Diagnosing the Cause of Abortion in Cattle

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The purpose of this paper is to discuss a practical approach by which the cause of abortions in cattle may be determined. This will be presented under the following headings; definitions, magnitude of reproductive failure, some general causes of reproductive loss, infectious causes, and diagnostic rate. Ways to improve the diagnostic rate will include areas of responsibility, clinical examination and samples to collect.

Definitions

For purposes of description, the stages of gestation failure in cattle are identified as: failures of conception (which of course occur immediately after breeding); early embryonic death (from conception to day 42); abortions (conception to day 260) and premature deliveries (from the 260th day of gestation to term) (Hubbert).

Magnitude of Reproductive Failure

Even under so-called normal conditions, the magnitude of reproductive failure is considerable, and has been stated by David et al. to be approximately 37 failures for every 100 cows bred once. The magnitude of loss varies greatly during the different periods of gestation. Fourteen of 100 breedings may result in failure of conception, 20 of 100 in early embryonic death, and two to four of 100 in spontaneous abortion in the last two trimesters of pregnancy (Sreenan and Diskin). Four to six percent of pregnancies terminate as stillbirths; of which 9.1% may have evidence of infection, 7-8% congenital anomalies, and 84% may die of neonatal asphyxia (Leipold).

Genetic Causes of Reproductive Loss

Reproductive wastage may be caused by genetic, noninfectious and infectious factors. Genetic causes are considered by some to be inevitable and are described as the "elimination of faulty genetic experiments at a low biological cost" (Bishop). Others believe these losses are not inevitable and may be reduced by careful selection of breeding stock (Bolet). Genetic wastage includes hereditary factors (e.g. mucopolysacchariodosis), errors in fertilization (e.g. trisomy) and mutations. In women, errors of fertilization account for about 41% of spontaneous abortions (Carr), and in cattle, an incidence of 3-5% is reported by Gustavsson using cytogenetic studies on bovine embryos. Others, studying 12-18 day embryos found this to be 1.9% and

suggested that earlier studies are required as many embryos with abnormalities die before this time (Hare et al.). Losses attributed to genetic causes generally occur early in gestation (less than 90 days) and are beyond the routine capability of most diagnostic laboratories. Genetic causes of reproductive loss which are manifest late in gestation occasionally have characteristic morphologic abnormalities and may thereby be recognized as genetic in origin. For example, "prolonged gestation" and so called "bull dog calves" are strongly suspected of having, respectively, an autosomal recessive and incompletely dominant inheritance (Jubb et al., A&B). In women, approximately 0.4% of pregnancies result in a baby born alive with a chromosomal abnormality (Carr). In cattle, chromosomal abnormalities in the dam may contribute to infertility. In one study, 13 of 71 heifers found not to be pregnant after two breeding seasons were identified as having chromosomal abnormalities. This is in contrast to no abnormalities in 71 contemporary females to which they were compared (Swartz and Vogt).

Non-infectious Causes

Hormonal asynchrony in the early postpartum period may be caused by infectious or noninfectious factors and is considered by many to be a major cause of reproductive wastage and prolongation of the calving interval. Noninfectious causes of reproductive failure encampass a very wide range of factors including the effects of age, nutritional deficiencies or excesses, noise, toxic plants and chemicals, and also certain environmental factors such as season and temperature. While these factors may be very important as a cause of reproductive failure, except in circumstances where large numbers of animals abort, they are infrequently recognized. An exception to this may be vitamin E and selenium deficiency which is presumptively diagnosed on the basis of the heart or skeletal muscle lesion in the individual animal (Miller and Quinn).

Infectious Causes

By far the most commonly recognized causes of abortion are those which are associated with infectious disease (Hubbert et al.). The actual proportion of reproductive wastage that is due to infectious causes, however, varies greatly and is dependant on a susceptible population and the presence of organisms capable of causing abortion. Before Brucellosis was controlled the proportion due to infectious causes could easily exceed 50%.

