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Case Report – Diagnosing Twin Pregnancies in Holstein Cows: Accurate Diagnosis by Transrectal Ultrasonography and Outcome of Twin Pregnancies

Billy I. Smith, *DVM, MS, DABVP*
Department of Clinical Studies, University of Pennsylvania, New Bolton Center, Kennett Square, PA 19348; e-mail address: bis@vet.upenn.edu

Abstract

Cattle are capable of carrying twins to term, and the pregnancy can be unicornual or bicornual. Risk factors associated with twinning include genetics, breed, parity, ovulation rate and milk production. Methods such as palpation *per rectum* and transrectal ultrasonography can be used to diagnose twin pregnancies in cattle. This case report demonstrates the usefulness of transrectal ultrasonography for early detection of twin pregnancies between 55 and 83 days of gestation over an approximately two-year period (2001-2003), and provides information on the outcome of 64 cows (case population) randomly identified with twin pregnancies. Of the 64 cows, 48 (75%) animals delivered twins, one (1.6%) animal delivered a single calf, 13 (20.3%) cows aborted the pregnancy and two (3.1%) animals died prior to calving. This study should support the importance of accurately diagnosing and understanding outcomes of twin pregnancies in cattle. Further research is warranted to better understand the dynamics surrounding twin pregnancies in cattle.

Keywords: bovine, twins, ultrasound, parity, embryo location

Résumé

Les bovins peuvent donner naissance à des jumeaux et la gestation peut prendre place dans une ou deux cornes de l'utérus. Les facteurs de risque de la gestation double sont reliés à la génétique, à la race, à la parité, au taux d'ovulation et à la production laitière. La palpation rectale et l'échographie transrectale peuvent être utilisées pour diagnostiquer la présence de jumeaux chez les bovins. Ce rapport de cas démontre l'utilité de l'échographie transrectale dans la détection précoce de jumeaux entre les jours 55 et 83 de la gestation sur une période de deux ans approximativement (2001-2003) et montre le résultat de la gestation chez 64 vaches (population de cas) identifiées aléatoirement comme étant gestantes avec des jumeaux. Parmi les 64 vaches, 48 (75%) ont mis bas à des jumeaux, une (1.6%) a mis bas à un seul veau, 13 (20%) ont avorté et deux (3.1%) sont mortes avant le vêlage. Cette étude devrait montrer l'importance de diagnostiquer adéquatement et de comprendre le résultat des gestations doubles chez les bovins. Plus de travaux sont nécessaires pour mieux comprendre la dynamique entourant les gestations doubles chez les bovins.

Introduction

Twinning in dairy cattle is considered an undesirable outcome of pregnancy that is not diagnosed at routine pregnancy evaluation conducted at 35 to 42 days post-insemination, but rather at the time of calving. Twinning is undesirable because cows carrying twins have a higher risk of reproductive disorders including abortion, dystocia, retained placenta and metritis, and are more likely to develop metabolic disorders such as displaced abomasum, periparturient hypocalcemia and ketosis.[4,12,16,21,35] Another important negative aspect of twinning is its potential to reduce the number of heifers available for use as replacements in the herd. The herd profile is mainly damaged due to an increased number of abortions[12,16] and increased newborn calf mortality.[8,17,28] Overall, the negative economic impact of twinning in dairy cattle due to the various disorders associated with the cow and also with the newborn calves makes twinning an unwanted event in dairy herds.

Various methods such as palpation *per rectum*, transrectal ultrasonography and blood testing for pregnancy-associated proteins (bovine pregnancy-specific protein B (bPSPB) and bovine pregnancy-associated glycoproteins (bPAG)) can be used to diagnose pregnancy (singlet or twins) in cattle.[10,46] Manual examination of the uterus via palpation *per rectum* is the most common method used by professionals in the industry. However, the accuracy of diagnosing twin pregnancies may be re-

duced when compared to transrectal ultrasonography.[10] Blood testing for bovine pregnancy-associated proteins provides a means for early, accurate and safe pregnancy diagnosis, however, it provides limited information regarding the viability of the pregnancy or the presence of twins when testing a single cow. Transrectal ultrasonography may be used both to diagnose a viable pregnancy and to accurately determine the presence of twins.[11,32]

Routine pregnancy examination by palpation *per rectum* in dairy cattle most often occurs between 35 and 42 days post-insemination (before the second estrus period).[55] At this time, a diagnosis of twins may be made possible only with a meticulous examination.[10] Many factors play a role in missed diagnosis of twins during routine pregnancy examination of dairy cattle. It is increasingly difficult to determine a positive sign of pregnancy (either singlet or twins) the earlier one performs palpation *per rectum*, possibly leading to decreased accuracy of diagnosis.[51] Further, more vigorous palpation *per rectum* during early pregnancy diagnosis may lead to embryonic death.[17,37] Accurately diagnosing twins by early (35 to 42 days post-insemination) palpation *per rectum* is obviously confounded.[10] Diagnosis of a twin pregnancy seems easier when the embryos are located in separate horns (bicornual pregnancy) versus two embryos occupying the same uterine horn (unicornual pregnancy). As with palpation *per rectum* to accurately diagnose a singlet pregnancy, the more specific diagnosis of twins by palpation *per rectum* is more accurately made when pregnancy examination is delayed by two to three weeks (50 to 60 days post-insemination).[1,51]

