Caroline Livestock Study

1991 – 2007

Cheryl Waldner DVM PhD

HISTORICAL DATA,
ANNUAL SUMMARY 2006 AND 2007
AND
COMPARISON AND FOLLOW UP
TO THE
WESTERN CANADA STUDY OF
ANIMAL HEALTH EFFECTS
ASSOCIATED WITH EXPOSURE
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NATURAL GAS FIELD FACILITIES

THE CAROLINE LIVESTOCK STUDY: HISTORICAL DATA, ANNUAL SUMMARY 2006 AND 2007 AND COMPARISON AND FOLLOW UP TO THE FINDINGS OF THE WESTERN CANADA STUDY OF ANIMAL HEALTH EFFECTS ASSOCIATED WITH EXPOSURE TO EMISSIONS FROM OIL AND NATURAL GAS FIELD FACILITIES

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Abstract:

The objective of the Caroline Livestock Study (CLS) study has been to examine productivity and health information from domestic livestock as one indicator of the potential long-term environmental impact of oil and natural gas developments in a rural area. Detailed biological accounting methods were developed to measure the health and productivity of cow-calf herds in an area with intensive oil and gas production. From 1991 through calving 2007, cow production records from 21,474 recorded bull contacts were examined from nine different area cow-calf herds. The median risks for non-pregnancy, abortion, calving late, stillbirth, and calf mortality for local herds did not differ substantially from other published reports.

One of the limitations of this study has been the relatively small number of herds and limited geographic scope. In 2006, the results of a much larger study known as the Western Canada Study of Animal Health Effects Associated with Exposure to Emissions from Oil and Natural Gas Field Facilities were released. Much of the protocol for this study was modeled after the CLS study. Reproductive performance in the two studies, as measured by the percentage of cows that failed to become pregnant, aborted, had a stillborn calf, or had a calf that died before weaning, was very similar.

The two studies provided important and complementary information on reproductive performance and calf survival – a large number of herds and geographic scope from the WISSA study and an opportunity to examine the role of year-to-year variation in the long-term CLS study. Between herd differences explained a relatively small but significant part of the variability in the data from both studies. While individual herd performance varied substantially between years in the CLS, there was no clear pattern of bad or good years across all study herds for the risk of non-pregnancy and abortion. However, there were significant year-to-year variations in the risk of stillbirth and calf mortality.

A detailed histopathology study was conducted in the CLS herds starting in the spring of 2004 and the results were compared to the results of the WISSA study. The same pathologist examined submissions from both studies. While the frequency of most of the findings were similar across both the CLS and WISSA samples, some types of pathology were potentially more common in calves examined from the CLS herds in 2004 and 2005. Samples collected in 2006 and 2007 were also examined by the same pathologist and the results reported and analyzed as for the WISSA study. The prevalence of most of the lesions of interest were either similar or considerably lower in the CLS samples from 2006 and 2007 than in either the 2004 and 2005 CLS samples or in the WISSA study samples.

With the assistance of the Alberta Beef Producers, additional analysis were done to examine the role of nutrition and calving difficulty in some of the more interesting pathology findings from the WISSA study including muscle and thyroid lesions. The lesions in the muscle tissue were of interest because they were observed in a high proportion of the calves that died during the WISSA study. Both the occurrence of these muscle lesions and the overall risk of calf mortality were associated with increasing exposure to

sulfur dioxide in the WISSA study. The changes in the thyroid gland were of interest because before the WISSA study they had not previously been described and the type of lesion observed could potentially affect calf viability particularly in cold climates. The occurrence of thyroid lesions was also associated with exposure to benzene in the WISSA study. Neither a history of calving difficulty nor the trace mineral status of these calves were associated with the occurrence of tissue lesions in 2006 and 2007; however, calves that were assisted at birth had significantly lower concentrations of the thyroid hormone T3 and higher levels of creatine kinase (CK) activity suggesting muscle damage. Calves with lower concentrations of vitamin E in their serum were more also likely to have higher activity of a serum CK.

No air monitoring was done in the CLS herds in 2006 and 2007. SO_2 concentrations were monitored in the herds that were part of the CLS study in 2005. Passive samplers were analyzed for monthly mean SO_2 concentrations using similar technology and methods to that applied in both the WISSA study and PAMZ air monitoring program. The SO_2 concentrations measured on pastures used by the CLS herds were higher than that from the PAMZ monitoring network in 2005, but were lower than those measured in the high exposure herds from the WISSA study and the PAMZ monitors in 2001-2002. SO_2 concentrations have consistently improved in the PAMZ area since 2000.

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All Caroline Livestock Study (CLS) data in this report collected between 1999 and 2007 were provided by the study participants or were collected by the local veterinary clinic and contracted animal health technician. The majority of CLS data included in this report were collected prior to June 30, 2007. This report is intended to summarize available information to the end of the 2007 calving season.

Data from the Parkland Airshed Management Zone (PAMZ) were obtained from Kevin Warren of Amarok Consulting and from the CASA website.

Finally the data from WISSA or the Western Interprovincial Science Studies Association are derived from a series of public reports released May 18, 2006. Information on how to obtain copies of these reports can be obtained through the WISSA website.

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INTRODUCTION

Since the first major developments of the petroleum deposits in Alberta in the mid-1900's, beef cattle have been pastured adjacent to oil and gas field facilities. During the rapid expansion of petroleum industry across rural areas of western Canada in the 1970's and 1980's questions were raised by some area residents about the potential effects of oil and gas facility emissions on human and animal health. Against a background of increasing industrial activity and associated environmental concerns from the local community, the Caroline gas field, one of the largest natural gas finds in Western Canada in 20 years, was discovered in 1986. The raw gas from the Caroline gas field was sour and contained approximately 35% H_2S.

A process of public consultation and regulatory hearings took place prior to development of the Caroline field. During this process it became apparent that local residents were interested in how additional sour gas production in their area might affect environmental health and, particularly, livestock productivity. Subsequently, a requirement to monitor potential impacts on livestock was appended to the terms and conditions of the ministerial approval from the Department of Environment for the Caroline Gas Project (Department of Environment, 1990). Attempts to develop a study that would meet the needs of both the Department of Environment and area ranchers began in 1991. The regulatory directive requiring the monitoring of livestock health was the first of its kind for the oil and gas industry in Canada. Consequently, there were no precedents or existing regulations to direct the design of this study nor were there any precedents in the literature for a prospective approach to using livestock in this type of environmental monitoring.

Other published examples where livestock had been used in environmental monitoring focused on measuring residues in the tissue or biomarkers in the blood of the exposed animals (Parada, et al., 1987; Gummow, et al., 1991; Halbrook, et al., 1992; Rubes, et al., 1992; Chamberland, et al., 1994). A review of the literature provided very little applicable information on the toxicology of sour gas processing emissions in cattle (Waldner, 1999). There were no expected tissue residues from exposure to sour gas emissions or known biomarkers for the potential toxicological effects of these emissions that could be measured and reliably interpreted in exposed cattle (Beck, 1992; Lodgepole Inquiry Panel, 1984).

In 1991, the documented investigations of the effects of sour gas on livestock were limited to two retrospective responses to accidental releases of sour gas during well blowouts (Lodgepole Inquiry Panel, 1984; Alberta Environmental Centre, 1986). In both examples, some of the livestock owners surrounding the blowouts expressed concerns about effects of the blowouts on the health and productivity of their herds. There were no strong conclusions from either study, however, that would provide a focus for monitoring the impact of the new sour gas plant.

The predominant livestock industries surrounding the new gas plant were commercial and, to a lesser extent, purebred cow–calf ranches. Area ranchers and interested government veterinarians and researchers were asked in the early part of 1991 to assist in defining the scope and objectives of a study that would meet the objectives of the Department of Environment licensing directive. The first task put before this group was to develop accurate baseline information on the health and productivity of area herds as well as some of the risk factors outside the petroleum industry that can affect cattle health.

Construction of the Caroline Gas Project and field facilities began in October, 1990. Operation of the Caroline gas project began in March 1993. After the requirement for livestock monitoring specific to the Caroline gas project had been completed (Waldner, 1997), the study was adopted by the Sundre Petroleum Operators Group and a committee of local livestock producers and has continued collecting data through 2007 with the support of various agencies. Past and current partners in this project include: Alberta Energy & Utilities Board (EUB), Alberta Environment, Canadian Association of Petroleum Producers (CAPP), Environmental Research Advisory Council (ERAC), Alberta Agriculture and Food (formerly Alberta Agriculture, Food, and Rural Development), the Alberta Agriculture Research Institute, the Western College of Veterinary Medicine, and the Alberta Beef Producers (formerly the Alberta Cattle Commission).

After the start of the CLS monitoring project, two studies were completed that attempted to investigate potential links between oil and gas industry emissions and adverse effects on livestock health (Scott et al., 2003a; Scott et. al. 2003b; Scott et al. 2003c; Waldner, 2001; Waldner et al., 2001a), but these studies left many important local concerns unanswered. Given both the remaining questions regarding the potential effects of emissions on animal health and the ongoing interest of area residents, the study has continued to monitor the health and productivity of area herds in the general vicinity of Sundre and Caroline, AB. The objective of this ongoing monitoring initiative was to identify substantial variation or consistent trends in the performance of this group of herds that might be associated with changing emissions patterns or new industrial development in the area. Emphasis was placed on accurate measurement of the indices of health and productivity that most directly affect the profitability of the beef cow-calf herd. The first objective of this 2007 report to the Caroline Livestock Committee is to update the last annual summary of long-term trends to include data collected through the end of calving season 2007.

The Caroline Livestock Study (CLS) was extended beyond the original term because of the interest in maintaining the study as a surveillance program using beef herds as sentinel "markers" of local environmental health. The data from these herds have been and could be used again as real-time benchmarks for situations where other herds experience some sort of unusual exposure event (Waldner et al., 1998). However, one of the criticisms of this ongoing monitoring project and subsequent studies using this data to examine the potential effects of emissions on cattle health was the limited number of participating herds and the limited geographic scope of the project. In 2006, the results of a much larger study were released which addressed many of these limitations. The larger study by the Western Interprovincial Scientific Studies Association modeled much of its approach after the work done on various aspects of the CLS project. This larger study examined cows from 205 herds across Alberta, Saskatchewan, and north-eastern British Columbia. However, the data from this study were collected for only one cow-calf production cycle. For completeness, this report includes an update of the previously published comparison of both the animal health data and the air monitoring data collected as part of the CLS study to the data collected as part of the WISSA study. The animal health data from the CLS herds provides important information to complement the data collected in the WISSA study by addressing questions about the potential effects of year-to-year variation on herd performance.

In addition to updating the CLS baseline data with the information collected during 2006 and calving season 2007, the second objective of this report was to use data collected from the CLS postmortem examinations during 2006 and 2007 to address some of the initial questions arising from the WISSA study relating to the occurrence of muscle and thyroid lesions in these calves. The specific objectives of this additional work were as follows: 1) Describe the gross post mortem and histopathologic lesions associated with calf mortality with emphasis on lesions of skeletal muscle, heart and thyroid gland. 2) Describe the micronutrient profile from fresh livers collected from stillborn calves and neonates. 3) Determine the association between calf serum thyroid hormones T3 & T4, muscle enzyme creatine kinase (CK), vitamin E, selenium and trace minerals and dystocia or prolonged parturition.

MATERIALS AND METHODS

Air monitoring data

A one-year limited air monitoring program was initiated in 2005 and was based on the approach to exposure monitoring designed for the WISSA study. The funding for this part of the monitoring project was provided by a grant from the Alberta government. Passive sampling technology was selected because of its reliability, simplicity, low cost, and ability to measure low concentrations of emissions by integrating exposure over a 1-month period. By simple diffusion, the removable samplers entrapped compounds on collection media which was later analyzed in the laboratory. The sampling program was managed by Kevin Warren (Amarok Consulting).

Each monitoring station consisted of a PVC rain shelter (200 mm or 8 inches in diameter) (Maxxam Analytics, Inc., Edmonton, AB) in which the sampler or samplers (50 mm or 2 inches in diameter) were attached to the underside, allowing undisturbed movement of air across the diffusion membrane. The entire shelter unit was placed 1.5 to 1.8 m (5 to 6 feet) high, on a steel pipe attached to a fence post. The samplers were deployed for one month, then collected and replaced.

The SO_2 sampling medium was a filter impregnated with sodium carbonate/sodium bicarbonate (Maxxam Analytics, Inc., Edmonton, AB). The sulphate ion was extracted from the media with a solution of hydrogen peroxide in ultra-pure distilled/deionized water. Ion chromatography, following US EPA method 300.1, was used to determine the sulphate ion concentrations. The sampling rate, controlled by the diffusion barrier, was calculated using an empirical relationship specific to SO_2 that considers the average temperature, wind speed and relative humidity associated with the monitoring period. The reported detection limit of 0.1 ppb (0.26 $\mu g/m^3$) is based on a 1-month exposure period. Tang et al. (1997) and Sembulak and Kindzierski (1999) provide additional information on this method.

The monitoring network was configured with 10% rotating duplicates, 10% field blanks, and the monitors were located where the cattle were pastured each month. Therefore the number of samplers deployed varied with the time of year and number of management or pasture groupings for each herd each month. There were more monitors in use during the summer when herds were dispersed on pasture than in the winter months when herds were confined to facilitate feeding and calving management.

Participant selection and loss to follow up.

The study area was originally delineated primarily by projected sulfur deposition zones as presented in the Environmental Impact Assessment (EIA), around the Caroline gas plant. The study was located in southwest central Alberta, in an area encompassing approximately eight townships around the Caroline and Sundre area.

Community meetings were held in the summer of 1991 and advertisements were placed in local newspapers notifying area farmers of the proposed study and of the need for participants. Local herd owners were then selected based on location, interest, size, availability of facilities and past records. Eight cow–calf operations were selected to participate in the study. The eight cow–calf herds included approximately 1300 cows and bred heifers. These beef herds varied from 30 to 250 head and included both commercial and purebred herds. Where suitable data were available, past records for these herds were analyzed back to breeding season 1987 in order to increase the pre-operation database for the project.

The owners of the smallest herd selected for the project sold most of their cattle in the early fall of 1993 and, therefore, the herd data were not included in this analysis. One more herd owner sold his operation in the fall of 1996. One new herd was added in the spring of 2003, a second herd was added in 2005 and one herd changed to a fall calving program and withdrew from the study in the summer of 2005. The total number of herds reported in any year, therefore, varies from six to eight.

Herd productivity and health data

A record system was devised to obtain accurate information for both participating herds and individual cows within these herds. To maintain producer compliance, the record system was customized when required to suit individual operations. Attention to individual animal identification was essential to maintain an accurate inventory. To this end, metal ear tags indicating the animal's year of entry into the herd and a three-digit number were provided during the first years of the study to those herd owners who did not tattoo their animals as insurance against plastic tag loss.

Participating producers were encouraged to use their regular herd veterinarian for post-mortem examinations, bull evaluations, and pregnancy examinations to minimize potential for perception of investigator bias. Cooperating producers were paid a small amount for each cow that was pregnancy checked and for each calving record to compensate them for the time involved with data collection and record keeping for the project.

The principal investigator began on-farm data collection just before pregnancy testing in the fall of 1991 and continued through the calving season of 1999. From the fall of 1999 through 2007 on-farm data were collected by the local veterinary clinic and a contracted animal health technician.

The pregnancy status of individual cows was determined by the herd veterinarian in the fall of each year. Risk of non-pregnancy (%) was determined as the number of females diagnosed non-pregnant divided by the number of females pregnancy tested in the fall of the year (x 100). However, this should not be interpreted as a true measure of the conception risk. Pregnancy checking was usually done between September and November when the cows were between two and six months of gestation. Thus, fetal loss could have occurred between conception and the time of pregnancy checking. Earlier pregnancy checking was not practical because of the difficulty in accessing cattle during the summer pasture period.

Background information was collected for each herd on factors known to affect non-pregnancy risk prior to 1999. These factors included breeding soundness examination results for all herd bulls, record of dates and duration of bull exposure, bull to cow ratios, herd breed composition, herd age distribution, source of replacement stock, feed and water analysis, pasture condition and stocking densities, body condition scoring, heifer pre-breeding weights, blood and liver micronutrient analysis, some infectious disease screening, and a record of vaccinations used. No systematic biological or feed sampling were done after 1996 except for post-mortem examinations. No replacement heifer or weaning weights were collected after 1998. Pasture evaluations were discontinued in 2003.

