

# Causes of Stillbirth and Time of Death in Swedish Holstein Calves Examined Post Mortem

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– This study was initiated due to the observation of increasing and rather high levels of stillbirths, especially in first-calving Swedish Holstein cows (10.3%, 2002). Seventy-six Swedish Holstein calves born to heifers at 41 different farms were post mortem examined in order to investigate possible reasons for stillbirth and at what time in relation to full-term gestation they had occurred. The definition of a stillborn calf was dead at birth or within 24 h after birth after at least 260 days of gestation. Eight calves were considered as having died already in uterus. Slightly less than half of the examined calves (46.1%) were classified as having died due to a difficult calving. Four calves (5.3%) had different kinds of malformations (heart defects, enlarged thymus, urine bladder defect). Approximately one third of the calves (31.6%) were clinically normal at full-term with no signs of malformation and born with no indication of difficulties at parturition or any other reason that could explain the stillbirth. The numbers of male and female calves were rather equally distributed within the groups. A wide variation in post mortem weights was seen in all groups, although a number of the calves in the group of clinically normal calves with unexplained reason of death were rather small and, compared with e.g. those calves categorised as having died due to a difficult calving, their average birth weight was 6 kg lower ( $39.9 \pm 1.7$  kg vs.  $45.9 \pm 1.5$  kg,  $p \leq 0.01$ ). It was concluded that the cause of stillbirth with a non-infectious aetiology is likely to be multifactorial and difficult calving may explain only about half of the stillbirths. As much as one third of the calves seemed clinically normal with no obvious reason for death. This is a target group of calves that warrants a more thorough investigation in further studies.

*Autopsy; congenital defects; dairy calves.*

## **Introduction**

In recent years, we have seen a remarkable increase in the incidence of stillbirths in the Swedish Black and White Breed (SLB) concurrent with increased imports of North American Holstein genes. Today, the SLB breed can be regarded as a Swedish Holstein Breed. Stillbirth is commonly defined as a calf that dies just prior to, during, or within 12-48 h of parturition. The problem is most pronounced in heifers and during the past 20 years an increase

from about 6% to 10.3% has occurred. In contrast, in older SLB cows and in heifers and cows of the Swedish Red and White Breed (SRB), the increase has been of much lower magnitude with an overall incidence of around 5% (Swedish Dairy Association, 2003). See Fig. 1 for a description of the stillbirth rate during 1982-2002.

An increasing stillbirth rate is probably due to a multifactorial cause. The problems appear to be

rather different today, compared with earlier experience, since stillbirths in calves born to heifers seem to be less closely related to high birth weight and to difficult calving than they used to be (Berglund & Philipsson 1992). It was concluded in that study that calf vitality appears to be of growing importance in the still-born syndrome. Sluiter et al. (1990) and Simpson (1990) also reported increasing incidences of stillbirths in heifer calvings in the Netherlands and in England, respectively. In the Dutch study it was found that the placenta was usually expelled together with the calf, which otherwise is a quite unusual feature. In the English study, evidence of trauma, often very severe with rib fractures, was found in the majority of dead calves.

During late bovine pregnancy several hormones are involved to maintain and develop a successful result with a live calf. Changes in endocrine

profiles in late bovine pregnancy have been related to abortions and stillbirths. Measurement of bovine pregnancy-specific protein B (bPSPB), pregnancy associated glycoprotein (PAG), and oestrone sulphate have been found useful for monitoring placental functions and indirectly also foetal viability (Dobson et al. 1993, Patel et al. 1997, Beckers et al. 1999, Kornmatitsuk et al. 2003).

The outcome of pregnancies can also be affected by pathogens causing stillbirths and abortions. Sweden is free from several serious infectious agents such as brucellosis, leptospirosis and IBR. Presence of Bovine Viral Diarrhoea Virus (BVDV) and *Neospora caninum* may play a role for neonatal mortality and calves that are very weak and die soon after birth. *N. caninum* infection causes stillbirth and abortion throughout pregnancy, but abortion at 5 to 6 months of gestation is most common