In addition, transrectal ultrasonography can aid in fetal sexing. The optimum stage of gestation for fetal sexing is 55 to 75 days post-insemination. Transrectal ultrasonography can be of benefit to the producer because of the increase in accuracy of determining a twin pregnancy and the added benefit of identifying the sex of the growing fetus. However, delaying the routine diagnosis of pregnancy in dairy cattle is economically unacceptable due to the added cost of days open in dairy cattle.[22] These added benefits of accuracy of twin detection and fetal sexing could be performed during a routine re-examination of pregnancy status in cattle.

This paper provides retrospective information on the use of real-time transrectal ultrasonography as a method of detecting (55 to 83 days post-insemination) twin pregnancies in Holstein cattle. Also, it provides information on the occurrence of twins in Holstein cattle, and the outcome of identified twin pregnancies.

Clinical Report

Current Diagnostic Procedure for Identifying Twins in Cattle by Ultrasonography

The use of real-time ultrasonography to diagnose pregnancies in the mare has been utilized since the early 1980s.[38] Prior to this, mares were identified pregnant via transrectal palpation. Today, transrectal ultrasonography is a common adjunct method utilized in diagnosing pregnancy in the mare. In contrast, the same acceptance and use of transrectal ultrasonography to identify pregnant cattle has not occurred in the bovine industry. There are several reasons for the reluctance to utilize real-time ultrasonography for pregnancy diagnosis in cattle. First, the time frame for diagnosis of early pregnancy (27 to 30 days) is very similar for palpation *per rectum* and transrectal ultrasonography, so that the increased cost and set-up time for equipment and the increase in time to perform the procedure make transrectal ultrasonography less appealing. During days 25 to 30, the embryo proper is seen as a small echogenic structure with a prominent "C" shape, which is the result of the cephalic and caudal flexures and general curvature of the vertebral column.[9] Second, unlike the mare's reproductive tract, the cow's reproductive tract is not as easily accessible. The bovine's uterus is more coiled and pendulous, thus producing many sectional images on the ultrasound screen at one time (Figure 1). Furthermore, in the early stages of pregnancy between 25 and 27 days, the fluid accumulation within the uterine lumen appears as a nonechogenic line similar to images obtained from cattle that are in or near to being in estrus (heat) (Figure 2).[28] Use of transrectal ultrasonography to diagnose twin pregnancy in cattle results in higher accuracy, especially when the examination is delayed beyond 50 days post-insemination.[11,32]

At 35 to 50 days post-insemination, ultrasonographically the echogenic bovine conceptus is surrounded by spherical, non-echogenic areas, making it readily visible (Figure 3).[9] The amnion can be seen as an echogenic line roughly encircling the embryo (Figure 3).[9] From days 55 to 65 of gestation, a clear, spherically shaped embryonic vesicle containing a fetus is repeatedly identifiable. Unlike in the mare, where the fetus becomes deeply situated in the endometrium and thus becomes increasingly more difficult to locate, visualization of the bovine fetus is not similarly hindered. The cotyledonary type of placentation in the bovine allows for easy identification of the fetus as it freely floats around in the spherical, amnionic vesicle (Figure 4).

Cattle are capable of carrying twins to term, either as a unicornual or bicornual pregnancy. In the bovine, the optimal time for determining twins by transrectal ultrasonography is days 55 to 65 of gestation.[11] Similarly, fetal sexing usually occurs 55 to 75 days post-insemination. During this optimum time frame, the uterus may be retracted onto the pelvic floor, the pregnant horns are readily accessible for bicornual pregnancies, and both fetuses can be readily visualized on the screen if the pregnancy is unicornual (Figure 5). To avoid errors in diagnosis, retraction of the bovine uterus may be necessary to allow complete access to both uterine horns.

Cattle Population Data

Examinations using transrectal ultrasonography to identify animals making up the case population were done at various times over a 28-month period beginning in August 2001 and extending through December 2003. Data presented in this case report constitute a retrospective look at a specific group of cows diagnosed with twins. Animals identified with twins were used for a separate research trial. Data were not recorded on cows diagnosed with a single fetus or no fetus.