Body condition scores (BCS) were regularly recorded during the study as condition scoring is a practical and reliable indicator of the risk associated with insufficient pre-calving and pre-breeding nutrition and resulting reduction in conception success (Wikse, 1988). Wherever possible, cows were condition scored before calving, before breeding, and again at pregnancy testing.

Condition scoring also provided an opportunity to verify and update herd inventory status. Visual inspection was utilized in place of palpation when the cows were not put through the chute for other routine processing. Visual appraisal of body condition score is as accurate as palpation of fat cover, except when cattle have long hair (Wikse, 1988). Body condition was scored from one, which was very thin, to five, which was very fat. The system uses the following descriptions to define each score (as excerpted from Alberta Agriculture, 1989 and modified from Wikse (1988)). This scale was designed to be increased in one-half increments producing a nine-category scoring system.

Score 1:

- individual short ribs fairly sharp to the touch and no fat around the tail head
- hip bones, tail head and ribs visually prominent

Score 2:

- short ribs identified individually when touched but felt rounded rather than sharp
- some tissue cover around the tail head and over the hip bones and the flank
- individual ribs no longer obvious

Score 3:

- short ribs only felt with firm pressure
- areas on either side of the tail head had a degree of fat cover, which could be easily felt

Score 4:

- fat cover around the tail head evident as slight "rounds" that were soft to the touch
- short ribs not felt even with firm pressure, and folds of fat beginning to develop over the ribs and thighs of the animal

Score 5:

- bone structure no longer noticeable and animal had a "blocky" appearance
- tail head and hip bones almost completely buried in fat and folds of fat apparent over the ribs and thighs
- short ribs completely covered by fat and the animal's mobility impaired by the large amounts of fat

An abortion was defined as an observed premature calving judged to be at least one month prior to full term, or a cow that was found to be pregnant by a veterinarian but subsequently failed to calve. The abortion risk was defined as the number of females aborting expressed as a percentage of the number of females exposed to breeding (McDermott et al., 1991a). Herd owners were asked to record the cow identification and date for every animal known to have or suspected to have aborted. The producer was asked to make every effort to locate the fetus and placenta for laboratory testing.

The date, identification, breed, and source of any introductions to the herd were to be recorded. All accessible cows were body condition scored before calving. Detailed individual calving records included the breed and age of each cow. Cow age was verified against the herd inventory records. Detailed information on cow age was available at the start of the study for all cows in four herds, for most of the cows in one herd, and the younger cows in the two remaining herds. In 2007 age was available for almost all breeding cows participating in the study except for some cows from the most recent herd to join the study and some recently purchased cows in other herds. Individual animal calving records were available for all project herds starting from 1989. Complete calving records were obtained for six of the seven initial project herds for 1988.

The herd owners were asked to record their diagnoses and treatments for all sick animals. The treatment record form requested information on the following: the date, animal identification, disease suspected, treatment used (i.e. the product, dose, frequency, and duration of use), response to treatment, and details of any veterinary consultation. Any herd level problem was to be reported as soon as possible to the project coordinator to permit necessary investigation.

The herd owner was also asked to record the identification and date for any animal that died and to have the animal examined following a suggested protocol. Whenever possible, post-mortem examinations were to be completed on farm by the herd owner's veterinarian. A written report of the complete post-mortem examination was required and was to include date of death, animal identification; estimated or reported age, sex, weight; and a record of the samples submitted for laboratory verification.

Laboratory submissions were made at the discretion of the examining veterinarian through to 2004 when an additional sample submission protocol was introduced. However, laboratory submissions were strongly encouraged for all post-mortem examinations from the start of the study. The original suggested sampling protocol included trachea, lung, liver, kidney, spleen, heart, lymph nodes, skeletal muscle, small intestine and spiral colon (fixed); lung, liver, kidney, rumen content (fresh), plus any tissues considered appropriate based on gross examination findings. The brain was to be removed and submitted when nervous signs were reported. Laboratory submissions were made to the laboratory of the veterinarian's choice.

In 2004, the post mortem protocol was updated and a list of tissues was requested from each case examined (Table 1).

Table 1. Tissues requested for microscopic examination.

Tissues requested:	
Brain	Placenta
Diaphragm	Parotid salivary gland
Duodenum	Sciatic nerve
Eyelid	Skeletal muscle
Heart	Spinal cord
Ileum (Peyer's patches)	Spiral colon
Jejunum	Spleen
Kidney	Thymus
Liver	Thyroid
Lung	Tongue
Lymph node	Trachea

All tissues were submitted in formalin to Prairie Diagnostic Laboratory in Saskatoon. One section was trimmed from each piece of submitted tissue (with the exception of the brain) and multiple tissue samples were placed on each slide. The tissues were processed routinely; slides were stained with hematoxylin and eosin and submitted to the pathologist for evaluation. Systems of interest were identified based on previous reports in the literature, producer concerns, and the results of the WISSA study. These systems were classified as either having or not having lesions in each case and the results from the CLS samples were compared to the data collected in the WISSA project.

Stillbirth risk was defined as the number of calves dead from birth to 24 hours after birth as a proportion of the number of calves born during the period that appeared to be within one month of full-term gestation (McDermott et al., 1991b). Calf mortality risk was the number of calves dead from 24 hours of age to weaning as a percentage of the number of calves alive at 24 hours of age (McDermott et al., 1991b).

Calving dates were utilized in addition to recorded times of bull exposure to generate a calving distribution pattern for each herd. The calving distribution pattern was the frequency of calvings that occurred during each successive 21-day period of the calving season. The percentage of the herd calved in the first 63 days of the calving season is presented here as a measure of the proportion of cows calving within the ideal calving season as compared to the proportion of late calving cows each year.

Micronutrient analysis for liver samples collected during postmortem examination

In 2006 and 2007, fresh liver samples were to be collected during postmortem exam from all calves that died at or within the first 3 days of birth. The liver samples were frozen at -20C and shipped to Saskatoon where they were frozen at -70C until laboratory submission. Prairie Diagnostics Services Toxicology Laboratory, Saskatoon, Saskatchewan, performed analysis of vitamin E and trace minerals.

High-pressure liquid chromatography (HPLC) methods were applied to determine the tocopherol (Catignani and Bieri, 1983) concentration in liver for the assessment of vitamins E. Briefly, 0.3 – 0.6 g. tissue was homogenized with KC1 (0.146 mol/L) made up to a volume 25 ml, 1.5 ml homogenate was added to a test tube.along with 0.5 ml. of 0.01 mol/L ascorbic acid; 1 ml. of ethanol and the internal standard were added and the tube was mixed on a vortex mixer for 45 seconds, covered with tin foil and then put in a 70^{o}C oven for 5 min. One mL of 10 mol/L KOH was then added, the tube mixed with a

vortex mixer and again covered with tinfoil and placed in the 70°C oven for 30 minutes. The tubes are allowed to cool at 4ºC, 4 ml of petroleum ether, was then added. The mixture was vortexed for another 45 seconds and centrifuged using an International Refrigerated Centrifuge PR - 6 (International Equipment Co., Needham, MA) at 550 g (ca. 2500 rpm) for 5 minutes. About ¾ of the ether phase was transferred to a clean 12 x 75 mm glass tube and evaporated to dryness under air. The residue was dissolved into 500 µl filtered HPLC grade methanol and vitamin was detected using a 5 µm UltrasphereTM ODS (4.6 mm x 15 cm) HPLC column (Beckman Coulter Canada Inc., Mississauga, ON) attached to a fluorescent detector and set for a 285 nm wavelength for vitamins E. Samples and standards were protected from light at all times.

The trace mineral concentrations were measured following an acid digestion technique. Trace minerals were determined immediately following acid digestion by analysing samples using a Trace Scan, Atomic Scan 16/25 Inductively-Coupled Plasma (ICP) spectrometer (Thermo Jarrell Ash Corporation, Franklin, MA).

Selenium concentration was determined from liver in a similar manner to that described for trace minerals. Selenium concentration was determined immediately following acid digestion by analysing samples using an ICP spectrometer coupled with a hydride generator device to assist formation of volatile selenium hydrides. Selenium analysis includes the use of a hydride generator to improve instrument sensitivity.

Case-control study of micrantrient and thyroid factors associated with difficult calving

A case-control study was set up to examine the association between dystocia and the thyroid hormones - T3 and T4, creatine kinase (CK – an indicator of muscle damage), vitamin E and selenium levels in serum. In 2006 and 2007, herd owners were asked to report any calvings that required assistance. Blood samples were then collected from the assisted calf and at least one more calf from the same herd that was not assisted within 48 hours of birth. The non-assisted calf selected for comparison was the first accessible calf of similar age to the assisted calf.

Serum micronutrient analysis

Prairie Diagnostics Services Toxicology Laboratory, Saskatoon, Saskatchewan, performed analysis of vitamins A and E and trace minerals.

Retinol (Milne and Botmen, 1986) and tocopherol (Catignani and Bieri, 1983) concentrations were determined by high pressure liquid chromatography (HPLC), in serum as measures of vitamins A and E, respectively. Although extraction and analysis of individual vitamins was conducted separately, the procedure was identical. Briefly, 1 ml of serum was added to a 15 ml glass stoppered centrifuge tube along with 1 ml of a 1% solution of bovine serum albumin (1%-BSA), 1.6 ml of ethanol, and 0.4 ml of internal standard. The sample was mixed using a vortex for 10 seconds, and a 4 ml aliquot of petroleum ether was added. The mixture was vortexed for another 45 seconds and centrifuged (International Refrigerated Centrifuge PR – 6, International Equipment Co., Needham, MA) at 550 x g (ca. 2500 rpm) for 5 minutes. About ¾ of the ether phase was transferred to a clean 12 x 75 mm glass tube and evaporated to dryness under air. The residue was dissolved in 500 µl filtered HPLC grade methanol, and vitamin was detected by HPLC using a 5 µm UltrasphereTM ODS (4.6 mm x 15 cm) column (Beckman Coulter Canada Inc., Mississauga, ON) and a fluorescent detector at 325 or 285 nm for vitamins A and E, respectively. Samples and standards were protected from light at all times.

Trace minerals, with the exception of Se, were measured in plasma samples, following acid digestion. Plasma samples were digested in a Microwave Accelerated Reaction System

(MARS-5, CEM Corporation, Matthews, NC) by adding 2.5 ml concentrated nitric acid and 2.5 ml of double distilled (DD) water to 1.0 ml of sample. Using the appropriate MARS-5 digestion vessels, the samples were placed into the microwave for a total of 20 minutes at 120˚C and 120 PSI. Digested samples were transferred to a 25 ml volumetric flask. MARS containers were rinsed with DD water and the rinsate was added to the flask. The flasks were brought to volume with DD water, covered and mixed. Trace minerals were determined immediately following acid digestion by analysing samples using a Trace Scan, Atomic Scan 16/25 Inductively-Coupled Plasma (ICP) spectrometer (Thermo Jarrell Ash Corporation, Franklin, MA).

Selenium concentration was determined in a similar manner to that described for other trace minerals. Selenium concentration was determined by analyzing samples using an ICP spectrometer with a hydride generator device to assist formation of volatile selenium hydrides.

Serum creatine kinase (CK) analysis

Serum creatine kinase (CK) activity was measured by the clinical chemistry laboratory at Prairie Diagnostic Services using a Hitachi 911 automated clinical chemistry analyzer. The reagents for the in vitro assay were supplied by Roche.

Thyroid hormone assay

Serum for thyroid hormone determination was collected in serum vacutainer tubes, separated and frozen at -70C until analysis. Thyroid hormone assays were performed by Prairie Diagnostic Services (Saskatoon SK) using commercially available chemiluminescent assay kits. These kits measured total circulating triiodothyronine (T3) and thyroxine (T4) respectively. The kits used were Total T3 (Siemens, Catalog Number: LKT31) for *in vitro* diagnostic use with the Immulite Analyzer and Total T4 (Siemens Catalog Number: LKT41) for *in vitro* diagnostic use with the Immulite Analyzer.

Run to run precision (CV) for the T3 kit ranges from 7.1% to 15.6% depending on the T3 concentration. Cross-reactions for the T3 kit with Reverse T3 are estimated to be 0.07%, L-Thyroxine (T4) - 0.24%, D-Thyroxine - 1.0%, and Triido-D-thyronine - 100%. The calibration range for the T3 assay is 0.61 - 9.2 nmol/L. For samples in excess of the calibration range, samples are diluted 1:3 with a diluent supplied by Siemens and re assayed. Analytical sensitivity is 0.54 nmol/L (Total T3, product monograph, Siemens Medical Solutions Diagnostics).

Intrassay precision (CV) for the T4 kit ranges from 6.7% to 9.8% depending on the T4 concentration. Cross reaction for the T4 kit with D-Thyroxine were 70%, Tetraiodothyroacetic Acid - 70%, Triiodo-L-Thyronine - 1.6%, and Triiodo-D-Thyronine - not detected. The calibration range for the T4 assay is 13-309 nmol/L. Analytic sensitivity is 5 nmol/L (Total T4, product monograph, Siemens Medical Solutions Diagnostics).

Data management and analysis

Individual herd records were maintained on two commercial software packages (CowChip$, ver. 2.0, AAFRD, Edmonton, Alberta and Epi Info ver. 6, Centers for Disease Control and Prevention, U.S.A). In later years data were transferred to a spreadsheet program (Microsoft Excel, Microsoft Corporation) for summary and reporting purposes and then to a custom built database (Microsoft Access, Microsoft Corporation). The patterns of the productivity outcomes within and among herds across the study period were summarized graphically. This report summarizes and adds the data from 2006 and 2007 to existing reports. The principal investigator was not involved in data collection during this period.

The importance of herd and year effects on the occurrence of non-pregnancy, abortion, stillbirth, and calf mortality, all categorical variables with 2 levels, were examined using mixed models with a binomial distribution and logit link function. The calculations were performed using penalized quasi-

likelihood estimates (2nd order PQL) (MLwiN version 2.02, Centre for Multilevel Modeling, Institute of Education, London, UK). A null model was created for each outcome variable before examination of any exposures or potential risk factors. Within herd clustering was accounted for as a random intercept in all models. Data from the null models were used to estimate the intraclass (i.e., intraherd) correlation coefficient ($\rho=\sigma^2_h/(\sigma^2_h+\pi^2/3)$) to measure clustering of each outcome within herds (Dohoo et al., 2003). Year was added to the models from the CLS data as a fixed categorical variable and the statistical significance of the fixed-effect for year and its impact on the ICC for herd were assessed.

Data from the Parkland Airshed Management Zone (PAMZ) were obtained from the CASA website and from Kevin Warren of Amarok Consulting. Data from the Western Canada Study of Animal Health Effects Associated with Exposure to Emissions from Oil and Natural Gas Field Facilities were obtained from reports described on the WISSA website.

All data from the micronutrient, enzyme, and hormone analysis were managed and summarized using commercial spreadsheet and database programs (Microsoft Excel, Microsoft Corporation, and Microsoft Access, Microsoft Corporation). Associations between the presence of specific pathologic lesions, measures of calving difficulty, micronutrient and thyroid hormone concentrations were examined using non-parametric methods including the Wilcoxon Rank sum test and Fischer's exact test because of the relatively small sample size and non-normal distribution of the data (Statistix for Windows 8, Analytical Software, Tallahassee FL).

RESULTS

Beef herd health and productivity data

Data are reported from 7 herds from 1991 through 1996 and from 6 herds from 1997 through 2002. A seventh herd was added starting with the breeding season in 2003; some historical data were included for this herd. An eighth herd was added in the spring of 2005 and one herd changed to a fall calving program and withdrew from the study in the summer of 2005. In the fall of 2006, there were 1623 beef cows and heifers pregnancy tested with complete records from 7 herds. Two of the herds sell purebred replacement stock; the other herds are primarily commercial cow-calf operations with a couple of herds feeding their calves for varying lengths of time over the winter depending on market conditions. Of the cows pregnancy tested in the fall of 2006, 15.2% were bred heifers, 15.6% were first calf heifers (~2 ½ years old), 6.9% were cows with their second calf (~3 ½ years old), 53.2% were mature cows, and 9.1% were cows greater than 10 years of age. In the fall of 2006, these herds consisted of a combination of purebred and crossbred animals with the following most apparent genetic influences: 41% British, 39% continental, and 20% crossbred and other.