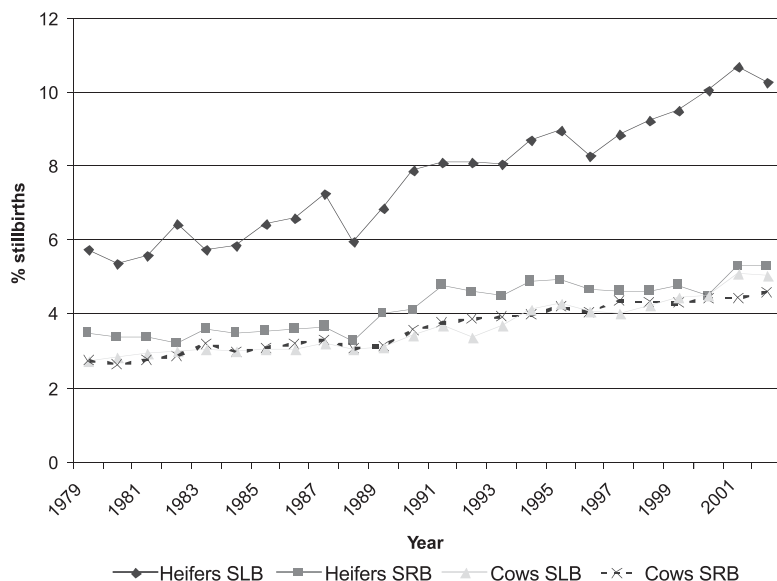


Figure 1. Annual statistics of stillbirth rate 1982-2002 in Swedish Holstein (SLB) and Swedish Red and White (SRB) breeds (Swedish Dairy Association).

(Dubey 1999). The prevalence of *N. caninum* is low in Sweden (Björkman *et al.* 2000). BVDV is important for early abortions (2-5 months) (Larsson *et al.* 1994). An eradication programme of BVDV started in 1993 (Lindberg & Alenius 1999) with a rapid decrease in herd prevalence, and today 95% of the herds are declared free from the infection. Giri *et al.* (1990) used endotoxins to induce abortions. The highest sensitivity for abortions was in the first trimester, but termination of pregnancy can occur at any stage of gestation.

Incompatibility between calf size and dam size, as well as pelvic and vulvar conformation, are factors likely to have an important impact on stillbirths. A prolonged but not necessarily difficult calving caused by, e.g. weak labour, might be a risk factor for calf mortality. For stillborn calves, the time interval from onset of labour until calving was completed was approximately twice as long as for liveborn calves (Berglund *et al.* 1987).

A large genetic variation in calving traits between daughter groups of SLB bulls is also evident (Berglund & Philipsson 1992, Steinbock *et al.* 2003). Genetic defects, e.g. a larger number of sublethal genes, lower the viability at birth and might be one explanation of the increased stillbirth rates. Some examples of recently identified genetic defects in the Holstein breed are Complex Vertebral Malformation (CVM), bulldog syndrome, Bovine Dilated CardioMyoPathy (BDCMP) and Bovine Leukocyte Adhesion Deficiency (BLAD). Other defects, which we do not know about yet, might be present and new defects will certainly turn up in future.

Furthermore, increasing average herd sizes, which most likely gives less time for supervision of calving, might result in a larger proportion of difficult calvings being recorded as stillbirths. The large number of stillborn calves is both an ethical and an economic problem. The

economic problem comprises loss of the calf, lower fertility of the dam at the next breeding, longer calving to conception interval, and a tendency for milk production to be decreased (Chassagne *et al.* 1999).

This study was initiated due to the observation of increasing and rather high levels of stillbirths, especially in first-calving Holstein cows. The objective was to investigate possible reasons for stillbirths and to establish the time they had occurred in relation to full term. Therefore, a post mortem examination of Swedish Holstein calves born to heifers was carried out. Increased knowledge of the background to these problems is important both for management and breeding purposes in the effort to reduce stillbirths and calving problems.

#### Materials and methods

Farmers from 200 Swedish Holstein herds were asked during the years 1994-1996 to send stillborn calves from primiparous cows for post mortem examination. The definition of a stillborn calf was dead at birth or within 24 h of birth after at least 260 days of gestation. Altogether 76 Swedish Holstein calves were examined for reasons of stillbirth, including establishment of the approximate time of death. The calves were born at 41 different farms and all were singletons except for 2 pairs of twins. The post mortem examinations were performed in 3 different regions in southern Sweden Halmstad (Svelab), Kristianstad (Svelab) and Skara (AnalyCen) at laboratories with the required competence and facilities for pathological/anatomical investigations. A further prerequisite was that the laboratories were located reasonably close to the herds.