The effects of infectious and noninfectious causes may be manifest indirectly through the cow or directly through the placenta and fetus. Causes of abortion such as hemoconcentration, circulatory failure, anemia, fever, endotoxemia or respiratory disease will not be detected by examining the placenta or fetus but will only be determined by examination of the cow. Abortion may occur some time after the initiating illness, and therefore health and milk production records become important in suggesting the diagnosis. Cows may contact infectious agents by many routes: through the respiratory tract as, for example with the viruses of BVD and IBR; through the mouth with BVD virus, Brucella, Listeria and Leptospira, through the vagina as with *Campylobacter fetus*. Some agents may be carried into the reproductive tract with the semen. *Ureaplasma diversum,* and IBR and BVD viruses may contaminate semen, and being resistant to the antibiotics commonly used in the treatment of semen, they survive there. Organisms may also contaminate embryo transfer fluids and be transmitted to the recipient animal in this way. In addition, there is evidence that some agents, such as *Leptospira hardjo* are maintained in the uterus and uterine tube (oviduct) of non-pregnant cattle for prolonged periods (Ellis et al. 1986). *Campylobacter fetus* commonly contaminates the vagina of cattle and as the hormonal influence changes from predominantly estrogen to progesterone, the organism may move from the vagina to the uterus producing early embryonic death and, less frequently, abortion (Van de plassche et al.) Other agents may act similarly. Some organisms may multiply in the conceptus for a very long period before abortion occurs. *Ureaplasma diversum,* for example, has been detected in amniotic fluid for up to 117 days before abortion (Miller et al.). Similarly *Aspergillus fumigatus* may multiply in the placenta for at least 25 days before producing abortion (Hill et al.).

Caution must be taken in interpreting the results of culturing the fetus, placenta or even the abomasal contents. For example bacteria may move from the vagina to the conceptus when the cervix dilates during an impending abortion due to another cause. These organisms may rapidly contaminate the placenta, grow in the fetal fluids and even be swallowed by a viable fetus thereby appearing in the abomasal contents. Under some circumstances two or more bonafide causes of abortion may be identified in the same fetus (Kendrick). Kirkbride (1984A) reports finding BVD virus in 3 of 16 fetuses with *Bacillus* sp., 6 of 30 with *C. pyogenes (Actinomyces pyogenes)* and 17 of 45 with fungal infection. The interaction and significance of those isolates can only be postulated at this point. In these animals immunosuppression associated with BVD virus infection may permit the organism to circulate in the dam and localize in the placenta or it may be, as just stated, the animal was aborting due to BVD virus infection and the other organisms are contaminants (Reggiardo and Kaeberle). In contrast, abortion may occasionally be classified as infectious because of histopathologic lesions observed, but with no organisms identified (Jerret et al.). This may be due to failure of

organism to grow because of improper handling of the samples or severe fetal autolysis. The most commonly diagnosed infectious causes of abortion in Southern Ontario cattle in 1985 included BVD virus (12%), *Ureaplasma diversum* (10%), various mycoses (6%), *Leptospira* sp. (6%), *Bacillus licheniformis* (5%) and IBR virus (1.5%) (Maxie). Percentages are of total submissions, not just of those diagnosed.

Improvement of the Diagnositc Rate

The diagnostic rate on aborted fetuses in many laboratories averages near 25% but this may be increased to 50% or more if considerable care and forethought is employed. As there are usually at least five persons involved (livestock manager, clinician, pathologist, bacteriologist, virologist), reaching a diagnosis depends on each of these persons not only doing their job but cooperating and communicating extensively with the next person in line. Eventually many other specialists such as nutritionists, toxicologists, botanists, and epidemiologists may be involved.