The total numbers of females exposed to breeding for the nine different herds included in this study from 1991 through 2007 have ranged from 1098 to 1703 (Figure 1). In 2006, 1703 cows were exposed to breeding. Data are available on herd management for 7 herds prior to the breeding season in 2006. Three of the 7 herds used a modified-live BVDV/IBR vaccine and the remaining herds did not vaccinate prebreeding in 2006, and in 2007 only 2 herds used a modified-live BVDV/IBR vaccine. Seventy-eight bulls were evaluated for breeding soundness in the spring of 2006 and 5 failed. Sixty-seven bulls were put out with the cows in 2006 for an average length of 134 days (s, 33). In 2007, 67 bulls were tested and 5 failed.

Individual herd data were summarized for 1987 through 2007 (Figure 2-6). For the 2005-2006 cycle, median herd risks for reproductive failure were as follows for the 7 herds with complete data: non-pregnancy risk (4.6%), abortion risk (1.3%), risk of calving late (9.7%), risk of stillbirth (4.1%), and the risk of calf mortality (6.2%). For the 2006-2007 cycle, median herd risks for reproductive failure were as follows for the 7 herds with complete data: non-pregnancy risk (5.8%), abortion risk (1.2%), risk of calving late (9.7%), risk of stillbirth (4.7%), and the risk of calf mortality to summer pasture turnout (7.0%). The median risk has remained reasonably consistent across the study period, but the some herd risks of non-pregnancy, calving late, abortion, stillbirth, and calf death loss did fluctuate outside established targets of performance for individual years. In a model accounting for herd effects, calendar year was not a significant predictor of the risk of non-pregnancy (P=0.18) or abortion (P=0.11), but was a significant predictor of stillbirth (P=0.01) and calf death before weaning (P=0.001) suggesting there was no clear pattern of good or bad years across all herds for pregnancy and abortion, but that there were more important year effects for stillbirth and calf mortality.

In the period 1992 to June 2007 there were 371 pregnancies reported to have terminated in abortion. Post-mortem examination reports were available for 45 abortions or 12% of these cases. Most of these animals were not examined post-abortion because the herd owner was unable to find the fetus or placenta, or because the cow was not easily accessible on winter pasture. For the abortions where a laboratory examination was reported, the diagnostic results were as follows: no diagnosis/non-infectious, 40.0%; bacterial abortion (including placentitis, pneumonia), 26.7 %; thyroid abnormality, 6.7%; congenital defects, 4.4%; neosporosis, 4.4%; mycotic abortion, 4.4%; and other, 13.3%.

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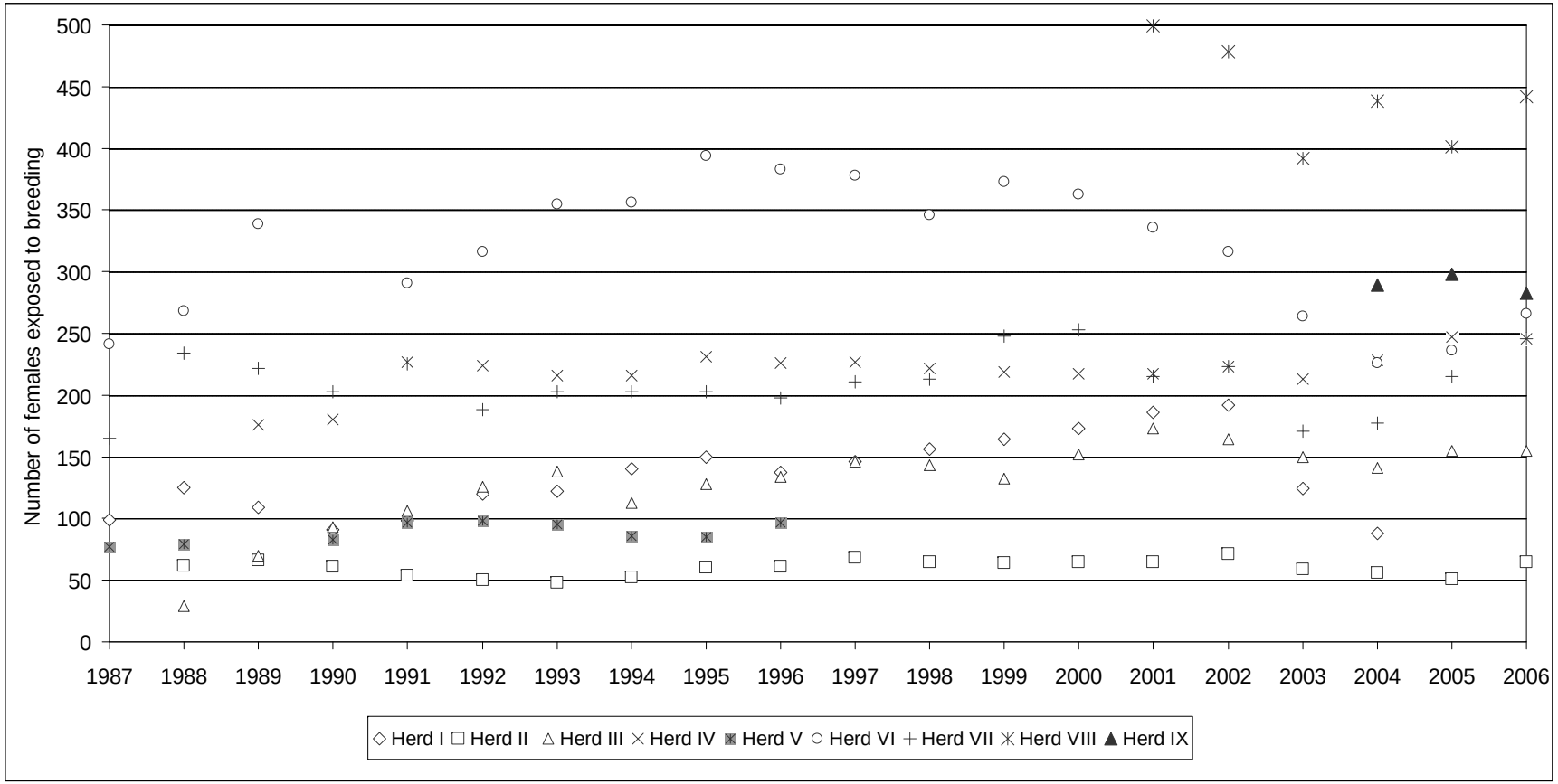

Figure 1. Number of replacement heifers and mature cows exposed to breeding for each of the nine study herds for the period 1987 through 2006. On farm data collection began in the fall of 1991. For the period 1991 through 2006, the total numbers of females exposed to breeding were 1098, 1122, 1177, 1166, 1251, 1236, 1176, 1145, 1200, 1223, 1691, 1667, 1373, 1643, 1603, and 1703.

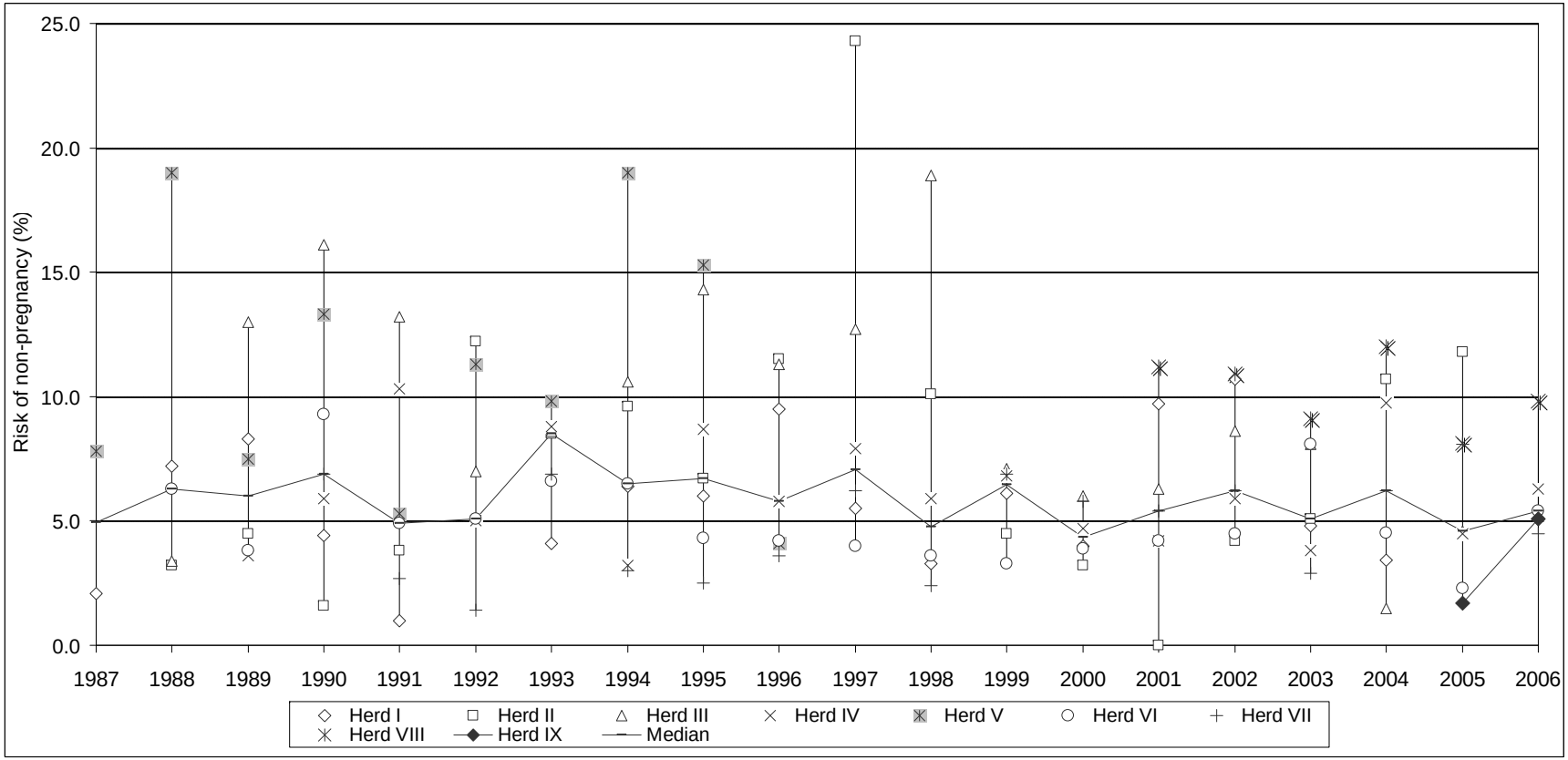

Figure 2. Individual herd non-pregnancy risks for the nine study herds for the period 1987 through 2006. The risk of non-pregnancy (%) was defined as the number of cows that were not pregnant at fall examination divided by the number pregnancy tested X 100. The overall median for the period 1991 through 2006 was 6.0% (IQR, 4.2% to 8.9%).

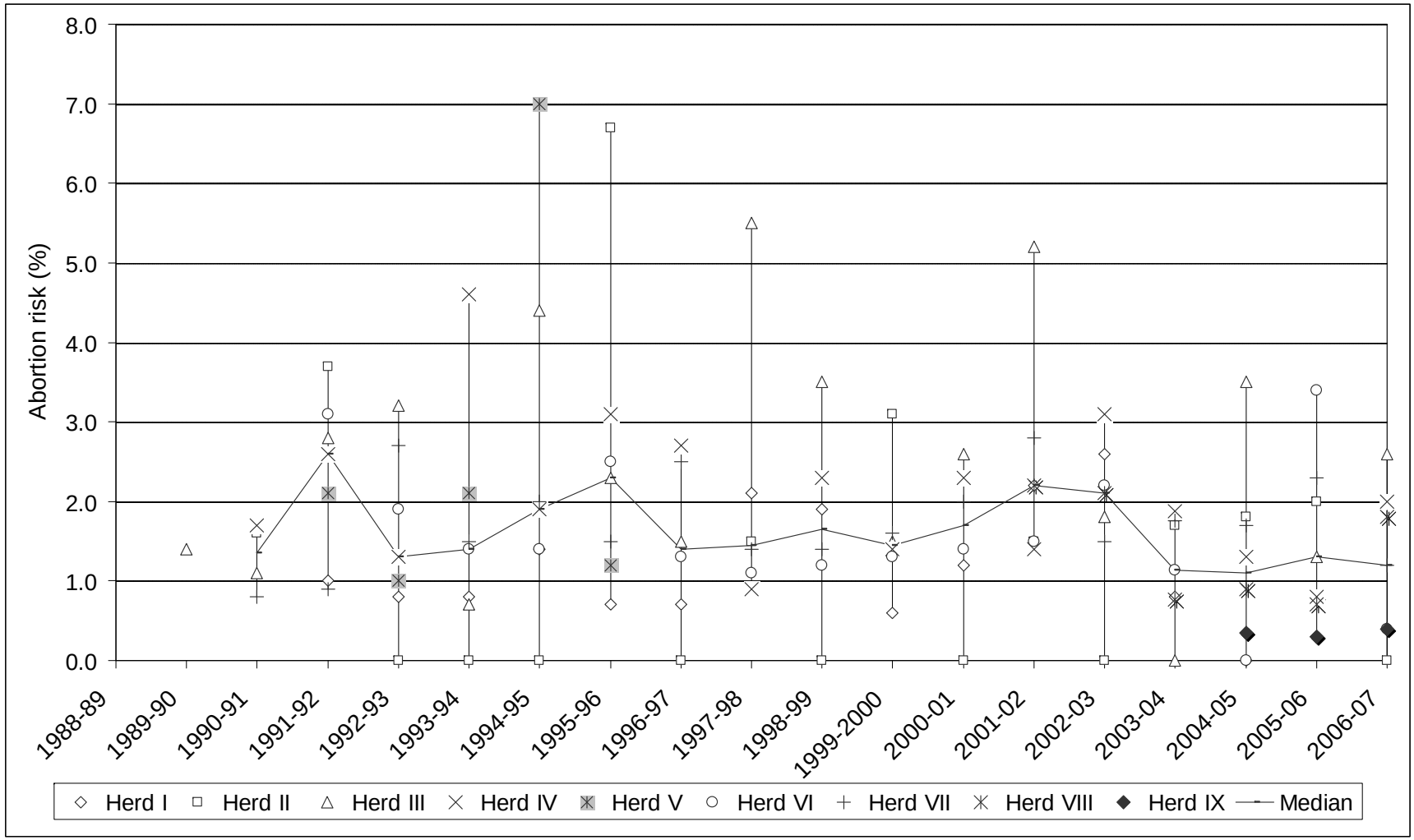

Figure 3. Individual herd abortion risks for the nine study herds for the breeding-to-calving season 1987-88 through 2006-07. The abortion risk (%) was defined as the number of cows observed aborting plus the number of cows pregnant at fall examination that later failed to calve divided by the number exposed to breeding x 100. The overall median for the period 1991 through calving 2007 was 1.5% (IQR, 0.9% to 2.3%).