A standard post mortem examination protocol was developed for this purpose. The protocol included information on sex, weight and length of foetus (crown rump length). An assessment was made if the foetus was alive during parturi-

tion or dead in uterus, and if so, approximately for how long before expulsion. Calves with signs of dehydration and mummification were regarded as having been dead for more than one week before expulsion and calves with subcutaneous oedema and hydropsies in the chest and abdominal cavity for less than one week. Calves that had died during the process of birth showed signs of partially inflated lungs, and/or subcutaneous, subdural or internal bleedings.

A macroscopic investigation of the most important organs (heart, liver, lungs, kidneys, spleen, brain and of navel region) was performed. Lungs were checked as to whether they were inflated or not. Samples from lungs, liver heart and muscle were routinely taken for histological examination when there was a reason to suspect changes. Visible signs of inflammatory changes were recorded and samples for relevant microbiological examination were taken. Placenta was checked when available. Congenital defects/malformations visible as severe anatomic deformities were recorded.

The study was part of a larger study where preparing for calving and the calving process of about 4000 first calving Swedish Holstein cows was followed by detailed observations recorded in calving reports (Berglund 1996). These contained information about age and pedigree of heifer, insemination dates and pedigree of bull, calving date, single or twin birth, calving ease

(1=easy, 2=normal, 3=difficult), attitude of calf, status of cow after calving and retained placenta. In addition, the calving reports also contained information from the herdsman on the sex of calf, birth weight, viability (born alive or dead/died within 24 h) and on any visible malformation.

Data from all sources were compiled to form cause and time of death categories based on the presence of one or several of the criteria in each category as follows:

#### *Difficult calving*

Trauma associated with technical assistance at delivery such as subcutaneous, subdural or internal bleedings, and external lesions. Abundant amounts of mucus in the respiratory tract which may be a sign of ceased placenta function before the birth process was completed, resulting in suffocation of the calf. In cases of uncertainty in interpreting the pathological findings, the report of a difficult calving from the herdsman was used to strengthen the assessment.

#### *Intrauterine death*

Subcutaneous oedema and hydropsies in chest and abdominal cavity or dehydration/mummification. The state of decomposition as an indicator of how long the calf has been dead in uterus.

Table 1. Cause and time of death categories and sex of calf for 76 stillborn Swedish Holstein calves.

Cause and time of death	N	%	Male calf	Female calf
Difficult calving	35 <sup>a</sup>	46.1	16	16
Intrauterine death	8	10.5	3	5
Clinically normal, unknown cause of death	24 <sup>b</sup>	31.6	10	12
Malformations	4	5.3	2	2
Unspecified infections	2	2.6	2	0
Unknown cause/not possible to categorise	3 <sup>b</sup>	3.9	0	1

<sup>a, b</sup> recording of sex missing for <sup>a</sup> 3 calves, <sup>b</sup> 2 calves

Table 2. Post mortem weights of calves (kg) in different cause-and-time of death categories.

Cause-and-time of death	N	Mean	SD	Min.	Max.
Difficult calving	32	44.9	7.6	32	62
Intrauterine death	6	44.7	11.4	31	60
Clinically normal, unknown cause of death	23	40.2	8.4	24	55
Malformations	4	45.0	15.0	23	56
Unspecified infections	2	47.5	3.5	45	50
Unknown cause/not possible to categorise	2	38.0	2.8	36	40

#### *Clinically normal at full-term, unknown cause of death*

Clinically normal (well-formed) calves. Lack of external signs of difficult calving. Foetal or partial neonatal atelectasis. No malformations.

#### *Malformations*

Calves with visible malformations. Apart from the specific malformation no consideration was paid to whether the calves were clinically normal at full-term or not.

#### *Unspecified infections*

Calves with unspecified infections (born after normal gestation length).

#### *Unknown cause/not possible to categorise*

Not fulfilling the criteria for clinically normal at full-term, unknown cause of death. Full information such as, e.g. age of calf missing.

The GLM procedure in the SAS package (SAS institute Inc., 2000) was used to analyse the effects of sex of calf and cause and time of death category on birth weights.