The case population animals were located on a commercial dairy farm in southeastern Pennsylvania composed of approximately 625 Holstein cows milked twice daily with a mature equivalent (ME) 305-day milk yield of 23,400 lb (10,636 kg), and bulk-tank fat and protein content of 3.5 and 3.0%, respectively. Cows were housed in a conventional six-row, free-stall barn and fed a total mixed ration (TMR) during the entire lactation. Cattle receive recombinant bovine somatotropin (rbST)[a] starting on or near day 85 postpartum and then every 14 days during the entire lactation.

The farm's routine reproductive program included two pre-synchronization treatments of prostaglandin $F_2\alpha$[b] given during the voluntary waiting period. The first treatment was given on days 40 to 46 post-calving, with the second treatment given two weeks later. Following

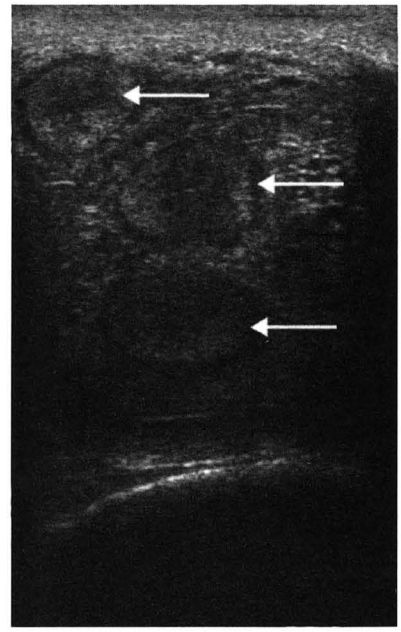

Figure 1. Image obtained by transrectal ultrasonography using a MyLab™ ultrasound machine (Biosound Esaote, Inc., Indianapolis, Indiana, US) and a 7.5-MHz transducer showing uterine cross-sections (arrows). Note the many cross-sectional images on the ultrasound screen at one time.

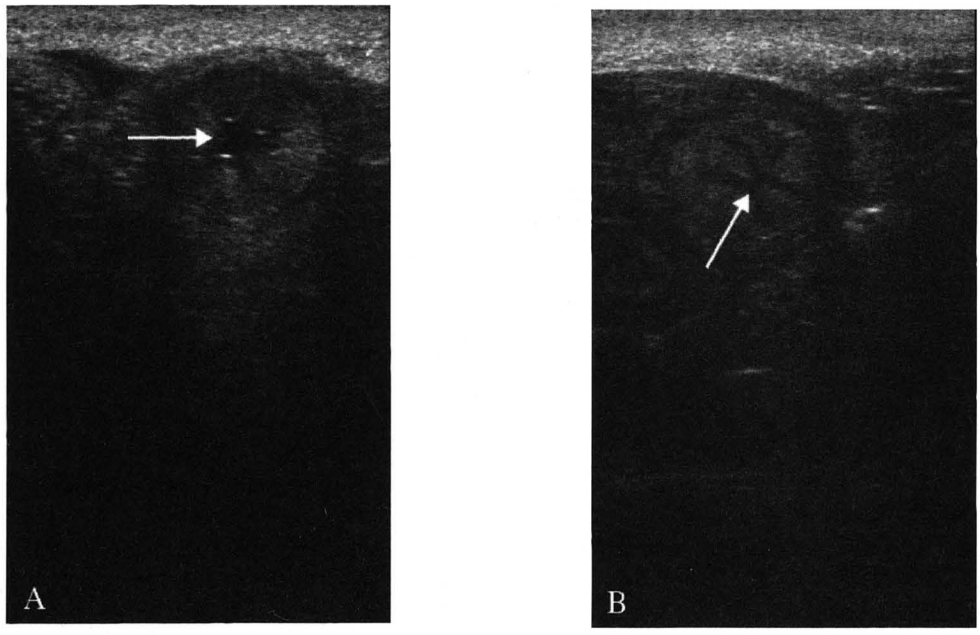

Figure 2. Images A and B obtained by transrectal ultrasonography using a MyLab™ ultrasound machine (Biosound Esaote, Inc., Indianapolis, Indiana, US) and a 7.5-MHz transducer showing the similarities of images regarding fluid accumulation within the uterine lumen between a cow diagnosed with an early pregnancy and a cow in estrus (standing heat). (A) Ultrasound image showing a cross-sectional view of a uterine horn of a cow diagnosed with a 27-day-old pregnancy. Note the fluid accumulation in the lumen of the uterine horn which appears as a non-echogenic area (arrow). (B) Ultrasound image showing a cross-sectional view of a uterine horn of a cow identified in estrus. Note the fluid accumulation in the lumen of the uterine horn which appears as a non-echogenic area (arrow).

