaks of Menangle and Nipah viruses has changed our appreciation of Paramyxoviruses as pathogens of swine. Fruit bats likely represent the initial source of infection for each of these viruses.

Porcine rubulavirus (La Piedad-Michoacan virus)

Outbreaks of encephalitis and corneal opacity were first reported in 1981 in central Mexico. The corneal changes lead to the term "blue eye disease," which is caused by La Piedad-Michoacan virus (LPMV), a member of the family Paramyxoviridae, genus Rubulavirus. Disease caused by LPMV is clinically distinctive and characterized by neurologic signs in young pigs, corneal opacity in pigs of all ages, epididymitis and orchitis in boars, and reproductive

failure in the breeding herd. Following experimental infection by a natural route, studies have demonstrated that LPMV can cross the placenta of non-immune females and cause fetal death and mummification. To date, the disease has not been identified outside of Mexico.

Clinical signs are variable and age dependent because of an apparent age-related resistance to severe clinical illness. Piglets 2 to 15 days old are most susceptible. These animals develop a fever, rough hair coat, and an arched back. This is quickly followed by progressive neurologic signs, which include ataxia, weakness, rigidity, muscle tremor, abnormal posture, and death, often within 48 hours. Morbidity in suckling pigs is high (20-50%) and mortality can reach 87-90% in affected animals. In the breeding herd, sows may be anorexic for 1-2 days and occasionally develop corneal opacity, but most infected sows remain clinically normal. Reproductive disease is characterized by signs of infertility manifest as increased returns to estrus, which may persist for up to 8 months, decreased farrowing rate, an increased wean-to-service interval, and an increase in nonproductive sow days. These signs are accompanied by stillbirths, mummified fetuses, and infrequent abortion.

Aborted litters often consist of a mixture of mummified fetuses, and those with dermal ecchymoses randomly interspersed with normal fetuses. Piglets with neurologic signs typically develop nonsuppurative interstitial pneumonia, anterior uveitis with corneal edema, and nonsuppurative encephalomyelitis characterized by lymphoplasmacytic perivascular cuffs, multifocal gliosis, neuronal necrosis, neuronophagia, meningitis, and choroiditis with intracytoplasmic neuronal inclusions.

Procedures to establish a herd diagnosis have been reported, but specific fetal diagnostics are not well described. The virus can be isolated on a variety of cell lines, but primary pig kidney or PK-15 cell lines are preferred. Virus has been recovered from brain, lung, liver, and placenta of aborted fetuses. LPMV was only isolated from 44% of litters from experimentally infected females and from 12-64% of fetuses from infected litters. Tonsil, lung, and olfactory bulb are the tissues of choice for virus isolation from piglets. In cell culture, LPMV causes cytopathic effect with syncytia formation. Direct immunofluorescence can be used to confirm LPMV.

Immunostaining for viral antigens in tissue impression smears has been used as a rapid diagnostic test in young pigs. Lung, olfactory bulb, and mid-brain are the tissues of choice in these animals. Virus isolation was demonstrated to be slightly more sensitive than immunostaining.

RT-PCR has been used to detect viral genomic RNA in brain and lung. PCR appears to be the most sensitive method for detecting LPMV in chronically infected animals. Viral genomic RNA was demonstrated in the midbrain of convalescent pigs with RT-nested PCR, while infectious virus or viral antigen could not be detected with virus isolation or immunostaining.

Serologic tests include hemagglutination inhibition, virus neutralization, indirect immunofluorescence, and ELISA assays. Each of these tests is able to detect seroconversion by the eighth day post-infection. A blocking

ELISA, based on a monoclonal antibody against the LPMV hemagglutinin-neuraminidase glycoprotein, has been developed that has a sensitivity of 99% and a specificity of 97%. This blocking ELISA currently represents the most suitable method for screening large numbers of samples. Because sows have generally seroconverted by the time reproductive wastage is evident, serology cannot be used to establish the cause of abortion in individual animals. Collection of acute and convalescent samples during the course of an outbreak can confirm participation by LPMV.

Menangle virus

Menangle virus, a member of the family Paramyxoviridae, genus Rubulavirus, was isolated from stillborn fetuses during a single outbreak of reproductive disease in Australia in 1997. Menangle virus produces subclinical infection in sows and growing pigs and distinctive reproductive disease in the breeding herd typified by poor farrowing performance, fetal mummification, abortions, and stillbirths with characteristic congenital deformities. Neutralizing antibodies against Menangle virus were detected in the serum of affected pigs, in two humans in contact with infected pigs, and in fruit bats found in the vicinity. The fruit bat is considered to be the reservoir host for Menangle virus.

Clinical signs during the outbreak were limited to reproductive problems characterized by decreased farrowing percentages, reduced litter size, and reduced number of live-born piglets, accompanied by increased numbers of mummies and stillborn fetuses, which often had congenital anomalies. Several litters contained only one or two mummified fetuses or stillborn piglets, indicating that many embryos may have died and been resorbed before 35 days of gestation. The most important effects on reproductive performance were due to reduced farrowing percentages, litter sizes, and numbers of piglets born alive.

Affected litters typically consist of a mixture of mummified fetuses, autolyzed and fresh stillborn piglets, and a few normal liveborn animals. Stillborn fetuses often had severe malformations including arthrogryposis, brachygnathia, kyphosis, a slightly domed cranium, pulmonary hypoplasia, and a variety of CNS abnormalities. Gross CNS lesions included a marked reduction in size of the cerebellum, cerebral hemispheres, brain stem, and spinal cord, as well as porencephaly and hydrancephaly. The main skeletal defects were arthrogryposis and craniofacial deformities, which included mandibular brachygnathia and doming of the skull. Excessive amounts of pale yellow or blood-tinged fluid in the body cavities, occasionally containing strands of fibrin, epicardial hemorrhages, and subcutaneous oedema were also observed.

Microscopic lesions in stillborn fetuses are most dramatic in the central nervous system. There can be extensive degeneration of both gray and white matter in the brain and spinal cord with associated inflammation. Microscopic lesions varied from degeneration and necrosis of individual neurons in the brain and spinal cord to extensive neuronal necrosis with liquefaction of the neuropil and infiltration of Gitter cells. Multifocal to locally extensive gliosis, perivascular cuffs of lymphocytes and macrophages in the brain and spinal

cord, and occasionally mild nonsuppurative leptomeningitis can be seen. Neurons and microglial cells of the brain and spinal cord may contain eosinophilic intranuclear and intracytoplasmic eosinophilic inclusion bodies. Multifocal nonsuppurative myocarditis and hepatitis are less common features.

The Menangle virus can be isolated on baby hamster kidney cells (BHK 21). Brain, lung, and heart from fresh stillborn fetuses with evidence of cerebral degeneration are the preferred materials for diagnostics. The virus induces CPE in culture. Virus isolation is less reliable than serology because it may take three to five passages before virus is recovered.

The presence of congenital malformations in stillborn piglets distinguishes Menangle virus from most other causes of reproductive wastage, with the exception of Japanese encephalitis virus. Because there has been only a single report of Menangle virus-associated reproductive disease, the most rapid method of assessing potential Menangle virus involvement is serology on aborting sows using serum neutralization assays. Females bearing affected litters will have high titers at the time of abortion. Although the diagnostic sensitivity of fetal serology has not been extensively evaluated, detection of specific antibodies in the fetal thoracic fluid of stillborn piglets is diagnostic for *in utero* infection.

Bacterial and fungal abortion in swine

A wide variety of bacteria, and occasional fungi, have been isolated from the products of porcine abortion. It cannot be assumed that all recovered bacteria and fungi are causative because contamination of the fetus and placenta can occur during passage through the birth canal, from the environment, or during postmortem examination. Isolation of numerous organisms in relatively pure population from several fetuses in a litter, along with the identification of incriminating lesions lends strong support for a causal link between an isolate and abortion. Conversely, it cannot be assumed that negative culture results exclude bacteria and fungi from consideration because there are a number of microbes that cannot be recovered using routine techniques.

Theoretical mechanisms of bacterial-induced abortion include the following: (1) preexisting low-grade metritis at conception; (2) ascension through the cervix; (3) fetoplacental infection following maternal bacteremia; and (4) the physiologic effects of bacteria-induced maternal illness. Of all the listed mechanisms, maternal bacteremia leading to infection of the fetoplacental unit is generally considered to be the most common. Excluding abortion due to the physiologic effects of maternal illness, the offending organism can potentially be recovered from the fetoplacental unit in all other mechanisms. If the sow is systemically ill at the time of abortion, samples from dead or aborting sows should be harvested to assess whether abortion was the result of systemic illness in the sow. Bacteria that cause sepsis in adult pigs, such as *Erysipelothrix rhusiopathiae* and *Actinobacillus suis*, have the potential to induce abortion as a result of either maternal illness or direct fetoplacental infection.

In a recent retrospective study, bacteria accounted for 6.4% of porcine abortions, including 21.8% of abortions in which an infectious agent was identified. (See Table 3 in Appendix A.) Fungi reportedly account for 0.3% of porcine abortions. Bacteria isolated from aborted fetuses can be broadly divided into three categories: (1) primary contagious causes of abortion (abortion is the principal sign of infection); (2) secondary contagious causes of abortion (abortion is a by-product of maternal infection); and (3) noncontagious bacterial causes of abortion. As can be inferred from the preceding categories, the herd health significance of various bacterial species differs considerably.

The majority of bacteria isolated from aborted porcine fetuses are category 3 organisms. In other words, they are not considered to be contagious pathogens of adult swine, are isolated from sporadic, individual animal abortions, and are generally of minimal herd health significance. Examples include *E. coli*, *A. pyogenes*, and *S. aureus*. *Leptospira* serovars and *Brucella suis* are the principal, category 1, primary, abortion-causing bacteria of swine. Reproductive disease associated with *Leptospira* infection in pigs is currently uncommon. Regulatory programs based on serologic testing and removal of seroreactive herds have led to the virtual elimination of *Brucella suis* from domestic populations in the US, northern Europe, and Australia.

Contagious bacteria of adult swine that are capable of causing a bacteremia may result in outbreaks of abortion. These include *E. rhusiopathiae*, *Actinobacillus suis*, and *Salmonella spp.* The herd health significance of additional contagious and potentially abortifacient bacteria can be difficult to classify because expression of disease in the breeding herd is dependent on both the pathogenic potential of the organism and the level of herd immunity. Management changes intended to produce high health pigs may result in breeding stock that lack immunity to contagious pathogens of nursery and grow/finish pigs. Naïve sows may suffer outbreaks of bacteremia/septicemia, with subsequent abortion, due to agents that have traditionally caused disease in younger swine. When faced with an outbreak of abortion, if a potentially contagious bacterium has been isolated (such as, *S. hyicus* ss. *hyicus*), but the herd health significance is unclear, then multiple aborted litters should be evaluated. An organism isolated from multiple abortions has the greatest herd health significance.

The majority of bacteria linked to porcine abortions are isolated with routine procedures. However, the identification or recovery of a number of bacterial species implicated as causes of porcine abortion, including *Leptospira*, *Campylobacter*, *Arcobacter*, *Mycoplasma*, and *Mycobacterium* species, require special procedures. In an abortion outbreak where routine diagnostics have failed to establish a cause and placentitis, fetal pneumonia, or both have been identified in more than one submission, it is advisable to broaden the search and employ additional diagnostic techniques.

Clinical signs vary depending on the bacteria involved. Organisms that cause sporadic bacterial abortion rarely result in noteworthy clinical signs in the sow, other than abortion. When abortion is a consequence of maternal illness, clinical signs may include fever, depression, anorexia, cutaneous erythema, diamond skin lesions (erysipelas), and possibly lameness due to arthritis. When a sow

aborts during the acute, febrile phase of the illness, diagnostics should focus on the sow, as fetuses are unlikely to be infected at this stage of the disease. When abortion is a consequence of fetal infection, signs of a mild systemic illness may have been observed 2–4 weeks prior to the abortion during the bacteremia phase of the infection.

Gross lesions are uncommon in bacterial abortions. Gross lesions reported in 50 randomly selected, bacterial abortion cases submitted to the ISU VDL from 1/2003 to 1/2010 were tabulated. In 3 cases (6%), a scant, fibrinous exudate was identified on the surface of the lung and/or heart. In 2 cases (4%), the placenta appeared slightly thickened and was covered by small amounts of cream-colored exudate. In a single case (2%), multiple, random, pinpoint white/tan foci were identified in the liver of an animal with evidence of systemic bacterial infection. This lesion is uncommon and necessitates including PRV as a differential (Figure 4.5).

Based on the same study referred to above, the most common microscopic lesions of bacterial abortion included suppurative placentitis, suppurative fetal bronchopneumonia, and the identification of monomorphic colonies of bacteria in fetal lung with or without associated inflammation. Heedless of the value of placenta in providing supportive evidence for a diagnosis of bacterial abortion, placentas were only submitted in 38% of these cases.

Preferred tissues for culture include fetal lung and stomach contents and to a lesser extent liver. When submitted, placenta should also be cultured. Isolation of bacteria from the placenta is problematic because it commonly becomes heavily contaminated. Recovery of bacteria from the placenta is unlikely to be significant in the absence of placentitis.

Leptospiral abortion

Leptospirosis is caused by a group of antigenically and genetically distinct aerobic spirochetes belonging to the genus *Leptospira*. Pathogenic leptospires cause disease in humans and a wide range of animal species, including swine. The chief manifestation of leptospirosis in swine is reproductive disease characterized by infertility, abortion, stillbirths, and the birth of weak piglets. The epidemiology of leptospirosis is potentially complex, due to the existence of numerous serovars that vary in their pathogenicity for different species, the ability of *Leptospira* to cause acute and chronic disease, the existence of host adapted strains, and the opportunity for incidental infection from reservoir species. Swine may act as maintenance hosts for a variety of serovars including the *Pomona*, *Australis*, and *Tarassovi* serogroups. Pigs can also become infected by serovars maintained in other species and serve as incidental hosts.

Leptospira persist and multiply in the kidneys, are shed in urine for long intervals, and can survive for extended periods in a warm, moist environment. The primary mode of transmission is contact with voided urine from an infected host. *Leptospira* infection can be introduced into a herd by the introduction of infected swine, contact with nonswine *Leptospira* carriers, or exposure to a contaminated environment or water source. Contact by

Leptospire with abraded skin or the mucous membranes of the eye, mouth, nose, or genital tract leads to infection, which is followed by leptospiremia. The organism then localizes in the kidney and the uterus of pregnant females.

The seroprevalence of *Leptospira* serovars in swine has decreased dramatically over the past 30 years. This is likely the result of management changes that have interrupted transmission of the organism. Raising pigs in confinement with attention to rodent control, limiting exposure to potential wildlife reservoirs, and the elimination of access to water runoff or unsafe water sources have all likely contributed to this decreased incidence. Since the introduction of carrier pigs is often the source of infection, *Leptospira* testing in quarantine prior to introduction into the breeding herd is an important biosecurity measure. In a recent outbreak of *Leptospira* abortions, the initial source was likely an unsafe water supply. Spread of disease to multiple herds was the result of movement of infected pigs.

Overall, *Leptospira* spp. are reported to contribute to 3-6% of porcine abortions. Analysis of ISU VDL records from 1/2003 to 1/2010 indicates that *Leptospira* abortion is currently uncommon, accounting for only 1.7% (24/1,376) of total abortions. Even this number may overrepresent the importance of *Leptospira* serovars as a cause of porcine abortion because 74% (17/24) of these cases were submissions from a single large outbreak.

During the period of leptospiremia, sows may exhibit transient fever, anorexia, and depression. In the breeding herd, reproductive disease is typically the only clinical manifestation, and abortion has been reported to occur from 19-73 days following maternal infection. Leptospirosis has been associated with infertility, mummies, abortion, stillbirths, and weakborn piglets, the specifics varying with the offending serovar. During outbreaks, abortion in up to 19% of pregnant sows has been reported.

Aborted fetuses are occasionally jaundiced, and may have patchy hepatic necrosis and increased amounts of serosanguinous fluid in the body cavities. Petechiation of the skin, lungs, and kidneys has been described.

The following microscopic lesions were reported in a series of 16 confirmed leptospiral abortions submitted to the ISU VDL. Lesions were most consistently observed in the kidney and consisted of multifocal cortical hemorrhage, mild nonsuppurative interstitial nephritis, and/or degeneration and necrosis of tubular epithelial cells with scant, intratubular accumulations of neutrophils. Lung, liver, and placental lesions were each identified in a quarter of the cases. Small accumulations of neutrophils were seen within alveolar spaces and small airway lumina. Lymphocytes, plasma cells, and lesser numbers of neutrophils formed focal infiltrates in placentas. A multifocal suppurative to necrosuppurative hepatitis was observed in four cases. Nearly one-third of the cases were without microscopic lesions.

Kidney is the tissue of choice for fetal leptospiral diagnostics, but leptospire may also be detected in the liver and placenta. Laboratory procedures commonly used to diagnosis leptospiral abortion include FA, IHC, and PCR. Autolysis, which is common in aborted fetuses, can negatively impact both FAs and IHC because the organisms may become fragmented. Reports indicate

that PCR is currently the most sensitive and specific test on clinical specimens. None of these testing methods currently identify the offending serovar.

Culture of *Leptospires* is time consuming, costly, not routinely available, and of limited sensitivity due to reduced viability of organisms secondary to autolysis. PCR-based assays have been developed for typing *L. interrogans*, which do not require isolation of the organism from clinical specimens and provide more rapid results than culture-based techniques.

Leptospires, like many other causes of abortion in swine, are not typically identified in all fetuses in a litter. In one study, *Leptospires* were only detected in 12 of the 22 piglets (55%) in three aborted litters, again emphasizing the need to submit tissue from more than one fetus to increase the likelihood of establishing a diagnosis.

The Modified Agglutination Test (MAT) is the most widely used serologic assay. Titers may reach 1:400 to 1:800 following vaccination, and accurate interpretation requires an understanding of vaccination history. Since antibody titers from immunization are short lived, typically decreasing below 1:400 within 60 days of vaccination, a titer of 1:400 in a pig that has not been recently vaccinated is considered positive for leptospiral infection. Following abortion due to acute infection, titers are typically much higher. Because of cross reactions, MAT titers to several serovars will generally be elevated, with the highest titers representing the offending strain (Table 4.5). Titers do not persist for long periods of time and may fall below 1:800 within several weeks.

It is proposed that abortion may result from acute or chronic infection. When abortion follows acute infection, sows will typically have high titers at the time of abortion. Serology is less useful in diagnosing abortion due to chronic infection of the urogenital tract where titers may be low ($\leq 1:100$) at the time of abortion.

Brucella suis

The genus *Brucella* is composed of a group of genetically similar Gram-negative, facultative, intracellular bacteria, which can infect over 80 domestic and wild animal species. *B. suis* was first isolated from swine, but has also been identified in reindeer, caribou (*Rangifer tarandus*), hares, and murine species. Two *Brucella* species (*B. suis*, *B. abortus*) and two vaccine strains (S19, RB51) have been isolated from naturally infected swine. In animals, *Brucella* spp. primarily infect the lymphoreticular system and the male and female reproductive tracts. Four members of the genus *Brucella*, including *Brucella suis*, are zoonotic and produce a disease in humans referred to as undulant fever.

Swine brucellosis has a worldwide distribution, occurring in both domestic and feral swine populations. In the US, porcine brucellosis was recognized as a major disease during the 1920s to 1950s. Changes in management, combined with regulatory programs based on serologic testing and removal of seroreactive herds, have lead to the virtual elimination of this disease from domestic populations in the US, northern Europe, and Australia. In both the

Table 4.5 Leptospira Modified Agglutination Test (MAT) titers in aborting sows from a herd with confirmed *Leptospira Pomona* infection (ISU VDL database, 2008).

Animal ID	Lepto Bratislava Titer/Result	Lepto Canicola Titer/Result	Lepto Grippio Titer/Result	Lepto Hardjo Titer/Result	Lepto Ictero Titer/Result	Lepto Pomona Titer/Result
Sow A	1,600 / *	<100 / Neg	800 / *	1,600 / *	400 / *	≥12,800 / *
Sow B	800 / *	<100 / Neg	800 / *	<100 / Neg	200 / *	≥12,800 / *
Sow C	200 / *	<100 / Neg	100 / *	100 / *	100 / *	1,600 / *
Sow D	800 / *	<100 / Neg	400 / *	200 / *	200 / *	≥12,800 / *
Sow E	400 / *	400 / *	200 / *	400 / *	100 / *	6,400 / *

A sample with the star (*) indicates it has an antibody titer that may indicate the animal was exposed to the pathogen or antigen.

southeastern United States and Australia, feral swine are heavily infected. Feral swine have been reported in 27 of 50 states in the US, and brucellosis has been documented in these populations in at least 14 states. Because the number of feral swine has increased dramatically in recent decades, due to natural increases and the establishment of new populations for the purpose of hunting, introduction of *B. suis* from feral reservoirs is a significant concern. All recent US *B. suis* outbreaks in domestic herds have been linked to feral swine.

Pigs of all ages can be infected with *B. suis*, and the alimentary tract is considered to be the most common portal of entry. *Brucella suis* may be present in the semen, sometimes in the absence of clinical signs, and can be spread by artificial insemination. In swine, venereal transmission is more common than with brucellosis in ruminants. Of the *Brucella* species, *B. suis* is most noteworthy for causing chronic and persistent infections.

Infection in many herds is inapparent. *Brucella suis* affects the female reproductive tract, the primary and secondary reproductive organs of the male, the lymphoreticular system, bone marrow, and joints. Clinical signs may include infertility, abortion at any stage of gestation, stillborn and weakborn piglets, orchitis, posterior paralysis, and lameness. Early abortions may appear clinically as infertility.

The placenta may be congested, with focal hemorrhages, edema, and a thin surface exudate. Subcutaneous edema and serosanguinous fluids in the body cavities may be observed in the fetus.

Microscopic lesions in the fetoplacental unit consist of a purulent placentitis with or without necrosis, a diffuse, mild to moderate, purulent interstitial pneumonia and purulent, portal hepatitis.

The diagnostic "gold standard" continues to be isolation followed by characterization. Theoretically, *Brucella suis* can be grown on blood agar in the absence of added CO₂. However, because the products of abortion are often contaminated by additional microorganisms, the use of *Brucella* selective media is recommended because this will diminish contaminant overgrowth of this slow-growing bacterium. Inoculated plates should be incubated at 37°C in 5% CO₂ for 7 days. Preferred tissues for culture include vaginal discharge, placenta, and most fetal tissues (stomach content, liver, lung, and pericardial fluid). The strong, almost immediate urease activity of *B. suis* easily allows its differentiation from *B. abortus* and most *B. melitensis* isolates.

PCR techniques have been employed as a rapid method of identifying cultured *Brucella* species, since classical typing methods are often time-consuming, hard to standardize, and require high-level biosafety containment. Several studies have evaluated the use of PCR to identify *Brucella* directly from tissue. To date, the sensitivity of PCR assays has generally been inferior to bacteriologic culture due to the co-purification of PCR inhibitors with DNA and interference by excessive host DNA. Assays have been developed that may exceed the sensitivity of direct culture, but these are generally not available in a routine veterinary diagnostic setting. Tissues similar to those described for culture are generally appropriate for PCR.

Immunohistochemistry and FA techniques have been used to diagnose *B. suis* infection in swine. These techniques are not widely available and have generally been found to be inferior to bacterial culture.

A variety of serologic tests have been used on swine, including fluorescence polarization, standard tube agglutination, and card agglutination assays. The serologic assays used in the diagnosis of *B. suis* infection use the O-polysaccharide side-chain of the lipopolysaccharide (LPS) molecule from *B. abortus*, and none are designed specifically for *B. suis*. However, in studies comparing serologic assays using *B. suis*-specific antigens to tests with standard *B. abortus* antigens, appreciable differences were not identified.

Serology is generally adequate as a herd diagnostic tool, but because of variations in the stage of disease in individual animals and low-test sensitivity and specificity, none of the conventional serologic assays are reliable for establishing a diagnosis in individual pigs. A recent study illustrates the limitations of *B. suis* serology. When serum samples collected at necropsy were compared with necropsy culture results, the combined sensitivity of the three serologic assays mentioned above was 54.1%. The sensitivity rates for individual assays were 13.1% for the card test, 44.6% for the standard tube test, and 42.6% for the fluorescence polarization assay.

***Chlamydia* and *Chlamydophila* spp.**

The family Chlamydiaceae consists of a diverse group of obligate intracellular bacterial pathogens. Several chlamydial species have been reported to infect pigs including *Chlamydia suis*, *Chlamydophila psittaci*, *Chlamydophila abortus*, and *Chlamydophila pecorum*. The impact of Chlamydiaceae on swine is controversial due to the high prevalence of chlamydiae in clinically normal swine herds and relatively few descriptions of acute clinical illness. Chlamydial infections in sows have been associated with abortion, infertility, the occurrence of MMA-syndrome (mastitis, metritis, agalactia), and perinatal mortality in piglets. Reports have also linked chlamydiosis in swine with enteritis, pneumonia, conjunctivitis, orchitis, and pericarditis. The detection of chlamydiae in the semen of boars suggests a potential for venereal transmission. The association between *Chlamydia* spp. and reproductive disease is largely retrospective, based on the identification of the organism in aborted fetuses, the reproductive tracts of sows with reproductive failure, a high seroprevalence on breeding farms experiencing reproductive problems, and a favorable response to antibiotic treatment in herds with reproductive disease. To the author's knowledge, neither infertility nor abortion has been experimentally reproduced with Chlamydia in swine.

Chlamydia is believed to be widespread in swine herds, often without causing clinical symptoms. The role of chlamydiaceae in reproductive disorders of

pigs is currently unclear, though investigators, primarily in Europe, have concluded that *Chlamydia* spp. may play an important role in reproductive disorders of swine.