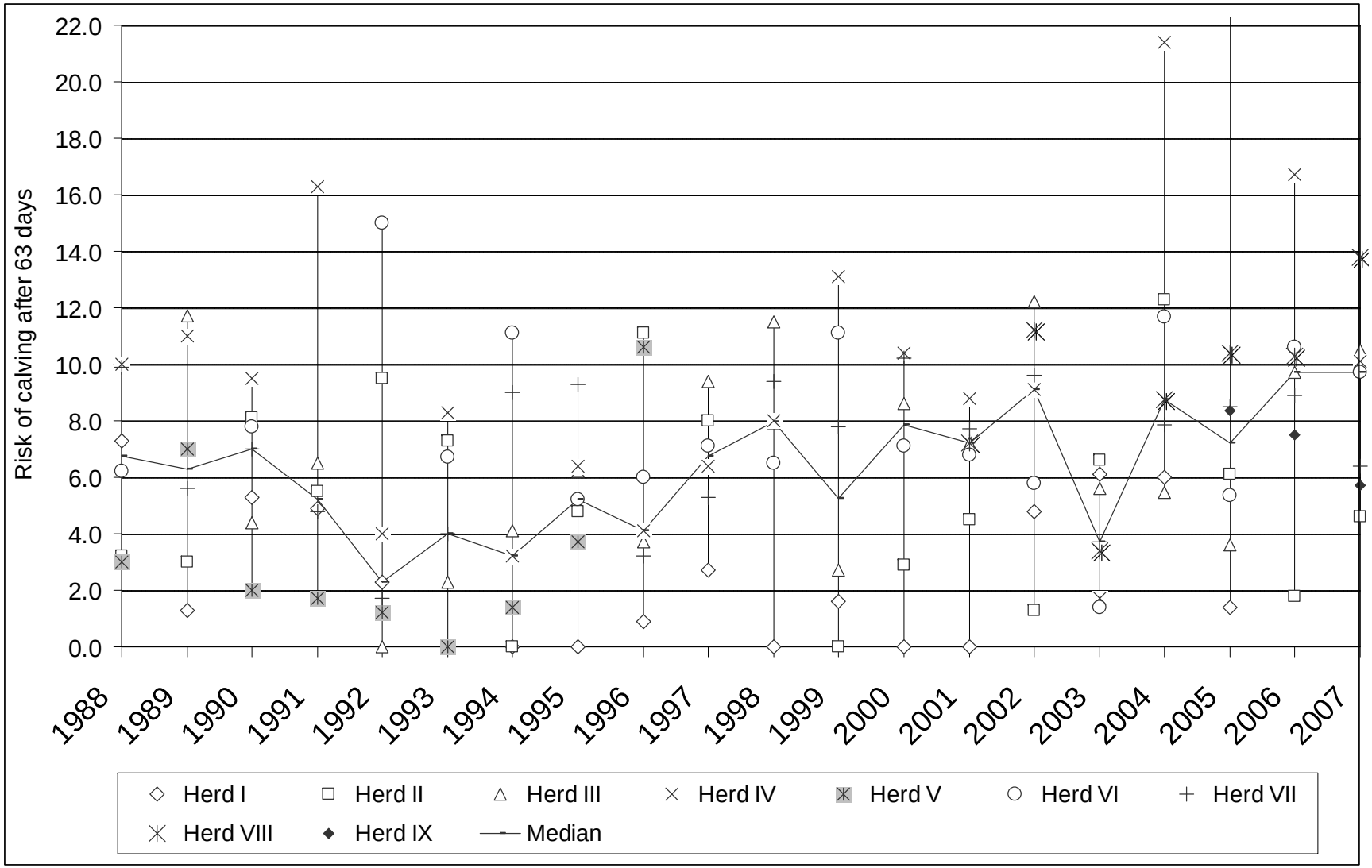

Figure 4. Individual herd risks of calving late, or the percentage of the herd calving greater than 63 days after the start of calving season, for the nine study herds for calving season 1988 through calving season 2007. The overall median for the period 1991 through calving 2007 was 6.4% (IQR, 3.5% to 9.4%).

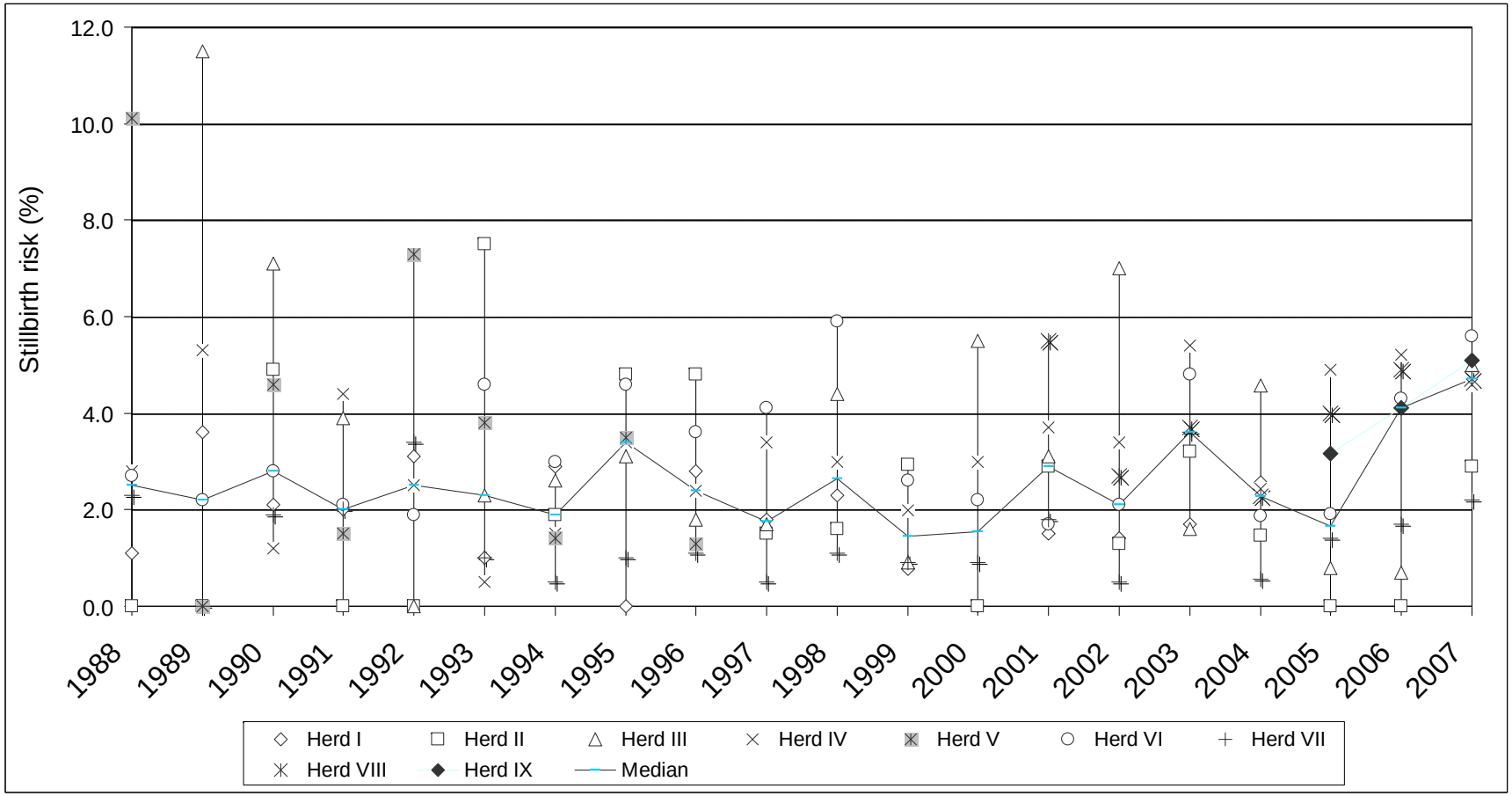

Figure 5. Individual herd stillbirth risks for the nine study herds for the period 1988 through 2007. The stillbirth risk was defined as the number of calves born after greater than 270-days of gestation that died before 24 hours after birth as a percentage of the total number of calves born dead or alive. The overall median for the period 1991 through calving 2007 was 2.4% (IQR, 1.4% to 3.7%).

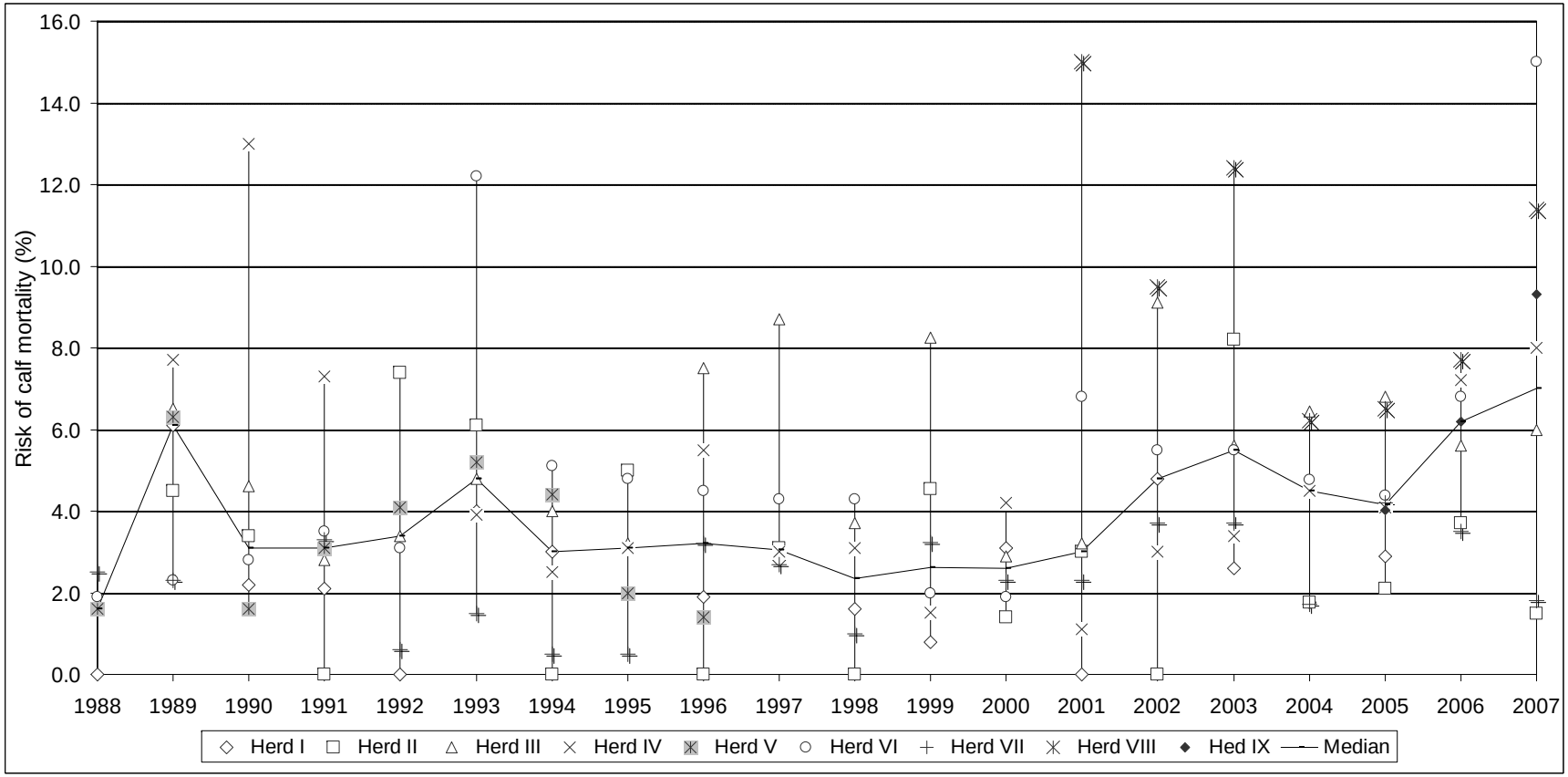

Figure 6. Individual herd calf mortality risks for the nine study herds for the period 1988 through 2007. The calf mortality risk was defined as the number of calves that died between 24 hours of age and weaning as a percentage of the number of calves alive at 24 hours after birth. The overall median for the period 1991 through calving 2007 was 3.6% (IQR, 2.1% to 5.5%). The 2007 data only include calf losses to the start of summer pasture.

In the period 1992 to June 2007, 528 calves died at or near full term and within the first 24 hours of life and were classified as stillbirths. Complete post-mortem reports were available for 359 or 68% of these cases. Some of these calves were not examined post-mortem, because the herd owner had attributed the cause of death to calving difficulty or an accident and had chosen not to have it examined. For the calves that died within the first 24 hours of birth and that were examined post-mortem to the end of June 2007, the proportional mortality risks were as follows: calving difficulty or hypoxia, 55%; unknown or no diagnosis, 19%; accident or trauma, 8%; congenital defect, 7%; exposure or hypothermia, 6%; bacterial infection, 2%; and other, 4%.

In the period 1992 to 2007, there were 802 calves that died between 24 hours and fall weighing. Complete post-mortem examination reports were available for 492 or 61% of these calves. Producer cooperation was better in ensuring all calves in this age group were examined post-mortem when the calf died during calving season and prior to summer pasture. The calves that were not examined in many cases were lost on summer pasture or scavenged by predators before they could be examined. For the calves that died after the first 24 hours of birth (and before fall weighing) that were then examined post-mortem, the proportional mortality risks were as follows: scours, 21%; accident or trauma, 15%; abomasal ulcer, 11%; unknown, 9%; septicemia or bacteremia, 8%; pneumonia, 7%; maternal neglect, 7%; intestinal accident, 6%; congenital defects, 5%; other, 4%; bloat, 2%; calving difficulty/hypoxia, 2%; heart failure, 1%; exposure, 1%; and navel infection, 1%.

Data were also collected on the number of cows culled from each herd and the reasons for culling. For the period from breeding season 1991 through to the start of breeding season in 2007, the median risk of culling across all herds across this period as a percentage of cows exposed to breeding was 18% (IQR, 15% to 22%). Of the animals culled or sold, the most common reasons were; not pregnant, herd reduction, reproductive problems, or poor performance. Other reasons for loss are itemized in Table 2.

Table 2. Reasons for culling or loss from herd.

Not pregnant	29.6%	Miscellaneous disease	2.9%
Breeding stock/herd reduction	17.5%	Udder problem	2.1%
Reproductive problem	13.0%	Lameness	1.9%
Poor performance	12.0%	Temperament	1.9%
Dead or missing	7.8%	Calving problem	0.9%
Aborted	5.8%	Cancer eye	0.4%
Old age	4.2%		

The median herd risk of cow mortality as a percentage of cows exposed to breeding from the start of one breeding season to the beginning of the next across all herds during the study period was 1.5% (IQR, 1.2% to 1.7%) (Figure 7). There were 263 cows reported dead or missing from 1992 to the start of breeding in 2007; of these 164 cows were examined post-mortem (62%). The reported reasons for death loss in mature animals are listed in Table 3.

Table 3. Cause of death amongst mature animals.

Trauma	11%	Uterine prolapse	3%	Lymphosarcoma	1%
Heart failure/complications	9%	Pyelonephritis	3%	Johne's disease	1%
Post-calving complications	8%	Unknown or no diagnosis	2%	Intestinal accident	1%
Pneumonia	6%	Cancer	2%	Haemophilus	1%
Peritonitis	5%	Liver failure	2%	Cancer eye	1%
Bloat	5%	Myopathy	2%	Other	13%
Clostridial disease	4%	Mastitis	1%	BSE Program	17%

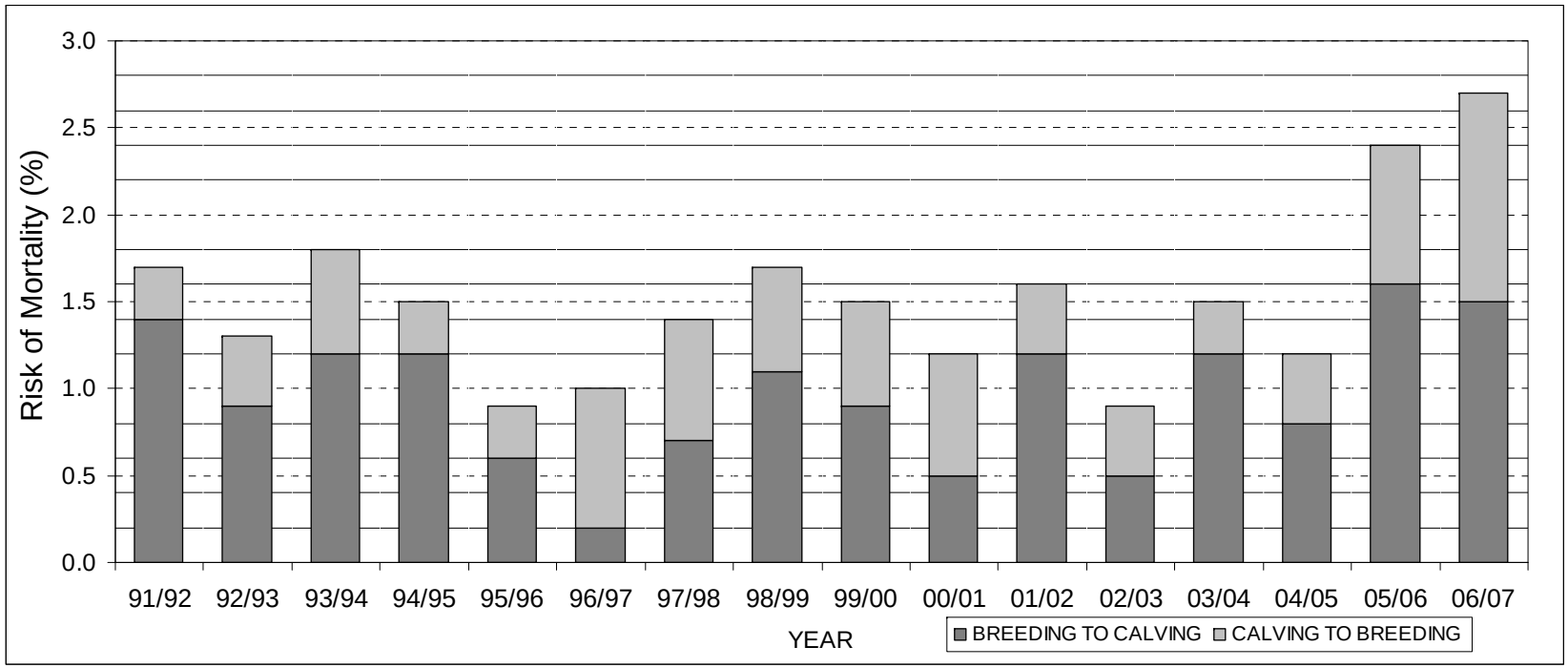

Figure 7. The risk of cow mortality was determined for the period from the start of the breeding season to the beginning of the calving season as the number of cows that died or were missing as a percentage of the number of cows exposed to breeding. The risk of cow mortality was also determined for the period from the start of the calving season to the beginning of the breeding season as the number of cows that died or were missing as a percentage of the number of cows present at the start of calving season.