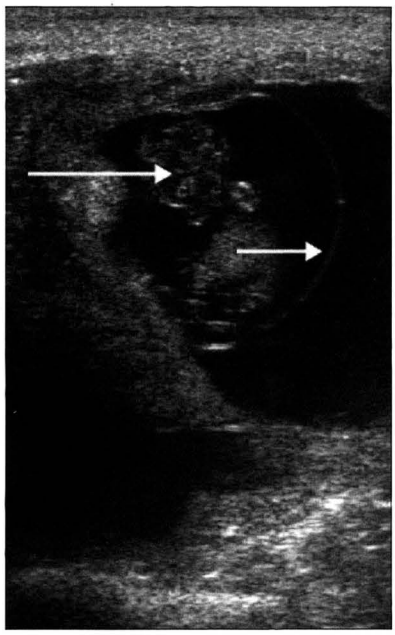

Figure 3. Image obtained by transrectal ultrasonography using a MyLab™ ultrasound machine (Biosound Esaote, Inc., Indianapolis, Indiana, US) and a 7.5-MHz transducer showing an echogenic bovine conceptus (long arrow) surrounded by spherical non-echogenic area. Note the amnion can be seen as an echogenic line roughly encircling the embryo (short arrow).

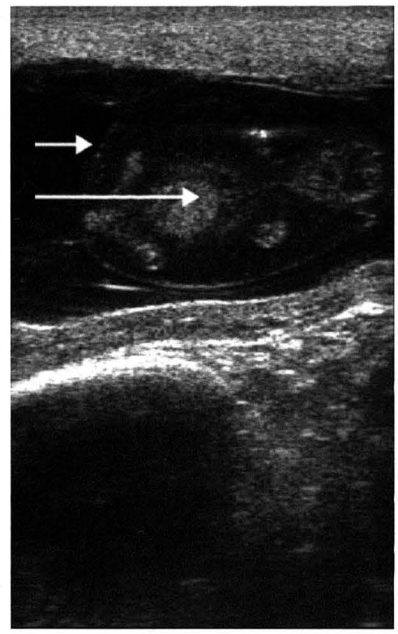

Figure 4. Image obtained by transrectal ultrasonography using a MyLab™ ultrasound machine (Biosound Esaote, Inc., Indianapolis, Indiana, US) and a 7.5-MHz transducer showing a 55-day-old bovine fetus (long arrow) as it freely floats around in the spherical amnionic vesicle. Note the amnion can be seen as an echogenic line roughly encircling the embryo (short arrow).

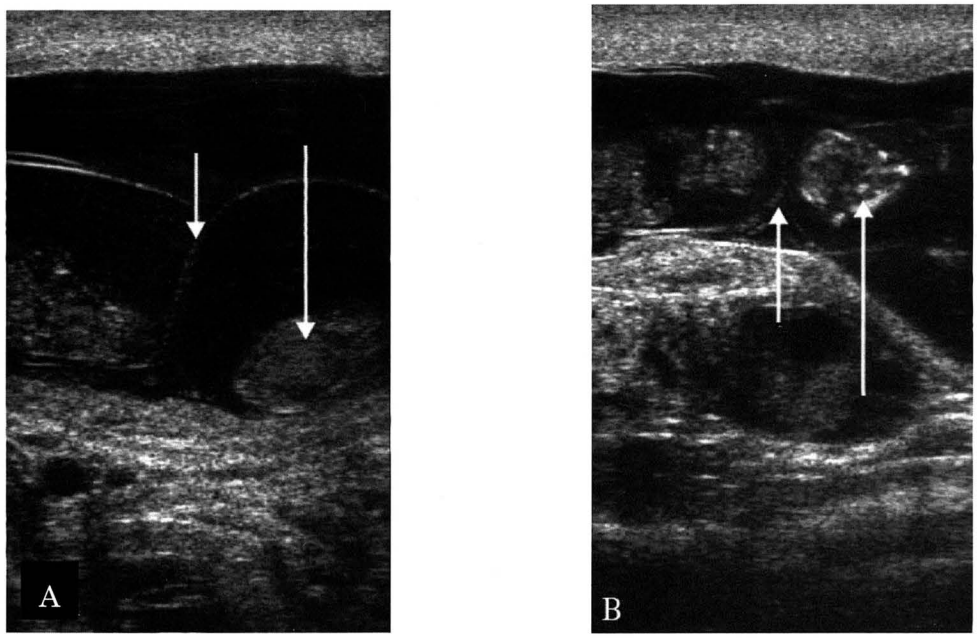

Figure 5. Images A and B obtained by transrectal ultrasonography using a MyLab™ ultrasound machine (Biosound Esaote, Inc., Indianapolis, Indiana, US) and a 7.5-MHz transducer showing both echogenic bovine fetuses (long arrows) of a 55-day-old unicornual twin pregnancy surrounded by spherical non-echogenic areas. Note the juxtaposition of the amnionic membranes from each conceptus, which can be seen as two echogenic lines roughly encircling the fetuses and eventually contacting each other to produce what appears as a single echogenic line (short arrow).

the second treatment, cattle identified in heat were inseminated. Cattle not observed in standing heat were then started on an Ov-synch[47,48] program, with the first treatment of gonadotropin-releasing hormone (GnRH)[c] administered 14 days after the last pre-synchronization treatment of prostaglandin $F_2\alpha$. Seven days after the GnRH treatment was administered, a prostaglandin $F_2\alpha$ treatment was administered, followed by a treatment of GnRH 48 hours later. Artificial insemination was then scheduled 16 hours after the second GnRH treatment. Inseminated cattle were scheduled for pregnancy examination by palpation *per rectum* at 38 to 42 days post-insemination. Cattle confirmed pregnant were then scheduled for re-examination using transrectal ultrasonography.