Gross lesions have not been described. Microscopic lesions consist of mild, periportal infiltrates of neutrophils and eosinophils in fetal livers. A predilection for the chorionic epithelium has been demonstrated with abortigenic chlamydia of ovine origin. Following experimental infection of pregnant sows with an ovine abortigenic strain, *Chlamydia* was identified in placental tissues accompanied by a placentitis, but reproductive consequences were not demonstrated.

Chlamydial diagnostics generally lack species specificity because most testing methods use genus-specific reagents. This is not necessarily a disadvantage because several chlamydial species have been isolated from pigs.

IHC has the advantage of allowing for a correlation between the presence of the agent and specific lesions. IHC has been used to identify *Chlamydia* in association with lesions in the liver and placenta. The use of genus-specific antibodies will allow for the detection of several species of *Chlamydia*.

Isolation in embryonated chicken eggs or cell culture has been regarded as the standard method for diagnosis. However, isolation has a number of drawbacks. Culture is time consuming. Many strains are difficult to grow and require special facilities and expertise. Degradation during fetal autolysis and transport may cause false negative culture results since recovery depends on the viability of this fragile organism. Because of these difficulties, culture is not routinely used.

Commercially available antigen capture ELISAs were developed and marketed for the detection of human *Chlamydia trachomatis*. These tests target chlamydial lipopolysaccharide (LPS) and will detect the genera *Chlamydia* and *Chlamydophila*. These tests have limited sensitivity, generally requiring 10^3 to 10^7 inclusion-forming units (ifu) for a positive result. Specimens of choice for these tests include uterine discharge and/or placental swabs.

Several studies have documented the superior sensitivity of PCR over all other testing methods. Primers have been developed for the *Chlamydia* genus outer membrane protein 1 (OMP1) that will detect all strains of *Chlamydia* of veterinary importance, including *Chlamydophila* (*abortus*, *psittaci*, *felis*, *caviae*, *pecorum*) and *Chlamydia* (*suis*, *trachomatis*, *pneumoniae*). More specific methods have been developed to differentiate the major groups among the nine species (*C. psittaci*, *C. abortus*, *C. felis*, *C. caviae*, *C. pecorum*, *C. pneumoniae*, *C. trachomatis*, *C. suis*, *C. muridarum*).

In general, serologic tests detect genus-specific antibodies, and seropositivity might be the result of a previous or concurrent extragenital chlamydial infection. Serologic tests appear to be of limited value for diagnosing chlamydial abortion unless seroconversion is demonstrated in paired samples, which is unlikely because seroconversion has generally occurred by the time of abortion. Comparative serum neutralization, in the presence of complement, may assist in species differentiation.

Miscellaneous causes of abortion and neonatal loss

Carbon monoxide

Carbon monoxide (CO) is a colorless, odorless, tasteless, non-irritating gas. It is produced by the incomplete combustion of hydrocarbon fuels when the supply of oxygen is inadequate to enable complete oxidation to carbon dioxide. CO combines reversibly with hemoglobin, with an affinity 200-230 times that of oxygen, to produce carboxyhemoglobin (COHb), decreasing the oxygen-carrying capacity of blood. The binding of carbon monoxide also increases the affinity of hemoglobin for oxygen, resulting in impaired oxygen delivery to tissues. It is assumed that the mechanism of CO toxicosis is systemic tissue hypoxia, but this mechanism, by itself, does not explain much of the published *in vivo* and clinical data, indicating that carbon monoxide has additional adverse effects for which the mechanisms are currently unclear.

Dissolved CO in the maternal bloodstream diffuses across the placenta and combines with fetal hemoglobin, which has a higher affinity for CO than maternal hemoglobin. Outbreaks of abortion and stillbirths have been associated with environmental CO concentrations between 120 and 260 ppm. Experimentally, stillbirths have been produced with CO concentrations at or above 250 ppm.

CO poisoning typically occurs in confinement facilities following the onset of cold weather and the initiation of supplemental heating in buildings with decreased ventilation. Carbon monoxide levels increase in a facility due to a combination of increased production (improperly maintained and/or adjusted, hydrocarbon-fueled heaters) and/or decreased elimination (improper venting of exhaust, decreased building ventilation).

Clinical signs in the sow herd are generally inapparent but may include paresis, inappetence, restlessness, vomiting, irritability, and occasionally aggression. A large percentage of sows may exhibit late-term abortions or increased numbers of stillborn and weakborn piglets. Liveborn piglets may be depressed, lethargic, ataxic, exhibit a weak, suckling reflex, and may be unresponsive to external stimuli.

Carboxyhemoglobin is bright "cherry-like" red, and cherry-red discoloration of the subcutaneous tissue, muscles, abdominal, and thoracic viscera are characteristic of carbon monoxide poisoning. These changes are typically accompanied by the accumulation of a large volume of serosanguineous pleural effusion.

Microscopic lesions associated with fetal carbon monoxide poisoning include multifocal hemorrhages and vacuolization of the neuropil throughout the cortical white matter and brainstem. Subacute lesions may be observed in weakborn piglets that survive for several days and include focal necrosis of the cortical white matter with extensive glial vascular proliferation, hemorrhage, and gitter cell infiltration in tissue adjacent to foci of malacia.

Cherry-red discoloration of fetal tissues is highly suspicious for carbon monoxide poisoning. A definitive diagnosis can be established by harvesting 5–10 mL of fetal thoracic fluid in a sterile, airtight container and analyzing the fluid for carboxyhemoglobin. Thoracic fluid should be chilled and not frozen, because freezing causes hemolysis, which markedly decreases carboxyhemoglobin levels. Carboxyhemoglobin levels >2% in fetal thoracic fluid are compatible with a diagnosis of carbon monoxide-induced fetal death. Carboxyhemoglobin levels of ≥10% in the whole blood of aborting sows are indicative of increased CO exposure. Studies have demonstrated that maternal carboxyhemoglobin levels of >23% are associated with an increase in stillbirths. Carbon monoxide can be measured in the farrowing facility; however, results may be misleading because levels can fluctuate with changes in ventilation or when personnel enter and exit the building. Environmental CO levels of 120 ppm and greater have been associated with abortions and stillbirths.

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Chapter 5

Disorders of Horses

Neil M. Williams

Introduction

Pathogenesis of abortion and neonatal loss

Abortion is a common reproductive problem in horses with an occurrence rate estimated at 10–15%. Two peaks in abortions are observed. The first is in early pregnancy before day 40. This is when most abortions occur and is referred to as early embryonic loss. The second peak in abortions is during the last several months of gestation. Although abortions do occur during midgestation, they are less common.

The terminology used to describe an equine offspring varies depending on whether it is normal and delivered around the anticipated due date, delivered at the wrong time of gestation, or if it is in an abnormal condition. These terms can be confusing and are often used improperly. These terms relate to particular events or processes in gestation, and the proper usage allows certain diseases to be considered and others to be discarded as possible causes of abortion or neonatal disease, making correct usage of these terms important.

The offspring is considered to be a fetus until delivery, becoming a foal upon birth at the end of gestation. An equine fetus at the completion of gestation is sometimes referred to as a term foal.

An abortion is the delivery of a dead foal at any time before the onset of normal parturition. Abortions are also called stillbirths. Technically, delivery of a dead offspring at any time of gestation is a stillbirth; however, the term is best reserved for delivery of a nonviable offspring after the time when external viability is possible. In mares, loss after about 310 to 320 days would be a stillbirth. Referring to the loss of a term fetus as a stillbirth and reserving the term abortion for earlier losses is helpful, although there are overlaps. This allows a different set of differential diagnoses to be considered in a stillbirth, many of which are related to the act of parturition. By contrast, abortions are

often caused by infections of the fetus or conditions affecting the membranes, such as placentitis or umbilical cord abnormalities.

Mares have a highly variable gestation length that makes horses unique from other domestic animals. The average length of gestation is between 320 and 370 days, and a mare will typically have her own normal gestation length. Therefore, a mare that normally delivers at 360 days may have a premature foal at 330 days, while a mare that normally delivers at 320 days may have a normal term foal at 318 days. The concept of a mare being overdue when gestation goes beyond the "average" 340-day interval is erroneous. Mares have to be considered on an individual basis. In general, births before 320 days are considered premature, and foals rarely survive if born before 300 days.

In addition to "premature" foals that are born early, there are instances where foals are born beyond their due date and are abnormal. These foals can be stillborn or viable. One example includes foals that are small and appear premature. These are dysmature foals. Dysmaturity is commonly associated with placental insufficiency. A second example is the foal with extended gestation that is normal to large in skeletal size but that has a thin body condition. These foals are called postmature. The classical cause of this is consumption of endophyte-infected fescue grass.

Equine abortions may result from primary maternal illness, infection of the fetus, and non-infectious fetal disease. Prompt examination of the fetus and fetal membranes is essential to determine the cause of abortion problems. Mechanisms of abortion from fetal disease include direct fetal infection by viruses and bacteria leading to fetal death. Infection with bacterial and fungal organisms may be limited to the fetal membranes. In these instances the infectious agents can cause abortion by inducing inflammatory changes and the production of cytokines and other mediators of inflammation. Another mechanism resulting from bacterial and fungal infection is placental insufficiency due to damage to a portion of the chorionic membrane. Non-infectious causes of abortion can produce abortion by fetal hypoxia and toxemia due to placental perfusion problems resulting from blockage of blood flow through the umbilical cord. The classic example of this is umbilical cord torsion. Other non-infectious causes of placental insufficiency include twinning, placental detachment, and placental infarction. Refer to Table 5.1 for a summary of gross and microscopic lesions and their associated causes.

Early gestation fetuses that are aborted commonly are expelled with no evidence or observation of abortion. The fetus is not recovered, and the mare is discovered to be nonpregnant on subsequent pregnancy examination. When recovered, early gestation fetuses often are autolyzed with no appreciable lesions or microbiological findings, and a fetal explanation for the abortion is not found. These idiopathic abortions are problematic and often lead to the suggestion that the abortion may have resulted from maternal or fetal genetic factors. Fortunately, there is a greater probability of elucidating a definitive diagnosis in late-gestation aborted fetuses; however, idiopathic abortions occur in this group as well.

Table 5.1 Possible causes of gross and microscopic lesions in equine fetuses.

Gross Lesions	Causes
Fresh, well-preserved fetus	Herpesvirus, dystocia
Pinpoint white foci in liver	Herpesvirus
Fetal pneumonia	Herpesvirus, Bacterial abortion, MRLS
Placentitis—cervical area	Bacteria, fungi
Placentitis—diffuse	<i>Leptospira</i> , <i>Salmonella</i>
Placentitis—base of horns	Nocardioform, Cellulosimicrobium
Nonruptured cervical star	Premature placental separation, herpesvirus
Large avillous area on chorion	Twinning, Nocardioform placentitis
Umbilical cord edema	Torsion, dystocia, MRLS, fescue toxicosis
Placental edema	Dystocia, fescue toxicosis, normal
Fetal mummification	PCV2
Microscopic lesions	Causes
Intranuclear inclusions	Herpesvirus
Vasculitis	Equine viral arteritis
Tubulonephritis	<i>Leptospira</i>
Multifocal hepatic and pulmonary necrosis	Herpesvirus

In cases where fetal abnormalities or pathogens are not found, a maternal condition or illness should be considered to be a potential cause. Unfortunately, while association of abortion with maternal illness and conditions can be made, proving an abortion to be the result of a maternal problem is often impossible. Studies have shown reduced fertility and embryo survival in aged mares. Evidence suggests that a depressed progesterone level in pregnant mares is associated with pregnancy loss. Endotoxemia is another potential cause of abortion, through the mechanism of prostaglandin-induced luteolysis in mares 55 days of gestation or less. Pregnant mares experiencing either severe medical or surgical colic were found to be at a slightly higher risk of associated abortion. It has been suggested that endotoxin and proinflammatory cytokines can also impair utero-placental circulation and metabolism and have a detrimental effect on pregnancy. Other maternal causes of abortion include uterine artery rupture, uterine torsion, and abdominal wall hernia formation.

Tissue sampling

Following initial dissection of the fetus and arrangement of the placenta, samples should be obtained for laboratory testing prior to detailed examination to minimize contamination. Samples of lung, liver, kidney, spleen, umbilical cord,

and cervical, anterior body, and the allantochorion from the pregnant or gravid horns should be taken and placed separately in appropriate sterile containers. Stomach contents, heart blood, and pericardial fluid are aspirated into sterile syringes and placed into sterile tubes. During examination, additional tissue samples are taken of any suspected abnormalities. Routine tissues, placenta, and suspected lesions are sampled and placed in buffered formalin for histopathologic examination.

Routine testing will vary by laboratory and region of the country. In general, aerobic cultures are performed on lung, liver, stomach content, and allantochorion. Fungal cultures are conducted on allantochorion if a fungal placentitis is suspected. Fluorescent antibody (FA) or PCR testing for *Leptospira* is performed on kidney and placenta, or on kidney and liver if placenta is not available. Tissues are pooled, and FA or PCR testing for equine herpesvirus is performed. Virus isolation is conducted on pooled tissue samples. Heart blood and pericardial fluid are tested for the presence of antibodies to *Leptospira*.

Table B.4 in Appendix B summarizes what tissues to collect, which tests should be requested, and lists additional ancillary tests that can be performed to maximize the diagnostic effort of attempting to confirm a diagnosis from a list of selected pathogens.

Gross examination of the equine fetus and placenta

A complete external and internal examination of the fetus should be performed as soon as possible after delivery. The body condition and degree of postmortem decomposition should be noted. The fetus should be weighed, its crown-to-rump length should be measured (Table A.4 in Appendix A), and its sex should be recorded. In addition, gross features such as length and texture of hair, if present, the presence or lack of teeth eruption, and development and descent of testicles in male foals helps determine gestational age, if breeding dates are unknown. The fetus is dissected in a routine fashion and abnormalities noted. The lungs are examined for inflation and the degree of aeration is recorded. The shoulder joints of late-gestation fetuses and term foals should be opened to determine if hemarthrosis is present. Hemorrhage into the shoulder joints is an indication of excessive trauma during delivery and suggests the possibility of a dystocia.

The placenta should always be examined in cases of abortion or stillbirth, but it should also be examined in apparently normal foalings to ensure that portions of the placenta are not retained as well as to check for any abnormality.

The placental membranes of the horse consist of the allantochorion, the amnion (allantoamnion), and the umbilical cord. The total placenta should be weighed, and, following examination, the allantochorion removed and weighed separately. The combined membranes are spread on an examination table with the allantoic surface outermost. The attachment of the umbilical cord to the allantochorion is examined and the location of the attachment between the horns noted. The cord is severed at its allantoic attachment and its length

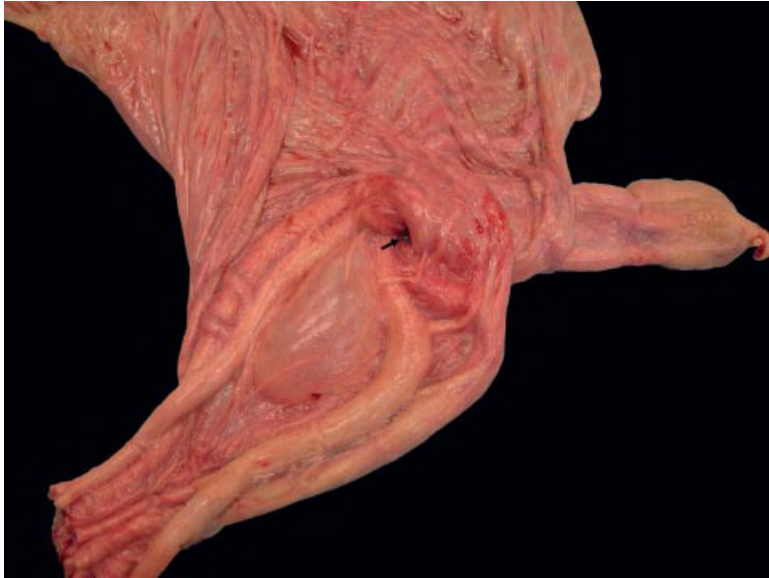


Figure 5.1 Urachal opening. The urachus opens into the allantoic cavity and represents the anatomic structure that helps distinguish allantoic from amniotic umbilical cord. (Courtesy of Dr. NM Williams, University of Kentucky.)

measured. The umbilical cord has amniotic and allantoic segments. The amniotic segment of the umbilical cord contains two arteries and a vein that arborize onto the amnion. They continue as multiple vessels in the allantoic segment of the cord with predominately two arteries and two veins with branches. The urachus courses within the cord from the fetus and empties into the allantoic cavity. The opening of the urachus is the anatomic landmark of the division of the amniotic and allantoic portions of the cord (Figure 5.1). During or following parturition, the umbilical cord arteries break or rupture within the abdomen of the foal, leaving two slightly coiled vessels extending from the foal end of the cord. This is normal. The equine umbilical cord is normally twisted, and the number of twists of the cord should be counted and any areas of potential torsion (crimping) of the cord evaluated. Sites of torsion are typically blanched and constricted and sometimes have tearing of the intima. Dilation of vessels and/or the urachus on the fetal side of the torsion is often present. The cord is examined for edema, focal swellings, roughening of the surface, plaques, and appearance of the vessels. An incidental finding is the variably sized (approximately 3 cm in length) collapsed sac-like structure present in the allantoic portion of the cord. This is the yolk sac remnant (Figure 5.2). It is attached at both ends by a thread-like cord that is the vitelline duct. Rarely, the yolk sac may be fluid filled, distended, and even calcified. It may expand to greater than 10 cm in diameter and protrude from the cord. The calcified yolk sac should not be confused for a twin fetus. A tan, flattened hippomane is often found in the allantoic cavity (Figure 5.3 and Figure 5.14).

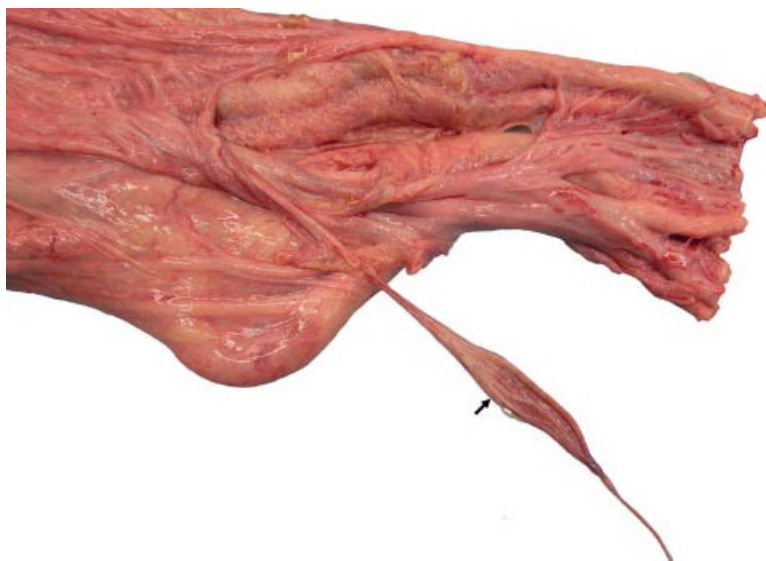


Figure 5.2 Yolk sac remnant. A common incidental finding in the umbilical cords of foals, the yolk sac remnant (*arrow*) remains attached by the vitelline duct (*arrowhead*). In this example, it is a collapsed, sac-like structure whereas in other examples, it may be fluid filled or contain mineralized concretions. (Courtesy of Dr. NM Williams, University of Kentucky.)



Figure 5.3 Hippomane. Within an opened allantoic cavity is this flattened, yellow-tan, soft, putty-like, free floating aggregate called a hippomane. In cross section (*inset*), they typically are formed by concentric deposition of urinary calculi about a central nucleus of desquamated fetal membranes. (Courtesy of Dr. NM Williams, University of Kentucky; inset courtesy of Dr. A Alcaraz, Cornell University.)

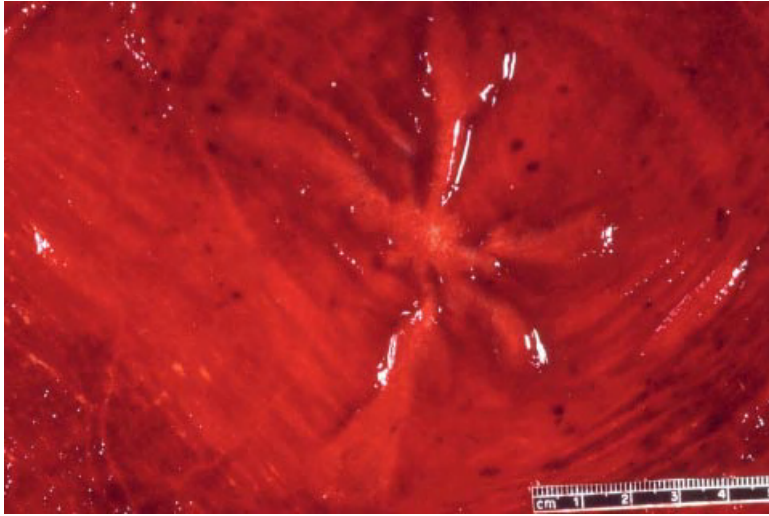


Figure 5.4 Cervical star. The interface of the allantochorion with the maternal cervix results in a focal stellate area known as the cervical star. (Courtesy of Cornell University.)

The allantochorion is next examined. With the allantoic surface facing outward, the allantochorion is placed in an “F” arrangement with the pregnant horn representing the top of the “F,” the nonpregnant horn the short arm, and the cervical area of the placenta representing the base of the “F.” The foal normally exits through the cervical area of the placenta causing rupture of the cervical star (Figure 5.4). Any abnormalities are noted, paying special attention to the allantoic vessels that are clearly visible. The allantochorion is repositioned with the chorion outward and placed again in an “F” arrangement. The chorionic surface is examined for abnormal color, thickness, loss of villous pattern, exudate, tears, and shape. The chorionic villi in the normal fresh equine placenta have a dark red, uniform color with a velvety texture. Normally there are regional differences in length and density of villi with the nonpregnant horn, base, and end of gravid or pregnant horn having longer, denser villi. The cervical, body, and greater curvature areas of the pregnant horn typically have shorter, more sparsely distributed villi. Small avillous areas are found at the cervical star, at the sites of the involuted endometrial cups, and where the allantochorion folds upon itself, usually along the course of a vessel. The chorionic surface becomes a uniform tan color as it autolyzes. Brown fluid, not to be confused with an exudate, is often present on an autolyzed chorion. The normal placenta is uniformly thin (approximately 3–5 mm); however, the tip of the pregnant horn is often mildly edematous. Focal or generalized edema of the placenta, while not normal, is nonspecific and can be observed in normal foalings. Following examination of the allantochorion, the amnion is examined for abnormal changes. It is normally thin and slightly transparent with prominent thick, cord-like vessels that arborize over the membrane.

Viral causes of abortion and neonatal loss

Equine herpesvirus

Equine herpesvirus (EHV)-1, and less commonly EHV-4, are well-recognized abortigenic agents. EHV-1-induced abortions occur as isolated events on a farm or can present as abortion outbreaks, rapidly affecting multiple mares over a short time. This may occur following recent introduction of new individuals into the herd, or the herd may be closed with no new introductions or movement of horses. Abortions due to EHV-1 typically occur suddenly with no outward signs of impending abortion in the mares. Abortions usually occur in mares in mid to late gestation, and the delivery is usually rapid.

Gross lesions are consistent and include a fresh, well-preserved fetus in good body condition. Internally, the fetus may have increased amounts of clear yellow fluid in the thoracic cavity, pericardial sac, mediastinum, perirenal area, abdomen, subcutaneous tissues, and along the fascial planes of the neck. There often are hemorrhages on the surface of the organs and tissues. The liver may contain myriad 1-2 mm whitish foci visible on the surface and within the parenchyma (Figure 5.5), and the lung typically is firm and variably edematous and congested. Rarely, fibrin plugs may be evident within airways and when observed are considered virtually pathognomonic for fetal EHV infection (Figure 5.6). Placental expulsion usually occurs with or shortly after the fetus, and the placenta usually lacks gross lesions.

Microscopically, EHV abortions have lesions in multiple tissues including the lung, liver, adrenal glands, spleen, thymus, and allantochorion. The lesions

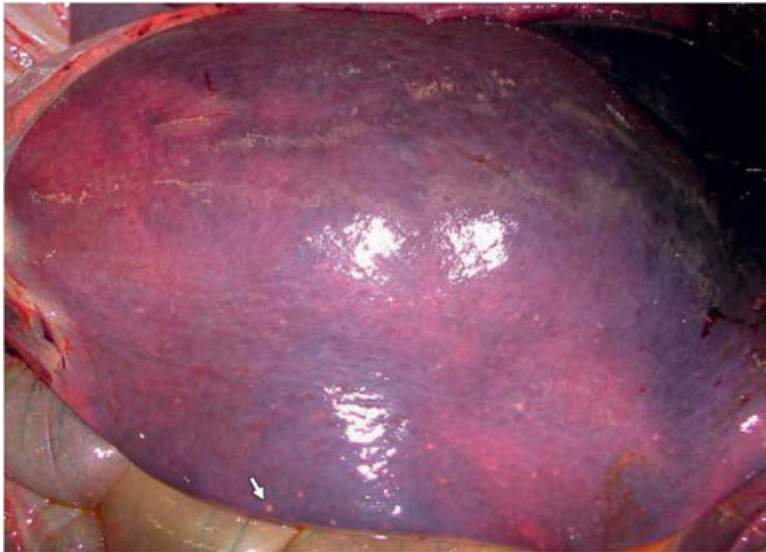


Figure 5.5 Equine herpesviral hepatitis. Multifocal pinpoint white areas of hepatic necrosis (arrow) are scattered throughout the liver. This is caused by fetal EHV infection. (Courtesy of Dr. NM Williams, University of Kentucky.)

consist of multifocal areas of necrosis with accumulation of cellular debris and eosinophilic intranuclear inclusion bodies within cells surrounding the necrotic foci. Occasionally, infected epithelial cells will form syncytia and may also contain herpetic intranuclear inclusion bodies (Figure 5.7). A small percentage of cases will exhibit necrosis of chorionic epithelial cells with some chorionic cells containing intranuclear inclusion bodies (Figure 5.8). Little to no inflammation is associated with the necrosis, and a placentitis usually is not observed.