## **Results**

Table 1 shows the distribution of calves in different cause-and-time of death categories. Slightly less than half of the calves (n=35) had signs of a difficult calving (subcutaneous, subdural or internal bleedings). Among these, 8 calves also had reports from the herdsman of a

difficult calving. Eight calves were regarded as already dead in uterus, one of them for more than one week. No macroscopic findings indicated the cause of death in this group. Four calves (5.3%) were malformed. The malformations found were: one calf with enlarged thymus (5cm×15cm), 2 calves with heart defects (chamber septum defect and persistent foramen ovale), and one calf with a urine bladder defect. Two calves died within 24 h of birth after full-term gestation due to peritonitis and gastro-enteritis. Approximately one third of the calves were clinically normal at full-term with no signs of malformations and born with no indications of difficulties at parturition or any other reason that could explain the stillbirth. The remaining 3 calves could not be categorised, partly due to the lack of certain data. The numbers of male and female calves within each cause and time of death category were rather equally distributed (Table 1).

From the calving reports a note made by the herdsman on the ease of calving was made in connection with 46 parturitions, 13 of which were classified as difficult. The post-mortem examination showed that 3 calves out of the 76 examined had fractured ribs (1) or fractured spinal column (2). There were no reports from the herdsman of expulsion of the placenta together with the calf. Seven cases of retained placenta were reported. These were rather evenly distributed over the different cause and time of death categories.

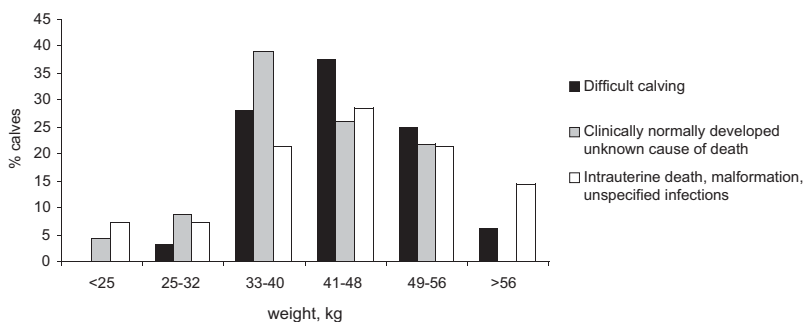


Figure 2. Frequencies (%) of calves in the different cause-and-time of death categories distributed over birth weight classes.

The examined calves weighed from 23 to 62 kg ( $n=69$ ), with an average body weight of 43.2 kg ( $SD=8.7$  kg). In Table 2, the average weight at post-mortem inspection of calves in the different cause and time of death categories are shown. Fig. 2 shows the frequencies of calves in different categories of death distributed over birth weight classes. Calves were grouped in 3 categories: difficult calving, clinically normal with unknown cause of death, and a third group comprising the other causes of death. A wide variation in post mortem weights can be seen for all groups. The clinically normal calves with unknown cause of death had a centre of gravity shifted to the left, indicating slightly lower weights.

The analysis of variance showed that the overall difference in birth weights between the different cause and time categories of deaths was non-significant. But when the group of calves categorised as clinically normal at full-term with unknown cause of death was compared with the category of calves with difficult calving, the former group was 6 kg lighter ( $39.9\pm 1.7$  kg vs.  $45.9\pm 1.5$  kg,  $p\leq 0.01$ ). There was a significant effect of sex of calf on birth weight, male calves weighing  $45.4\pm 2.0$  kg and female calves  $38.3\pm 2.4$  kg ( $p\leq 0.001$ ).

## Discussion

The classification of calves in different cause and time of death categories was primarily based on the pathological findings in the post mortem examination. Since data on the calving process were collected under field conditions in several different farms, all calvings were not supervised. This means that the finding of a dead calf does not necessarily mean that the calf was born dead. This is also why the commonly used definition of a stillborn calf includes a certain time period after birth. Furthermore, the criteria for the judgement of calving difficulty is likely to vary between herdsmen. This is probably also an explanation of why not all calves from calvings reported as difficult by the farmer fell into this category as a result from the pathological investigation. Thirteen of all calvings were reported as having been difficult by the farmer. Based on pathological findings eight calves from these 13 calvings were classified in the category difficult calving. Slightly less than half of the examined calves were classified as having died due to a difficult calving. This corresponds well with what has been recorded by herdsmen in larger field materials where less than half of the stillborn calves were from an easy or a normal calving

(Berglund & Philipsson 1992, Steinbock *et al.* 2003). Genetic analysis of stillbirths also shows that half of the variation in stillbirth still remains after adjusting for calving difficulties (Steinbock *et al.* 2003). Thus, only about half of the variation in stillbirths is explained by difficult calving. Difficult calving is normally more common in connection with the birth of male calves (e.g. Steinbock *et al.* 2003). In the present study, the distribution of sexes in this category of death was even but the large difference in birth weights between male- and female calves was mainly due to some very large male calves in this category.