Animals were sorted weekly and placed in a herringbone palpation rail. Restraint was obtained by fully loading the palpation rail with cattle so that side-to-side contact of the cows within the rail prevented movement of the animals. Fecal material was removed from the rectum prior to insertion of the handheld ultrasound transducer. A Shimadzu-350 XL[d] ultrasound machine with a 5.0-MHz transducer was used for the pregnancy reexamination between 55 and 83 days of gestation. During this window, the fetus could be easily accessed and the umbilical cord could be observed free floating in amnionic fluid surrounded by the amnionic membrane. After retraction, the entire uterus, including both horns and body, was carefully scanned. No adverse outcomes following transrectal ultrasonography were reported in these cattle.

Record Analysis of Herd

All calving events were recorded and managed by use of Dairy Comp 305 herd management software.[e] Cows delivering twins were recorded as an event with the code TW. The herd's annual rate of twinning during the study period was 5.3% of all calvings (Table 1).

Further analysis of twinning events revealed a higher (χ^2 test $P<0.001$) incidence rate of 7.5% for second and greater lactation numbers, and 1.8% for first-lactation animals (Table 2).

Twin Findings and Outcomes from Case Population Cattle

A total of 64 cows were identified with twins, and they became the case population (Table 3).

Time from Diagnosis to Abortion in Case Population Cattle that Aborted

Thirteen cows (20.3%) diagnosed with twins aborted after 55 days in gestation. Abortion dates were recorded when cows were observed in heat, and the abortion was confirmed by palpation *per rectum* or when a fetus or fetal membranes were seen delivered by the cow. Post-abortion transrectal ultrasound examination was not performed. The range of time from conception to diagnosis of abortion (date recorded in

Table 1. Number of calving and twinning events by year, and annual incidence rates (%) of twinning in the dairy herd were determined.

Year	Calving events	Twinning events	Incidence rate (%) ± SD
2000-2001	652	37	5.7
2001-2002	683	44	6.4
2002-2003	652	20	3.0
2003-2004	749	44	5.8
Cumulative	2736	145	5.3 ± 1.5

Table 2. Descriptive and summary data of twinning events in the dairy herd from which the case population originated during 2000-2006.

Time period	Outcome variable	Lactation		*P*
		2nd and greater	1st lactation	
2000-2006	Twins			
	Yes	134	17	
	No	1656	929	
	Incidence rate	7.5%	1.8%	<0.001

Table 3. Descriptive data on the number of animals and outcomes of confirmed pregnancies affected by twinning.

Outcome	Number	Percent of total
Delivered twin calves	48	75.0
Delivered single calf	1	1.6
Aborted	13	20.3
Cows died prior to calving	2	3.1
Total	64	100

records) in this case group varied from 86 to 245 days of gestation.

Discussion

Highlighted points include: 1) this case report demonstrates the use of transrectal ultrasonography for early detection of twins in cattle at a time frame beyond where early embryonic death contributes to pregnancy loss; 2) a review of the association between the complexity and risk factors of twinning in dairy cattle was demonstrated; and 3) 20.3% of cows in our case population diagnosed with twins aborted after 55 days in gestation, and a majority of the abortions (62%) were late-term, as identified from records.

Transrectal Ultrasonography for Detection of Twin Pregnancies

Until relatively recently, the most common method to determine a cow was pregnant with twins was the observed delivery of two calves at parturition. However, a diagnosis of twins in cows can be made by palpation *per rectum* alone or with the use of ultrasonography.[10,11,32] This case report is unique in that data were gathered in a time frame beyond where early embryonic death contributes to pregnancy loss. In the bovine, several factors including parity, number of days post-insemination and operator experience may affect the accuracy of pregnancy diagnosis by transrectal ultrasonography.[2] The accuracy of detecting a single conceptus pregnancy by ultrasound has been reported to be between 62 and 100%, with accuracy improving to 100% in younger animals and decreasing in older animals.[2,24,25,40] The accuracy of pregnancy examination by ultrasound prior to 25 days,[24,40,54] and even prior to 35 days,[2] post-insemination decreases and results can be variable and inconsistent. Most likely, the same factors affecting the accuracy of diagnosing singlet pregnancies by transrectal ultrasonography in cattle would also affect accurate diagnosis of twin pregnancies.