Herpesvirus abortion may be presumptively diagnosed based on characteristic gross lesions. Diagnosis is confirmed by microscopic examination of the lesions demonstrating characteristic intranuclear inclusions. Laboratory

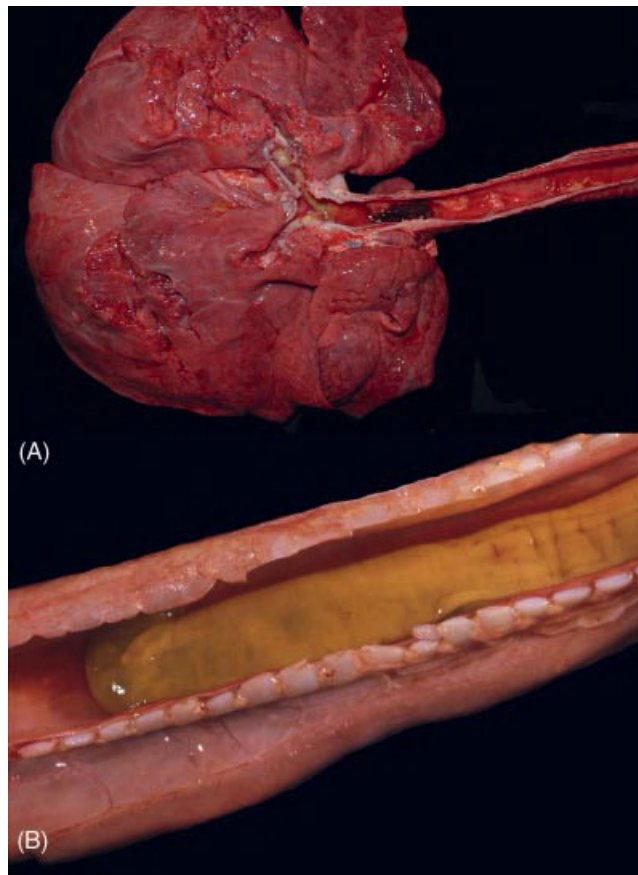


Figure 5.6 Equine herpesviral bronchial and tracheal fibrin plugs. (A) within the trachea and main stem bronchi are yellow aggregates of fibrin mixed with blood clots. (B) is a higher magnified view of another fetus with a very large, yellow, fibrin clot partially filling the lumen of an opened trachea. This rare lesion is virtually pathognomonic for fetal EHV infections. (A courtesy of Dr. BL Njaa, Oklahoma State University; B of courtesy Dr. BL Njaa, Cornell University.)

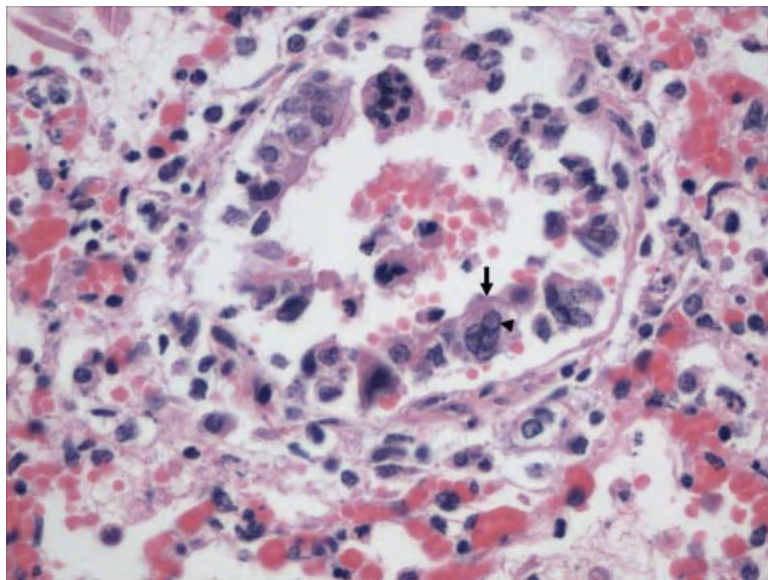


Figure 5.7 Histologic section of equine herpesviral bronchiolitis. Within the lumen of this bronchiole are several multinucleated syncytial cells (arrow). Within the nuclei of several epithelial cells are prominent, purple viral intranuclear inclusion bodies consistent for EHV infection. H&E stained section. (Courtesy of Dr. BL Njaa, Cornell University.)

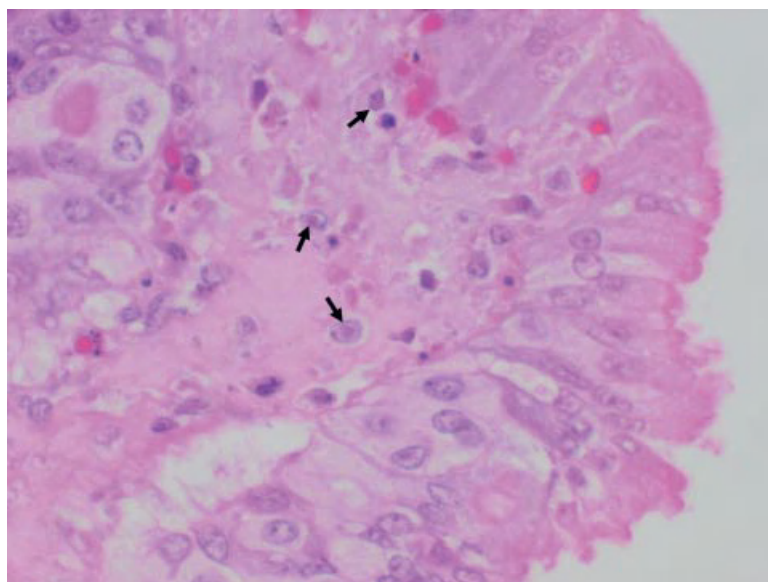


Figure 5.8 Histologic section of equine herpesviral chorionitis. Herpetic intranuclear inclusion bodies are located within chorionic epithelial cells (arrows) of a chorionic villus with minimal to no associated inflammation. H&E stained section. (Courtesy of Dr. NM Williams, University of Kentucky.)

confirmatory tests include FA or IHC stains on fetal tissues, PCR testing, and virus isolation using susceptible cell lines. Maternal serology can be performed; however, the common practice of vaccinating mares repeatedly against EHV1 and the overlap between titers resulting from vaccination and natural infection makes interpretation of a single sample problematic.

Equine viral arteritis

Equine arteritis virus infection usually presents as an outbreak of influenza-like illness in adult horses. Some outbreaks are limited to respiratory signs in the herd, but other outbreaks also have mares abort due to the infection. The aborted fetuses are usually late in gestation, may be aborted in either an autolyzed or fresh state, and are infected by arteritis virus. Gross examination of the aborted fetuses may reveal pulmonary edema but usually no other lesions. Microscopically, aborted fetuses may have no lesions or there may be inflammatory changes in sections of brain, liver, spleen, heart, placenta, and other tissues. Lesions consist of a variable vasculitis with mononuclear cells and rare neutrophils infiltrating the vessel walls and forming small perivascular cuffs.

Presumptive diagnosis can be made by the presence of vasculitis in fetuses during an abortion outbreak. The diagnosis is confirmed by virus isolation from the fetal tissues, PCR testing, and serum neutralization showing maternal seroconversion.

Bacterial and fungal causes

Bacterial infections of the fetoplacental unit are an important cause of equine abortion. These infections may be restricted to the fetal membranes or they may involve the fetal organs or fluids. Most bacterial abortions are associated with a placentitis, the result of either ascending or hematogenous spread. Occasionally bacteria will be isolated from fetal organs, and there will be associated inflammation without evidence of placentitis. These are typically diagnosed as bacterial abortions. Bacteria are capable of breaching the placental barrier and infecting the fetus without producing a placentitis.

Ascending placentitis

Ascending placentitis is the most common form of placentitis in the mare. As the name implies, the bacterial or fungal organisms gain access to the placental membranes by traversing the lower reproductive tract and passing through the cervix. The cervical star region of the chorion is initially infected, and the process may extend cranially along the body of the allantochorion toward the horns. Concurrently, the infectious agent may gain access to the fetal fluids, other placental structures, and the fetal organs. One study reported that bacteria were cultured from both the placenta and fetal organs in approximately



Figure 5.9 Ascending mycotic placentitis. Tan, caseous exudate is present on the chorionic surface of an equine placenta in the region of the cervical star. (Courtesy of Cornell University.)

60% of the cases, demonstrating that dissemination of infection from the membranes is a common occurrence. The most commonly isolated organisms are *Streptococcus zooepidemicus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus equisimilis*, *Enterobacter agglomerans*, *Klebsiella pneumoniae*, and alpha-hemolytic *Streptococcus* spp. These bacteria are common isolates from the lower reproductive tract of the mare; therefore, it is believed that these bacteria are opportunistic in nature. Fungal isolates were usually *Aspergillus* spp. and mucoraceous fungi. Mares with ascending placentitis may spontaneously abort with no evidence of problems, but some will develop premature mammary gland enlargement and have a vaginal discharge. Ultrasound examination may demonstrate thickening of the membranes, membrane separation, and fluid accumulation in the area of the cervical star.

Gross lesions are typically observed and include irregular thickening and roughening of the allantochorion in the area of the cervical star with brown or tan discoloration and mild exudate on the chorionic surface (Figure 5.9). The affected area may be hemorrhagic or congested resulting in areas of reddish-black discoloration. The cranial extent of the placentitis is often sharply demarcated from the unaffected chorion, and there may be a thin intervening hemorrhagic zone. The remainder of the allantochorion is usually grossly normal. If the infection has gained access to the fetal fluids or extraembryonic coelom, there may be concurrent hemorrhages, plaques, or roughening on the allantoic surface, amnion, or umbilical cord. Fetal organs and membranes may have petechial or ecchymotic hemorrhages. The lungs may be firm, and the liver is sometimes swollen, soft, and brown or brownish-yellow.

Microscopically, the changes may be acute or chronic. The allantochorion is usually infiltrated by variable numbers of neutrophils, macrophages, and

lymphocytes. These cells surround stromal blood vessels and infiltrate into the subchorionic stroma and chorionic villi. Inflammatory cells may be on the chorionic surface admixed with debris and fluid. Bacteria may be observed in the surface exudate or in the allantochorion. There is degeneration and necrosis of the chorionic epithelium, and areas may exhibit hyperplasia and, on occasion, squamous metaplasia. Blood vessels are often congested, and there may be stromal hemorrhage. Stromal fibrosis, neovascularization, and allantoid cystic hyperplasia are sometimes seen in chronic cases. Within the fetus, there may be histopathologic evidence of pneumonia, and the liver may have swollen vacuolated hepatocytes.

Specific diagnostic testing should include bacterial and fungal cultures of the affected allantochorion, and bacterial culture of the lung, liver, stomach contents, fetal fluids, placenta, and any of the lesions observed grossly.

Hematogenous (diffuse) placentitis

Hematogenous placentitis is less commonly diagnosed in the mare. This type of placentitis is the result of systemic infections in the mare that also involve the uterus with the infection extending to the chorion. Pathogens associated with this type of placentitis include certain bacteria (*Leptospira* spp. and *Salmonella* spp.) and rarely other agents such as *Histoplasma* spp. and *Encephalitozoon* spp.

Gross changes, if present, are seen in the chorion and include diffuse, patchy, or randomly distributed multifocal areas of discoloration, thickening, hemorrhage, and possibly exudate accumulation. The fetal lung may be firm, the liver may be swollen and discolored, and the kidneys may be swollen and hemorrhagic, depending on the pathogen.

Histologic lesions of the allantochorion include mixed inflammation, hemorrhage, degenerative changes of the chorionic epithelium, and possibly the causative microorganisms.

Diagnostic testing should include bacteriologic and fungal culture, as well as, special histochemical stains, and specific PCR and serologic testing as indicated.

Leptospirosis

Leptospirosis has emerged over the last two decades as an important example of hematogenous placentitis and abortion. The causative agents in horses are typically *Leptospira interrogans* serogroup pomona serovar kennewicki or *Leptospira kirschneri* serogroup grippotyphosa serovar grippotyphosa. These organisms typically cause mid- to late-term abortions. The mare will usually abort with no premonitory evidence of infection and no signs of illness. The fetuses may be fresh but are more often moderately autolyzed.

Gross lesions are observed in approximately 80% of the fetuses. Lesions include icterus, hemorrhages, hepatomegaly with mottling, and renal edema with pale streaks. The placenta shows a range of changes varying from no

obvious gross lesions to multifocal or diffuse placentitis characterized by necrosis, discoloration, and superficial exudate. There may be cystic allantoic hyperplasia.

Microscopic lesions in the kidney and liver are the most severe. The kidneys contain microabscesses with giant cells, dilated tubules, fibrosis, and mononuclear interstitial nephritis. Liver lesions include infiltration of the portal triads by lymphocytes and macrophages and giant cells distributed throughout the parenchyma. Less commonly, microscopic changes are observed in the lung, heart, lymphoid organs, and brain. The allantochorion has vasculitis, thrombosis, and infiltration by inflammatory cells. Silver stains may demonstrate the presence of leptospire in affected tissues. These bacteria break down rapidly in the postmortem period and are often difficult to recognize. The renal tubules and chorionic membrane are the best sites for detection of bacteria. Other helpful diagnostic tests include fetal and maternal serology, FA testing, and PCR. Most fetuses at the time of abortion will be seropositive. Likewise, mares will be seropositive, and the titers will often be extremely high. At the University of Kentucky, a maternal titer of $\geq 6,400$ is considered highly suggestive of active infection. Lower titers in maternal sera may simply indicate prior exposure and should not be considered to be diagnostic of leptospiral abortion. Any fetal leptospiral titer is considered to be significant. An FA test using a multivalent *Leptospira* fluorescent antibody conjugate is best conducted on impression smears of placenta and either fetal kidney or liver. Positive fluorescence with typical spirochete morphology is highly suggestive of *Leptospira* abortion. PCR testing can also be employed to help determine abortion due to leptospirosis.

Atypical (nocardioform) placentitis

A third form of placentitis is recognized. This atypical type of placentitis has been named nocardioform placentitis. Nocardioform placentitis has a unique location and appearance that is different from classical ascending placentitis, and the site and solitary nature of the lesion does not fit a hematogenous distribution. The pathogenesis is not known but the portal of entry is likely via the mare's lower reproductive tract. Nocardioform placentitis was not reported prior to the mid-1980s. This type of placentitis is associated with the presence of Gram-positive filamentous branching bacteria. Initially these organisms were unclassified which led to the designation "nocardioform" based on their morphology and characteristics. This form of placentitis is not associated with a single bacterial species but rather with a group of similar organisms. Several of the most common nocardioform organisms have been characterized and are now classified as *Crossiella equi*, *Amycolatopsis kentuckyensis*, *Amycolatopsis lexingtonensis*, and *Amycolatopsis pretoriensis*.

Confounding the situation, cases with a gross appearance identical to nocardioform placentitis are encountered in which other types of bacteria are isolated with no evidence of nocardioform type of bacteria present. Bacteria isolated from these cases include *Staphylococcus* spp., *Pantoea agglomerans*,

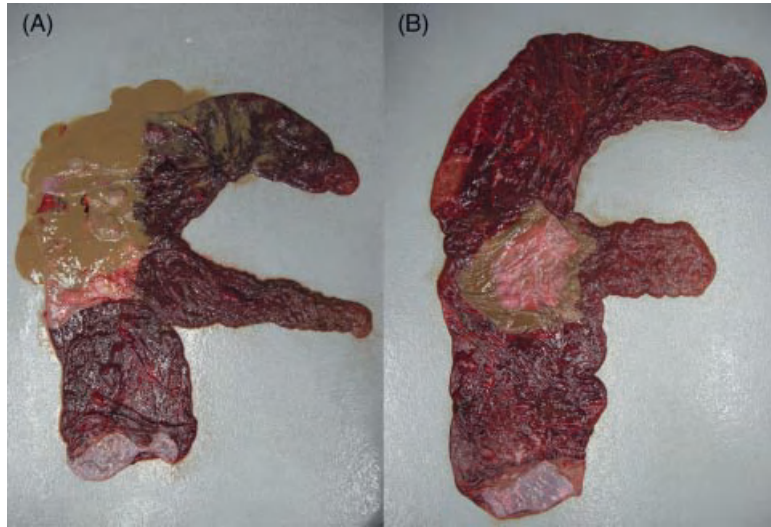


Figure 5.10 Equine nocardioform placentitis. (A) There is a large amount of pale brown exudate partially obscuring an area of placentitis affecting the chorionic surface at the junction of the body and horns of the placenta. (B) Placenta from a similarly affected mare with a chorionic lesion affecting an area at the junction of the body and horn after the exudate has been washed away.

(A and B courtesy of Dr. NM Williams, University of Kentucky.)

Cellulosimicrobium cellulans, *Enterobacter* spp., and others. This would indicate that the nocardioform bacteria are not essential for the development of this type of placentitis and that many different organisms can, through some common pathogenic mechanism, cause the same pattern of placentitis. Although most cases have been diagnosed in Kentucky, confirmed cases have been diagnosed in other parts of the United States and several foreign countries. Nocardioform placentitis cases typically involve late-gestation fetuses or term foals with the occurrence of one of three possible outcomes: (1) the fetus may abort, (2) the foal may be born premature or full term and alive but weak and compromised, or (3) the foal may be normal at birth.

The gross lesions are basically pathognomonic for nocardioform placentitis. The placenta typically contains a large amount of exudate on the chorionic surface. This material usually is brownish-green, thick, and tenacious. The affected area on the chorion is a solitary, variably sized, but often large lesion (Figure 5.10). This type of placentitis is usually located over the cranial body of the placenta at the base of the horns. The placentitis often extends onto one or both horns. The affected chorion often is thin and pale in the central portion of the lesion with the margins being thickened and tan. There is a sharp line of demarcation between the affected and normal chorion. Chronic changes such as cystic adenomatous allantoic hyperplasia may concurrently be present. The placentitis does not involve the cervical portion of the allantochorion (cervical star area), and there usually is no history of vaginal discharge in the mare.

Microscopically, sections from the center of the lesion tend to be chronic with areas of necrosis and both blunting as well as loss of chorionic villi. The inflammation is predominately mononuclear with fewer neutrophils. The margins of the placentitis exhibit active inflammation with more inflammatory cells and a larger population of neutrophils. The inflammatory cells infiltrate the subchorionic stroma and villi. There may be degeneration and necrosis of the chorionic epithelium with areas of hyperplasia and sloughing. On the surface is a large amount of eosinophilic-cellular debris and fluid mixed with inflammatory cells and the characteristic long filamentous branching nocardioform bacilli. The nocardioform bacteria may be observed in chorionic epithelial cells, but invasion of the stroma is not typical.

Diagnosis is based on the gross and histopathologic changes and the culture of typical bacteria from the allantochorion. The bacteria in this group have similar culture characteristics. They are aerobic, catalase-positive, nonacid-fast, Gram-positive, filamentous bacteria that branch extensively on artificial media. Growth is on tryptic soy agar with blood (blood agar plate [BAP]) or without added blood. Forty-eight to 72 hours of incubation at 36°C are usually required for colonies to be visible. PCR tests have been developed to detect *Crossiella* and *Amycolatopsis* species. PCR testing can be conducted on placental tissue or swabs.

Miscellaneous causes

There are a number of important miscellaneous (non-infectious) causes of abortion and stillbirth in horses. Complications during the delivery process are the most common non-infectious causes of fetal death. These cases can present a diagnostic challenge because of the lack of definitive lesions in some of the fetuses; however, the diagnosis is facilitated by consideration of the history, signalment, gross and microscopic lesions, and absence of pathogens.

Dystocia, delayed delivery, perinatal asphyxiation

Compared to other domestic animals, the delivery of the foal is rapid and forceful. The average time of second-stage delivery is about 17 minutes with a range of 10 to 70 minutes. If this process is prolonged or if excessive trauma occurs to the foal, its viability can be compromised. A delayed delivery or dystocia can cause fetal hypoxia due to inability of the foal to inspire effectively, impairment of blood flow through the umbilical cord and placenta, or foal trauma. As a result, the following three outcomes can happen: (1) foal may be dead upon delivery, (2) it may be unresponsive with diminished or absent respiratory efforts and cardiac contractions, or (3) it may be viable but hypoxic and compromised. The second group expires immediately following delivery, and the last group often develops complications related to the respiratory or nervous systems and dies during the neonatal period.

The history will often indicate a dystocia or delay in delivery, but occasionally the foal is found dead on pasture or in a stall following an unobserved delivery. Some cases of neonatal asphyxiation will occur during what is reported to be a normal uncomplicated delivery.

The appearance of the foal is usually normal, but its overall size may be large. Internal examination may reveal non-aerated or poorly inflated lungs. Bruising can be observed on the ribs and the surface of the ventricles of the heart. Most commonly the right ventricle is affected, but both may be involved. The tracheal and bronchial lumens may contain frothy fluid. Examination of the shoulder joints will often reveal a hemarthrosis. Rib fractures, often multiple, may be present. The fourth through ninth or tenth ribs or some combination are typically fractured. Jagged rib ends commonly penetrate the parietal pleura, and there may be a hemothorax from laceration of the lungs or myocardium. Other less common lesions include longitudinal tearing of the pulmonary artery 4-5 cm from the origin of the artery on the right ventricle, rupture of the liver, and diaphragmatic perforation with visceral herniation. Microscopic changes are few and nonspecific and include hemorrhages and pulmonary atelectasis and congestion of the alveolar capillaries.

A key feature of asphyxiation is that the foal was viable at the start of stage 2 labor or when delivered. The poor expansion of the lungs, traumatic lesions, and lack of other findings (infection, inflammation, and anomaly) support the diagnosis of asphyxiation in these cases where the history did not indicate a dystocia.

Premature placental separation (red bag delivery)

Premature placental separation indicates a partial or complete detachment of the chorion from the endometrium before or during delivery resulting in a dead or compromised foal. These occur around the time of anticipated delivery and are referred to as “red bag” deliveries. This is because the cervical star of the allantochorion does not rupture and, instead of the amnion or front feet being initially presented, the chorion bulges from the vulva, appearing as a red bag. The causes of this are multiple and sometimes idiopathic. Causes include processes that result in thickening, scarring, or detachment of the allantochorion. Placentitis, fibrosis, and placental edema of any cause can result in premature separation. Ingestion of endophyte-infected fescue grass is a recognized cause of placental edema and red bag deliveries. Occasionally, explosive deliveries are reported with the foal being presented with, and enclosed in, the allantochorion. These will sometimes occur with herpesvirus abortions, and were common with late-term abortions associated with the mare reproductive loss syndrome (MRLS). Premature placental separation may involve the entire allantochorion detaching and passing with the foal, or there may be a partial detachment with tearing of the allantochorion through the body and presentation of the caudal allantochorion before or with the foal (Figure 5.11).



Figure 5.11 Premature placental separation. The allantochorion is torn through the anterior portion of the body resulting in separation. The cervical star was not ruptured. This is called a red bag delivery. In addition, the umbilical cord is twisted multiple times with various areas of torsion and fluid accumulation in the cord. (Courtesy of Dr. NM Williams, University of Kentucky.)

Often the history will indicate the occurrence of a red bag delivery, but, when examined by the pathologist, this is not obvious due to the foaling assistant tearing the cervical star area of the chorion to deliver the foal. Findings to suggest or confirm premature separation in these cases include tearing of the allantochorion through the body, and the allantochorion being presented with the chorion outermost, rather than inverted as is normally the case. Obviously, the examiner has to know how the placenta was handled by the foaling assistants.

The microscopic appearance will vary depending on the cause. Some cases will have no significant microscopic changes.

Twinning

Twinning in the past was a major cause of equine abortion; however, the use of ultrasound examination has enabled early intervention and a reduction in the number of abortions due to this condition. The occurrence of twins in mares is a result of the tendency for mares to have double ovulation (up to 20%), and the twins are dizygotic. The majority of twin pregnancies abort with loss of both fetuses. Even when one or both are born alive, there is a high death rate

in the neonatal period. The appearance of fetuses can vary from both foals being similar in size, but small; one being more developed and the other small; or one being developed and the other mummified. This is usually the result of the manner in which the fetuses are implanted in the uterus. Death and abortion is attributed to placental insufficiency. Often the larger fetus is in a fresh postmortem state while the twin is autolyzed.

If both fetuses are presented, the diagnosis is apparent. Occasionally, it is not clear to the farm personnel if the fetuses represent an abortion by one mare or whether it is two separate abortions. Additionally, sometimes only one fetus from a twin abortion is recovered and presented.

Examination of the placental membranes is characteristic. There will be two separate, although often fused, placentas. The area of contact between the two chorions will be thin and avillous due to lack of contact with the endometrium. Often the allantochorions will have to be pulled apart to reveal this. Once separated, in addition to the avillous area, the allantochorion will lack the normal "F"-shaped conformation since the membranes of each fetus do not occupy the entire uterus. There will be two separate amnions and umbilical cords. Typically, the fetuses are disproportionate in size, thin, and vary in postmortem preservation. Internal examination of the fetuses reveals few lesions other than possible hepatic enlargement. Microscopic examination of the allantochorion demonstrates villus aplasia in the areas of contact with the other chorion.