Table 4 is a comparison of herd performance data between the CLS herds and the WISS herds. Most indices of herd performance are similar for the two studies.

Histopathology findings for the calves that were aborted or died for the two studies are summarized in Table 5; formal statistical comparison of the two studies with regards to the histopathology results was not completed because of the low numbers of calves and tissues submitted in the CLS during this period. Detailed assessment (Tables 6 and 7) of the histopathology of calves that died and were examined post mortem is limited by the low numbers of animals and tissues submitted from the CLS herds.

Liver trace mineral and vitamin E concentrations measured in calves that died within 24 hours of birth or between 24 hours of birth and 90 days of age are presented in Tables 8 and 9. There was no association between liver selenium or vitamin E and the presence in any lesions in muscle tissue (Table 10) nor was there a relationship between liver selenium, vitamin E, copper or molybdenum and thyroid lesions (Table 10). There was no association between whether or not the calf was assisted and whether the calf had a thyroid lesion (with dystocia: 22% or 4/18) or not (no dystocia: 32.5% or 49/151) (P=0.43). There was also no association between dystocia and the occurrence of myopathy in any of the tissues examined from these calves (with dystocia: 0% or 0/25; no dystocia: 12.8% or 24/187; P=0.09).

Serum (blood) concentrations of micronutrients, creatine kinase (CK) activity, and thyroid hormones (T3 and T4) for calves that were sampled before 48 hours of age are presented in Table 11. There were no associations between the concentration or activity of serum selenium and vitamin E and calving difficulty (Tables 12 and 13), nor was there an association between vitamins A or E and thyroid hormones (Table 14). However, T3 concentrations were significantly lower in calves that had been assisted than in calves born without any aid. T4 levels were also slightly lower in these animals although the difference was not significant. CK activity was significantly higher in the serum of calves that were assisted at birth than in those that were not assisted. There was also an association between serum vitamin E and CK activity; those calves with high CK activity (greater than the median, 215 U/L) had lower serum vitamin E concentrations than did those calves with low CK activity (Table 15).

Comparison of data from CLS study to WISSA project report

Table 4. Comparison of reproductive performance data collected from the CLS herds from 1987 through 2007 and herd performance data collected in the WISSA study in 2001-2002.

Performance measure	Study	n	Median	5th percentile	25th percentile	75th percentile	95th percentile	% variation explained by herd[e]	% of herd variation explained by year[e]
Non-pregnancy									
	CLS 1987-2006	127 obs. (9 herds)	5.9%	0.0%	3.8%	8.6%	13.5%	3.1%	0.0%
	WISSA 2001	205 herds	6.3%	2.0%	4.2%	8.5%	13.0%	6.3%	
	WISSA 2002	197 herds	7.2%	2.1%	5.1%	10.0%	16.9%	7.3%	
Abortion									
	CLS 1989-2007	113 obs. (9 herds)	1.5%	0.0%	0.9%	2.3%	4.0%	3.6%	29%
	WISSA 2001-2002	203 herds	1.3%	0.0%	0.6%	2.4%	4.0%	7.5%	
Stillbirth									
	CLS[a] 1988-2007	136 obs. (9 herds)	2.4%	0.0%	1.4%	3.8%	5.7%	5.8%	15%
	WISSA[b] 2002	203 herds	2.5%	0.0%	1.3%	3.9%	6.4%	6.1%	
Calf mortality									
	CLS[c] 1988-2007	134 obs. (9 herds)	3.5%	0.0%	2.1%	5.5%	9.4%	8.0%	22%
	WISSA[d] 2002	203 herds	2.9%	0.0%	1.6%	5.1%	8.2%	9.0%	

[a] The CLS definition of stillbirth includes all full-term calves born dead and those dead within 24 hours.
[b] The WISSA definition of stillbirth includes all full-term calves born dead and those dead within 1 hour.
[c] The CLS definition of calf mortality includes all full-term calves born alive that died from 24 hours to weaning.
[d] The WISSA definition of calf mortality includes all full-term calves born alive that died between 1 hour to 3 months of age.
[e] The % variation explained by herd and by year was calculated for the period 1996-2007 for the CLS herds. The % variation explained by year was not calculated for the WISSA study as data were reported from only one season for all measures but pregnancy success.

Table 5. Comparison of histopathology data from selected systems for samples collected from the CLS in 2004 and 2005, from the CLS in 2006 and 2007, and samples collected in the WISSA study in 2002. The number of animals with tissues examined for each system is given with the percentage of animals that had a lesion in at least one tissue from that system.

Age category	Study	Immune system	Nervous system	Respiratory system	Muscle and heart	Thyroid
Abortion						
	CLS 06/07	0% (n=6)	0% (n=7)	14% (n=7)	0% (n=7)	50% (n=6)
	CLS 04/05	40% (n=5)	20% (n=5)	60% (n=5)	40% (n=5)	40% (n=5)
	WISSA	39% (n=146)	5% (n=142)	26% (n=145)	18% (n=146)	17% (n=115)
Stillbirth						
	CLS 06/07	17% (n=76)	0% (n=71)	11% (n=76)	0% (n=77)	37% (n=60)
	CLS 04/05	53% (n=36)	24% (n=37)	58% (n=36)	27% (n=37)	46% (n=37)
	WISSA	31% (n=525)	6% (n=519)	13% (n=523)	27% (n=521)	26% (n=442)
Neonate						
	CLS 06/07	38% (n=24)	0% (n=24)	21% (n=24)	21% (n=24)	25% (n=24)
	CLS 04/05	79% (n=28)	57% (n=28)	79% (n=28)	71% (n=28)	43% (n=28)
	WISSA	65% (n=355)	15% (n=355)	43% (n=345)	55% (n=355)	22% (n=304)
Calf < 90 days						
	CLS 06/07	37% (n=101)	0% (n=100)	6% (n=102)	17% (n=106)	28% (n=79)
	CLS 04/05	76% (n=46)	19% (n=47)	34% (n=47)	53% (n=47)	20% (n=46)
	WISSA	73% (n=505)	11% (n=504)	33% (n=509)	52% (n=505)	13% (n=406)

Table 6. Histological diagnoses and descriptions of tissues from respiratory, immune, and nervous systems.

	Calf age classification at time of death			
Histological findings	Abortions	Stillbirths	Neonates	4 to 90 days
Respiratory system				
Tracheitis	0%(0/6)	0%(0/65)	0%(0/26)	3%(3/87)
Pneumonia				
Any pneumonia	20%(1/5)	11%(8/73)	19%(5/27)	8%(8/99)
Interstitial	20%(1/5)	1%(1/73)	7%(2/27)	1%(1/99)
BronchoInterstitial	0%(0/5)	1%(1/73)	0%(0/27)	1%(1/99)
Broncho	0%(0/5)	0%(0/73)	0%(0/27)	4%(4/99)
Suppurative	0%(0/5)	0%(0/73)	4%(1/27)	1%(1/99)
Fibrino-Broncho	0%(0/5)	0%(0/73)	0%(0/27)	1%(1/99)
Aspiration:				
Meconium	0%(0/5)	8%(6/73)	0%(0/27)	0%(0/99)
Abomasal Contents	0%(0/5)	0%(0/73)	4%(1/27)	0%(0/99)
Milk	0%(0/5)	0%(0/73)	4%(1/27)	0%(0/99)
Blood vessel lesion – lung	0%(0/5)	0%(0/73)	7%(2/27)	2%(2/99)
Nervous system				
Hypomyelination:				
Any tissue	0%(0/7)	0%(0/71)	0%(0/28)	0%(0/98)
Immune system				
Atrophy, depletion, or hypoplasia				
Lymph node	0%(0/4)	3%(2/61)	14%(3/22)	14%(11/80)
Thymus	0%(0/5)	20%(13/66)	72%(18/25)	59%(44/75)
Spleen	0%(0/6)	0%(0/65)	14%(3/21)	12%(10/82)
Ileum/Peyer's patches	0%(0/3)	28%(16/57)	80%(20/25)	67%(55/82)

Table 7. Histological diagnoses and descriptions of tissues from thyroid gland, skeletal muscle, heart, and liver

	Calf age classification at time of death			
Histological findings	Abortions	Stillbirths	Neonates	>3 days
Thyroid gland				
Any thyroid lesion	50%(3/6)	37%(22/59)	25%(6/24)	28%(22/79)
Hypoplasia	0%(0/6)	10%(6/59)	0%(0/24)	9%(7/79)
Degeneration/necrosis	0%(0/6)	8%(5/59)	0%(0/24)	11%(9/79)
Hyperplasia	17%(1/6)	2%(1/59)	0%(0/24)	1%(1/79)
Hyperplastic goiter	0%(0/6)	2%(1/59)	4%(1/24)	1%(1/79)
Colloid goiter	0%(0/6)	2%(1/59)	0%(0/24)	1%(1/79)
Abnormal colloid only	50%(3/6)	36%(21/59)	25%(6/24)	22%(17/79)
Normal Colloid, Other lesion	0%(0/6)	2%(1/59)	0%(0/24)	6%(5/79)
Abnormal Colloid, Other lesion	17%(1/6)	17%(10/59)	4%(1/24)	16%(13/79)
No Colloid	100%(6/6)	100%(59/59)	100%(24/24)	100%(79/79)
Decreased Colloid	0%(0/6)	8%(5/59)	0%(0/24)	11%(9/79)
Decreased Colloid Staining	0%(0/6)	5%(3/59)	0%(0/24)	5%(4/79)
Decreased Lipofuscin	0%(0/6)	0%(0/59)	0%(0/24)	0%(0/79)
Skeletal muscle				
Any skeletal myopathy or necrosis	0%(0/7)	5%(4/76)	21%(6/28)	15%(15/100)
Myopathy				
Any skeletal muscle	0%(0/7)	5%(4/76)	21%(6/28)	14%(14/100)
Appendicular, postural or unspecified muscle	0%(0/7)	3%(2/72)	4%(1/26)	8%(7/91)
Diaphragm	0%(0/4)	3%(2/59)	5%(1/21)	5%(4/76)
Tongue	0%(0/6)	6%(4/64)	19%(5/26)	13%(11/88)
Eyelid	0%(0/2)	0%(0/21)	0%(0/7)	0%(0/25)
Esophagus	N/A(0/0)	0%(0/16)	0%(0/2)	8%(1/12)
Any muscle – severe myopathy	0%(0/7)	3%(2/76)	4%(1/28)	5%(5/100)
Any skeletal necrosis	0%(0/7)	0%(0/76)	0%(0/28)	5%(5/100)
Any skeletal mineralization	0%(0/7)	0%(0/76)	0%(0/28)	4%(4/100)
Heart				
Any cardiac myopathy or necrosis	0%(0/7)	0%(0/72)	0%(0/27)	1%(1/93)
Cardiac myopathy	0%(0/7)	0%(0/72)	0%(0/27)	0%(0/93)
Cardiac myopathy – severe	0%(0/7)	0%(0/72)	0%(0/27)	0%(0/93)
Myocardial necrosis	0%(0/7)	0%(0/72)	0%(0/27)	1%(1/93)
Cardiac muscle mineralization	0%(0/7)	0%(0/72)	0%(0/27)	1%(1/93)
Blood vessel lesion – heart	0%(0/7)	0%(0/72)	4%(1/27)	0%(0/93)
Liver:				
Hemosiderosis	0%(0/7)	7%(5/67)	4%(1/27)	3%(3/94)
Hemosiderosis – severe	0%(0/7)	1%(1/67)	0%(0/27)	2%(2/94)

Summary of the tissue micronutrient data from CLS herds (2006-2007)

Table 8. Concentration of micronutrients (ppm wet weight) in livers from calves reported to be dead at birth or within 24 hours of birth (n=43):

	Reference	Mean	SD	Min	25th percentile	Median	75th percentile	Max
Selenium	0.25 – 0.50	0.36	0.14	0.13	0.26	0.33	0.45	0.84
Vitamin E	12 – 20	2.0	1.1	0.1	1.3	1.7	2.8	5.0
Copper	25 – 100	72	31	37	50	64	86	195
Iron	50 – 450	273	211	22	105	190	430	742
Magnesium	100 – 250	129	24	73	111	133	147	169
Manganese	2.5 – 6.0	0.99	0.37	0.48	0.69	0.96	1.18	1.94
Molybdenum	0.16 – 1.60	0.41	0.15	0.21	0.27	0.37	0.50	0.72
Zinc	25 – 100	107	51	12	66	98	153	241

Table 9. Concentration of micronutrients (ppm wet weight) in livers from calves reported to be dead between 24 hours and 90 days after calving (n=36):

	Reference	Mean	SD	Min	25th percentile	Median	75th percentile	Max
Selenium	0.25 – 0.50	1.00	1.65	0.21	0.30	0.42	0.85	9.13
Vitamin E	12 – 20	12.9	32.3	0.1	1.6	2.6	4.4	172
Copper	25 – 100	81	33	27	57	71	109	173
Iron	50 – 450	257	344	20	70	105	371	1624
Magnesium	100 – 250	174	24	121	162	175	192	220
Manganese	2.5 – 6.0	1.70	1.03	0.64	1.08	1.24	1.98	5.04
Molybdenum	0.16 – 1.60	0.53	0.16	0.26	0.40	0.50	0.64	0.88
Zinc	25 – 100	106	57	38	61	95	141	259

Liver micronutrient concentrations	% deficient	Reference range (neonatal calves) (wet weight)*
Selenium	13.8%	<0.25 ppm
Copper	18.8%	<50 ppm
Vitamin E	85.0%	<4 ppm
	% high	Reference range
Molybdenum	0.0%	>1.6 ppm

* Puls, R. 1994. *Mineral Levels in Animal Health*. 2nd ed. Clearbrook, British Columbia: Sherpa International. and Puls, R. 1994. *Vitamin Levels in Animal Health*. 2nd ed. Clearbrook, British Columbia: Sherpa International.

Table 10. Association between liver micronutrient concentrations and selected histopathology findings (skeletal muscle and thyroid lesions).

	Selenium (ppm wet weight)		**Vitamin E (ppm wet weight)**	
	Myopathy		Myopathy	
	No	Yes	No	Yes
N	70	7	70	6
Mean	0.69	0.34	7.6	2.6
SD	1.22	0.12	23.6	0.8
Minimum	0.13	0.21	0.1	1.6
25th percentile	0.28	0.26	1.5	1.7
Median	0.37	0.29	2.0	3.0
75th percentile	0.49	0.45	3.0	3.2
Maximum	9.13	0.54	172	3.5
	P=0.24		*P*=0.27	

	Selenium (ppm wet weigh)		**Vitamin E (ppm wet weight)**		**Copper (ppm wet weight)**		**Molybdenum (ppm wet weight)**	
	Thyroid lesion		Thyroid lesion		Thyroid lesion		Thyroid lesion	
	No	Yes	No	Yes	No	Yes	No	Yes
N	53	8	52	8	53	8	53	8
Mean	0.76	0.33	9.1	2.4	74	82	0.48	0.51
SD	1.39	0.05	27.2	0.7	31	22	0.16	0.17
Minimum	0.13	0.27	0.1	1.4	27	60	0.21	0.35
25th percentile	0.28	0.28	1.6	1.7	55	61	0.36	0.38
Median	0.39	0.32	2.1	2.6	67	78	0.47	0.49
75th percentile	0.54	0.37	3.1	3.0	93	103	0.62	0.56
Maximum	9.13	0.42	172	3.2	195	116	0.85	0.88
	P=0.24		*P*=0.71		*P*=0.25		*P*=0.67	

Summary of serum micronutrient status, CK, and thyroid hormone concentrations

Table 11. Summary of serum analytes for 92 calves sampled within 48 hours after calving in 2006 and 2007.