Transrectal ultrasonographic examination with a 5.0-MHz transducer in this case report allowed for accurate diagnosis of twin pregnancies 55 to 83 days post-insemination. Only one cow of 64 total animals diagnosed with twins by transrectal ultrasonography delivered a single calf. Although the specificity in this report is high (98.5%), it was impossible to determine the sensitivity and overall accuracy due to incomplete information on all animals scanned by the author. However, sensitivity, specificity and overall accuracy for detecting single fetus and twin pregnancies in the bovine by palpation *per rectum* and transrectal ultrasonography have been reported. In a prospective study reported by Day *et al*,[10] the average sensitivity of twin pregnancy diagnosis by palpation *per rectum* was 49.3%, resulting in less than half of the twins born being correctly diagnosed at pregnancy examination. This poor sensitivity resulted from a twin pregnancy being misdiagnosed as a single pregnancy. The study involved multiple veterinarians who routinely diagnosed pregnancies as early as 30 days post-insemination and twins by palpation of two amnionic vesicles. The overall reported specificity and accuracy for all veterinarians were 99.4% and 96.0%, respectively. Davis *et al*[11] reported an improvement in accuracy of detecting multiple fetuses by transrectal ultrasonography in beef cattle at 51 days versus 43 days post-insemination. The time frame post-insemination for which the ultrasound examination occurs is important to reduce the variabilities in accuracy of transrectal ultrasonography when diagnosing twin pregnancies.[12] Accurately identifying twin pregnancies at 50 days or more post-insemination is the main benefit for using transrectal ultrasonography.

In this case report, the one cow delivering a single calf either was misdiagnosed with twins by ultrasonography or embryonic/fetal loss of one conceptus occurred. Regardless, this is a very low occurrence following use of transrectal ultrasonography to initially diagnose a twin pregnancy beyond 50 days insemination. The author's personal experience would suggest that clear visualization of the embryonic vesicle containing the fetus is necessary for confirmation of pregnancy. I postulated that visualization of twins could be hindered by twin approximation and location in the uterus. If the twin pregnancy is bicornual, the operator may find it difficult to scan the entire reproductive tract due to the pendulous and coiling nature of the tract *in situ*. The confusion created by multiple cross sections visualized on the screen could result in the diagnosis of twins when only one fetus is present. This error is less likely to occur in unicornual twin pregnancies where the two embryonic vesicles are juxtaposed and easily differentiated from each other (Figure 5). Indepth knowledge of the bovine reproductive tract, sufficient practice or skill in ultrasonography, and an adequately restrained, cooperative

animal provide additional assurance that cows carrying twins will be detected accurately.

The incidence of a single birth from a twin pregnancy diagnosed by transrectal ultrasonography in this report was 1.6%, which is extremely low when compared to other reports in the literature. One study conducted in Spain on Holstein cows reported a reduction in conceptus number (6.2%) in cattle diagnosed with twins by transrectal ultrasonography when diagnosed at 36 to 42 days post-insemination and compared to re-examination at 90 days.[32] However, unlike the current report, no further information regarding final outcome at parturition was reported. In the current case report, the low (1.6%) occurrence of a single birth from a twin pregnancy may be due to delay in diagnosing the twin pregnancies. Diagnosis occurred 55 days or more post-breeding at a time frame beyond when early embryonic death may contribute to pregnancy loss. In addition, the birth of a single female calf later determined to be a freemartin has also been reported.[5,36,52]

Risk Factors for Twinning

There is a concern that the twinning rate continues to increase over time, thereby forcing dairy producers to rethink how they handle these animals. In 1974, Scanlon *et al*[44] reported a twinning rate of 2.8% in over 2,000 pregnancies. Various researchers from several different countries published a range (1.4 to 4.8%) in twinning rates between 1978 and 1994.[4,13,29,33,35,39] Recently, Day *et al*[10] reported a twinning rate of 6.86% in over 4,400 pregnancies in Holstein dairy cattle in the United States. The analysis of the records for the case population delivering twins during the time frame of this report revealed a cumulative twinning rate of 5.3%. The incidence rate for the farm in this study fluctuated over time, varying from a low value of 3.0% to a high value of 6.4%, and was similar to reports in the literature. The incidence of twins could be higher in this study than actually reported because not all cattle were re-examined via ultrasound during the time frame permitting diagnosis of twins.