Umbilical cord torsion

The equine umbilical cord is normally a twisted structure. This is the result of fetal mobility beginning around 60 days of gestation when the cord lengthens, and proceeding until about the seventh month of pregnancy when the fetus becomes positioned in an anterior presentation. Twists are normally observed in both the amniotic and allantoic portions of the cord, indicating movement of both the fetal-amniotic unit within the allantoic cavity and fetal movement within the amniotic sac. An average number of 4.4 twists has been reported. Examination at our laboratory of a group of over 130 cords from normal foalings suggests a lower average number of twists.

Twisting of the equine umbilical cord is normal and does not automatically equate to fetal problems. However, some cords will be excessively twisted to the extent that blood and urine flow is compromised; this condition is referred to as torsion of the cord. The published range of normal cord lengths is 35 to 84 cm. Cords over 85 cm in length are considered to be excessively long, and these foals are at risk for cord-related problems. The umbilical cord in cases of torsion of the cord is usually excessively long and multiply twisted with tightly constricted blanched areas of torsion (Figure 5.12). The cord at the sight of constriction is pale, and there may be areas of intimal tearing. On either side of the torsion, there is often marked congestion of the cord. Proximal to the tight constrictions there can be dilated, sacculated areas representing distended vessels or urachal swelling (Figure 5.11). Umbilical

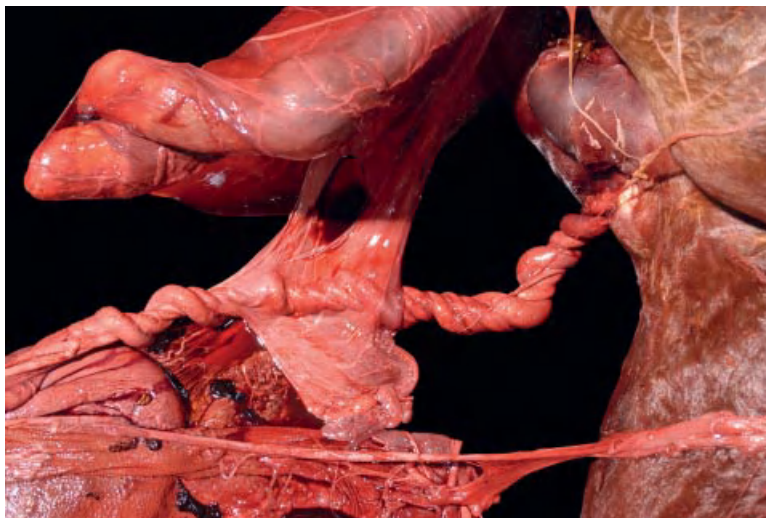


Figure 5.12 Umbilical cord torsion. The umbilical cord is twisted more than normal with at least eight twists evident in this image. There are sac-like dilations in the cord due to obstruction from torsion. (Courtesy of Dr. AW Stern, Oklahoma State University.)

cords with these changes obviously represent a torsion and are easy to diagnose. Some cords are more challenging in that they are long with excessive twisting but with no obvious compromise of blood flow. The dilemma in these is whether there was sufficient partial obstruction of flow over time to cause fetal compromise ultimately leading to death and abortion. It is not unusual to observe highly twisted cords in normal-term deliveries with normal foals. Also twisted cords are seen in fetuses in the uterus of mares at necropsy. Most cases of cord torsion occur around 6 to 8 months of gestation but can also be diagnosed late term. The fetus is often moderately autolyzed and may be thin.

Microscopically, there are few lesions. Mineralization within the lumens of the stromal vessels in the allantochorion may be observed and is considered to be an indication of cord torsion. However, cases of obvious cord torsion may lack this change, and vessel mineralization may occur in the allantochorion of cases without torsion.

Placental infarction

On rare occasion, areas of infarction are seen in the allantochorion. These areas are pale, thin, often transparent, and are usually sharply demarcated from the adjacent unaffected chorion. These usually occur in the body of the allantochorion in the area of the cervical star and have been referred to as cervical pole necrosis (Figure 5.13). These have also been associated with excessively long umbilical cords.

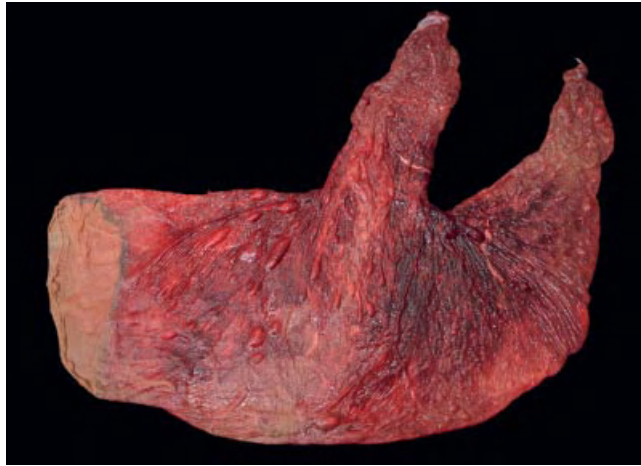


Figure 5.13 Placental infarction. There is an area of tan discoloration in the area of the cervical star that corresponds to infarction of the allantochorion. This is sometimes referred to as cervical pole necrosis. (Courtesy of Dr. NM Williams, University of Kentucky.)

Body pregnancy

Body pregnancy, though rare, often results in abortion and presents to necropsy with a thin, small fetus and a placenta that appears abnormal. The placenta has an enlarged body area, but both horns are small and resemble the nongravid horn of a normal placenta. This represents a body pregnancy where the fetus was located in the body of the uterus and fails to also occupy a horn. As a result the horn and consequently the placenta do not enlarge normally. The fetus dies and is aborted due to placental insufficiency. Why this occurs is not known. It is believed that contact of the conceptus with the turgid horn normally results in relaxation and accommodation of the uterine wall allowing the fetus to elongate into the pregnant horn. Obviously, some failure of the process occurs in the production of a body pregnancy. There is no indication that mares in which this occurs have a tendency to repeat this with subsequent pregnancies.

Fescue toxicosis

Fescue pastures are commonly infected with an endophytic fungus. Late-term gestation mares grazing endophyte-infected tall fescue grass may suffer reproductive losses in the form of abortions, stillbirths, and the birth of weak foals. The fungus, *Neotyphodium (Acremonium) coenophialum*, produces ergot alkaloids, principally ergovaline. These alkaloids have multiple effects on the mare and fetus including decreased prolactin secretion, inhibition of adrenocorticotrophic hormone secretion, decreased tissue binding of estradiol, and vasoconstriction.

The history often includes prolongation of gestation beyond the anticipated due date and lack of mammary gland development. Many times the presentations are described as being red-bag deliveries. Gross examination reveals a large but thin postmature (dysmature) foal. The lungs are usually poorly aerated, and there may be lesions indicating a difficult delivery. The allantochorion and sometimes the amnion are often heavy and edematous. There may be failure of the foal to rupture the cervical star.

Mare reproductive loss syndrome

In April and May of 2001 a previously unrecognized abortion epidemic occurred in central Kentucky and was reported to occur to a lesser extent in other areas of the Ohio Valley. The epidemic was repeated on a slightly smaller scale during the same time in 2002. Both late-gestation abortions and early fetal losses were observed during the epidemic. This was a reflection of the timing of the epidemic relative to the foaling season and rebreeding in the Thoroughbred industry. The central Kentucky fetal losses were estimated to be 25-30% of pregnancies in 2001 with an estimated economic loss on the order of \$330 million. This abortion event of unknown cause was named mare reproductive loss syndrome (MRLS). Initial epidemiologic investigations revealed an association of abortions with elevated populations of eastern tent caterpillars (ETC) (*Malacosoma americanum*), presence of wild black cherry trees, and unusual weather patterns. The nature of the outbreak suggested a point-source epidemic (that is, multiple cases occurring simultaneously in an area over a short time period indicating a common exposure). Multiple experimental studies demonstrated abortigenic activity associated with the ingestion of ETC, specifically the exoskeleton portion. Control measures directed at decreasing exposure of pregnant mares to ETC resulted in dramatically fewer abortions the second year on those farms compared to farms with no control efforts. Attempts to confirm a role of other incriminated causes of abortion were unsuccessful.

Early fetal losses were observed in the 40- to 150-day gestation range fetuses. In many instances, mares previously diagnosed as being pregnant were discovered to be open or nongravid. Other early fetal loss cases exhibited abnormal findings on ultrasound of the fetus. These findings included hyperechoic material within the amniotic fluid and signs of decreased viability of the fetus or death. In the viable fetuses, over the course of several days, the amount of hyperechoic material increased, and the fetal viability (heart beat and movement) diminished until death. A number of the affected fetuses and their placentas were examined and were usually in an advanced state of autolysis with no significant gross or microscopic changes. Bacterial culture often yielded non-beta-hemolytic *Streptococcus* or *Actinobacillus* spp., and bacteria were commonly observed microscopically in the membranes and fetal tissues. Cytology of the flocculent material in the amniotic fluid revealed it to be composed of desquamated fetal cells and clumps of bacteria.

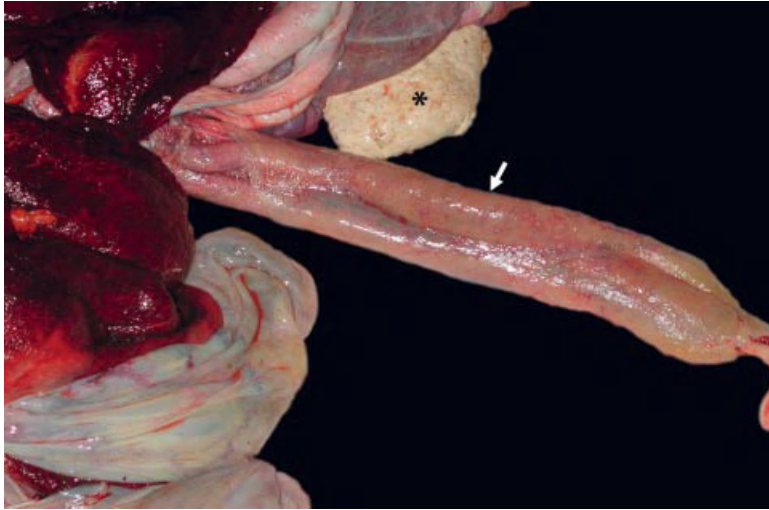


Figure 5.14 Funisitis associated with mare reproductive loss syndrome. Only the amniotic portion of the umbilical cord becomes affected. In this example, the cord is edematous, discolored yellow-tan, and roughened due to surface exudation (arrow). A large dough-like hippomane is present adjacent to the affected umbilical cord (asterisk). (Courtesy of Dr. NM Williams, University of Kentucky.)

Approximately 550 late-gestation fetuses were necropsied in Kentucky in 2001 during the MRLS epidemic. Multiple breeds and all ages of mares were affected. Most of the fetuses were delivered at term or were aborted several weeks prior to the due date, usually with no premonitory signs in the mares. The delivery was described as “red bag” in about one-third of the cases, indicating that the allantochorion separated from the endometrium and was presented and passed concurrently with the fetus. Often the abortion was described as explosive. Placentas were often edematous. The fetuses were normally developed and were usually presented in good postmortem preservation. The lungs were variably inflated and were sometimes firm, suggestive of fetal pneumonia. Hemorrhages were commonly observed on the surface of organs and placental membranes. The most unusual lesion occurred in the umbilical cord. Approximately 75-80% of the cases had funisitis characterized by edema and thickening of the umbilical cord, irregular roughening of the cord surface, hemorrhages, and dull yellow to gray discoloration. Only the amniotic portion of the umbilical cord was affected (Figure 5.14). Histologically, the lungs often contained numerous intra-alveolar cornified squamous cells of amniotic fluid origin along with low-to-moderate numbers of neutrophils and macrophages within the alveolar spaces. Occasionally, multinucleated cells were observed. Bacteria were often associated with the intra-alveolar cells and debris. Microscopic examination of the umbilical cords showed colonization of the surface by bacteria with loss of

the surface epithelium and infiltrates of neutrophils and macrophages that were concentrated near the surface. Placentitis (allantochorionitis) was less commonly seen and usually consisted of mild microscopic inflammation within the stroma of the allantochorion. Amnionitis was commonly observed, but lesions in other tissues and organs were not consistently present.

Bacterial culture of the aborted fetuses yielded bacteria in many of the fetuses. Routine sites of culture included the placenta, lung, liver, and stomach contents. In many of the cases, the umbilical cord was also cultured. Significant bacteria were isolated from 75% of the fetuses. The most frequently isolated bacteria were non-beta-hemolytic *Streptococcus* spp. (51.8% of fetuses) and *Actinobacillus* spp. (14.4% of the fetuses). These bacteria were not typical isolates from fetuses and had only rarely been associated with equine abortions previously.

The means by which ETC ingestion induces abortion has not been elucidated. Experimental data has suggested the possibility of damage to the alimentary tract of the mare by the hairs (setae) of the ETC with induction of a bacterial infection in the mare and spread to the fetus. However, the precise pathogenesis has not been determined.

Congenital anomalies

Developmental anomalies are sometimes found upon examination of aborted fetuses or stillborn foals. The most common is the contracted foal. This is manifest as severe flexor contraction with fixation of usually the distal joints of all legs in flexion, resulting in a flexor deformity. Attempts to deliver a contracted foal often result in dystocia causing death of the fetus due to delayed delivery and asphyxiation. Even if delivered live, these foals are often euthanized due to the severe contraction and poor prognosis. Many of the foals, in addition to flexor deformity, have severe scoliosis of the spine and less commonly may have facial deviation (maxillofacial deformity) and are referred to as contracted foal syndrome.

Various other congenital anomalies occur infrequently. Sometimes these are believed to be the cause of abortion or they are incidental findings unrelated to the abortion. Cytogenetic study is difficult on aborted fetuses and not often attempted.

Idiopathic abortion

A major study of abortion and perinatal death found that 16% of the cases did not have a diagnosis. In these, pathogens were not isolated, and no significant gross or microscopic lesions were detected, suggesting that these are not the result of infection of the fetoplacental unit. These abortions are often attributed to vague maternal factors, although there is often no history of maternal problems. Suggested maternal factors include hormonal abnormalities, stress, and illness.

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Chapter 6

Disorders of Dogs and Cats

Claus D. Buergelt

Introduction

Mechanism of abortion and neonatal loss

The duration of pregnancy in the bitch is generally considered to be 63 days postmating. A range of 56 to 72 days from first mating to birthing has been suggested. In the queen the duration of pregnancy generally is 66 days with a range of 64 to 69 days.

Abortion is defined as the expulsion of a developing conceptus at any period of gestation. The term "stillborn fetuses" refers to term fetuses that are born dead. Viable fetuses that survive despite being born before their due date are called premature births. There are generally non-infectious (toxins, drugs, metabolic, chromosome abnormalities) and infectious causes for gestational disturbances. Non-infectious causes of abortions are most difficult to determine.

Toxins, drugs and infectious agents in carnivores usually hematogenously cross the placental membranes to affect the fetus. The fetal organs are most susceptible to injury at specific gestational times. The reactions to injurious principles are different to perinatal, postnatal, and juvenile animals. Abortions ensue secondary to fetal death whether this is due to damage to the pregnant uterus, placental membranes, or fetus.

There are three principal manifestations in which abortions can occur:

1. Maternal illness due to metabolic, hormonal, or infectious causes (such as, feline rhinopneumonitis).
2. Uterotropic (placental) pathologic changes as with brucellosis.
3. Direct injury to the fetus as with certain viruses.

Infectious agents may reach the fetus by one of three basic mechanisms:

1. Direct infection by way of the vagina and cervix (ascending the birth canal).
2. Transplacental transmission (hematogenous) from the mother to the fetus.
3. Infection of the ovum (transovarian transmission).

Tissue sampling

Specialized techniques

Hormone assays

Pregnant bitches do not produce pregnancy-specific gonadotropin such as equine chorionic gonadotropin in mares. In its absence, other circulating hormones, such as prolactin (PRL) and relaxin should be measured. Relaxin is the only specific pregnancy-associated protein identified in the dog. A relaxin enzyme immunoassay is available and can be used for the diagnosis or to assess viability of fetuses *in utero*. However, there can be false-positive results following abortion. Circulating levels of placental relaxin are elevated from day 21 to 24 of pregnancy. Relaxin reaches peak concentrations (5 ng/mL) in late pregnancy (40 to 50 days of gestation). The levels decrease after parturition but remain detectable during lactation until day 30. PRL levels rise in midgestation with a peak at day 35 of between 15 to 20 ng/mL with levels up to 50 ng/mL near parturition. Measurement of PRL is done via radioimmunoassay or enzyme-linked immunosorbent assay (ELISA) from commercial kits. Individual animal variations in the serum PRL concentrations make it somewhat unreliable for pregnancy diagnosis.

Cytology

Cytology or culture is a helpful tool to check for evidence of infection and infectious agents during vaginal discharge. Impression smears also should be done from placental tissue, fetal organs, and fluids when lesions are observed.

Fetus karyotyping

This technique could be performed to determine chromosomal abnormalities, including sex chromosomal abnormalities. The tests are typically done on proliferating lymphocytes harvested from peripheral blood or cultured dermal fibroblasts and include Giemsa staining, fluorescence *in situ* hybridization (FISH) or chromosomal microarrays. Laboratories performing these tests are at North Carolina State's College of Veterinary Medicine; Pennsylvania School of Veterinary Medicine; Veterinary Diagnostic Laboratory, Michigan State University; or Veterinary Genetics Lab, Davis, California. Depending on the test, costs range between \$150 to \$300 per sample.

Serology

Depending on the body size and gestational period of the carnivore, fetal heart blood should be collected for serologic evaluation and submitted together with the blood of the aborting bitch or queen. The determination of titers is especially important for certain bacterial and virus diseases such as canine brucellosis or feline calicivirus. Results of the serologic examination of a single sample from an animal that aborted must be interpreted with caution. There is no specific titer for any infection that will with certainty differentiate an infected from a vaccinated, uninfected animal. Likewise, paired serum samples may be inconclusive because the first titer is usually determined at the time of abortion when the serum titer might have already reached its maximum. In cases of animal colonies or several animals in a household with an abortion history, paired samples from several animals may give reliable diagnostic results. A fourfold or higher increase in serum titer or animals with first negative and then positive titers in the second sample may indicate active infection in the colony or household. Serologic results should always be supplemented with history, ancillary laboratory results, and when possible, necropsy results.

Pooling of samples

It is recommended at the time of necropsy to collect samples of multiple organs from all aborted fetuses in a litter. Sample pooling should be discouraged because not all fetuses in a litter are equally affected particularly when it relates to infectious agents. Thus, fresh tissues may be saved and frozen pending further use for identifying or better characterizing an infectious agent.

Table B.5 in Appendix B summarizes what tissues to collect, which tests should be requested, and lists additional ancillary tests that can be performed to maximize the diagnostic effort of attempting to confirm a diagnosis from a list of selected pathogens.

Fetus and fetal membranes

During necropsy of the fetus, a standard set of tissues should be taken and fixed for histopathology. Samples from vital organs for microbiology, virology, or toxicology should be collected. Fetal fluids and stomach contents for ancillary analyses should be collected as well.

Fetal and placental tissues should be fixed in 10% neutral buffered formalin. Because many fetal organs are blood tinged, care should be taken to avoid mixing the formalin with excessive blood or fetal fluids. This will significantly lower the fixation strength. The fixative should be changed at least once within the first 12 hours.

Selected organs should be collected for bacteriology, virology, or toxicology or frozen at -70°C if examinations are to be performed at a later date.



Figure 6.1 Canine neonatal necropsy. The body cavities have been opened as part of a complete necropsy examination allowing visualization of possible lesions as well as collection of tissues. (Courtesy of Dr. CD Buergelt, University of Florida.)

Aseptically swab lung and stomach contents for bacteriologic examination. Collect two sets of tissues aseptically from lung, liver, kidney, spleen, and thymus for direct virus isolation, molecular biology, or FA work in Ziploc® bags. Collect liver, kidney, brain, and lung for toxicological or nutritional analyses.

Gross examination of the fetus

Like in adult animals, a complete necropsy should be performed on the fetus, be it the smallest and most autolyzed (Figure 6.1). Determine sex, and measure and record weight and crown-rump length to estimate gestational age (Table A.5 and Table A.6 in Appendix A). Fetal organs contain a high percentage of water, thus giving many structures an almost edematous appearance. Breakdown of erythrocytes during autolysis stains surfaces of internal organs with hemoglobin, an event that should not be confused with antemortem bleeding perhaps from septicemia. In addition, autolysis distorts and weakens the texture of individual organs and fills body cavities with fluid. Autolysis quickly affects kidneys, liver, and brain, but lungs and heart stay relatively stable as to texture for a longer period of time. Include the umbilicus and placenta if available for ancillary examinations.

Gross examination of fetal membranes

The normal placenta is dark brown in the dog; it is red-brown in the cat.

The fetal membranes are expelled, and about 10 minutes after the birth of the pup, the bitch usually eats the membranes. She also breaks the umbilical cord. The fetal membranes either are expelled between fetuses, or one fetus may be expelled with its own placenta. This species behavior makes it difficult to harvest placental tissue for macroscopic and microscopic examination.

In the queen, the birth of the first kitten usually takes 30 to 60 minutes, and the interval between delivering subsequent kittens varies from 5 to 60 minutes.

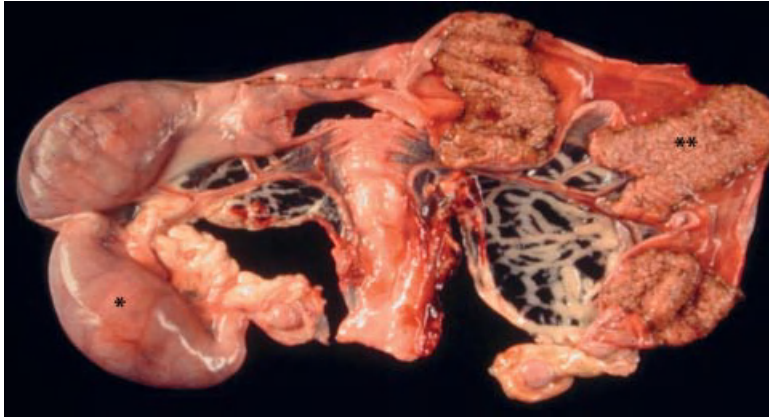


Figure 6.2 Feline gravid uterus. Three fetal endometrial attachment sites for its zonary placenta are evident in a partially opened gravid uterus. Two fetuses remain attached in the unopened section of the left uterine horn. (Courtesy of Dr. CD Buergelt, University of Florida.)

The expulsion of the placenta usually occurs after each kitten is delivered. Queens will sever the umbilical cords and eat the placentas.

Otherwise, as a matter of principle, placental examination is critical in evaluation of perinatal morbidity and mortality. A careful placental examination may reveal clues to events occurring throughout the gestation. Clinicians, breeders, and owners should be educated to submit not only the fetus, but also any available fetal membranes. A detailed history about the circumstances of the abortion should be obtained from the owner or clinician.

Placental tissues should always be handled with gloves. Both allantoic and endometrial surfaces should be visualized after spreading the placenta out on an even surface. Fresh samples should be collected aseptically. Several slab sections particularly from the labyrinth should be fixed from each placenta and examined histologically. If possible, digital images should be obtained.

Carnivore placentation

According to gross classification, the carnivore placenta is zonary because of the shape of the circumferential placentation site (Figures 6.2 and 6.3). Zonary placentas are a complete girdle in dogs and an incomplete girdle in cats. Histologically, the type of the maternofetal interface is lamellar, with fetal projections interdigitating with maternal septa in a folded fashion. Chorionic attachment to the endometrium occurs through the formation of a placental labyrinth. The labyrinth is a deciduate, endothelio-chorionic configuration with four tissue layers at the fetomaternal interface (Figure 6.4). Fetal capillaries are embedded in fetal connective tissue containing cytotrophoblasts and syncytiotrophoblasts. Cytotrophoblasts express Ki-67 antigen.

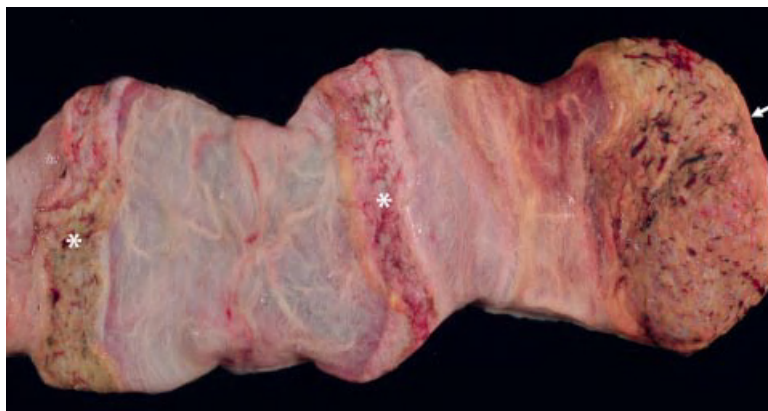


Figure 6.3 Canine zony placenta. One horn of a canine uterus has been evaginated, exposing the endometrial surface with three zony placental sites (asterisk). At the tip of the horn (*arrow*) is a broader area of placental. (Courtesy of Dr. BL Njaa, Oklahoma State University.)