It is up to the clinician and livestock manager to provide a complete and accurate history. While the history seldom provides information pointing directly to the cause of abortion, clues may be found that will indicate what needs to be done to determine the diagnosis. It is important not to eliminate a disease from the list of possibilities because of a history of vaccination. Many diseases may occur and result in abortion in spite of previous vaccination, e.g. BVD and IBR and of course, vaccination with certain live vaccines during pregnancy may cause abortion.

While gathering the history it is important for the clinician to carefully examine the cows which aborted and perhaps even more importantly examine a representative portion of the rest of the herd. Much information may be obtained by examining the other cows in the herd. Vaginal discharges from animals with impending abortions are much less likely to be contaminated by extraneous organisms than samples from an animal who has aborted several days previously. Serology is more easily interpreted on a herd basis than it is on an individual animal. At least ten animals, or 10% of all animals, should be sampled. Two samples from a cow which has just aborted may give no useful information. Many agents which subsequently cause abortion have produced their highest antibody titer long before abortion occurs, and the titers may actually be within the normal range at the time of abortion. In addition many animals may not respond to the agent with the production of antibody and yet the animal may be carrying the organism and abort because of it. Up to 22% of animals aborting because of *Leptospira hardjo* may not have a significant titer (microscopic agglutination test) at the time of abortion (Ellis et al, 1982). The serum antibody titer of an animal as indicated on a sample collected at the time of pregnancy diagnosis, subsequently compared to the results of serology conducted on a blood sample taken at the time of abortion may be very useful, especially if sampling

has been conducted on a herd basis.

As there are many causes of abortion and because the act of abortion rarely points to a definite cause, it is important to collect every bit of information possible. In addition, it is important for the clinician to maximize sample collection initially, thereby permitting the use of all the necessary diagnostic aids as the cause is gradually revealed. Unnecessary samples may be easily thrown out later but are usually impossible to collect or are of less value when collected long after the fact.

The pathologist must also maximize sample collection at the time of necropsy. In conditions other than abortion the pathologist is usually presented with a carcass in which the system involved is indicated in the history. This is not true in abortion. As the cow, placenta or any organ system in the fetus may be involved it is important that samples of each be collected. If the placenta is retained, portions of it should be removed and submitted. The part protruding between the vulvar lips usually has few lesions, and is heavily contaminated by organisms from feces and the environment. The portion that is retained internally should be submitted as it frequently has the most severe lesion (It may be the reason it is adherent to the caruncle.) and is less contaminated by extraneous bacteria. If the animal has passed the placenta and it cannot be found or is unfit to use, a caruncle should be removed and submitted. It is very important to have at least a portion of this interface between the dam and fetus as lesions pointing to the diagnosis may only be present in the placenta. The larger the portion of placenta that can be examined the better, as it is a very large organ and much of it may be normal.

As many laboratories have specific forms to fill out for abortions and will supply preservatives and containers on request, a discussion with the regional laboratory pathologist before submitting specimens is desirable. Samples should include serum from the cow and herd, swabs or samples from the uterus, caruncles, placenta and fetal tissues. Tissues should not be frozen but should be refrigerated. If the fetus is too bulky to save or transport in its entirety, samples may be removed and one portion placed in fixative and the other in plastic bags on ice for culture.

Portions of chilled kidney are useful for the fluorescent antibody detection of IBR virus, BVD virus and leptospira. Stomach content is useful for bacterial or mycological culture and direct examination for bacteria, fungi and coxiella. Certain organisms are extremely fragile and care in handling samples becomes very important. Samples to be cultured for *Ureaplasma diversum* for example, should be collected aseptically, chilled to 4-10°C and delivered to the laboratory immediately. Leptospires may be equally fragile. Tissues for fixation should always include appropriate portions of caruncle or placenta, skin, lung, heart, liver, kidney and intestine. Fetal serum or thoracic fluid samples for antibody detection may be collected but the antibody titers, as in the dam, must be interpreted with caution. The fetus is capable of responding to a variety of agents with antibody production relatively early in gestation and the presence of specific antibody in fetal fluids may be considered presumptive evidence of fetal infection. It has been shown, however, at least in sheep, that placental vascular damage may allow antibodies to cross the placenta from the dam to the fetus thereby making interpretation difficult (Poitras et al.). When this occurs concentrations in the fetus will presumably be lower than in the dam.