Twinning in cattle can be classified as monozygous (identical) or dizygous (fraternal). Monozygous twins result infrequently when a zygote cleaves during development, resulting in identical twins, and is reported to be less than 0.3% of all twin births.[42,43] In contrast, dizygous twinning accounts for the majority of twin births in dairy cattle.[14,27,43] Dizygous twins arise from the ovulation and subsequent fertilization of eggs from two separate ovulations. The incidence of double ovulation in dairy cattle is reported to be 14%, and there is strong genetic association of this trait and twinning.[18,19,34,49]

Twinning in cattle is a complex trait that may be influenced by several risk factors including genetics, breed, parity, ovulation rate, milk production and the use of recombinant bovine somatotropin (rbST).[3,4,6,18,27,29,53]

Genetics

It has been reported that the previous incidence of twinning is a risk factor for subsequent twin births.[3] Silva *et al*[43] reported that cows that had twinned once or twice before had an increased chance of delivering twins in subsequent pregnancies. Experimental herds comprising mainly beef cattle with a history of the foundation animals being Holstein cows with a history of twinning have been created for the purpose of research to better understand this complex trait.

Breed

The incidence of twinning is higher in dairy breeds than beef breeds.[53] The Holstein dairy breed has been reported to have the highest incidence of twinning when compared to the six major dairy breeds.[4] The population herd in this case report was made up completely of black and white Holsteins.

Parity

The incidence of double ovulation increases with parity in lactating dairy cows.[19,31] Similar to reports in the literature,[49,53] primiparous cows in this case report had a significant decrease in twinning incidence of 1.8% (17/946) when compared to 7.5% (134/1790) in multiparous cows.

Ovulation Rate

Older lactating cows are more likely to have twin pregnancies because of an increased incidence of double ovulation in multiparous cows versus non-lactating heifers.[41,53] In addition, non-lactating heifers more often abort the twin pregnancy before calving.[50]

Milk Production

Kinsel *et al*[29] reported an increased risk of twinning as milk production increased, and on average cows delivering twins had 5.95 lb (2.7 kg) greater ($P<0.05$) peak milk production. The increase in twinning over a 10-year period was shown to be associated with the increase in peak milk production.[29] The annual increase in milk production seen by the dairy industry over the years is mainly due to genetic selection.[29] The relationship between milk production, ovulation rate and twinning requires further research.

(rbST) Recombinant Bovine Somatotropin[a]

Initially in 1994, the FDA-approved label included a statement that an increase in twinning rates may be observed in cattle treated with rbST.[6] However, the label was modified in 1997 and the statement was removed after subsequent research trials revealed a

lack of evidence to support the positive effect of rbST on twinning rates.[7]

Abortion of Twins

In the equine species, the majority of twin pregnancies are bicornual and abortion of twin pregnancies is common.[20] The high rate of abortions in mares is due to a lack of placental area to properly nourish both foals.[26] Consequently, the growth of one foal is retarded, leading to its death and eventual abortion of both twins. Cattle have a different type of placentation than horses, but the lack of uterine space in unicornual twin pregnancies may contribute to abortions. It is not known whether this is an important factor in cows carrying twins to term, and further research is needed.

A major problem with cows carrying twins is abortion.[17] Cattle typically bear a single fetus. Being forced to carry twins causes excessive multi-system physiological demand, leading to weight loss.[24,32] Cows may respond by aborting the twins, thereby eliminating excess energy drain and returning to homeostasis. Energy requirements for cows carrying twins are 70% higher than for cows carrying a single fetus.[30] Abortions after three months' gestation due to twin pregnancies in cattle have been reported to be as high as 40% versus a 12% incidence of abortion in cows carrying a single calf.[8,15] In the study from Spain[32] where twin pregnancies were only followed up to 90 days post-insemination, a 24% incidence of abortion was reported. In this case report, 13 of the 64 cattle (20.3%) diagnosed with twins by transrectal ultrasonography aborted. This is especially noteworthy information because all of the cattle were confirmed pregnant by ultrasonography after 55 days in gestation, eliminating the contributions of early embryonic death to overall abortion rates.

There is limited information in the literature reporting the outcome of twin pregnancies after diagnosis by transrectal ultrasonography. In this case report, the exact time to abortion was impossible to determine on the 13 cows aborting their twins. For dairy cattle, not only is the abortion itself traumatic, but the time frame for peak incidence of abortion is critical. Abortions that occur late in gestation when milk production is declining rapidly are especially problematic. If a cow aborts late in lactation, it is difficult or impossible to reestablish milk production at an economically viable level. Consequently, cows aborting in late term tend to be removed from the herd without a rebreeding opportunity. More information describing the time frame for abortion in cows carrying twins is needed to help determine the best management practice to handle cows diagnosed with twins. It is possible more frequent palpations *per rectum* at various times throughout gestation after initial diagnosis of twins is needed.