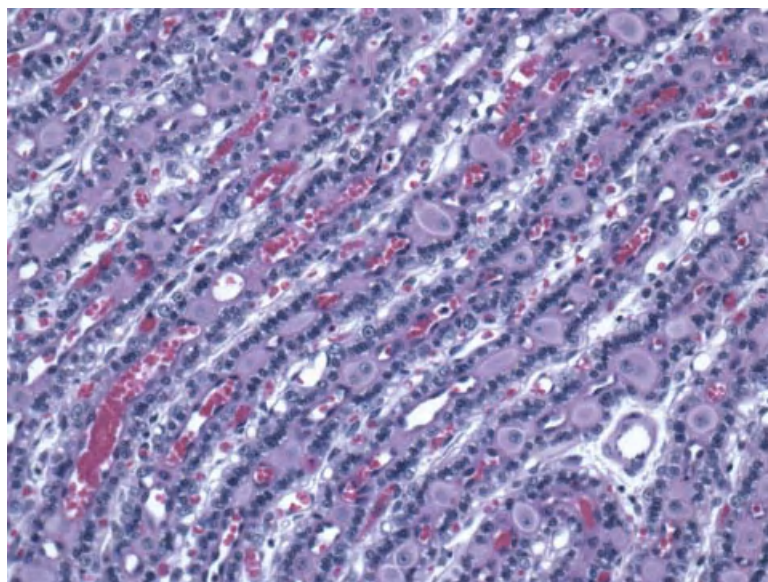


Figure 6.4 Feline chorion. Labyrinthine placenta at term pregnancy. The thickness of the labyrinth varies according to gestational age. It interdigitates with the surface of the endometrium. H&E stained section. (Courtesy Dr. BL Njaa, Oklahoma State University.)

The proximity of the fetal circulation to the mother classifies the placenta as endotheliochorial at the microscopic level. The thickness and length of the labyrinth varies according to gestational age. Maternofetal blood flow is of crosscurrent type.

A third classification divides placentation types into deciduate or conjoined and indeciduate or apposed. The true deciduate type is present in primates and rodents. It exists as a modified form in dogs and cats.

Placental development and maturation are complex in carnivores. Implantation involves apposition, adhesion, and intrusion of the blastema. The yolk sac is essential in early pregnancy (first 20 days) and rests ventral to the embryo containing prominent blood vessels. At day 20 of pregnancy, a choriovitelline placenta supplies the embryo, and afterward the formation of a chorioallantoic placenta begins. After 45 days of pregnancy until term, the lamellar organization and the density of capillaries increase as adjustment to the nutritional requirements of the fetus. Up to day 45 of gestation, the allantoic sac becomes prominent due to developing vasculature. The amnion surrounds the conceptus, containing fluid and remaining poorly vascularized. At day 53 of pregnancy, the fetus is totally enveloped by and inside the amnion, which is totally enveloped by the allantois.

Perizonary hematomas, also known as marginal hematomas, have the structural function of transferring iron to the fetus. They start to develop between days 22 and 25 of pregnancy, and are visible as black-green pigment at the border of the placental girdle as a result of extravasation of blood (vascular lakes) from maternal capillaries and metabolism of hemoglobin to uteroverdin (Figure 6.5). The development of perizonary hematomas can happen during the entire period of placentation and should not be confused with pathologic hemorrhage into the placenta (Figure 6.6). In cats, the labyrinthine portion of the placental girdle is similar to dogs, but the marginal hematoma is much less obvious when compared with canine placentas (Figure 6.7).

Viral causes of abortion and neonatal loss

Dogs

Canine herpesvirus (CHV)

This alpha-herpesvirus of dogs causes abortions or neonatal death, but it also is associated with stillborn puppies and infertility. At the gross level, renal cortical hemorrhages and ecchymoses are prominent in aborted, stillborn, or perinatal pups (turkey-egg kidney) (Figure 6.8). Similar areas of hemorrhage may also be grossly evident in the liver and lung. The leptomeninges may reveal punctate, red foci (petechiae) randomly distributed over its surface. Microscopic examination of tissues reveals foci of necrosis with intranuclear inclusion bodies in the liver, spleen, adrenal gland, kidney, and lung. If placental tissue is available for examination, focal necrosis with inclusion bodies in trophoblast epithelium can be seen. A more usual presentation of herpesvirus infection is a clinical manifestation of weakness in viable neonatal puppies infected by either passing through the birth canal at whelping or from oronasal secretions from the bitch or infected littermates. Such infection of puppies up to 3 weeks old is aided by the subadult temperature and incompletely

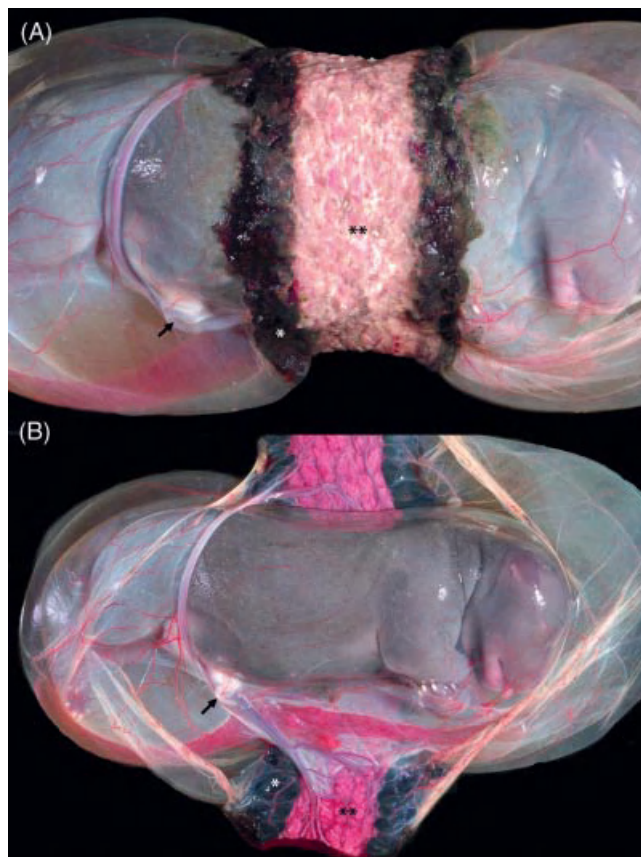


Figure 6.5 Canine zony placenta. (A) This central zone of placentation is composed of the central pale tan-pink labyrinth (*double asterisk*) peripherally flanked by dark green marginal hematomas (*asterisk*). The umbilical cord (*arrow*) is visible through the allantochorion. (B) The zony placenta and allantochorion are incised to expose the allantoic surface. The bright red central region of the placentation site corresponds to the labyrinth (*double asterisk*) with two dark black rims that represent the marginal hematoma (*asterisk*). The umbilical cord vessels (*arrow*) feed various portions of the allantoic surface of the zony placenta. (A and B courtesy of Dr. BL Njaa, Oklahoma State University.)

developed thermoregulatory capability of the neonate. Clinical signs in such postnatally infected puppies are hypothermia, anorexia, abdominal pain, and crying and paddling of the limbs.

Diagnosis of herpesvirus infection is made via virus isolation, molecular biology tests, or serology. Virus isolation from infected tissues is done on canine cell monolayers. Serology uses an ELISA with antigen prepared by solubilized infected cells. Other less-sensitive tests are microplate serum neutralization test and plaque reduction assays.

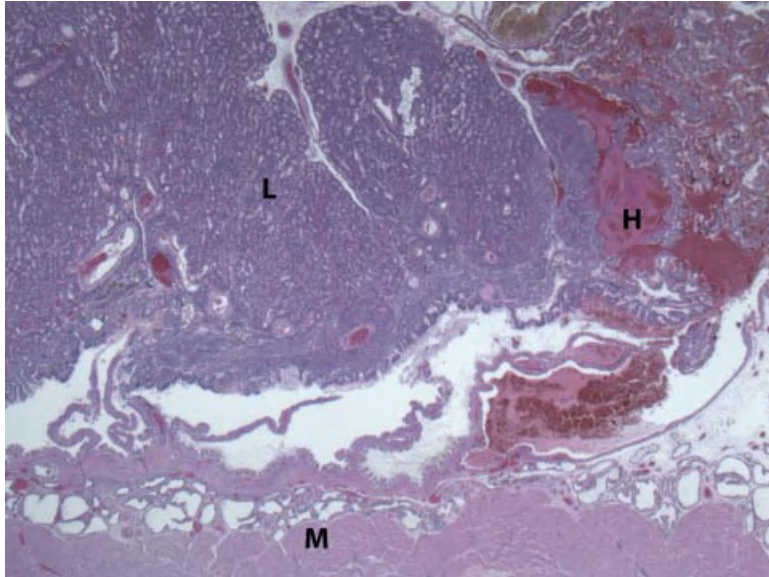


Figure 6.6 Canine marginal hematoma. In this section the marginal hematoma is present (*H*) in a perizonary position relative to the labyrinth (*L*). The placental attachment site comprises the maternofetal labyrinth as it interfaces with the uterus (*M*). (Courtesy of Dr. BL Njaa, Oklahoma State University.)

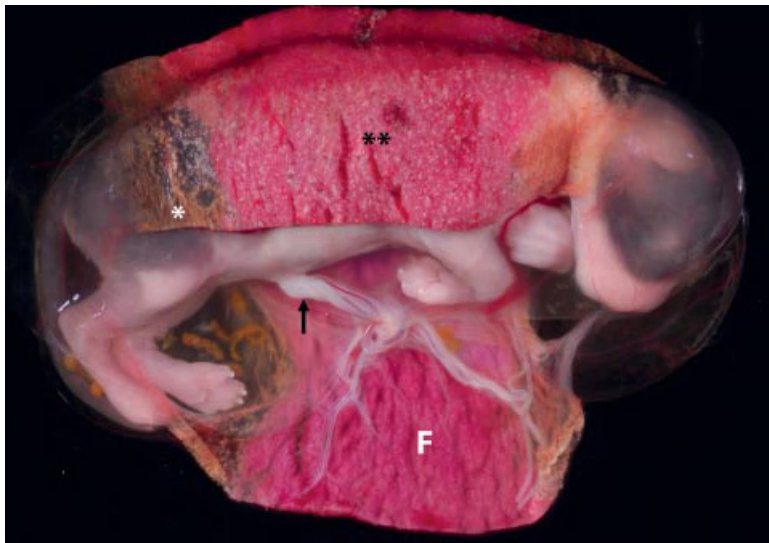


Figure 6.7 Feline placentation. Similar to the canine placental girdle, the endometrial surface (*double asterisk*) interfaces with the uterine site of placentation and allatonic surface (*F*) is fed by the umbilical blood vessels (*arrow*). The marginal hematoma (*asterisk*) is pale brown and thinner when compared to the canine. Within the amniotic compartment near the caudal part of the feline fetus are variably sized, yellow-orange aggregates that represent released meconium. (Courtesy of Dr. BL Njaa, Oklahoma State University.)

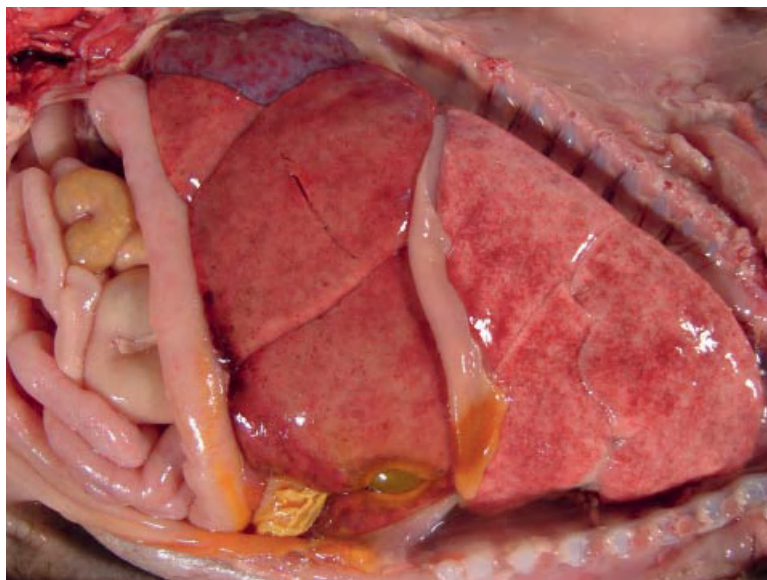


Figure 6.8 Canine herpesviral (CHV) infection. Lesions include lungs that fail to collapse and petechial to ecchymotic hemorrhages scattered throughout the lungs, kidney, and liver. These lesions are virtually pathognomonic for CHV infections. (Courtesy of Dr. BL Njaa, Cornell University.)

Molecular biology assays include PCR, conventional, or nested from a wide range of fresh or formalin-fixed tissues, and *in-situ* hybridization.

Minute virus of canines (MVC)

This canine parvovirus-type 1 is widely distributed among domestic animals in the US and is a pathogen of neonatal puppies. The virus is closely related to the bovine parvovirus as to DNA homology. Transplacental infection occurs in infected dams resulting in fetal death, weak puppies, or early neonatal death. Neonates may develop necrotizing enteritis with eosinophilic inclusion bodies present in enterocytes of villi (Figure 6.9). Crypt necrosis, as seen with parvovirus-type 2 infection, is not a feature of minute virus of canines.

Diagnosis of canine parvovirus type-1 is done by hemagglutination inhibition (HI), PCR or virus isolation from especially susceptible canine cell lines, designated as WRCC/3873D and MDCK. Virus strain GA3 is used as a reference strain.

Canine adenovirus type 1

The virus may cross the placenta during pregnancy resulting in the birth of dead pups, weak pups, or pups with systemic infection. Diagnosis is done via virus isolation. Bitches should be vaccinated prior to mating.

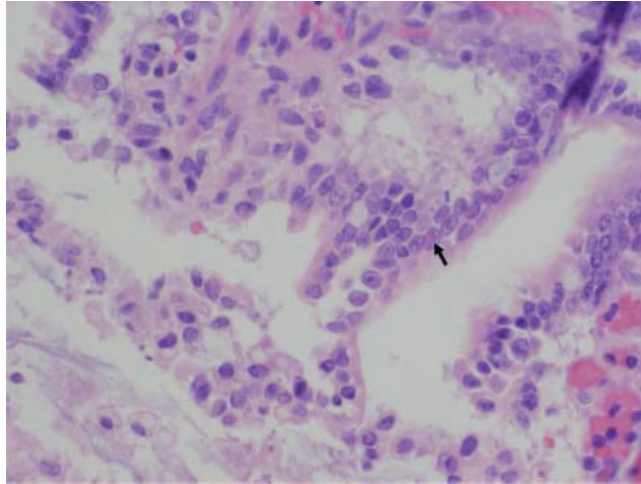


Figure 6.9 Minute Virus of Canines (MVC) infection. In this section of intestinal mucosa, villi are blunted, and many infected enterocytes contain purple intranuclear inclusion bodies (arrow). A diagnostic feature for this canine parvovirus-type 1 infection is intranuclear viral inclusions in enterocytes. H&E stained section. (Courtesy of Dr. CD Buergelt, University of Florida.)

Canine distemper virus (CDV)

This virus most likely is not involved in transplacental abortions. After experimental inoculation of virulent CDV virus into pregnant dams resulted in clinical signs of distemper, the virus could not be isolated from the aborted fetuses. The literature does not contain information of abortion in naturally infected pregnant bitches.

Bluetongue-virus contaminated canine vaccine

Abortion and death of pregnant bitches have been reported after vaccination with a bluetongue-virus contaminated modified-live multi-component canine vaccine, targeted against canine distemper, adenovirus, parvovirus, and parainfluenza virus. An experimental inoculation with bluetongue virus serotype 11 resulted in abortion and even death of pregnant animals. Bluetongue viral nucleic acid was detected by *in-situ* hybridization in endothelial cells from various tissues.

Cats

Feline herpesvirus-1 (FHV)

Feline herpesvirus-1 is primarily a respiratory pathogen in postnatal cats, and abortions are rare in infected pregnant cats. Kittens may, however, die of generalized infection in the first 3 weeks of life. It has been suggested that if abortions happen after FHV-1 infection, the event is due to severe respiratory maternal rhinopneumonitis and generalized viremia.

Virus has been directly recovered from tissues such as uterus, placenta, and vagina in experimental conditions. In natural situations, virus isolation does not discriminate between infected and carrier cats. The sensitivity of PCR, indirect immunofluorescence, and ELISA likewise is compromised and is of limited diagnostic value because positive results can be obtained from clinically normal, healthy carrier, and diseased cats. PCR cannot differentiate between vaccine strains and wild-type virus. In general, a high detection rate of FHV-1 does not correlate with high diagnostic sensitivity.

Feline leukemia virus (FeLV)

FeLV virus is mainly transmitted from cat to cat via saliva. Susceptibility is highest in young kittens. This retrovirus, when transplacentally transmitted, can lead to late-term abortions or neonatal death in catteries. Control of the virus through preventive management such as vaccination will end episodes of abortions.

Commercial ELISA for antigen (PetCheck®) on whole blood or serum, virus isolation and PCR, including real-time PCR for provirus detection on fetal blood cells, fetal tissues, and fetal fluids have been described.

Feline immunodeficiency virus (FIV)

The virus is mainly transmitted via saliva after bites or fights. This virus causes decreased litter size and pregnancy losses in queens. The transplacental transmission mode is unclear. Evidence suggests that some kittens acquire infection *in utero* in natural situations. After experimental infection, FIV could be isolated from almost all placental membranes and fetuses. FIV is definitively transmitted horizontally by direct contact and excreted into milk and semen.

A real-time PCR assay is used to analyze for provirus on blood cells. Conventional PCR apparently is unreliable to detect FIV epitopes. FIV in tissue sections can be assayed for viral RNA using *in-situ* hybridization. Immunohistochemical detection of antigen can be done using high antibody titer serum from cats chronically infected with FIV. Mouse monoclonal or rabbit polyclonal antibodies are not considered suitable for FIV detection in tissues because of low sensitivity and lack of specificity. Serologic ELISA methods to check for FIV antibody also are unreliable because they may yield false-positive and false-negative results.

Feline enteric coronavirus (FCoV)

The virus that causes feline infectious peritonitis (FIP) is a mutated form of feline enteric coronavirus. It may cause abortion and stillbirths in catteries with high titers for FCoV. Regular testing and removal of seropositive cats from premises and eliminating parents and offspring developing FIP from breeding programs are commonly performed management strategies.

Commercial kits test for serum antibodies against FCoV infection are available. Direct or indirect immunofluorescence can be used to test for antibodies or

virus antigens. Blood samples can be tested for FCoV messenger RNA (mRNA) by reverse transcriptase polymerase chain reaction (RT-PCR) assay.

Feline calicivirus (FCV)

Feline calicivirus has been recognized as cause of upper respiratory disease and of ulcerative stomatitis with viremia ensuing after initial infection. Abortion from calicivirus is rather uncommon. There is one report in the literature where FCV was isolated from fetuses with petechial hemorrhages in the skin. Little is known about transplacental transmission. In addition to virus isolation, real-time reverse transcription PCR is used for the specific diagnosis of FCV. Also the titer to the virus can be determined serologically.

Feline panleukopenia virus (FPLV; feline parvovirus)

Infection with this feline parvovirus or vaccination with a modified-live vaccine causes abortions or birth of kittens with pathognomonic cerebellar abiotrophy depending on the stage of gestation when viral exposure occurs (Figure 6.10).

The virus can be isolated in cell cultures. Restriction fragment length polymorphism (RFLP) of PCR-amplified fragments can differentiate between field isolates and live FPLV vaccine strains. Serum neutralization or hemagglutination inhibition methods test for antibody titers, but they cannot differentiate between infection or vaccination status.

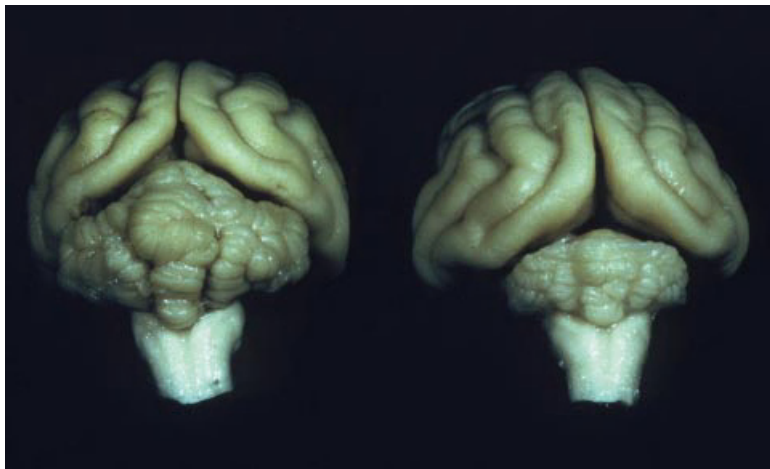


Figure 6.10 Feline panleukopenia-induced cerebellar abiotrophy. The brain on the right has a cerebellum of reduced size, affecting the vermis and both cerebellar cortices. The brain on the left is from a normal, age-matched control. (Courtesy of Dr. RJ Panciera, Oklahoma State University.)

Bacterial causes of abortion and neonatal loss

Dogs

Brucellosis

First isolated in 1966 from puppy losses in kennels, canine brucellosis is a contagious disease with venereal and oral modes of transmission. It produces abortion in females as a result of placental infection (Figure 6.11) and epididymitis and prostatitis in males. It leads to infertility in both sexes.

The disease is caused by *Brucella canis*, a Gram-negative coccobacillus. Infection mainly occurs through vaginal secretion. Both sexes secrete bacilli through urine. Male dogs secrete through semen. Milk can be a vehicle of transmission. After the organisms gain entrance into the animal, they are phagocytized by macrophages and taken to lymph nodes and the spleen. Generalized lymphadenitis occurs in adults. From there bacteremia develops. This is a zoonotic pathogen with human infections reported. Infected humans respond well to antibiotics.

Abortion occurs between 30 and 57 days of pregnancy. Aborted puppies appear autolyzed but may show lesions of septicemia such as serosal petechiations. Weak puppies may be born. Embryo absorption is possible.

Diagnosis is difficult because of unstable serum antibody titers. Serologic tests comprise tube agglutination, rapid slide agglutination test, AGID, ELISA,

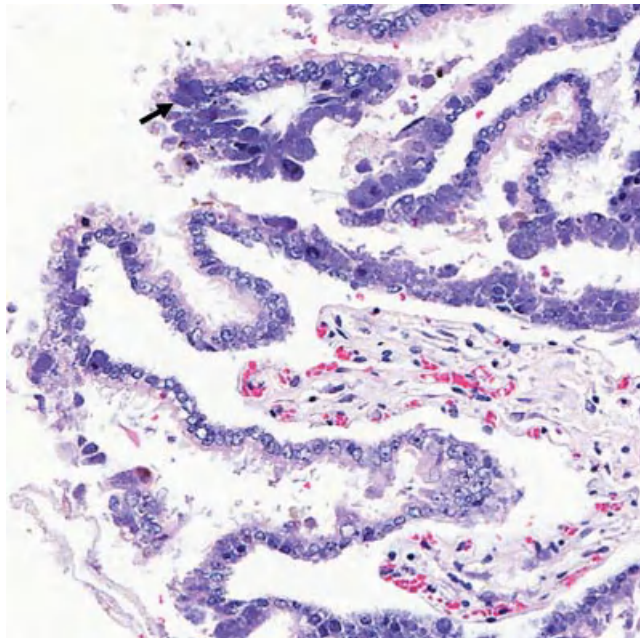


Figure 6.11 *Brucella canis* abortion. *Brucella* bacteria are present within the cytoplasm of the trophoblast cells in the labyrinth of the placenta (arrow). H&E stained section. (Courtesy of Dr. DH Schlafer, Cornell University.)

and IFA tests. Routine testing in kennel situations is recommended. A positive culture of the organism makes the diagnosis definitive.

E. coli* and *Staphylococcus aureus

Enterobacter species and *Staphylococcus* spp. can be frequently isolated from aborted fetuses, especially in kennels (Figure 6.12). Caution is advised as to relating them to causation. *E. coli* is a common aerobic bacterium present in the vagina of the normal bitch. *Staphylococcus* organisms are common opportunistic pathogens or environmental contaminants.

Diagnosis can be made by bacterial culture. Strain classification is done by examination of virulence factors such as somatic antigens, capsular antigens, and flagellar antigens and by PCR to identify bacterial virulence factor genes. Serologic assays analyze for serotropic markers such as toxins, adherence factor, or lysins.

Beta-hemolytic *Streptococcus* spp.

Streptococci are common inhabitants of the vagina of the normal bitch. They have been cultured from puppies with neonatal septicemia. *S. canis*,



Figure 6.12 Multiple aborted and stillborn canine fetuses. In this canine abortion, various-sized fetuses with evidence of decomposition indicate fetal death occurred at different times of gestation with the fresher fetuses suggesting they were stillborne. *E. coli* was cultured from placental membranes in this case. (Courtesy of Dr. CD Buergelt, University of Florida.)

S. dysgalactiae, *S. equisimilis* and *S. equi* subsp. *zooepidemicus* are the most common pathogenic streptococcal species in the dog. Spread from the vagina to the uterus may result in endometritis and subsequent spread to the placenta. Abortion or neonatal deaths are common outcomes.

Diagnostic confirmation of these pathogens is done via bacterial culture.

Campylobacteriosis

Campylobacter jejuni in pure culture was isolated from vaginal discharge of three dogs after late-pregnancy abortions.

Mycoplasmosis

Mycoplasma canis is part of the normal flora of the canine urogenital tract. It is unclear whether the bacillus contributes to canine reproductive diseases including abortion. The contribution of *M. canis* to disease should be made by culture.

Leptospirosis

Unlike in other domestic animal species, *Leptospira* spp. cause more systemic disease in the dog than pregnancy loss.

Cats

Abortions due to *Salmonella typhimurium*, *E. coli*, beta-hemolytic *Streptococcus* spp., and *Bartonella henselae* have been reported in individual situations. Identification is achieved by bacterial culture.