		Mean	SD	Min	25th percentile	Median	75th percentile	Max	Reference range
Selenium	ppm	0.053	0.071	0.015	0.027	0.037	0.065	0.670	Deficient <0.025 ppm Marginal <0.080 ppm
Copper	ppm	0.38	0.16	0.22	0.28	0.34	0.45	1.30	0.60 – 1.50 ppm
Molybdenum	ppm	0.14	0.13	0.01	0.05	0.11	0.20	0.98	0.01 – 0.10 ppm
Magnesium	ppm	21.5	4.7	1.6	19.0	21.3	24.1	34.8	1.8 – 3.0 ppm
Manganese	ppb	3.7	3.8	0.0	1.4	3.0	5.0	26.5	6 – 70 ppb
Zinc	ppm	1.22	0.77	0.32	0.73	1.01	1.50	5.28	0.80 – 1.40 ppm
Vitamin A	ppm	0.14	0.06	0.02	0.10	0.14	0.17	0.35	0.2 – 0.25 ppm
Vitamin E	ppm	0.87	0.74	0.10	0.32	0.71	1.20	3.68	0.8 – 1.2 ppm
T3	nmol/L	10.2	5.2	1.0	6.0	9.9	14.6	22.5	Not available
T4	nmol/L	195	85	26	133	199	240	447	Not available
CK	U/L	495	1189	42	146	215	369	9980	64 – 344 U/L

Proportion of calves sampled within 48 hours after calving 2006 and 2007 classified as deficient based on existing guidelines (n=92).

Serum micronutrient concentrations	% deficient	Reference range (neonatal calves)*
Selenium	19.6%	<0.025 ppm
Copper	82.6%	<0.5 ppm
Vitamin A	83.7%	<0.2 ppm
Vitamin E	56.5%	<0.8 ppm
	% high	Reference range
Molybdenum	53.3%	>0.1 ppm

* Puls, R. 1994. *Mineral Levels in Animal Health*. 2nd ed. Clearbrook, British Columbia: Sherpa International. and Puls, R. 1994. *Vitamin Levels in Animal Health*. 2nd ed. Clearbrook, British Columbia: Sherpa International.

Serum analytes associated with calving difficulty

Table 12. Serum thyroid hormone concentrations and association with calving difficulty

	T3 (nmol/L)		**T4 (nmol/L)**	
	Calving difficulty		Calving difficulty	
	Yes	No	Yes	No
N	50	42	50	42
Mean	9.0	11.7	181	211
SD	4.8	5.2	78	91
25th percentile	5.3	7.5	130	133
Median	8.9	12.5	172	213
75th percentile	12.1	15.6	223	259
	***P*=0.02**		*P*=0.07	

Table 13. Serum CK, micronutrient concentrations, and association with calving difficulty

	CK (U/L)		**Selenium (ppm)**		**Vitamin E (ppm)**	
	Calving difficulty		Calving difficulty		Calving difficulty	
	Yes	No	Yes	No	Yes	No
N	50	42	50	42	50	42
Mean	579	394	0.049	0.057	0.83	0.91
SD	1410	861	0.033	0.010	0.82	0.66
25th percentile	148	127	0.028	0.024	0.18	0.47
Median	279	198	0.040	0.032	0.58	0.77
75th percentile	412	287	0.062	0.065	1.18	1.24
	***P*=0.04**		*P*=0.41		*P*=0.20	

Serum vitamin concentrations associated with changes in changing concentration of thyroid hormones and serum enzymes associated with muscle damage.

Table 14. Serum vitamin concentration and association with serum thyroid concentrations (T3 and T4)

	Vitamin A (ppm)		**Vitamin E (ppm)**	
	T3		T3	
	High*	Low	High*	Low
N	45	47	45	47
Mean	0.14	0.14	0.87	0.87
SD	0.06	0.07	0.55	0.89
25th percentile	0.11	0.08	0.50	0.10
Median	0.13	0.14	0.76	0.58
75th percentile	0.16	0.18	1.19	1.24
	P=0.88		*P*=0.17	

* High = T3 greater than median concentration 9.9 nmol/L.

	Vitamin A (ppm)		**Vitamin E (ppm)**	
	T4		T4	
	High*	Low	High*	Low
N	46	46	46	46
Mean	0.14	0.14	0.83	0.91
SD	0.05	0.07	0.61	0.87
25th percentile	0.11	0.08	0.44	0.22
Median	0.13	0.14	0.73	0.65
75th percentile	0.16	0.18	1.10	1.34
	P=0.89		*P*=0.73	

* High = T4 greater than median concentration 199 nmol/L.

Table 15. Serum selenium and vitamin E concentration and association with creatine kinase (CK) activity

	Selenium (ppm)		**Vitamin E (ppm)**	
	CK		CK	
	High*	Low	High*	Low
N	46	46	46	46
Mean	0.044	0.062	0.70	1.04
SD	0.025	0.097	0.71	0.75
25th percentile	0.027	0.028	0.21	0.51
Median	0.031	0.040	0.53	0.92
75th percentile	0.061	0.067	0.89	1.49
	P=0.38		***P*=0.01**	

* High = CK greater than median concentration 215 U/L.

Comparison of air monitoring data from CLS to WISSA

Monthly SO_2 data comparisons from data collected the CLS, the Parkland Airshed Management Zone and WISSA study are presented in Table 16 and compared with reported emissions (Figures 8 and 9).

Table 16 shows that mean monthly concentrations in the CLS herds in 2005 were lower than those observed at either the PAMZ monitors in 2001-2002 or the upper half of concentrations measured during the WISSA study. Table 16 also shows that the distribution of concentrations measured at half of the WISSA sites was consistently higher than that observed in the PAMZ area in 2001-2002 in all but two months. In those months, the concentrations observed in the highest quarter of the WISSA sites (94 monitors in March 2002 and 157 monitors in April 2001 and 2002) were higher than those measured by the PAMZ network.

Figure 8 is a graphic representation of emissions and background SO_2 and suggests that mean annual SO_2 concentrations have decreased in the PAMZ area since 2000 and that reported emissions also decreased each year from 2000 through 2004.

Figure 9 demonstrates that concentrations measured in participating CLS herds were slightly higher on average than mean concentrations observed at the PAMZ monitoring sites for each month in 2005.

Table 16. Comparison of SO_2 data from samples reported from the CLS in 2005 (n=521 from 9 herds) and samples reported in the WISSA study in 2001 and 2002 (n=13,991 from 205 herds) and the Parkland Airshed Management Zone during the same period as the WISSA study (see explanation of study groups below).

Month	Study	n	Median	5th percentile	25th percentile	75th percentile	95th percentile
January							
	CLS 2005	23	0.3	0.2	0.3	0.4	0.4
	PAMZ 2002	33	1.0	0.1	0.6	1.3	1.7
	WISSA 2002 all stations	430	1.0	0.4	0.6	1.4	2.3
	WISSA 2002 high exp. group	212	1.4	1.0	1.2	1.9	2.9
February							
	CLS 2005	21	0.5	0.4	0.4	0.6	0.6
	PAMZ 2002	33	0.9	0.2	0.7	1.4	1.6
	WISSA 2002 all stations	403	0.8	0.3	0.5	1.1	2.2
	WISSA 2002 high exp. group	201	1.1	0.8	0.9	1.5	2.6
March							
	CLS 2005	24	0.5	0.4	0.4	0.6	0.6
	PAMZ 2002	33	1.9	0.8	1.5	2.3	2.9
	WISSA 2002 all stations	380	1.4	0.7	1.0	1.8	2.6
	WISSA 2002 high exp. group*	188	1.9	1.5	1.6	2.2	2.8
April							
	CLS 2005	39	0.4	0.3	0.4	0.5	0.6
	PAMZ 2001-2002	65	0.8	0.1	0.6	0.9	1.2
	WISSA 2001-2002 all stations	665	0.5	0.2	0.3	0.7	1.2
	WISSA 2001-2002 high exp. group**	326	0.7	0.5	0.6	1.0	1.5
May							
	CLS 2005	39	0.5	0.3	0.4	0.5	0.6
	PAMZ 2001-2002	66	0.4	0.1	0.3	0.6	0.8
	WISSA 2001-2002 all stations	1703	0.3	0.1	0.2	0.5	0.9
	WISSA 2001-2002 high exp. group	835	0.5	0.4	0.4	0.7	1.2
June							
	CLS 2005	57	0.3	0.2	0.3	0.4	0.5
	PAMZ 2001-2002	66	0.6	0.1	0.4	0.7	1.0
	WISSA 2001-2002 all stations	2550	0.4	0.1	0.3	0.7	1.2
	WISSA 2001-2002 high exp. group	1247	0.7	0.5	0.5	0.9	1.5

Table 16 (continued). Comparison of SO_2 data from samples reported from the CLS in 2005 (n=521from 9 herds) and samples reported in the WISSA study in 2001 and 2002 (n=13,991from 205 herds) and the Parkland Airshed Management Zone during the same period as the WISSA study (see explanation of study groups below) (continued).

Month	Study	n	Median	5th percentile	25th percentile	75th percentile	95th percentile
July							
	CLS 2005	62	0.4	0.2	0.3	0.5	0.6
	PAMZ 2001-2002	66	0.6	0.2	0.5	0.7	0.9
	WISSA 2001-2002 all stations	1997	0.6	0.2	0.4	0.8	1.5
	WISSA 2001-2002 high exp. group	998	0.8	0.6	0.7	1.0	1.8
August							
	CLS 2005	62	0.4	0.3	0.3	0.4	0.7
	PAMZ 2001-2002	66	0.6	0.1	0.4	0.7	0.9
	WISSA 2001-2002 all stations	2010	0.5	0.2	0.4	0.8	1.4
	WISSA 2001-2002 high exp. group	987	0.8	0.6	0.6	1.0	1.6
September							
	CLS 2005	62	0.4	0.2	0.3	0.5	0.7
	PAMZ 2001-2002	66	0.6	0.1	0.4	0.8	1.0
	WISSA 2001-2002 all stations	1309	0.5	0.2	0.3	0.7	1.3
	WISSA 2001-2002 high exp. group	652	0.7	0.5	0.6	1.0	1.7
October							
	CLS 2005	57	0.4	0.2	0.3	0.5	0.7
	PAMZ 2001-2002	65	0.5	0.1	0.3	0.7	0.9
	WISSA 2001-2002 all stations	1167	0.4	0.1	0.3	0.7	1.3
	WISSA 2001-2002 high exp. group	582	0.7	0.5	0.6	1.0	1.8
November							
	CLS 2005	44	0.4	0.2	0.4	0.5	0.7
	PAMZ 2001-2002	65	0.6	0.1	0.4	1.0	1.4
	WISSA 2001-2002 all stations	779	0.6	0.3	0.4	1.0	1.8
	WISSA 2001-2002 high exp. group	383	1.0	0.7	0.8	1.3	2.2
December							
	CLS 2005	31	0.6	0.4	0.5	0.7	0.9
	PAMZ 2001-2002	65	0.6	0.1	0.3	1.1	1.8
	WISSA 2001-2002 all stations	598	0.9	0.3	0.6	1.4	2.5
	WISSA 2001-2002 high exp. group	295	1.4	0.9	1.1	1.8	2.9

Explanation of differences in numbers and timelines for each study listed in the air concentration tables above (Table 16):

CLS 2005 – All monitoring stations on CLS pastures for each month of 2005.
PAMZ 2001-2002 – All data from PAMZ monitoring sites for each month of 2001-2002. Data from both 2001 and 2002 were included for these months to make it directly comparable to the WISSA data which were also available for both 2001 and 2002 for these months.
PAMZ 2002 – All data from PAMZ monitoring sites for each month of 2002. Data were restricted to 2002 to make it directly comparable to the WISSA data which were only available for 2002 for these months.
WISSA 2001-2002 all stations – Data from all air monitoring stations in the WISSA project. Data were available for both 2001 and 2002 for these months.
WISSA 2001-2002 high exposure group – Data from air monitoring stations in the WISSA project from which the SO_2 values were above the median SO_2 concentration for that month (upper half or 50% of the recorded measurements). These stations represented the high exposure areas within the WISSA study for that month.
WISSA 2002 all stations – Data from all air monitoring stations in the WISSA project. Data were available for 2002 only for these months.
WISSA 2002 high exposure group – Data from air monitoring stations in the WISSA project from which the SO_2 values were above the median SO_2 concentration for that month (upper half or 50% of the recorded measurements). These stations represented the high exposure areas within the WISSA study for that month. Data were available from only 2002 for these months.

* There were 94 observations in the WISSA group with the highest measured values (upper quarter or 25% of SO_2 measures) for March 2002 (median, 2.2 ppb; 5^{th} percentile, 1.9; 25^{th} percentile, 2.0; 75^{th} percentile, 2.5 ppb, 95^{th} percentile, 3.3 ppb).
* There were 157 observations in the WISSA group with the highest measured values (upper quarter or 25% of SO_2 measures) in April 2001-2002 (median, 1.0 ppb; 5^{th} percentile, 0.8; 25^{th} percentile, 0.9; 75^{th} percentile, 1.2 ppb, 95^{th} percentile, 1.8 ppb).

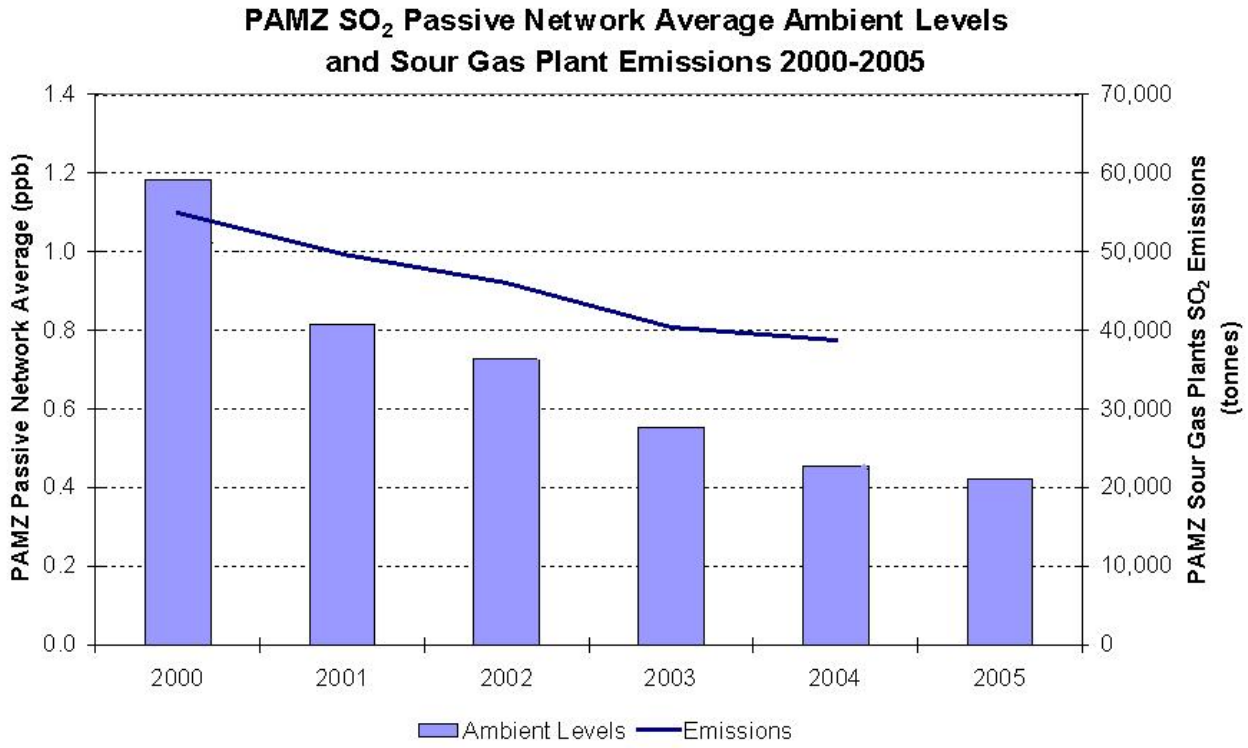

Figure 8. Summary of emissions and background air quality data for the area surrounding the study (summarized by Kevin Warren, Amarok Consulting)..