Conclusion

A correct diagnosis of twin pregnancies in dairy cattle requires a patient, skillful ultrasound operator and cooperative animal. This case report demonstrates that an accurate diagnosis of twins in dairy cows can be made at 55 to 83 days post-insemination. The incidence of twinning in the case population herd was 5.3%, and the majority of cattle diagnosed with twins were multiparous. The data demonstrated that the majority of cattle diagnosed with twins at a time frame beyond when early embryonic death contributes to pregnancy loss can successfully carry twins to term, but this condition is not without complication. A total of 13 of 64 (20.3%) cows in our case population diagnosed with twins aborted. This study may help shed light on the importance of accurately diagnosing and understanding the outcome of twin pregnancies in cattle. Further research is warranted to better understand the dynamics surrounding twin pregnancies in cattle.

Endnotes

[a] POSILAC®, Monsanto Company, St. Louis, MO
[b] Lutalyse®, Pfizer Animal Health, Exton, PA
[c] Cystorelin®, Merial, Iselin, NJ
[d] Shimadzu-350 XL, Shimadzu Corporation, Columbia, MD
[e] Dairy Comp 305, Valley Agricultural Software, Tulare, CA

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VETERINARY PERSPECTIVE

SRP® vaccine stopped a Salmonella outbreak in a Vermont dairy herd

Seven to eight years ago, several of Dr. Kent Henderson's clients began experiencing severe outbreaks of salmonellosis caused by *Salmonella* Typhimurium. In herds with the worst cases, clients were losing large numbers of high-producing cows. Henderson's primary prevention and control program, in addition to increased hygiene on the dairy farm, was vaccination with an autogenous bacterin cultured and produced from the bacteria found on the farm. Production and delivery of autogenous bacterins took considerable time and their use often produced mixed results for Henderson's practice.

When AgriLabs introduced the new *Salmonella* Newport Bacterial Extract vaccine (SRP vaccine) in 2004, Henderson and his associates were excited about the opportunities it afforded them and their clients. Henderson said, "We believed that the SRP vaccine's unique mode of action might provide cross protection against multiple serotypes of salmonella. We were hoping this was going to be a tremendous advantage in our salmonella prevention programs. So we discontinued the use of autogenous vaccines and relied solely on the SRP vaccine with most of our clients."

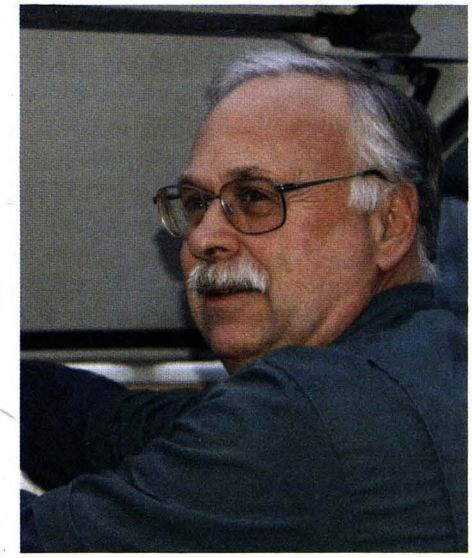
"On a Sunday afternoon in April of 2006, I received a call from one of my clients who had not been vaccinating with SRP. She had five critically ill cows in her herd of 500 milk cows. These fresh-sick animals had high fevers and all five animals were suffering with watery, "lemonade-like" diarrhea, typical in salmonella infections. Plus, the fecal discharge had a unique odor, characteristic of salmonellosis."

In an effort to prevent a salmonellosis outbreak in the entire herd, Dr. Henderson vaccinated 300 of the 500 cows in the herd that afternoon, exhausting the supply of SRP vaccine he had on hand, and completed vaccinating the herd the next day. Henderson said, "I had heard from other veterinarians that, in addition to prevention, SRP vaccine can help treat animals suffering from salmonella infection. So, in addition to fluid and NSAID therapy, I elected to vaccinate the five critically ill, possibly terminal, animals that afternoon. Three of the five showed dramatic signs of improvement within 18 hours of vaccination and all five survived and went back into production. We revaccinated the entire herd with SRP vaccine per label instructions and had only two additional mild cases of salmonella in the herd."

The laboratory at Cornell University identified the cause of infection to be the C_1 strain of *Salmonella* Infantis which is normally considered to be an avian strain of the bacteria. Dr. Henderson is not sure if wild birds were the vector or if the bacteria were brought to the dairy by a farm worker who picked them up at poultry facilities on the premises.

Since the outbreak, the producer has made a concerted effort to clean up the premises. Dr. Henderson feels that the clean up, together with the SRP vaccination program, has prevented further salmonella problems in both the cows and baby calves in this herd. Henderson sums it up this way, "Our client was pleased with our rapid response and the overall outcome our intervention and the SRP vaccination program has had on her dairy."

For more information about this case study or SRP vaccination programs, please call your AgriLabs representative or AgriLabs technical services at 800-542-8916.



Kent Henderson, DVM
Northwest Veterinary Associates
St. Albans, Vermont

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AgriLabs®
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P.O. Box 3103
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