Protozoal causes of abortion and neonatal loss

Dogs

Toxoplasma gondii* and *Neospora caninum

Toxoplasma gondii and *Neospora caninum* are vertically transmitted from the bitch to the pups during pregnancy and may cause fetal death. Neosporosis can cause encephalomyelitis and polymyositis in neonatal pups infected *in utero*.

Diagnosis of these protozoa is made via serology, dye-test for *T. gondii*, immunohistochemistry on tissue samples, or other molecular diagnostic tests.

Leishmaniasis

The literature contains a report of placentitis due to *Leishmania donovani*. The single case report affected a young Coonhound from Maryland that aborted

seven fetuses. One fetus was examined microscopically. *Leishmania* amastigotes were only identified in placental trophoblasts. The protozoa were not found in fetal tissues.

Members of the *L. donovani* complex are intracellular parasites of the mononuclear-phagocytic system and are typically transmitted by the sand fly. The disease occurs as cutaneous, mucocutaneous, or visceral forms. Dogs, cats, and rodents serve as reservoirs of infection for human beings.

Cats

Toxoplasma gondii

Cats are the definitive host for *Toxoplasma gondii*. Reproductive effects of toxoplasmosis develop in pregnant queens that initially may develop neurologic signs of the disease with subsequent abortion. Transplacentally infected kittens die shortly before or after birth.

Diagnosis of toxoplasmosis is made via serology or molecular biology. Serologic tests include modified agglutination test (MAT), indirect fluorescence antibody test (IFAT), the Sabin-Feldman dye test (DT), indirect hemagglutination test (IHAT), and ELISA. PCR methods are used for fecal and fluid-based *T. gondii* epitope detection.

Cytauxozoonosis

Cytauxozoon felis is an ixodic tick-transmitted protozoan infecting domestic and wild felids typically in the south, central, and southeastern parts of the United States. When clinically evident, the disease is usually fatal in domestic cats, although surviving cases have been reported. Bobcats (*Lynx rufus*) are considered to be the reservoir host.

Cytauxozoon felis was present in a young pregnant cat that died after aborting three kittens. In the queen, numerous schizont organisms were found in macrophages lining endothelial surfaces and occluding blood vessels. Organisms were not detected in the placenta or in the two fetal tissues (skeletal muscle, bone marrow) examined.

Miscellaneous causes of abortion and neonatal loss

Maternal diseases causing abnormality of pregnancy

These could be the result of metabolic disorders, hormonal deficits, or non-infectious diseases affecting the uterus or abdomen.

Dog

Diabetes mellitus

Progesterone, the hormone of pregnancy, causes insulin resistance and stimulates secretion of growth hormone, an insulin antagonist. Glycosuria and

hyperglycemia (less than 300 mg/dL) are clinicopathologic changes observed in affected pregnant bitches. Pups born to bitches with DM are unusually large and may cause dystocia.

Pregnancy toxemia

Pregnant bitches with a large litter present with anorexia and depression. Hepatic lipidosis and hypoglycemia with ketonuria in the absence of glucosuria may be present.

Cat

Eclampsia

Eclampsia results from hypocalcemia. It occurs prenatally and perinatally in nursing queens. Clinical signs include trembling, muscle fasciculation, dyspnea, and bradycardia. Diagnosis is made by analyzing for serum calcium. Calcium levels are usually between 3.5 to 5 mmol/L.

Trauma

Blunt trauma to the abdomen in late pregnancy may lead to preterm delivery or uterine rupture. Of course other vital abdominal organs may suffer from the same insult potentially leading to demise of the bitch from an event such as hemoabdomen. Generally, trauma in pregnancy may impede the mother's condition relative to oxygenation deficits, circulatory hypovolemia, and developmental abnormalities. Maternal trauma may increase the incidence of premature placental separation and preterm delivery.

Torsion of the uterus

Torsion is defined as a rotation along the long axis of a hollow organ. Uterus torsion in the queen occurs during the later phase of gestation. Affected queens show signs of valvular discharge, abdominal pain, and distension. Diagnosis is made via abdominal radiology or ultrasonography. Uterine torsion may also appear in pregnant dogs (Figure 6.13).

Ectopic pregnancy

Ectopic pregnancy is defined as extra-uterine development of one or more fetuses. Affected fetuses are located within the abdominal cavity. There are primary and secondary forms. Primary ectopic pregnancy probably does not occur in domestic animals. For this condition to develop, there must be a primitive trophoblast and/or functional primitive placentation created by the omentum or visceral peritoneum that guarantees connection to the maternal circulation. Secondary abdominal pregnancy may develop after a rupture of uterine tubes or uterus. Because placentation is incomplete and insufficient,

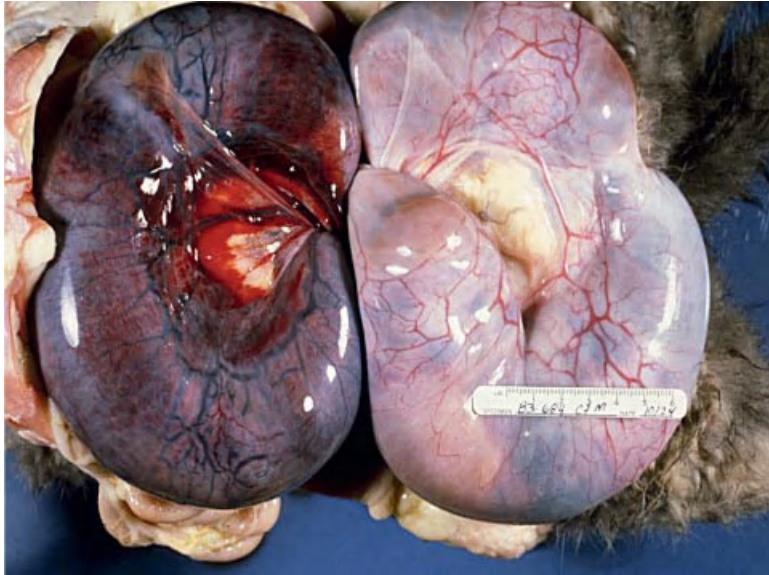


Figure 6.13 Torsion of single uterine horn in a dog. A twist along the longitudinal axis of a pregnant uterine horn resulted in severe, diffuse venous congestion and dark purple discoloration of the affected uterine horn. (Courtesy of Dr. CD Buergelt, University of Florida.)

the embryo or early fetus dies, and the dead conceptus is treated as a foreign body by the host. Viable fetuses should not be expected; instead mummified, aseptic global structures without fetal membranes attached to omentum or mesentery are seen (Figure 6.14). Various structures, including bone, are visible grossly (Figure 6.15).

Microscopically, ghosts of other tissue structures can be present. In some instances, clinical signs other than failure of parturition or abdominal extension do not occur during ectopic pregnancy; in other instances, urinary or gastrointestinal disturbances and general depression and lethargy can be expected. Diagnosis is made by abdominal radiography or ultrasound.

Chromosome errors

These will affect zygote, blastocyst, and the early embryo. It will result in early embryonic death and resorption. The early conceptus disintegrates, and estrous cycle will ensue. Dams and sires producing litters with chromosomal defects should not be bred to each other. Fetal loss may be caused due to congenital defects, lethal traits, or inherited traits due to faulty genes. Anatomic abnormalities can be expected in 20% of kittens that are stillborn. Laboratories performing karyotyping have been listed in the preceding chapter on fetal karyotyping.

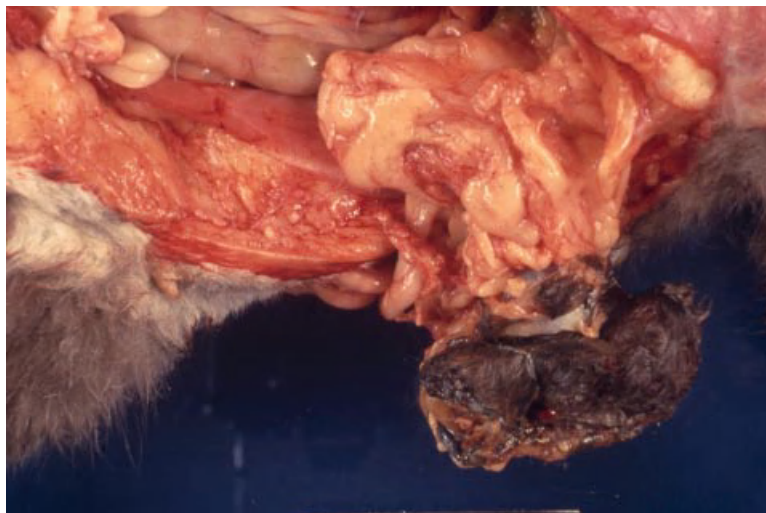


Figure 6.14 Feline ectopic pregnancy. An elongated fur-covered body is attached to intestinal mesentery and encapsulated by omental tissue. It was an incidental finding at necropsy. (Courtesy of Dr. CD Buergelt, University of Florida.)

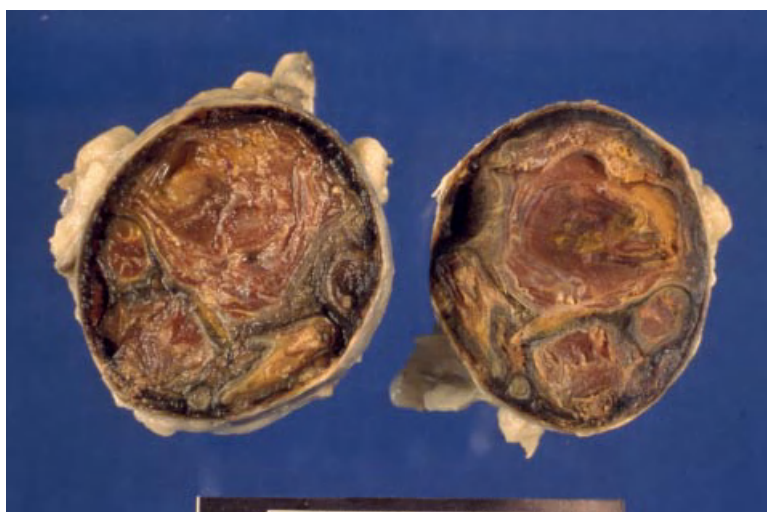


Figure 6.15 Canine ectopic pregnancy. Various inspissated tissue structures, including bone, are visible on cut section through the mesentery. (Courtesy of Dr. CD Buergelt, University of Florida.)

Drugs

Drugs may have toxic effects on the zygote or early conceptus with similar outcomes as described with chromosomal abnormalities. Administration of antifungal drugs such as griseofulvin or ketoconazole is contraindicated in pregnant cats because they may exert teratogenic effects on fetal facial

bones, eyes, and the central nervous system leading to deformed kittens. Nonsteroidal anti-inflammatory drugs such as indomethacin and aspirin given during late stage of pregnancy may cause delay of delivery. Glucocorticoids, tetracyclines, and chloramphenicol are medications known to interrupt pregnancy. Mechanisms of action are unknown. Certain antineoplastic agents have been reported to cause fetal death during pregnancy as they have a teratogenic effect.

Hormone deficiencies

Hypoluteoidism defined as decline of serum progesterone levels to less than 5 ng/mL due to insufficient production and secretion by the corpora lutea can cause pregnancy loss due to placental insufficiency in bitches and queens. In these animals, serum concentration of progesterone should be monitored. Causes for such corpora lutea dysfunction are not known because endometrial prostaglandins seem not be involved in hypoluteoidism.

Hypocalcemia

Elevated serum calcium can cause uterine inertia and atonia and may be involved in puppy mortality at birth. Serum calcium levels are below 7 mg/dL.

Hypoglycemia

The condition rarely causes reproductive problems in preparturient animals. Glucose levels range between 7 to 40 mg/dL.

Nutrition

Malnutrition can lead to *in utero* fetal death and expulsion. Specifically in cats, taurine deficiency in the diet has been reported to lead to resorption or abortion of fetuses, an increased incidence of near-term fetal death or kittens with low birth weights. Commercial diets have been adjusted for taurine deficiency, and therefore, this condition is largely of historical significance.

Parturient and postparturient abnormalities of pregnancy

Dystocia in cats and dogs

Mating of disproportional parents, breed peculiarities such as brachiocephalic breeds, litter size, and monster formation are causes for dystocia with subsequent delivery of weak or dead fetuses. Litter size may have a direct effect on length of pregnancy and size of puppies at parturition.

Subinvolution of placental sites in dogs

The condition develops in the process of placental degeneration and endometrial reconstruction following whelping. The cause remains unknown. Not all placental sites are affected. Persistent discharge of blood from the vagina of young bitches following parturition can be the result of retention of cytotrophoblast-like cells presumably of fetal origin. These cells implant into the myometrium and serosa of the uterus mimicking uterine neoplasia. Diagnosis is made via history and ultrasonography revealing echogenic foci in the endometrium. Recovery may be spontaneous, but peritonitis may be a complication. Ovariohysterectomy may be a treatment option if there is no recovery.

Uterine bleeding during canine pregnancy

The uterus of presumably pregnant dogs may be entirely filled with sterile blood and cellular debris. After removal of the blood, placentation sites may be visible, but no fetuses. These might have died *in utero* with subsequent resorption.

Canine pseudopregnancy (pseudocyesis) and canine deciduoma

The issue of uterine endometrial proliferative changes in nonpregnant, typically virgin bitches showing clinical signs of pregnancy is confusing, and the etiopathogenesis is not well understood. Terms such as pseudo-placental endometrial hyperplasia (PEH), endometrial hyperplasia in pseudocyesis, maternal placental-like endometrial hyperplasia and deciduoma have been implied for the characterization of the changes. Likely they are one and the same entity. The alternative of early pregnancy with subsequent resorption of the conceptus should be discarded for this condition because there is no morphologic evidence.

Pseudocyesis is considered to be an excessive physiologic syndrome observed in nonpregnant bitches at the end of diestrus (metestrous). The syndrome leads to clinical signs of pregnancy such as weight gain, nesting, mammary enlargement, lactation and maternal behavior. A mucoid, sometimes bloody, vulvular discharge may occur. It is postulated that rising circulation levels of prolactin or increased sensitivity to prolactin play a central role in the etiopathogenesis. Inhibition of prolactin release by ergot derivatives such as bromocriptine (10–100 mg/kg per day for 10–14 days) has proved to be effective as treatment of pseudocyesis. Side effects such as nausea, vomiting, or constipation have been reported during treatment. Ovariohysterectomy is a permanent preventive measure.

Morphologically and in the absence of fertilized ova within the endometrium, macroscopic focal areas of endometrial thickening develop in animals with clinical signs of pseudo-pregnancy. These decidua-like, segmental endometrial proliferations also have been referred to as deciduoma in the literature, a term that constitutes a misnomer because the endometrial changes are non-neoplastic histologically.

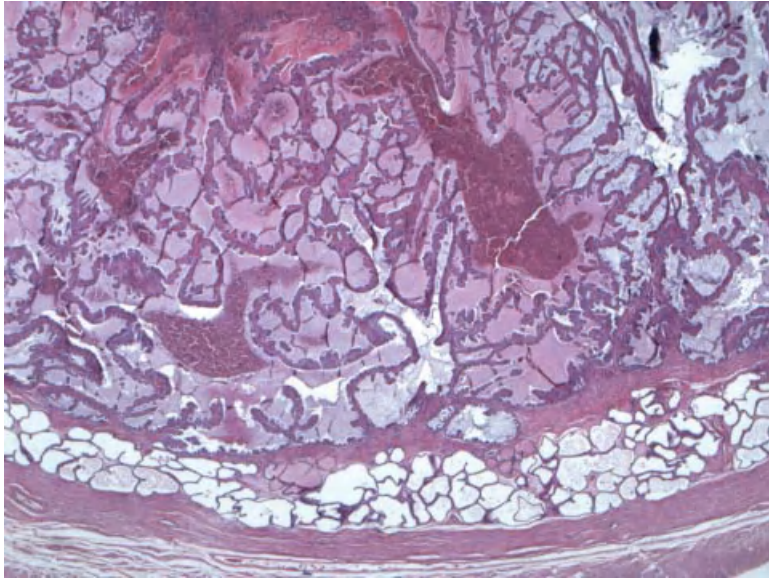


Figure 6.16 Canine pseudocyesis. In this uterine cross section, there is marked luminal proliferation endometrium forming long, tortuous papillary projections, crypts filled with proteinaceous fluid, and very conspicuous endometrial glands that are markedly dilated. H&E stained section. (Courtesy of Dr. BL Njaa and JM Cramer, Cornell University.)

The endometrial proliferation consists of polypoid projections of surface and cryptal epithelium into the uterine lumen nearly occluding it (Figure 6.16). Crypts are typically filled with mucoïd fluid and papillary projections are covered by similar mucus. Endometrial glands in the bottom of the proliferation are evenly distended and lined by cuboidal epithelium. The observed three-layer composition of proliferative endothelium has been proposed as endometrial remodeling. The top layer close to the uterine lumen contains a spiral-shaped configuration of long villous folds of endometrium mixed with endometrial secretions, a middle more dense layer contains folded endometrial epithelial cells embedded in a dense band of connective tissue with the third layer close to the myometrium containing uniformly distended endometrial glands. When regression of the segmental endometrial proliferation occurs, it starts with necrosis at the tip of the lesion, and the necrotic material will be sloughed into the uterine lumen.

Bitch-induced neonatal death (“Puppicide”)

Abnormal postparturient behavioral changes in individual unsupervised young bitches may result in neonatal mortality. The bitch may slowly kill and possibly cannibalize individual puppies or inflict rapid, fatal trauma of the entire litter usually by crushing their skulls (Figure 6.17). This may not be completely



Figure 6.17 Canine puppicide. Skin covering the heads of three canine neonates has been removed to reveal severe submucosal and muscular hemorrhage. Underlying bones are fractured due to crushing bites by the dam. (Courtesy of R Irvine, University of Glasgow.)

recognized by the breeder if the bitch is left unsupervised in the immediate postparturient period until her subsequent litters are born alive but die soon afterward.

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Chapter 7

Disorders of Nondomestic Mammals

Bruce A. Rideout

Introduction

Pathogenesis of abortion and neonatal loss

One of the most helpful principles in dealing with zoo and wildlife diseases is the use of taxonomic analogies. Domestic bovids are the best model for understanding the pathogenesis of abortion and neonatal loss (as well as other disease issues) in exotic bovids; domestic carnivores are the best model for exotic carnivores, etc. However, this approach has limitations. For example, some taxonomic groups have their own repertoire of pathogens that might either be different from what is found in their domestic counterparts or be similar to domestic animal agents but not detectable in conventional diagnostic tests. Conversely, some of the most important causes of abortion in domestic animals are rare or not reported in zoo animals, such as bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV), and porcine reproductive and respiratory syndrome virus (PRRSV). There are also many wildlife species that do not have a domestic counterpart or whose closest domestic relative may not be obvious. Despite these caveats, taxonomic analogies are still very useful in wildlife disease investigations, particularly for the nonspecialist. Having a reference on mammalian taxonomy can therefore be very helpful.

Other general principles for zoo animals include the following:

- Stillbirths are much more common than abortions.
- Noninfectious causes of stillbirths and abortions are much more common than infectious causes.
- Abortions and stillbirths are generally sporadic; case clusters or abortion storms are rare.
- Lethal congenital anomalies are more often sporadic and spontaneous than inherited or due to infectious disease or toxin exposure.

Kirkbride's Diagnosis of Abortion and Neonatal Loss in Animals, Fourth Edition.
Edited by Bradley L. Njaa.
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The avian counterparts to mammalian abortions and stillbirths (embryonic death and failure to hatch) are important subjects for zoos and conservation programs but are beyond the scope of this chapter. An overview of avian embryo pathology has been published in limited distribution. Resource materials on this subject can be obtained from the author.

Tissue sampling

Sample collection from nondomestic animal fetuses and placentas is similar to that for domestic animals. A complete diagnostic sample set would consist of stomach content for cytology and microbiology; liver, lung, kidney, spleen, brain, and placenta frozen at -70°C for microbiology; fetal heart blood and fetal fluids (thoracic and abdominal) for bacterial culture and serology; and a complete set of formalin-fixed tissues for histologic evaluation. Because the diseases of wildlife are less well known than the diseases of domestic animals, it is important to collect complete sets of tissues from all cases whenever possible. This not only enables thorough investigation of each individual case, it enables tissue archives to be created that facilitate much-needed research. It would be a great service to the wildlife disease community if diagnostic laboratories that periodically discard their paraffin blocks would return blocks from zoo and wildlife cases to the source institution for archiving.

For investigation of chromosomal anomalies, fresh tissues are generally collected for *in vitro* cultivation and cytogenetic analysis. It is worth noting that even when the fetus is autolyzed, the placental surface is still useful for cytogenetic sampling.

It may be difficult to obtain serum samples from dams immediately post-abortion because of difficulties with restraint or immobilization, but most zoos maintain serum banks for their animals, facilitating retrospective serologic investigations of abortion cases. Although we recommend collection of fetal heart blood at necropsy for fetal serology as well, such serologic testing can be problematic in wildlife for two reasons. First, diagnostic tests may not perform well in nondomestic species for which the tests have not been validated, particularly if they use species-specific reagents. The lack of validated tests for wildlife is a significant and often underappreciated problem, but the use of nonspecies-specific tests can help minimize problems. Second, maternal antibodies cross the placenta in some species. The different types of maternal-fetal barrier in placentas are a good indicator of whether or not maternal antibody transfer occurs. Taxa listed in bold in Table 7.1 transmit maternal antibodies across the placenta. Fetal serology will reflect the serologic status of the dam in these taxa.

Pooling of samples is not generally needed because abortions and stillbirths are rarely epizootic, but in any event, pooling should be avoided because of the lack of validation of the methods and potential loss of sensitivity.

Table 7.1 Placental types. Entries in bold indicate taxa in which maternal antibodies cross the placenta.

Order	Family	Example Species	Placental type	Maternal-fetal separation
Insectivora		Scalopus spp. moles Scapanus spp. moles	Discoid Diffuse	Endotheliochorial Hemochorial
Chiroptera		Bats	Discoid	Endotheliochorial Hemochorial in some
Prosimians		Tupaia spp. shrews Galago	Bidiscoid Diffuse	Endotheliochorial Epitheliochorial
Primates	New World Monkeys Old World Monkeys Great Apes	Marmoset Rhesus monkey Gorilla	Bidiscoid Bidiscoid Discoid	Hemomonochorial Hemomonochorial Hemomonochorial
Lagomorpha		Rabbit, hare	Discoid	Hemochorial
Rodentia		Guinea pig Rat, mouse Beaver	Discoid Discoid Discoid	Hemomonochorial Hemotrichorial Hemodichorial
Hyracoidea		Hyrax	Zonary	Hemochorial
Carnivora		Dog, cat	Zonary	Endotheliochorial
Artiodactyla		Pig, alpaca Sheep Cow, goat Camel	Diffuse Cotyledonary Cotyledonary Diffuse	Epitheliochorial Epitheliochorial Epitheliochorial
	Cervidae	Most deer Musk deer	Cotyledonary Diffuse	Epitheliochorial Epitheliochorial
Perissodactyla	Equidae, Tapiridae, Rhinocerotidae	Horse, tapir, rhinoceros	Diffuse	Epitheliochorial
Proboscidea		Elephant	Zonary	Endotheliochorial
Edentata		Armadillo Sloth	Discoidal Discoidal	Hemochorial Endotheliochorial

Adapted from Benirschke, Kaufmann, and Baergen 2006.

Placentas can generally be collected and submitted from most hoofstock species housed in zoos, but recovery of carnivore and primate placentas can be more difficult. It is important to carefully evaluate the placenta for completeness. It is not unusual for many species to have problems with retained placental fragments, so it is important to alert zoo veterinarians to this occurrence as early as possible.

Gross examination of the fetus and placenta

Procedures for gross examination of nondomestic animal fetuses are generally similar to those for domestic animal fetuses, with a few exceptions. First, examination of primate fetuses requires a higher level of personal protective equipment, such as an N95 mask and face shield or use of a biological safety cabinet. Second, as mentioned above, most zoos strive to create a tissue archive for research and retrospective investigations. Therefore, it is generally recommended that complete sets of tissues be taken, regardless of their diagnostic value to the case at hand.

The procedures for gross placental examination in nondomestic animals are likewise similar to those for domestic animals, with a few exceptions. The placenta is normally washed of dirt and debris, weighed, spread out in its *in-utero* configuration, measured, and evaluated for completeness. The length of the umbilical cord should also be measured. If the placenta is cotyledonary, the number and average size of cotyledons should be determined.

For discoidal placentas, the size and shape of the placental disk(s) and location of cord insertion should be recorded. Insertion of the cord in the fetal membranes or the margin of the disc increases the risk of vascular trauma and disruption relative to more normal insertion sites near the center of the disc. The maternal surface of the disk should be carefully examined for adherent blood clots that could indicate placental abruption (placental separation). The use of membrane rolls allows more thorough histologic evaluation of the fetal membranes. Although this technique can be used for fetal membranes of any species, it is primarily used for primate placentas. The membranes are normally spread out with the fetal (amnion) side up, then the edge is grasped with hemostats and rolled toward the disk, with the amnion on the inside of the roll. After fixation, the roll is transversely sectioned (Figure 7.1).

In situations where particular dams or breeding groups are experiencing persistent problems with abortions and stillbirths, karyotype studies can be helpful in ruling out genetic causes. (See the section on miscellaneous causes of abortion.) Viable cells are required for such chromosome studies, so previous arrangements need to be made to ship fresh tissue in culture medium to an appropriate laboratory. Fetal surface membranes of the placenta are good sampling sites. Sometimes viable cells can be recovered from tissues frozen in liquid nitrogen or at -70°C .