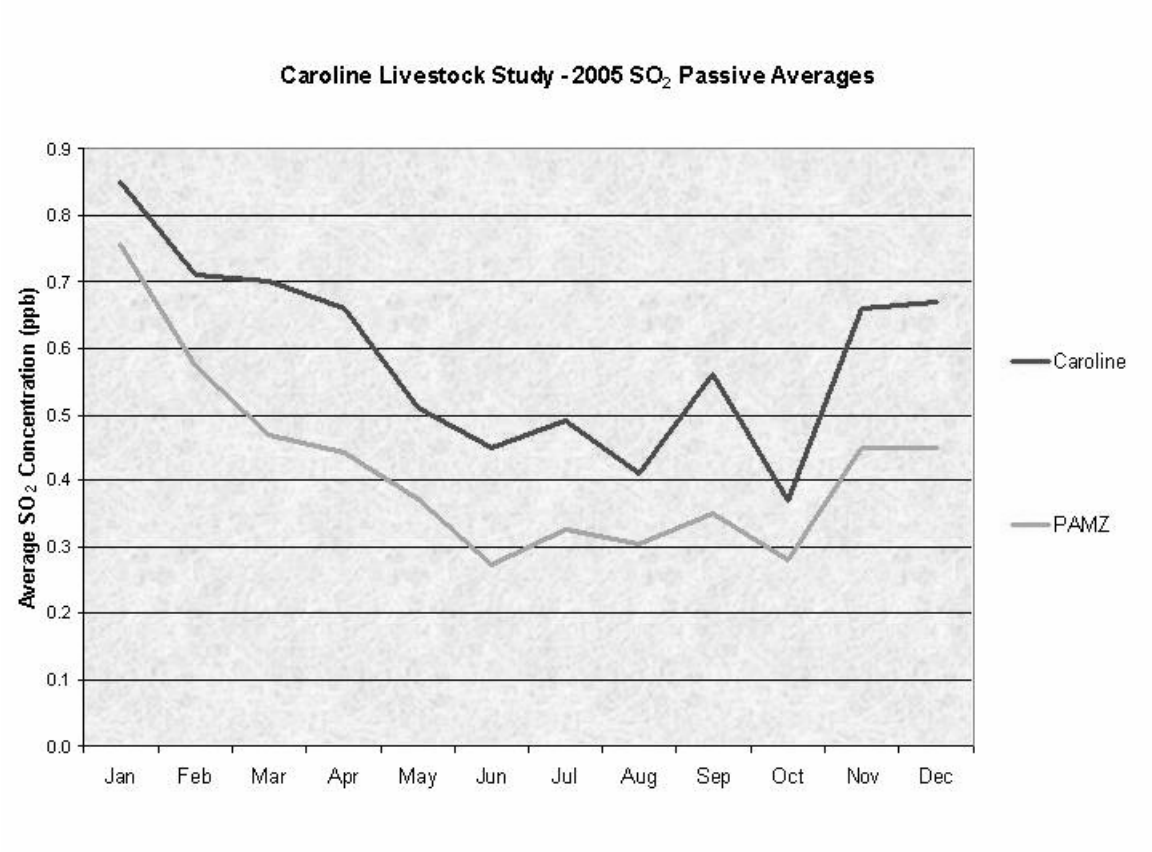

Figure 9. Comparison of monthly average SO_2 concentrations measured in all CLS herds (521 measurements at varying locations in 9 herds) to monthly average SO_2 concentrations from all monitors in the PAMZ network in 2005 (n=34 sites each month) (summarized by Kevin Warren, Amarok Consulting).

DISCUSSION

The primary objective of this study was to develop a baseline for animal health and productivity data for the beef herds within the boundaries of the Sundre Petroleum Operator's Group. More than 16 years of supervised data have been collected from area herds. The ongoing commitment of the participating herd owners and local veterinary clinic throughout this period is unprecedented.

In this report the available data from the Caroline Livestock Study (CLS) were graphed and then data collected following the 2003 breeding season when BSE was first detected in Alberta were compared to data from previous years and other databases. The time after 2003 is used as a separate comparison group because herd owners reported changing many of their management practices as a result of changing market conditions in the post BSE era. In this discussion herd performance for the period after calving season 2003 is compared to:

a.) information gathered between 1987 and the 1993 calving season when operations began at the Caroline Gas Plant,
b.) data collected between the breeding season in 1993 and calving season 2003,
c.) to other data reported in the literature, and
d.) comparable results from the WISSA study.

Comparisons of the results for the CLS herd data and WISSA studies are limited to examination of the animal health and performance data. As no directly comparable exposure data were collected in the CLS herds during 2001 and 2002, a direct comparison of whether or not there is a similar effect of emissions on reproductive performance and calf health was not possible. However, SO_2 concentrations were monitored in the CLS herds in 2005. These data were informally compared to both the WISSA data for 2001-2002 and PAMZ data for the period 2001 through 2005.

Finally, this report examines the occurrence of specific types of microscopic pathology highlighted in the WISSA report to determine how often these lesions occurred in the CLS herds during the period from 2004 to 2007. The investigation focuses on the occurrence of lesions in muscle and thyroid tissue. The lesions in the muscle tissue were of interest because they were observed in a high proportion of the calves that died during the WISSA study. Both the occurrence of these muscle lesions and the overall risk of calf mortality were associated with increasing exposure to sulfur dioxide in the WISSA study. The changes in the thyroid gland were of interest because before the WISSA study they had not previously been described and the type of lesion observed could potentially affect calf viability particularly in cold climates. The occurrence of thyroid lesions was also associated with exposure to benzene in the WISSA study. This report investigates the role of calving difficulty and nutrition in the occurrence of these lesions, and the role of micronutrients in thyroid function and serum enzyme indicators of muscle damage during the 2006 and 2007 calving seasons.

Herd fertility

Herd fertility is probably the most significant factor affecting the profitability of the beef cow–calf operation. The overall median risk of non-pregnancy (5.3%; IQR, 4.5% to 8.1%) for the period 2003 through 2006 after the diagnosis of BSE in Alberta did not differ from that reported for the period from 1987 to 1992 (5.6%; IQR, 3.8% to 9.1%) before the start of operations at the Shell Caroline Plant, the period 1993 to 2002 (6.2%; IQR, 4.2% to 9.3%), a previous Alberta survey (6.5%) (Mathison, 1993), published targets of performance (5%) (Radostits et al., 1994), or the median percentage of cows reported not pregnant for herds in the WISSA study in 2001 (6.3%) or 2002 (7.2%).

The median abortion risk (1.2%; IQR, 0.4% to 1.8%) for all project herds has also remained relatively constant for the period from breeding season 2003 to calving season 2007 as compared to the reported median abortion risk (1.6%; IQR, 1.0% to 2.7%) for the period from breeding season 1987 to calving season in 1993 and the risk (1.5%; IQR, 1.3% to 2.3%) from breeding season 1993 to calving

season 2003. This risk was within the established 2.0 % target of performance for commercial beef herds (Wikse et al., 1992; Radostits et al, 1994) and was similar to the 1.3% median herd risk of abortion reported in the WISSA study.

Difficulty in obtaining either the fetus or placenta was the major limitation to the success of the abortion investigation protocol in the present study. In many cases the fetus and/or placenta could not be found because of the size of the area where the cows were managed and presence of brush and trees. Often coyotes and other predators would scavenge the abortus before it could be recovered. Cows were usually only checked once a day at feeding during peak times for abortion risk. Many cows were not noticed aborting. The aborted cows were often noticed by the owner some time later exhibiting signs of estrus or simply failed to calve in the spring. The percentage of aborted calves recovered and submitted for laboratory diagnosis was substantially lower in the CLS herds (13%) than in the WISSA study (35%).

Calf death loss between birth and weaning

Perinatal calf deaths result in major economic losses to the beef cattle industry, second only to lost production due to pregnancy failure. Costs to the producer resulting from neonatal disease include: veterinary treatment costs, the producer's labor, any post-mortem examinations, breeding costs, lost income, the cost of maintaining dry cows, and decreased weaning weights (Mathison, 1993). In both the CLS and WISSA studies perinatal calf losses were measured as the percentage of calves that were stillborn as well as the risk of calf mortality. However, both stillbirth and calf mortality were defined slightly differently in the two studies. Calves were considered stillborn if they died within 24 hours in the CLS study and with 1 hour of birth in the WISSA study. Calf mortality was measured to weaning in the CLS study and to 3 months of age in the WISSA study.

The median risk of stillbirth (2.6%; IQR, 1.5% to 4.6%) remained relatively constant from 2004 through 2007 in the CLS herds as compared to the risk reported from 1988 to 1993 (2.3%; IQR, 1.1% to 3.9%) or from 1994 to 2003 (2.4%; IQR, 1.4% to 3.4%) and was consistent with previous Alberta (2.3%)[1] (Mathison, 1993), Ontario reports (3.6%)[2] (McDermott et al., 1991b), and the median herd risk of stillbirth reported in the WISSA study (2.5%). The median calf mortality risk for the period from 2004 through 2007 (4.8%; IQR, 3.5% to 6.8%) in the CLS herds was slightly higher than that from 1987 through 1992 (3.1%; IQR, 2.1% to 4.5%) or from 1993 through 2003 (3.2%; IQR, 2.0% to 4.8%) and just above expectations based on earlier studies from Alberta (2.6%) [3] (Mathison, 1993), Ontario (2.7%) [4] (McDermott et al., 1991a), and the median percentage of calves reported to have died in each herd before 3 months of age in the WISSA study (2.9%).

There were no other published precedents for a long term survey of causes of neonatal loss based on post-mortem examination. Calving difficulty, scours, accident or trauma, abomasal ulcers, bacterial infection and pneumonia, maternal neglect and exposure, and congenital defects were the most commonly reported causes of calf loss in the CLS study. Where large fluctuations in individual herd calf mortality were reported, most of the unusual losses were associated with scours.

The most common causes of perinatal calf mortality reported in the literature are: dystocia (calving difficulty), diarrhea (neonatal enteritis or scours), pneumonia, and the effects of exposure during inclement weather. The most important infectious disease associated with herd outbreaks is neonatal diarrhea. Morbidity rates of 30% to 50% of newborn calves affected with scours are common, and case fatality rates can reach 30% or higher (Townsend, 1994). In a study of Saskatchewan beef herds in 1974-1975, Acres reported that in any given year 10% of herds experienced scour outbreaks in which 4% or greater of the calves die (Acres, 1976). Other reports in the literature suggest that in any given year, 4% to 16% of cow-calf operators will experience unacceptable calf mortality losses.

Most of the previous reports in the literature are based on herd owner reported cause of death and no post mortem examinations were done. The cause of death for the CLS herds was primarily based on gross postmortem examination with tissues submitted for histopathology or other confirmatory testing at the discretion of the veterinarian. Only between 2004 and 2007 were complete tissue sets submitted for histopathologic evaluation. Both the earlier published reports and the information reported in the CLS

herds differed from the cause of death results in the WISSA study which were based not only on gross postmortem examination, but also on a systematic evaluation of tissue samples collected from all postmortem examinations.

The success in establishing a cause of death in the WISSA study increased with the age of the calf. A cause of death or the identification of potentially significant histological lesions was reported for 52% of aborted fetuses, 68% of stillborn calves, 89% of calves 1 to 3 days of age and 94% of the older calves examined. Noninfectious disease was more common than infectious disease in all age categories. Cardiac failure, thyroid abnormalities, and congenital anomalies were the most frequent lesions associated with noninfectious causes of abortions. Infectious or inflammatory disease associated with placentitis was present in 13% of abortions. Problems associated with parturition were the most common reason for calf loss in stillborn calves (30%). Dystocia (8%) was also an important cause of neonatal calf loss, second only to cardiac failure (13%).

Infectious disease was uncommon in stillborn calves (2%) examined as part of the WISSA study, but was more frequently associated with mortality in neonatal calves (20%) and calves older than 3 days (33%). Starvation, which was often secondary to or found in association with some other disease process, was the immediate cause of loss in another 20% of older calves. Abomasal lesions, including inflammation, ulcers, torsions, and perforation, accounted for 15% of older calf losses. Microscopic lesions in cardiac and skeletal muscle and thyroid glands were common in all age categories.

Treatment records were excluded from this report because of the level of inconsistency in reporting across herds. Variations in recording consistency among herd owners, and even within herds throughout the calving season, limited the potential to interpret calf morbidity or treatment records. Some herd owners maintained thorough individual calf records, while others reported approximate numbers treated. In addition to the practical problems in ensuring consistent treatment recording in large commercial operations, differences between veterinary and producer case definitions were also recognized as a possible limitation of herd owner generated calf treatment records (Van Donkersgoed et al., 1993).

Cow herd population dynamics, culling and death loss

The age distribution of a population provides some information on the dynamics of movement into and out of the group. The expected life of the average cow in a beef herd did not usually exceed eleven years prior to export restrictions associated with BSE being reported in Canada in 2003. The optimal culling age has been suggested to be between 8 to 10 years (McDermott et al., 1992a). The average cow produces only five to seven calves before she is culled. Because of this, a minimum of 15 % of the cows will be replaced per year in most herds. In the fall of 2005, 18% of the animals that were pregnancy tested in all CLS herds were bred replacement heifers.

The median risk of mortality for cows in the CLS herds was 1.3% and ranged from 0.8% to 1.7%. McDermott et al. (1991b) reported that 86 % of herds in the Ontario survey had no cow deaths and only 2.8 % of herds had death losses in cows of 5.0 % or greater. The average mortality rate for cows from calving to weaning was 0.5 % and the average for heifers was 0.3 %. The CLS study found an average mortality rate for breeding females slightly higher than reported for Ontario beef herds. Note that missing animals presumed dead were also included in the calculations from the CLS herds. Also, because many of these herds are managed on large tracks of forested pasture during the summer, a higher incidence of missing cows, as compared to a more intensively managed area, was not unexpected. The risk of mortality for the cow herds from the Caroline study was consistent across the period from 1991 to 2005, and the results were within performance targets suggested in the literature (Randle, 1993). Heart failure, pneumonia, bloat, peritonitis, and injury were the leading cause of death reported.

The median risk of culling in all CLS herds was 20% of the cows from each herd exposed to breeding that year. The suggested goal for herd culling rate is 15 to 20 % per year (Randle, 1993). The Ontario study, in contrast, reported a breeding-to-calving culling rate and a breeding-to-calving sold for breeding rate (McDermott et al., 1991b). The average culling rate for the cows in the Ontario study was

11.4 % and the sold-for-breeding rate was 3.4 %. The average culling rate for the heifers in that study was 3.4 % and the sold-for-breeding rate was 4.2 %. No cows were culled in only 14.4 % of herds. In the CLS study, the disposal risk was calculated from the start of breeding one season through calving to the start of the next breeding season. This differed from the Ontario study. The Caroline total disposal rate was higher than the sum of the culling rate and the sold-for-breeding rate in the Ontario study. The disposal rate for the Caroline study, however, also included cows that died and cows removed from the herd between calving and breeding. Neither of these losses were included by the Ontario culling rate.

In order of importance, the most common reasons for loss from inventory for breeding females from the Caroline study group were non-pregnancy, sales of breeding stock or herd reduction, reproductive problems including late pregnancy or calving problems, poor performance (based on calf weaning weight), dead or missing, abortion, old age, and lameness. In 123 of the Ontario herds, individual level data on culling and potential risk factors for culling were available (McDermott et al., 1992). The following individual cow culling risk factors: non-pregnancy, age, weaning weight index, calf outcome, abortion, prolapsed vagina, prolapsed uterus, calving injury, lameness, and mastitis or udder problems were significantly associated with culling.

Histopathology findings

Beginning in 2004 and continuing through calving season 2007, local veterinarians were asked to collect a consistent set of tissue samples from all postmortem examinations done for the CLS herds. While all major organ systems were sampled, examined and the results recorded, the histopathology findings from five target systems were summarized in this report: the respiratory system, the immune system, the nervous system, skeletal muscle and heart, and the thyroid gland. These systems were chosen to facilitate comparisons to the results of the WISSA study.