Successfully preventing further abortions during an outbreak after determining the cause of abortion is only rarely achieved. The greatest value in reaching a diagnosis is to facilitate prevention of future problems either through management or immunization procedures. Occasionally however, prevention of further abortions during an outbreak may be achieved. Control of abortions due to leptospirosis may be obtained by using a combination of antibiotic therapy (dihydrostreptomycin, 25 mg/kgm) and bacterins containing appropriate leptospira serovars (South and Staenner). Selenium deficiency may be corrected by its addition to the feed or by intramuscular injection. When abortion due to *Listeria monocytogenes* infection are occurring the feeding of ensilage may be stopped and antibiotics injected (Kirkbride et al. 1985B). It is not considered useful to vaccinate against IBR virus infection during an outbreak as the period from infection to abortion is so long that most of the indigenous population will already have been exposed by the time the first abortions occur.

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Pathogenesis of Bovine Laminitis (Diffuse Aseptic Pododermatitis) Experimental Models

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Summary

Digital intraarterial infusion of endotoxin resulted in clinical symptoms of endotoxemia, mild laminitis and sero-fibrinous tenosynovitis. The direct effect of endotoxin on the digital microcirculation corresponded well to the effect of endotoxin in other capillary beds and resulted in disorientation, vacuolization, and reduction in the staining properties of the horn-producing epidermal cells. Grain overfeeding of 1 calf produced rumen acidosis, mild clinical symptoms of laminitis, and light serofibrinous tenosyno-vitis. Reduction of the thrombocyte count indicated that sequestration and DIC took place. In the calf exposed to sublethal infusions of endotoxin on 3 days prior to the overfeeding clinical symptoms of endotoxemia of varying severity occurred on those 3 days. After the overfeeding a progressive, serofibrinous polysynovitis developed. Histo-pathological examination of the digits revealed an extensive perivascular cell infiltration not seen in any of the other 3 experimental calves or in 1 control calf. The presence of fibrin-stars in digital veins 3 days after the overfeeding could indicate that the lesion in the digits was still in progression and that a generalized Sanarelli-Schwartzman phenomenon rather than tolerance to endotoxin prevailed. Both experimental models designed could be useful for further pathogenetic and therapeutic investigations as laminitis was induced by digital intraarterial infusion of endotoxin as well as by rumen acidosis.

The Effects of *Haemophilus somnus* on Bovine Embryos by Day 8 Postbreeding

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Summary

A study using 23 healthy, mature, virgin Holstein-Friesian heifers was designed to determine if *H. somnus* caused detrimental effects in early bovine embryos and the mechanism(s) by which these effects were induced. Superovulated heifers were artificially inseminated 12 and 24 hours after standing estrus using high quality, Haemophilus-free semen from a single ejaculate of one bull. Treatment heifers (n=12) were exposed by intrauterine infusion, 12 hours after the second insemination, to approximately 1.5 x 10^9 *H. somnus* organisms (Iowa strain 1229) suspended in 10 ml of 0.85% sterile phosphate buffered saline (PBS). Control heifers (n=11) were inseminated and infused using sterile PBS as a placebo. Embryos were recovered 8 days from the second insemination using non-surgical technique and evaluated microscopically and graded on their estimated survivability. Representative embryos were also examined for their *in vitro* culture survival time, histopathological changes, vital stain uptake, and bacterial contamination. Results to date indicate that *H. somnus* had a detrimental effect n early bovine embryos. A significantly larger (P<0.01) number of dead embryos were recovered from *H. somnus*-infected heifers compared to embryos recovered from control heifers. Embryos from *H. somnus*-infected heifers survived in culture media for a significantly (P<0.01) shorter time than embryos from control heifers.

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