If exposure to toxic plants is suspected, surveillance for toxic plants in hoofstock exhibits generally requires placement of an anchored wire enclosure box that will allow plants to grow enough to allow identification.

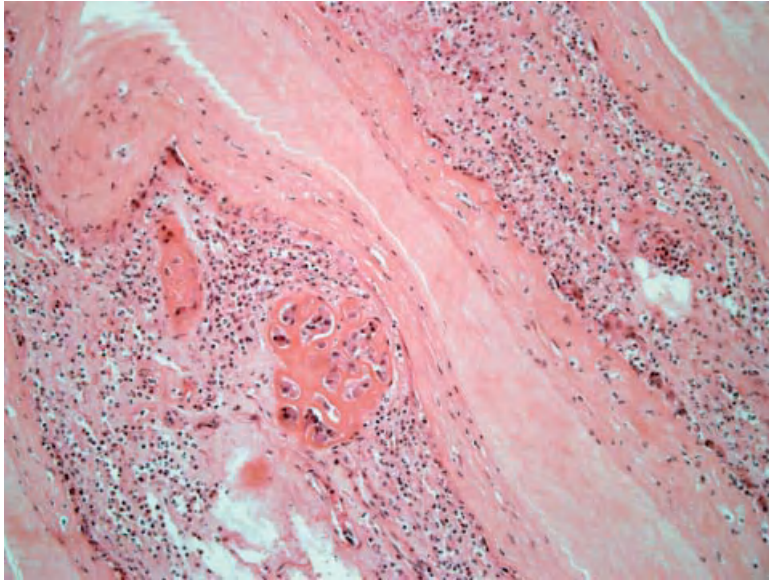


Figure 7.1 Fetal membrane (amnion) roll from a douc langur (*Pygathrix nemaeus*) stillbirth associated with preeclampsia, dystocia, and ascending chorioamnionitis. There are neutrophilic infiltrates throughout the amnion, with fibrinoid change in blood vessels. (Courtesy of Dr. BA Rideout, San Diego Zoo Global.)

Viral causes of abortion and neonatal loss

Bluetongue virus and epizootic hemorrhagic disease virus

Seropositivity to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) is common in nondomestic hoofstock, but disease is rare. Stillbirths, abortions, and perinatal deaths associated with BTV were reported in addax (*Addax nasomaculatus*), Cape buffalo (*Synceros caffer*), Cape hartebeest (*Alcelaphus buselaphus caama*), gemsbok (*Oryx gazella*), Grant's gazelle (*Gazella granti*), sable antelope (*Hippotragus niger*), and Nubian ibex (*Capra ibex nubiana*) in an outbreak at a single zoo. Fetuses and calves had no reported lesions, but serotype 11 BTV was identified by isolation or indirect immunofluorescence in 10 cases. European wildlife species are now being reported as being seropositive to several emerging strains of BTV. Some of these wild ruminant species are likely to be susceptible to clinical disease, but no abortions or reproductive problems have yet been reported.

Equine Herpesvirus 1 (EHV-1)

A unique herpesvirus closely related to EHV-1 was reported to have caused a late-gestation abortion in a Persian onager (*Equus hemionus onager*). Gross

fetal lesions included serosanguineous thoracic and abdominal effusions, splenomegaly, and white foci in the liver. Histologically, there was multifocal hepatic and splenic necrosis, with intranuclear inclusion bodies in hepatocytes, splenic lymphocytes, bronchiolar epithelium, thymic medullary, and adrenal cortical cells. Virus was isolated from multiple tissues. DNA fingerprinting revealed the isolate was closely related to, but distinct from, EHV-1. The source of the virus was not determined, but it was first identified in zebras.

Elephant endotheliotropic herpesvirus (EEHV)

This novel herpesvirus typically causes sudden death in young Asian elephants (*Elephas maximus indicus*), but a surveillance study in Europe identified the virus by PCR in three stillborn Asian elephants. Lesions were not reported, but young elephants are reported to develop acute edema of the head and thoracic limbs, oral ulcers, and cyanosis. Gross lesions include pericardial effusion and petechial hemorrhages throughout the heart and abdominal cavity. Characteristic histologic lesions consist of microhemorrhages in multiple organs with amphophilic to basophilic intranuclear inclusion bodies in capillary endothelial cells. Histopathology is generally diagnostic, but confirmation can be pursued by PCR or enzyme-linked immunosorbent assay (ELISA). (See <http://elephanttag.org>.)

Bovine viral diarrhea virus (BVDV)

There is evidence of exposure to BVDV or related pestiviruses in a wide range of captive and free-ranging wild species from at least seven families (Antilocapridae, Bovidae, Camelidae, Cervidae, Giraffidae, Tragulidae, and Suidae), but evidence of disease is rare. Apparently novel pestivirus species have been identified in a wild pronghorn antelope (*Antilocapra americana*) and a wild giraffe (*Giraffa camelopardalis*), but the pathogenicity of these viruses remains unknown. Naturally occurring abortions associated with BVDV infection have only been reported in captive Old World and New World camelids, but persistent infections are being documented in a growing number of species. There are reports of mummified fetuses and stillbirths in white-tailed deer experimentally inoculated with a cytopathic strain of BVDV isolated from white-tailed deer, but most experimental infections in wildlife do not result in disease. If abortions due to BVDV or related pestiviruses were to occur in non-domestic ruminants, they would presumably have clinical features and lesions similar to those reported in domestic livestock, with abortion at any stage of gestation, variable autolysis or mummification, and a variety of dysplastic lesions possible. Because these findings are not specific for BVD, diagnosis should be confirmed in every case by fluorescent antibody testing on fetal organs or lymph node, or by some other nonspecies-specific diagnostic method.

Measles virus

Abortions have been reported in rhesus monkeys (*Macaca mulatta*) infected with the human measles morbillivirus. The cause of abortion is a viral

placentitis, with formation of syncytial cells and intranuclear inclusion bodies. Diagnosis can be confirmed by immunohistochemistry (IHC) on placental lesions, or reverse transcriptase-polymerase chain reaction (RT-PCR).

Bacterial causes of abortion and neonatal loss

Brucellosis

Brucella spp. have been identified by serology and/or culture from a variety of wildlife species, but disease is rare in free-ranging animals and has not been reported in North American zoos. However, the potential for spillover of any *Brucella* spp. into a new wildlife host should always be considered. The most well-known examples of wildlife brucellosis include *Brucella abortus* in North American bison (*Bison bison*) and elk (*Cervus canadensis*), and African buffalo (*Syncerus caffer*); *B. abortus* and *B. melitensis* in camels (*Camelus dromedarius*) from North Africa and the Mediterranean region; and *B. suis* in reindeer (*Rangifer tarandus*) in the Arctic, feral pigs in the United States and Australia, and wild boar in Europe (both *Sus scrofa*). *B. ceti* and *B. pinnipedialis* have recently been identified as a cause of abortion and reproductive failure in cetaceans (whales, dolphins, and porpoises) and seals, respectively. In most species, brucellosis causes a necrotizing placentitis with late gestation abortion. Histologically, there is suppurative inflammation, often with abundant organisms in chorionic epithelial cells. The fetus may not have any lesions, or have turbid or flocculent fluid in the stomach and suppurative bronchopneumonia. Diagnosis can be confirmed by bacterial culture or PCR. Conventional serologic tests may not detect the marine mammal *Brucella* spp.

Enteric pathogens

Any of the enteric bacteria, such as *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *E. coli*, and *Yersinia enterocolitica* have the potential to cause placentitis and abortion in a wide variety of nondomestic species. Clinical signs, lesions, and diagnostic procedures are generally similar to those for domestic mammals.

Coxiella burnetii (Q fever)

Placentitis and stillbirth or abortion due to *Coxiella burnetii* has been reported in Cuvier's gazelles (*Gazella cuvieri*) and a greater kudu (*Tragelaphus strepsiceros*). It has also been seen in a Javan rusa (*Rusa timorensis*) and Malayan sambar (*Rusa unicolor*) and is reported to have caused placentitis in a wild Pacific harbor seal (*Phoca vitulina*). Other than evidence of fetal distress, there are often no gross or microscopic lesions in the fetuses. Placentas typically have pale areas in cotyledons. Histologically, there is extensive necrosis

with vasculitis and blue-gray granular intracytoplasmic material in chorionic epithelial cells. The diagnosis can be confirmed by immunohistochemistry. The potential host range is likely much broader than is indicated by the reported cases.

Listeria monocytogenes

Listeria has a broad host range and should be considered whenever there is evidence of infectious placentitis, but the only published reports of abortions or stillbirth in exotic animals include one late-term abortion in a llama (*Llama glama*); stillbirths in a colony of macaques (*Macaca mulatta* and *M. radiata*); and abortion and retention of macerated fetuses in European brown hares (*Lepus europaeus*). We have also seen abortions due to listeriosis in a breeding group of rock hyraxes (*Procavia capensis*). Placentas may have no gross lesions, but often have tan to brown areas of necrosis with variable amounts of fibrin on the surface. Typical histologic lesions include fibrinopurulent to necrotizing placentitis. Fetuses may have no lesions, or suppurative pneumonia and meningitis. Diagnosis is generally made by isolation. Cold enrichment increases the likelihood of successful isolation, so laboratories should always be alerted to the possibility of a *Listeria* case.

Miscellaneous causes of abortion and neonatal loss

Toxoplasma gondii

There is one report of a late-term abortion in a Greenland muskox (*Ovibos moschatus wardi*) and a reported stillbirth in a reindeer (*Rangifer tarandus*) due to *Toxoplasma gondii*. Tachyzoites can be found in the placenta. The diagnosis can be confirmed with immunohistochemistry. Dams may have high *Toxoplasma* titers.

Neospora caninum

Several surveys have revealed seropositivity to *Neospora* in a wide variety of captive carnivores and hoofstock. There are reports of late abortions or stillbirths due to *N. caninum* in Eld's deer (*Cervus eldi siamensis*) and lesser kudu (*Tragelaphus imberbis*). Typical lesions of nonsuppurative encephalitis, with or without tissue cysts, are seen. Diagnosis has been made by immunohistochemistry, fetal serology, and PCR. We have also documented abortion of a markedly autolyzed early second trimester fetus with necrotizing nonsuppurative leukoencephalitis in a pudu (*Pudu puda*), a small South American deer. Protozoa were not evident in H&E stained sections of brain, but immunohistochemistry was positive for *Neospora* spp. and negative for *Toxoplasma gondii*. The placenta had mild suppurative inflammation with protozoal zoites in the stroma.

***Encephalitozoon* spp.**

Microsporidia have caused placentitis with abortion, premature birth, and neonatal death in a wide range of species, including emperor tamarins (*Saguinus imperator*), rock hyraxes (*Procavia capensis*), and alpacas (*Lama pacos*). There is typically extensive necrotizing placentitis with $1\mu\text{m} \times 2\mu\text{m}$ organisms in lesions and trophoblasts. Encephalitis can sometimes be detected in fetuses if not obscured by autolysis. In neonates, widespread arteritis may be present. The organisms are Gram-positive and PAS positive, distinguishing them from protozoa. The diagnosis can be confirmed by immunohistochemistry, electron microscopy, or polymerase chain reaction. With such a broad host range, Microsporidia should be considered in cases of necrotizing placentitis in any species.

Abortion due to maternal illness

Maternal illnesses that cause abortions and stillbirths must be defined broadly for zoo animals. There are many conditions in the dam that can contribute to abortions and stillbirths that are not illnesses in the traditional sense. The following are some of the more common examples.

Body condition

It can be difficult to regulate individual animal food consumption in herds or large groups of animals, particularly in mixed species exhibits. This can lead to obesity in some animals and under-condition in others. Animal caretakers may not always be aware of these conditioning problems. Body condition can be difficult to assess in many species, particularly exotic hoofstock, where fat stores can be almost completely depleted while the animals maintain good muscle mass. In either case, complications of pregnancy can arise. For over-conditioned animals, dystocia is common, particularly if pelvic fat deposits have become saponified. Lesions in the fetus will be typical for dystocia, including hemorrhage and edema of the head and upper body. Under-conditioned animals can abort at any stage of gestation. If the pregnancy is carried to term, both under-conditioned and over-conditioned animals may die during parturition. Careful inquiry will be required if only an aborted fetus or stillborn animal is submitted.

Immobilization or relocation

Another maternal factor that can contribute to abortions and stillbirths is the chemical immobilization or physical relocation of a pregnant dam. Matings are often not observed, and pregnancy can be difficult to diagnose in many species, which increases the risk that inadvertent immobilization or relocation of a pregnant female will occur. Xylazine administered during late pregnancy can induce abortion, without gross or histologic lesions. The problem with physical

relocation (or addition of new enclosuremates) is that it can upset social hierarchies, leading to sufficient stress or trauma to cause abortion. Aborted fetuses typically have no lesions, or only nonspecific changes such as hemorrhage and body cavity effusions. In some cases, there is maternal retention of a dead fetus, which may be expelled later in severely autolyzed or macerated condition.

Chromosomal errors

An abnormal chromosome number in the mother (such as trisomy or triploidy) is a common cause of human abortion but has rarely been investigated in zoo animals. Such chromosomal errors may not produce any detectable phenotypic or behavioral abnormalities, so these errors are not often considered. If a particular dam has recurring problems of abortions or stillbirths, karyotypic analysis may be warranted. Detection of chromosomal anomalies has accounted for unexplained abortions in several species, such as the douc langur (*Pygathrix naemaues*) and Siberian tiger (*Panthera tigris altaica*).

Age of the dam

Over-age or under-age pregnancies can occur because it is not always possible to control breeding, especially in herd settings. In either case, abortion or stillbirth is a possible outcome. Generally there will be no lesions in the fetus, other than possible evidence of dystocia. Few references include information about normal breeding ages, so careful inquiry might be required to determine if the female was over-age or under-age. Normal breeding ranges can vary significantly across even closely related taxa, so careful attention should be paid to this issue. For example, some ruminant species, such as the royal antelope (*Neotragus pymaeus*), reach sexual maturity by about 6 months of age, while some female gaur (*Bos gaurus*) might be considered under-age at 18 months. The most useful reference in this regard is *Walker's Mammals of the World* (Nowak 1991).

Nutrition

Vitamin and mineral deficiencies or excesses are relatively uncommon in zoo animals because diet quality and composition are carefully regulated. Situations in which nutritional problems can arise include housing of animals with specific dietary requirements in mixed species exhibits. There is evidence that chronic copper deficiency in exotic hoofstock can increase the incidence of abortions, stillbirths, and perinatal death. Diagnosis is best obtained by measuring hepatic copper levels in the fetus or neonate. Hypomyelination in the central nervous system can be seen in severe cases, as with enzootic ataxia in sheep.

Twinning

Multiple births are a relatively common contributing factor in abortions and stillbirths in nondomestic animals, particularly exotic hoofstock. The

pathogenesis of fetal demise in these cases is not often evident. Presumably some cases are due to placental insufficiency, while others are related to dystocia. The biggest problem is determining which species normally twin, and would therefore be expected to suffer fewer complications due to a twin pregnancy. Table 7.2 lists the taxonomic groups for which twins or triplets are common. For species that typically twin, it is not unusual for a primiparous dam to give birth to a singleton, with twin or triplet births in subsequent years. Table 7.3 list species for which twinning is uncommon and often problematic.

Genetics

Few hereditary genetic conditions have been identified in zoo animals that affect pregnancy. Diagnosis of a hereditary condition should be based on adequate pedigree analysis and evidence of recurring malformations with particular pairings, not simply the apparent occurrence of a condition that is hereditary in domestic animals. The management implications of lethal hereditary traits for endangered species breeding programs are significant. Even when hereditary conditions are identified, carriers cannot always be eliminated from the breeding population. Instead, populations can sometimes be managed to reduce allele frequency without complete elimination of carriers.

The chief genetic concern in most populations is the effect of inbreeding depression on reproductive success. It is not always possible to calculate inbreeding coefficients for animal populations because of uncertainty over parentage, founder representation, and relatedness of wild caught founders. However, for individuals or populations facing low reproductive success, it can be helpful to obtain as much information as possible about the level of inbreeding.

An additional, but less commonly appreciated, problem is outbreeding depression. Outbreeding depression can be defined as reduced reproductive fitness as a result of breeding between individuals from different populations. In some cases, different populations of the same species can face different adaptive pressures, leading to genetic differences that can reduce fitness when interbreeding occurs. This can be an issue when wild-caught founder animals come from geographically distinct populations, as has occurred with dik diks (*Madoqua kirkii*) and Soemmerring's gazelles (*Gazella soemmerringi*).

Another genetic issue can occur when two distinct species are almost indistinguishable morphologically. This can lead to inadvertent cross breeding and varying degrees of reproductive failure. An extreme example is the muntjac, where *Muntiacus reevesi* has a diploid chromosome number of 46, while the morphologically similar *Muntiacus muntjac* has a diploid chromosome number of 6 for females and 7 for males (the lowest chromosome number for any mammal).

In other cases of recurring abortion or stillbirth, karyotype studies can reveal chromosomal anomalies that explain the reproductive failure, thus

Table 7.2 Taxa for which twinning is common.

Family	Genus	Common name	Twins	Triplets
Tragulidae	Hyemoschus	Tragulus	Chevrotains	Occasional
Cervidae	Multiple genera	Most deer	Occasional	Occasional
	Hydropotes	Chinese water deer	Common	Occasional
	Elaphurus	Pere David's deer	Common	Occasional
	Elaphodus	Tufted deer	Occasional	
	Oidocoileus	Whitetail and mule deer	Common	Occasional, rare quadruplets
	Caproleus	Roe deer	Common	Occasional
	Alces	Moose	Common	Occasional
	Antilocapra	Pronghorn	Common	Occasional
	Boselaphus	Nilgai	Common	Occasional
	Gazella	Cuvier's gazelle	Common	Occasional
Bovidae	Saiga	Saiga	Common	
	Capra	Ibex, Tur, Markhor	Common	Occasional
		Cretan wild goat	Occasional	
	Bos	Banteng, gaur, yak	Occasional	
	Procapra	Asian gazelles	Occasional	
	Nemorhaedus	Goral	Occasional	
	Rubicapra	Chamois	Occasional	
	Hemitragus	Tahr	Occasional	
	Pseudois	Bharal (blue sheep)	Common	Occasional
	Ammotragus	Barbary sheep	Common	Occasional
	Ovis	Urial, Argali, Mouflon		
		Desert bighorn sheep		
Callitrichidae	Many genera	Marmosets Tamarins	Common	Occasional
Lorisidae	Loris	Slender Loris	Common	
	Nycticebus	Slow Loris	Occasional	
	Galago	Galago or bush baby	Common	Occasional
	Otolemur	Greater bush baby	Common	Occasional
Cheirogaleidae	Multiple genera	Dwarf lemurs Mouse lemurs	Common	Occasional Rare quadruplets
Lemuridae	Multiple genera	Lemurs	Occasional to common	Occasional to rare

Source: Nowak 1991.

Table 7.3 Taxa for which twinning is uncommon and potentially problematic.

Order	Family	Genus	Common name	
Perissodactyla	Equidae, Rhinocerotidae, Tapiridae	Multiple genera	Horses, zebras, asses, rhinoceroses, tapirs	
Proboscidae	Elephantidae	Elephas, Loxodonta	Asian and African elephants	
Artiodactyla	Hippopotamidae Camelidae Tragulidae Cervidae	Hippopotamus, Choeropsis Multiple genera	Hippopotamus Camels, guanacos, llamas, alpacas, vicunas Chevrotain (mouse deer)	
		Axis	Axis deer	
		Cervus	Wapiti (red deer), Eld's deer,	
		Dama	barasingha, sambar, sika	
		Blastocerus	Fallow deer	
		Ozotoceros	Marsh deer	
		Mazama	Pampas deer	
		Pudu	Brocket deer	
		Giraffidae Bovidae	Rangifer	Pudu
			Giraffa, Okapia	Reindeer
	Tragelaphus		Giraffe, Okapi	
	Taurotragus		Bongo, sitatunga,	
	Bubalus		bushbucks, nyalas, kudus	
	Syncerus		Eland	
	Bison		Asian water Buffalo	
			African Buffalo	
			American and European bison	
	Artiodactyla		Bovidae	Cephalophus
		Kobus		Waterbuck, Lechwes, kob,
		Redunca		puku
Pelea		Reedbuck		
Hippotragus		Rhebok		
Oryx		Roan and sable antelope		
Addax		Oryx, gemsbok		
Damaliscus		Addax		
Alcelaphus		Hunter's hartebeest,		
Sigmoceros		bontebuck, blesbok		
Connochaetes		Hartebeest		
Oreotragus		Lichtenstein's hartebeest		
Raphicerus		Wildebeest (gnu)		
Ourebia		Klipspringer		
Neotragus		Steenbok, grysbok		
Madoqua		Oribi		
Antilope		Dwarf antelope, Royal antelope		
		Dik dik		
		Blackbuck		

(Continued)

Table 7.3 (Continued)

Order	Family	Genus	Common name
		Aepyceros	Impala
		Litocranius	Gerenuk
		Gazella	Most gazelles
		Antidorcas	Springbok
		Capricornis	Serow
		Oreamnos	Mountain goat
		Budorcas	Takin
		Oribos	Muskox
		Ovis	Bighorn (except Desert bighorn), Dall's sheep, snow sheep
Xenarthra	Multiple families	Multiple genera	Anteaters, sloths, armadillos
Primates	Lorisidae	Perodicticus	Potto
	Cebidae	Many genera	New World monkeys
	Cercopithecidae	Many genera	Old World monkeys
	Hylobatidae	Many genera	Lesser apes
	Pongidae	Many genera	Great apes
Chiroptera	Many families	Many genera	Most bats (some species have one–three young, rarely four)

Source: Nowak 1991.
Taxa not listed in Table 7.2 or Table 7.3 generally produce litters of two or more.

enabling affected individuals to be removed from the breeding population. Chromosomal anomalies cannot be managed at the population level the way that deleterious recessive alleles can be through reduction in allele frequency. This has occurred with a number of taxa, including primates such as the pygmy chimpanzee (*Pan paniscus*) and douc langur (*Pygathrix naemaesus*), and carnivores such as the Siberian tiger (*Panthera tigris altaica*).

Domoic acid

Certain marine algae, such as *Pseudo-nitzschia* spp., produce domoic acid, a glutaminergic or excitatory neurotoxin. Marine mammals are exposed to domoic acid through feeding on planktivorous fish. The neurotoxin crosses the placenta and can cause abortions and premature birth in California sea lions (*Zalophus californianus*). The only lesions reported are placental abruption (placental separation) and brain edema in fetuses. The diagnosis can be confirmed by analysis of stomach fluid, urine, feces, or amniotic fluid, but testing is only available at certain marine laboratories.

Toxic plants

Problems with toxic plants are rare in zoos, presumably because of attention to the quality of hay and minimal access to pasture. However, the potential for exposure cannot be easily ruled out by inquiry alone. Plants tend to be eaten as they spring up, making it difficult to identify the species being consumed. Placement of an anchored wire enclosure box on the pasture will allow plants to grow enough to allow identification.

Saponification of pelvic fat

Dystocia due to obstruction of the pelvic canal by large deposits of saponified and/or mineralized fat has been seen in exotic ruminants, particularly large deer species (e.g., Eld's deer, *Cervus eldi thamin*). Fat saponification can occur spontaneously or be caused by consumption of endophyte-infected fescue, and can be an individual animal or herd problem. By the time dystocia is recognized, the fetus may already be dead. Cesarean section is often required to save the dam. Affected females need to be identified so they can be treated with contraceptives or removed from the breeding population.

Fetal arthrogryposis

Arthrogryposis is a relatively common finding in stillborn hoofstock in zoos, particularly in dystocia cases. These cases occur sporadically and appear to be spontaneous (not hereditary), and no cases have been linked to toxic plant exposure.

Fetal cyclopiian malformation

Rare abortions of fetuses with cyclopiian malformations occur in zoos, primarily in exotic bovids such as the gaur (*Bos gaurus*), but none have been linked to toxic plant exposure (e.g., *Veratrum californicum*) (Figure 3.11).

Preeclampsia

This condition occurs occasionally in primates and is characterized by maternal proteinuria, edema, and hypertension. It can occur at any time during gestation or in the immediate postpartum period. The pathogenesis is uncertain, but it probably involves an abnormality of placentation, leading to placental ischemia. Very old or very young primiparous females are predisposed, as are those with underlying renal disease. The condition spontaneously resolves with termination of pregnancy. Aborted or stillborn fetuses have no specific lesions, but may be growth retarded. Placental lesions include atherosclerosis and fibrinoid arteriopathy, infarction, and basal hemorrhage (abruptio placenta). The critical issue is identifying affected dams so they can be more closely monitored in subsequent pregnancies.

Contraceptive implants

Melengesterol acetate implants are used for contraception in a variety of species. They have caused abortions when accidentally placed during early gestation in golden lion tamarins (*Leontopithecus rosalia*). Synthetic progestins have also been implicated in abortions in bears, prosimians, and other species. Aborted fetuses have no specific lesions. Diagnosis is made through careful inquiry and exclusion of other causes.

Table 7.4 Possible causes for gross and microscopic lesions in nondomestic mammalian fetuses.