The systems chosen for detailed reporting were based on historical concerns reported by cattle producers and the limited toxicological information available in the literature. The airborne contaminants associated with emissions from oil and gas field facilities are known to have diverse effects on humans and laboratory animals at concentrations associated with acute, high level exposures like those reported in some occupational toxicology studies. Much less is known concerning potential toxicological effects of chronic low-level exposure. For example, several studies have demonstrated adverse effects of chronic exposure to SO_2, H_2S and other malodorous sulphur compounds on respiratory health of oil and gas workers, sewer and water treatment workers, and the general public living in close proximity to sources such as pulp mills and areas of high geothermal activity. Commonly reported symptoms include increased incidence of respiratory infections, bronchial hypersensitivity, coughing and wheezing, and effects on airway resistance and lung function (ATSDR 1998, 1999). Chronic, relatively low level exposure to H_2S has also been associated with evidence of neurobehavioral impairment, and increases in the incidence of diseases of the nervous system and sense organs (ATSDR 1999). Inhalation of SO_2 and H_2S may impair pulmonary immune defenses by suppressing alveolar macrophage function (ATSDR 1998, 2006).

Inhalation of VOCs such as benzene and toluene is associated with respiratory tract irritation, but few other direct effects on the lungs are expected at commonly encountered airborne concentrations. Central nervous system dysfunction is the most important toxic effect reported in people chronically exposed to toluene. Neurotoxic symptoms, including decreased performance in tests of cognitive and neuromuscular function, hearing and visual impairment have been observed in occupationally exposed populations in numerous studies (ATSDR 2000). Benzene is a potent immunotoxicant and carcinogen, acting through suppression of bone marrow function to inhibit both cell-mediated and humoral immune responses, leading to decreased disease resistance in many species (ATSDR 1997). Like toluene, benzene also produces significant neurological abnormalities at relatively low airborne concentrations.

Within the range of concentrations examined in the WISSA study, exposures to SO_2, VOCs measured as benzene and toluene, and H_2S were not associated with the risk of lesions to tissues of either the immune or nervous system in calves that were aborted or died in spring 2002. Exposures to SO_2 and H_2S

were not significantly associated with the risk of lesions to respiratory tissues in calves that were born alive in spring 2002. Increasing postnatal exposure to VOCs measured as benzene and toluene were, however, associated with increased odds of respiratory lesions. The association between VOCs measured as benzene and respiratory lesions was significant for calves older than 3 weeks.

The association between exposure and the presence or absence of lesions in the thyroid gland and either skeletal or cardiac muscle were also examined in the WISSA study in response to interest generated by histopathological findings. The findings from the WISSA study suggested that during gestation, increasing exposure to SO_2 was associated with increased odds of lesions in either the skeletal muscle or myocardium, and exposure to the highest measured concentrations of VOCs measured as benzene was associated with increased odds of lesions in the thyroid gland.

The percentage of animals examined that had lesions in one or more tissues was similar between the CLS herds and calves examined as part of the WISSA study for most systems and age groups. Lesions in some of the systems examined were potentially more common in a few age groups in the CLS herds in 2004 and 2005, but not in 2006 and 2007. The types of lesions described were also different in 2006 and 2007. For example, most of the thyroid lesions in this period were simply described as abnormal colloid and most affected calves did not exhibit hypoplasia, degeneration, or necrosis. The proportion of calves examined histologically with evidence of skeletal myopathy was also considerably lower than in 2004 and 2005 or than in the WISSA study. Formal statistical comparisons were not done because of the small numbers of samples available in the CLS study. The data do suggest that the occurrence of these lesions may change from year to year and that future research should focus on identifying risk factors that might be associated with annual fluctuations in risk.

Factors associated with the occurrence of muscle and thyroid lesions

Many of the stillborn calves and neonates examined in the WISSA study that died within 3 days of birth had changes in the thyroid gland, skeletal muscle and heart. Large numbers of both stillborn calves and dead neonates had microscopic evidence of thyroid problems including follicular hypoplasia and lack of colloid, follicular degeneration or necrosis, or follicular hypertrophy (goiter). There were also a large number of calves in this group with evidence of degeneration in the skeletal and heart muscles or myopathy and necrosis. Prior to the WISSA study, the nature and extent of these tissue changes had not been previously documented in beef calves from Western Canada. Further work was necessary to determine if these lesions would be seen in other calves in years after the WISSA study and whether these lesions are responsible for some of the clinical signs attributed to the 'weak calf syndrome'.

The thyroid gland is the source of thyroid hormones (T3 and T4), which are responsible for regulating metabolic rate (Capen, 2000). The metabolic rate in calves with underdeveloped (hypoplastic) or degenerative thyroid glands would be affected, compromising the calf's ability to function and survive. The thyroid lesions described as hypoplastic or degenerative are different from that seen with iodine deficiency induced goiter. The causes of hypoplastic or degenerative thyroid lesions in full term calves are not documented; however, in utero stresses including nutritional factors such as vitamin A or E deficiencies are being investigated.

White muscle disease, or nutritional myopathy, is the best known and most commonly reported manifestation of vitamin E and/or Se deficiency in livestock. However, vitamin E deficiency, alone or in conjunction with selenium deficiency, has been linked to several other diverse clinical syndromes including increased risk of abortion and stillbirth, neonatal weakness, ill thrift and impaired immune function. In the WISSA study, lesions of skeletal myopathy were identified that differ microscopically from the classic lesions seen in white muscle disease. The age of the calves affected with these unusual lesions was also different than previously reported; the affected calves were primarily under 3 days of age. Most calves affected with classical white muscle disease are a few months old and on pasture.

Very active muscles, such as the tongue and diaphragm, are often the first and most severely affected. The calf could be unable to suck if the tongue is affected, increasing their risk of succumbing to starvation

or exposure. The prevalence of myopathy lesions was higher in the tongue in the 2006 and 2007 CLS samples than in any other muscle tissue. The association between damage to other muscles and the ability of the calf to position itself properly for parturition is unknown. After birth, affected calves could have difficulty rising. The prevalence of skeletal muscle lesions were similar to or higher in the CLS herds in 2004 and 2005 than in the WISSA study, but were lower in 2006 and 2007. Cardiac muscle in the heart can also be affected. Calves that succumb to cardiac failure due to myocardial necrosis, often die acutely, with no visible explanation on post mortem examination. Both selenium and vitamin E can account for these changes. The prevalence of heart lesions in the 2006 and 2007 CLS was very low (<2%).

With the cooperation of the Alberta Beef Producers a series of studies were done examining liver and serum micronutrient status, thyroid hormones, muscle damage as indicated by increased serum muscle enzyme activity, and calving difficulty. Laboratory results were difficult to evaluate in these samples because normal values are not well documented in this age of calf. The proportion of animals in these herds classified as deficient was much lower based on liver results than based on the serum analysis. No obvious risk factors were identified to account for the occurrence of either muscle or thyroid lesions in these calves.

There was no difference in the proportion of calves with muscle lesions that were or were not assisted at birth. There was no association between the occurrence of myopathy in these calves and liver concentrations of either selenium or vitamin E. Serum levels of an enzyme known as creatine kinase were also measured as an indication of muscle damage in live calves. Calves that were assisted at birth had significantly higher CK activity than calves that were not assisted. There was no association between serum selenium concentration and the serum CK activity. However, low serum vitamin E levels were associated with significantly higher CK concentration. This could suggest that calves with lower levels of vitamin E are more susceptible to muscle damage; however, it might be a result of failure of calves with muscle damage to ingest adequate amounts of colostrum. Colostrum is an important source of vitamin E to newborn calves.

Reduced blood flow to the thyroid gland was considered as one potential factor contributing to the histological lesions reported in this study. Reduced thyroid function has been shown to occur in human infants subjected to hypoxia prior to parturition (Avry et al., 1999; Pereira and Procianoy, 2003). There was no difference in the frequency of lesions in calves that either were or were not assisted at birth. However, T3 concentrations were significantly lower in calves that were assisted at birth compared with those that were not. T4 concentrations also tended to be lower but the difference was not significant.

Another potential explanation for the thyroid lesions is abnormal thyroid development and function *in utero*. This would be consistent with the lack of colloid and the pale staining colloid in the thyroid follicles of aborted and stillborn calves, and a decrease in lipofuscin production in follicular epithelial cells as was observed in samples from the WISSA study. The accumulation of lipofuscin in follicular cells with age is responsible for the progressive red-brown darkening of the thyroid (Jubb et al., 1985). Unlike the WISSA study, none of the calves in the 2006 or 2007 CLS samples had reduced levels of lipofuschin.

Nutritional deficiency is one possible explanation for abnormal development. In the few cases where hyperplastic goiter was diagnosed, the most likely explanation for the thyroid lesion is iodine deficiency (Jubb et al., 1985). There was no association between serum selenium, vitamin E, copper, or molybdenum concentrations and the occurrence of thyroid lesions. Serum T3 and T4 concentrations within 48 hours of birth were not associated serum vitamin A or E concentrations. The association between vitamin A and E concentrations and thyroid activity was examined because of concern that the drought in 2001 might have contributed to poorer quality feed and decreased availability of fat soluble vitamins (limited green grass in summer 2001 and old hay fed winter 2002) and the unexpected and relatively high occurrence of these lesions.

Long-term monitoring of herd health and productivity

The objective of the CLS was to establish a long-term baseline for beef herd health and performance in the SPOG area. This long-term database has provided very important complementary information to the WISSA study which examined a large number of herds over a large geographic region, but only looked at one production cycle. The herd performance measures from the two studies show a very similar pattern. There were no substantial long-term trends noted in the CLS study. While individual herd productivity in the CLS study did vary dramatically for some herds from year-to-year, there were no substantial measurable year effects in either the risk of non-pregnancy or abortion. There was, however, significant annual variation across the CLS study with recent peaks in the risk of both stillbirth and calf mortality in 2003, 2006, and 2007.

The idea of a productivity surveillance program was initially attractive to all stakeholders, particularly as an alternative to the previously reported methods of utilizing "high-tech" toxicological tests to measure the presence of suspected toxins in the meat or blood of an animal and then attempting to interpret the health and economic implications (Rubes et al., 1992; Chamberland et al., 1994). There are no currently available biomarkers in animals that are sufficiently sensitive and specific to allow for a simple toxicological assessment of the potential exposure-effect relationship in field investigations (Beck, 1992; Lodgepole Blowout Inquiry Panel, 1984) particularly with long-term exposure to emissions from oil and gas field facilities.

Surveillance of locally owned herds was originally chosen over an experimental study design to address the questions of area residents and interested regulatory agencies because all of the potential exposures could not be accurately predicted and modeled in a laboratory setting or adequately represented by studying one or two experimental herds. There were a large number of potentially interacting exposure variables complicating the effect of emissions from the oil and gas industry on animal health beyond what could be reasonably duplicated under laboratory restraints including: changing meteorological conditions; variation in the concentration and composition of raw gas and liquid from potential releases, leaks or fugitive emissions; the dynamic composition of incomplete combustion products potentially present in the plumes of the flare or incinerator stacks; and the potential for unpredictable interactions of combinations of these chemicals (Bott, 1993).

Monitoring of completely unexposed control herds was not used in the present study design for several reasons. A valid control group would have to be similar to the Caroline study herds for most, if not all, important determinants of productivity except for potential exposure to the petrochemical industry. It was not possible to locate a nearby area containing a sufficient number of interested herd owners with similar feeding and management practices, climate, topography, and vegetation that had no exposure to oil and gas activity with sufficient air-quality data to verify this assumption.

Historical controls or comparisons must, however, be interpreted with caution (Martin et al., 1987). There are many potential variables, some of which are difficult or impossible to measure, that could have resulted in productivity changes from one year to the next. The other potential concern with a long term surveillance project is that the data made available to the participants affected other herd production parameters. No biological data beyond post-mortem examinations have been collected in recent years. Most of the data are collected by the herd owners themselves; very little new information is provided that could influence herd management.

Intensive long-term studies of this type are difficult and costly. The benefits of this approach are, however, significant. Measurement error and recall bias are minimized because data are collected as soon as information becomes available and are reviewed for herds and individual animals by both the participants and study personnel. Herd health problems are identified early during regular visits to the herds while thus providing the opportunity for a complete investigation. As the regular herd veterinarian does the pregnancy checking, bull evaluations, and post-mortem examinations, the study should not interfere with the relationship between the herd owner and veterinarian. An additional benefit of the herd

veterinarian's participation is that any potential for observer bias by study personnel is minimized by using professional diagnoses independent of the study team.

The biggest limitation of active surveillance is the cost. There is no evidence, however, that this approach is more expensive than other strategies — particularly laboratory toxicological assessment. Therefore, the primary disadvantage of this type of long-term monitoring project is that the expense and time required with such a protocol places a practical limit on the number of herds that can be monitored. However, the same degree of accuracy and accountability achieved through this study is not possible through phone or mail surveys. A local veterinarian or technician must visit the herd on a regular basis to collect valid health, production and exposure data. So, while this type of design has been criticized for limited generalizability because of the small number of herds, it is the only method that allows for the attention to detail in data collection that is so critical to ensure the validity of the results.

Air monitoring data

No air monitoring data were collected as part of the CLS study in 2006 or 2007. SO_2 concentrations were monitored in the herds that were part of the CLS study in 2005. Passive monitors were placed on study herds and provided new information in addition to that from the existing monitors from the PAMZ network which overlaps much of the study area. Monitors were placed on each location containing cattle from study herds for each month of the 2005 calendar year. Passive samplers were analyzed for monthly mean SO_2 concentrations using similar technology and methods to that applied in both the WISSA study and PAMZ air monitoring program.

The SO_2 concentrations measured from the CLS herds were higher than that from the PAMZ monitors in 2005, but were consistently lower than those measured in the high exposure herds from the WISSA study and the PAMZ monitors in 2001-2002. The SO_2 measurements from the half of the WISSA sites with the highest reported concentrations were also consistently higher than those of the PAMZ sites in all but two of the months examined. For all months, the upper 25% of SO_2 measurements from the WISSA sites were substantially higher than those observed at the PAMZ sites. This suggests that at least a quarter of all of the WISSA sites had SO_2 measurements that exceeded the data from PAMZ in 2001 and 2002.

The long-term baseline information from the PAMZ network was necessary to interpret the differences between the CLS and the WISSA data. These data suggest that while SO_2 exposure at the sites of the PAMZ monitors and the CLS herds were not equivalent to the most highly exposed herds in the WISSA study, that most of the CLS herds are in relatively highly exposed areas within the local air-shed management zone.

Part of the difference in the CLS (2005) and WISSA numbers (2001-2002) appears to be associated with a consistent improvement in the mean annual SO_2 concentrations in the PAMZ area since 2000. Although the technology used to monitor air concentrations was the same, the approach to locating the equipment for exposure monitoring for the CLS and WISSA studies was different from a fixed network such as is used in the PAMZ model where the monitoring sites are fixed at specific locations and the number of monitors does not change during the year. That model provides data that can more easily be used to look at seasonal and long-term trends. However this network approach does not provide us with specific information on the concentration of substances to which individual groups of cattle are exposed during any period of time. The model used in the CLS and WISSA studies, where the monitors are located with the cattle, was intended to measure the exposure of all groups of cattle as closely as possible at each point in time rather than giving a more general picture of regional air quality.

CONCLUSIONS

Livestock health and productivity data can be used as a measure of environmental health. Beef cattle may be particularly useful environmental sentinels as they are more consistently exposed to potential sources of environmental contamination than many other species. In contrast to both humans and other more intensively managed livestock species, beef cows are housed almost exclusively outside and are fed primarily locally grown forage. Several productivity and health indices can be objectively and accurately measured. Other risk factors known to affect productivity can also be measured or minimized by good management practices.

Long-term accurate collection of data from designated sentinel herds is possible and can provide a valuable new tool for environmental monitoring programs. The analysis provides important information on general health of livestock in the region under study as compared with other areas. The data can also be used to monitor for sustained and substantial changes in health and productivity of the sentinel herds. This type of local program can be a valuable tool in community-based environmental monitoring. Data from large, outside studies may not be perceived by the general public as being applicable to local conditions. The information from this type of monitoring project is locally generated and audited and, therefore, may be perceived as being relatively credible to area residents and may better address community concerns.

Footnotes

[1] Calculated as a 40.5% proportional loss at birth of a total death loss of 5.6% (Mathison, 1993; page 47).

[2] Calculated based on 15% first calf heifers (7.9%) and 85% mature cows (2.8%) (McDermott et al., 1991a or b?).

[3] Calculated as a 924.4+17.0+5.2) 46.6% proportional loss from day 1 to weaning of a total death loss of 5.6% (Mathison, 1993; page 47).

[4] Calculated based on 15% first calf heifers (3.3%) and 85% mature cows (2.6%)(McDermott et al, 1991a or b?).

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