Gross Lesion	Causes
Placental necrosis	<i>Listeria</i> , <i>Chlamydophila</i> , <i>Coxiella</i> , <i>Brucella</i> , microsporidia, other bacteria
Fetal subleptomeningeal hemorrhage (especially primates)	Birth hypoxia
Fetal subdural hemorrhage	Birth trauma
Fetal growth retardation	Poor maternal condition, maternal old age, preeclampsia (primates), twinning, placental insufficiency
Basal placental hemorrhage (primates)	Preeclampsia
Extensive placental infarction (primates)	Preeclampsia
Recurring abortions in the same dam	Karyotype errors, cryptic species crosses (infertile hybrids)
Fetal brain edema (California sea lions)	Domoic acid
Microscopic Lesion	Causes
Multifocal hepatic necrosis	EHV-1, other herpesvirus
Nonsuppurative encephalitis	<i>Neospora</i> , <i>Toxoplasma</i> , <i>Encephalitozoon</i>
Myocarditis	<i>Neospora</i>
Arteritis	<i>Encephalitozoon</i>
Intranuclear inclusion bodies	Elephant herpesvirus, EHV-1, measles (primates)
Placentitis	<i>Chlamydophila</i> , <i>Coxiella</i> , <i>Brucella</i> , <i>Listeria</i> , <i>Encephalitozoon</i> , measles (Old World primates)
Interstitial nephritis	<i>Leptospira</i>
Pneumonia	<i>Listeria</i> , <i>Brucella</i>
Hepatitis	<i>Leptospira</i> , <i>Chlamydophila</i>
Fetal hypomyelination (ruminants)	Copper deficiency

Placental separation (abruption), placental insufficiency, fetal hypoxia, and brain hemorrhage

Premature placental separation can cause acute fetal hypoxia and death, leading to abortion or stillbirth. This presumably could occur in a variety of species but is most commonly diagnosed in primates. Diagnosis is typically based on the presence of a significant retroplacental hematoma. In some cases there is atherosclerosis or thrombosis of the maternal blood vessels surrounding the area of separation, but often there are no histologic lesions in the placenta (Table 7.4).

Prolonged fetal hypoxia can occur as a result of placental abnormalities (for example, irregular villous surfaces), maternal anemia, hypertension, preeclampsia, or high altitude. The characteristic histologic lesion in the placenta is increased in numbers of syncytiotrophoblasts (syncytial knots), primarily near villus tips (Figure 7.2).

Typical fetal lesions of hypoxia and distress include meconium staining, aspiration of meconium, and brain hemorrhage. In humans, and possibly other primates, subleptomeningeal hemorrhage is considered to be evidence of fetal hypoxia and must be distinguished from subdural hemorrhage, which is

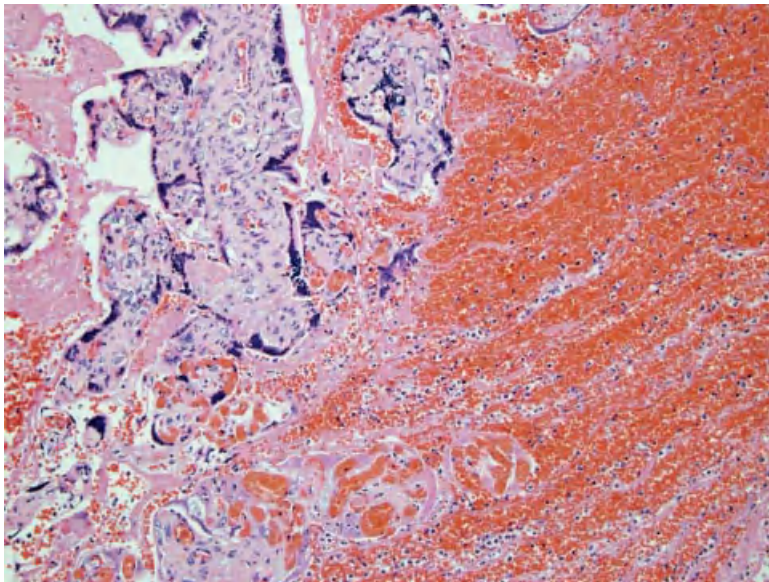


Figure 7.2 Placenta from a stillborn Angolan colobus monkey (*Colobus angolensis*) with characteristic lesions of placental abruption. Prominent syncytial knots are present in the left side of the figure, with extensive hemorrhage (hematoma) on the right. (Courtesy of Dr. BA Rideout, San Diego Zoo Global.)

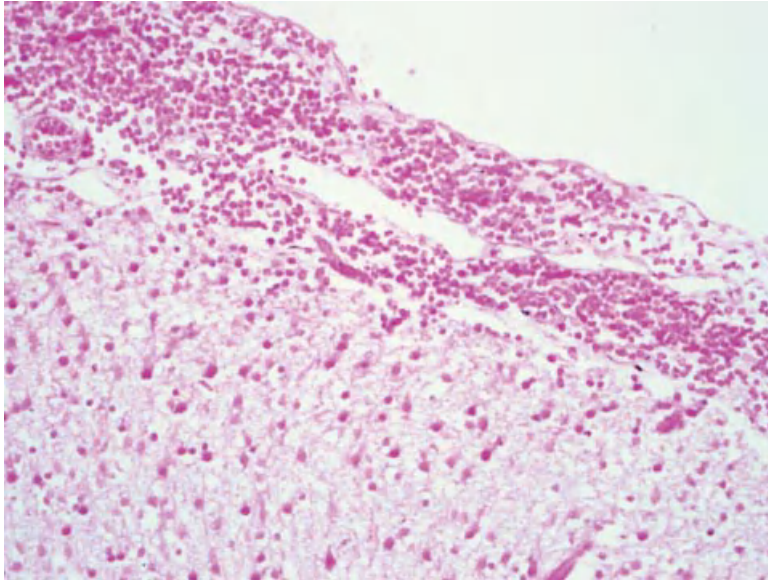


Figure 7.3 Cerebrum from a white-faced saki monkey (*Pithecia pithecia*) with subleptomeningeal hemorrhage associated with fetal hypoxia. (Courtesy of Dr. BA Rideout, San Diego Zoo Global.)

interpreted as evidence of trauma (Figure 7.3). Parenchymal or intraventricular hemorrhage in the brain is also generally attributed to hypoxia or prematurity rather than trauma.

Cases of fetal death due to hypoxia can present as a dystocia, but careful investigation will often reveal lesions of hypoxia and distress.

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Appendix A

Gestational Age Estimation Based on Fetal Measures and Phenotypic Characteristics

As has been outlined in various chapters of this book, certain pathogens have a tendency to affect fetuses prototypically. Some affect the fetus by inflicting predictable lesions whereas other pathogens are known to affect the fetus within a particular gestational age range. Therefore, it is important to have scientifically sound data to help determine the age of a fetus when a breeding or conception date is unknown. Therefore, this appendix includes a series of tables that aid the diagnostician in determining a gestation age of a fetus in a particular species. Fetal measures that may be included are crown-to-rump lengths, fetal weights, size comparisons, and phenotypic or physical characteristics that, in aggregate, help the diagnostician arrive at an estimated gestational age.

Table A.1 Gestational age estimates of bovine fetuses.

Age	Relative size	C-R Measurement	Weight	External fetal characteristics
2 mo.	Mouse	6 to 8 cm	8 to 30 gm	Claw buds and scrotum present
3 mo.	Rat	13 to 17 cm	200 to 400 gm	Hair on lips, chin, and eyelids
4 mo.	Small cat	22 to 32 cm	1 to 2 kg	Fine hair on eyebrows, claws developed
5 mo.	Large cat	30 to 45 cm	3 to 4 kg	Hair on eyebrows and lips, testes in scrotum, teats developing

(Continued)

Table A.1 (Continued)

Age	Relative size	C-R Measurement	Weight	External fetal characteristics
6 mo.	Small dog, beagle	40 to 60 cm	5 to 10 kg	Hair on inside of ear and around horn pits, tip of tail and muzzle
7 mo.	Dog	55 to 75 cm	8 to 18 kg	Hair of metatarsal, metacarpal, and phalangeal region of extremities and beginning on back, long hair on tip of tail
8 mo.	Large dog	60 to 85 cm	15 to 25 kg	Fine short hair all over body, incisor teeth not erupted

(From Veterinary Obstetrics and Genital Diseases Theriogenology, 3rd Edition, 1986, S. Roberts, p. 19).

Table A.2 Gestational age estimates of ovine and caprine fetuses.

Gestational Age	Crown to Rump Length	Fetal Detail
>3 to 4 weeks	0.3 to 2 cm	Head, body, and limbs are discernable
5 to 6 weeks	2 to 9 cm	Hoofs are visible at the end of digits
7 to 9 weeks	9 to 15.5 cm	No hair; rumen development near the end of this gestational period
10 to 13 weeks	15 to 35 cm	Large tactile hairs appear on lips and upper eyelids
14 to 18 weeks	35 to 40 cm	Eyelashes are well developed, some hair on tail and head
19 to 21 weeks	40 to 48 cm	Fetus becomes fully developed with the body covered with hair; hoofs complete but soft

Table A.3 Gestational age of porcine fetuses.

Crown-to-rump length (mm)	Approximate fetal age (days)
20	25
27	30
46	40
89	50
135	60
170	70
207	85
270	110

Table A.4 Gestational age of equine fetuses.

Weight, C-R Length, and Characteristics of the Equine Fetus			
Age in days	Weight	Fetus Length	Fetal and Placental Characteristics
16		0.32 cm	
20		0.66 cm	
25		0.6–0.85 cm	
30	0.2 gm	0.9–1.0 cm	Eye, mouth, and limb buds visible, chorionic vesicle present only in uterine horn.
35		1.5 cm	
40		1.8–2.2 cm	Eyelids and pinnae have appeared.
45		2.0–3.0 cm	
50		3.0–3.5 cm	
60	10–20 gm	4–7.5 cm (1–3/4–3 in.)	Lips, nostrils, and beginning development of feet observed, eyelid partially closed. Placenta not attached but beginning to go into the body of the uterus.
90	100–180 gm (3–6 oz)	10–14 cm (4–5–3/4 in.)	Villi of placenta present but without firm attachment, mammary nipples and hooves visible, body and horn of uterus both involved and enlarged.
120	700–1,000 gm (1.5–2 lb)	15–20 cm (6–8 in.)	External genitalia formed but scrotum is empty, placenta attached, ergots and orbital areas prominent.
150	1,500–3,000 gm (3–6 lb)	25–37 cm (10–14–1/4 in.)	May or may not have fine hair on orbital arch and tip of tail, prepuce not yet developed.
180	3–5 kg (6.5–12.5 lb)	35–60 cm (14–24 in.)	Hair on lips, orbital arch, nose, eyelashes, and fine hair, no mane.
210	7–10 kg (15.4–22 lb)	55–70 cm (22–28 in.)	Hair on lips, nose, eyebrow, eyelids, edge of ear, tip of tail, back, and mane.
240	12–18 kg (26.4–39 lb)	60–80 cm (24–32 in.)	Hair on main and tail, back and distal portion of extremities.
270	20–27 kg (44–59.4 lb)	80–90 cm (32–36 in.)	Short fine hair over entire body.
300	25–40 kg (55–88 lb)	70–130 cm (36–52 in.)	Body completely covered with short hair, prepuce developed, hair in mane, and tail increased.
330	30–50 kg (66–125 lb)	100–150 cm (40–60 in.)	Complete hair coat and hair coat gets its final color, testes descended.

Adapted by Brad Njaa from:
Bergin WC, Gier HT, Frey RA, Marion GB. 1967. Developmental horizons and measurements useful for age determination of equine embryos and fetuses. Proc. Am. Assoc. Equine Pract. pp. 179–196.
Roberts SJ. Veterinary Obstetrics and Genital Diseases. 1971. Ann Arbor, Michigan, Edwards Brothers, Inc.

Table A.5 Gestational age estimates of canine fetuses.

Age (days)	Crown-to-rump length (mm)	External fetal characteristics
20	8	First and second branchial arches present
30	20	Eyelids forming; grooves between digits; pinnae partly cover external acoustic meatus; five pairs of mammary primordia present; intestines herniate into umbilical cord
35	35	Eyelids partly cover eyes; pinnae covers acoustic meatus; digits separated distally; external genitalia differentiated
38	55	Tactile hairs appear on upper lip and above eyes
40	60	Eyelids fused; intestines returned to abdominal cavity; claws formed
45	80	Body hair forming; color markings appear; digits widely separated
50	118	
53	140	Hair covering complete; digital pads present
57	160	
57-63	160-185	Gestation complete; birth

(Table extrapolated from graphical data presented in, Evans HE and Sack WO, Prenatal Development of Domestic and Laboratory Mammals: Growth Curves, External Features and Selected References, *Anat. Histol. Embryol.* 2:11-45, 1973.)

Table A.6 Gestational age estimates of feline fetuses.

Age (days)	Crown-to-rump length (mm)	External fetal characteristics
18	8	Forelimb bud present; otic vesicle present
20	10	Head touches heart bulge; olfactory pits formed
24-26	18-20	Forelimb digits separate distally; mammary primordial present; pinnae triangular, projecting rostrally; eyelids forming; hind limb digits separating distally
27	25	Tongue visible; all digits widely spread; pinnae almost cover acoustic meatuses
30	35	Claws forming; eyelids almost closed; pinna covers acoustic meatus
37	60	Tactile hairs present on face
43	80	
46	95	Fine hairs appear on body; nose pigmented; claws hardening at the tips
50	110	Fine hairs cover body; claws white and hard; skin pigmented
55	125	
60	143	
60-63	143-150	Gestation complete; birth

(Table extrapolated from graphical data presented in, Evans HE and Sack WO, Prenatal Development of Domestic and Laboratory Mammals: Growth Curves, External Features and Selected References, *Anat. Histol. Embryol.* 2:11-45, 1973.)

Appendix B

Summary Tables of Diagnostic Tests for Infectious Causes of Abortion and Neonatal Loss

These summary tables were created to help the reader maximize their diagnostic acuity by selecting the correct tissues and ordering the best diagnostic tests relative to the particular infectious cause of concern. Both the choice of specific ancillary diagnostic tests and the significance of those test results depend on the presence of appropriate pathologic changes in the fetus or placenta. Each table summarizes the most common causes of abortion and neonatal loss affecting one or two species, focusing on which tissues should be collected, which tests are available for confirming a diagnosis and additional tests that may help confirm a cause. The table order follows what is set out in the main text.

Table B.1 Summary of diagnostic tests for bovine abortifacient pathogens.

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
Bovine Herpesvirus-1 (BHV-1)	Kidney, adrenal gland, liver, lung	FA, IHC, VI	IHC of placenta if there is placentitis
Bovine Viral Diarrhea Virus (BVDV)	Lung, kidney, heart, placenta, skin	FA, IHC, VI, PCR	Antigen capture ELISA, RT-PCR
Blue Tongue Virus (BTV)	Brain, spleen	PCR, VI	Fetal serology

(Continued)

Kirkbride's Diagnosis of Abortion and Neonatal Loss in Animals, Fourth Edition.
Edited by Bradley L. Njaa.
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Table B.1 (Continued)

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
Bunya Virus	None	None	Fetal or precolostral neonatal serology; fetal congenital abnormalities
<i>Brucella</i> spp.	Placenta, lung, abomasal contents, uterine fluid	Bacterial culture	Culture of milk from dam; Dam and Fetal serology
<i>Listeria</i> spp.	Placenta, lung, brain, abomasal contents	Bacterial culture, IHC	Gram staining of fresh aborted tissues; PCR
<i>Salmonella</i> spp.	Placenta, liver, lung, abomasal contents	Bacterial culture	
<i>Yersina pseudotuberculosis</i>	Placenta, liver, lung, abomasal contents, intestines	Bacterial culture	
<i>Leptospira</i> spp.	Kidney, placenta	FA, IHC, PCR	PCR of fetal urine; fetal and maternal serology
<i>Ureaplasma</i> spp.	Lung, abomasal contents, placenta	Ureaplasma culture, PCR	
<i>Campylobacter</i> spp.	Lung, abomasal contents, placenta	Campylobacter culture, IHC, Darkfield microscopy	Silver staining of tissues; PCR
Enzootic Bovine Abortion	Thymus, spleen, LN,	Modified Steiner's silver staining, IHC	Elevated fetal serum immunoglobulins
<i>Chlamydophila</i> -related spp.	Placenta	PCR, IHC, FA	Macchiavello's, Gimenez or modified acid fast stains
<i>Coxiella burnetii</i>	Placenta	PCR, IHC	Macchiavello's, Gimenez or modified acid fast stains
Mycotic causes	Placenta, abomasal contents, lung, skin lesion	Fungal culture, H&E histopathology	Direct identification of fungi by KOH wet mounts of skin or placenta or GMS and PAS stains of histologic sections

(Continued)

Table B.1 (Continued)

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
<i>Trichomonas foetus</i>	Placenta, abomasal contents, lung	Trichomonas culture, Bodian's silver stain, IHC, H&E histopathology	Darkfield microscopy of abomasal contents; PCR
<i>Neospora caninum</i>	Brain, lung, kidney, skeletal muscle, liver, placenta	IHC, PCR	Fetal serology (IFA, microagglutination titer, and ELISA's)
<i>Sarcocystis</i> spp.	Brain, lung, liver, kidney, skeletal muscle, placenta	IHC	Genomic probe for <i>S. cruzi</i> ; ribosomal RNA assays

Table B.2 Summary of diagnostic tests for ovine and caprine abortifacient pathogens.

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
Bluetongue Virus*	Brain, spleen	PCR, VI	Fetal and precolostral serology
Border Disease Virus*	Brain, heart, skin, placenta	VI, PCR, FA, IHC	Fetal serology
Caprine Herpesvirus-1	Adrenal glands, liver, lung, kidney	PCR	Visualization of intranuclear inclusion bodies in tissues
Cache Valley Virus*	Brain, skeletal muscle, heart	PCR, VI	Fetal and precolostral neonatal serology
Rift Valley Fever Virus	Liver, spleen, lymph node, brain, fetal fluids	VI, FA, PCR	EM of liver; ELISA
Akabane Virus*	Skeletal muscle, placenta, brain, spinal cord	VI, PCR	Fetal or precolostral neonatal serology
Nairobi Sheep Disease Virus	Lymph node, spleen, thymus, brain, kidney	PCR, VI	Fetal or precolostral neonatal serology
Wesselsbron Disease Virus	Liver, spleen, brain	PCR, VI	Fetal or precolostral neonatal serology

(Continued)

Table B.2 (Continued)

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
<i>Brucella</i> spp.	Placenta, lung, abomasal contents, uterine fluid	Bacterial culture, IHC	Fetal serology (Complement fixation test; ELISA; serum agglutination tests, rose Bengal test); PCR
<i>Campylobacter</i> spp.	Placenta, lung, liver, abomasal contents	Bacterial culture, FA, IHC	Darkfield and phase contrast microscopy
<i>Chlamydomphila</i> spp.	Placenta, liver	FA, IHC, PCR	Gimenez/Giemsa or modified acid fast stains of cotyledon impression smears and/or tissue sections; fetal serology
<i>Coxiella burnetii</i>	Placenta, lung, liver, kidney	FA, IHC, PCR	IFA on fetal fluids; ELISA; Compliment fixation tests, microagglutination tests, radioimmunoassays; modified acid fast stain on cotyledon impression smears and or tissue sections
<i>Flexispira rappini</i>	Placenta, lung, liver	Bacterial culture (requires specialized conditions)	Giemsa and crystal violet staining; Darkfield or phase contrast microscopy
<i>Francisella tularensis</i>	Placenta	Bacterial culture, IHC, PCR	This is a select agent so very few labs are able to perform bacterial cultures
<i>Leptospira</i> spp.	Kidney, placenta,	FA, IHC, PCR	Fetal/dam serology; Warthin-Starry staining, PCR on dam urine
<i>Listeria</i> spp.	Placenta, abomasal contents, lung, liver	Bacterial culture, IHC	Gram-positive organisms in placental impressions, abomasal contents or in histologic tissue sections
<i>Salmonella</i> spp.	Placenta, lung, liver, abomasal contents	Bacterial culture	Bacterial culture of vaginal discharge (aerobic and Salmonella enrichment broth)
<i>Sarcocystis</i> spp.	Brain, lung, liver, kidney, skeletal muscle, placenta	IHC, PCR	EM ultrastructural examination
<i>Neospora caninum</i>	Brain, lung, liver, kidney, skeletal muscle, placenta	IHC, PCR	Fetal and precolostral neonatal serology

(Continued)

Table B.2 (Continued)

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
<i>Toxoplasma gondii</i>	Brain, lung, liver, kidney, skeletal muscle, placenta	IHC, PCR	Fetal and precolostral serology

* Virus may have cleared the fetus at the time of the abortion, and tissues may be negative for the agent. Fetal serology or precolostral serology in newborns may be only way of determining infection.

Table B.3 Summary of diagnostic tests for porcine abortifacient pathogens.

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
PRRSV	Thoracic fluid pooled from 4 to 6 late-gestation fetuses	PCR	PCR on serum from sows that are ill when aborting
PCV2	Heart and lymphoid tissues	IHC, PCR	
PPV	Lung and heart from mummies	FA, PCR	Fetal serology documents in utero exposure
PRV	Lung, liver, spleen, brain and kidney	FA, VI, PCR	Eradicated from commercial herds in the US. Positive serology documents herd infection
SIV	None	None	PCR, VI, antigen capture ELISA on nasal swabs from acutely ill sows. Paired serology
TGE	None	None	PCR on feces from affect sows. Paired serology
EMCV	Heart, lung, spleen, kidney, brain	VI, PCR	Fetal serology
PEV/PTV	Lung	FA, VI, PCR	Fetal serology
Pestivirus's	Tonsil, kidney, spleen, and lung	PCR	
PRNSV	Lung, spleen, heart and kidney	VI, FA	
JEV	Brain, liver, spleen, lung and placenta	VI, IHC, PCR	Fetal serology
PCMV	Lung	PCR, FA, VI	

(Continued)

Table B.3 (Continued)

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
LPMV	Brain, lung, liver, placenta	VI, FA, PCR	
Menangle	Brain, lung, heart,	VI	Fetal and sow serology
<i>Leptospira</i> spp.	Kidney	PCR	Sow serology
<i>Brucella suis</i>	Placenta, stomach contents, liver, lung	Culture on <i>Brucella</i> selective media	Sow serology only appropriate for herd diagnostics
<i>Chlamydia</i> spp.	Placenta, liver	PCR, IHC	

Table B.4 Summary of diagnostic tests for equine abortifacient pathogens.

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
Equine herpesvirus	Liver, lung, adrenal glands, spleen, thymus, placenta	FA, IHC, VI, PCR	Intranuclear inclusion bodies in affected tissues (epithelium, endothelium); maternal serology should be carefully interpreted relative to previous natural infection or vaccination
Equine viral arteritis virus	Brain, liver, spleen, heart, placenta	VI, IHC, PCR	Serum neutralization showing maternal seroconversion
<i>Leptospira</i> spp.	Kidney, liver, placenta	FA, PCR	Silver staining for leptospires in kidney, placenta, or liver smears; fetal and maternal serology
Nocardioform placentitis	Placenta	Bacterial culture, PCR	Gross lesions of placentitis are virtually pathognomonic; use tryptic soy agar with blood and without added blood
Bacterial/fungal abortion	Placenta, lung, liver, stomach contents, fetal fluids	Bacterial and fungal culture, PCR	Maternal and fetal serology may be useful.

Table B.5 Summary of diagnostic tests for canine and feline abortifacient pathogens.

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
Canine			
Canine herpesvirus (CHV)	Kidney, adrenal gland, liver, lung, spleen	ELISA, VI, PCR, ISH, IHC	
Canine minute virus	Intestine	HI, VI, PCR	
Canine adenovirus	Lung, liver, spleen, placenta	VI, PCR, ELISA	
<i>Brucella canis</i>	Placenta, lung, stomach contents, uterine fluid	Bacterial culture	Serologic tests available: TA, RSAT, AGID, ELISA, IFA
Bacterial abortions	Placenta, lung, liver, spleen, lymph nodes, stomach contents	Bacterial culture,	Serologic assessment of virulence factors; PCR can identify virulence genes; strain classification can identify other virulence factors
Feline			
Feline herpesvirus	Kidney, adrenal gland, liver, lung, spleen, placenta	ELISA, IFA, VI, PCR	
Feline leukemia virus (FeLV)	Lymphoid tissue,	PCR	ELISA serology of whole blood or fetal fluids
Feline immunodeficiency virus (FIV)	Lymphoid tissue,	ISH, IHC	PCR of blood cells; ELISA-based serological tests
Feline enteric coronavirus (FoCV)	Lung, liver, kidney, spleen	ELISA, IFA	
Feline calicivirus (FCV)	Lung, liver, kidney, spleen	RT-PCR	Fetal serology
Feline parvovirus (FPV) (Feline panleukopenia virus)	Brain, lung, liver, spleen, kidney	VI, IFA	Restriction length polymorphism of PCR amplified fragments (RFLP); serology using SN or HI test methods
Bacterial abortions	Placenta, lung, liver, lymph node, spleen, stomach contents	Bacterial culture	Serotyping available

(Continued)

Table B.5 (Continued)

Agent	Preferred fetal tissues	Preferred fetal diagnostic test	Additional diagnostics
<i>Bartonella henselae</i>	Liver, lung, kidney, spleen, stomach contents	IHC, PCR	
<i>Toxoplasma gondii</i>	Brain, liver, spleen, lymph node	PCR	Fetal serology using MAT, IFAT, DT, IHAT, ELISA

AGID = Agar gel immunodiffusion
ELISA = Enzyme-linked immunosorbent assay
DN = Sabin-Feldman dye test
HI = Hemagglutination inhibition
IFA = Indirect fluorescence test
ISH = *In-situ* hybridization
IHC = Immunohistochemistry
IHAT = Indirect hemagglutination test
MAT = Modified agglutination test
PCR = Polymerase chain reaction
PR = Plaque reduction
RT-PCR = Reverse transcription polymerase chain reaction
RFLP = Restriction fragment length polymorphism
RSAT = Rapid slide agglutination test
SN = Serum neutralization test
TA = Tube agglutination
VI = Virus isolation

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