One of the signs used to classify a difficult calving was subcutaneous bleedings as these might be a sign of trauma in connection with the expulsion of the calf. Another possibility might also be that the bleedings had already started in the uterus due to a sub-optimal environment.

About one tenth of the stillborn calves were classified as dead in uterus. The cause of death is not known and could only be speculated upon as dysfunction of the placenta, hormonal changes or a nonvisible defect in the calf, or some combination of these. These calves had about the same average birth weight as calves in the total material. Thus, generally there was probably no intrauterine growth retardation, which often is the case when there is a dysfunction of placenta (except for possibly one calf weighing 31 kg classified as having been dead less than one week in uterus). Smyth *et al.* (1999) found that calves with leptospiral antigens detected in the placenta were significantly lighter by an average of 6 to 10 kg than calves with no antigen in the placenta.

The incidence of malformed calves was considered rather normal and in accordance with what has been reported in earlier studies. In a Swedish investigation of 104 aborted calf foetuses (from 2.5-9 months of gestation) during 1987-1988, 6 calves (5.8%) were malformed

(Elvander 1991). The various types of malformations both in that study and in the present study were in line with what has previously been reported.

There was a wide variation in birth weight of the stillborn calves, although they were probably, on average, slightly heavier than live born calves are. In the inquiry study, stillborn calves (n=261) were only 1.6 kg heavier than the average weight of all calves (n=2825). Calves born at a parturition classified as difficult (n=345) were as much as 4.0 kg heavier than calves born at a normal calving (unpublished results).

For as many as one third of the calves, no obvious cause of death could be seen. They were apparently born clinically normal after full term. A number of these calves had a rather low birth weight. Dysfunction of the placenta might be one reason for small, but otherwise apparently normally developed, calves. In a recent Swedish study (Kornmatitsuk *et al.* 2003) comprising 96 calvings no differences in the morphology of the foetal membranes could be observed, however, between cows with viable calves compared to cows with stillbirths. A weak calf syndrome characterised by the birth of full-term calves which either failed to breathe or did not breathe for longer than 10 min was recorded by Rice *et al.* (1986). This syndrome was suggested to be called stillbirth/perinatal weak calf syndrome (SB/WCS) by Smyth *et al.* (1992). However, in these studies from Northern Ireland many of the weak calves were born to multiparous cows, and consequently this situation differs from ours. An abnormal thyroid is a common finding in SB/WCS, but McCoy *et al.* (1995) found that iodine and selenium supplementation did not lower the incidence of SB/WCS. Nor could an experimental iodine deficiency induce SB/WCS (McCoy *et al.* 1997), and it was concluded that other factors were likely to contribute. It is not known if the cause of death in the present study was due to any hor-

monal deficiencies since endocrinology was not specifically studied and thus beyond the scope of this study.

Calves with the genetic defect CVM have a reduced viability and a lower body weight compared with normal calves. Embryonic mortality occurs soon after conception and throughout the entire gestation period, and recent Danish studies (Nielsen et al. 2003) show that very few calves are born alive at full-term gestation. According to our recordings, we had no case of CVM in our material.

There were very few calves with signs of severe trauma such as rib fractures in our material, and we had no reports of placentas expelled together with the calves, and thus our conditions (e.g. herd management) also appear to differ from those described in connection with weak calves in heifer calvings from England (Simpson 1990) and from the Netherlands (Sluijters et al. 1990).

Stillbirth rates for primiparous Holstein cows in Sweden are now close to the levels recently reported from the United States by Meyer et al. (2001). They also reported a slightly negative, but non significant, genetic trend in perinatal survival. Increased knowledge of genetical as well as non genetical possible causes of stillbirths is therefore important. Furthermore, it is important to consider the calving traits in the breeding evaluation of bulls and to use bulls that inherit a low rate of stillbirth and calving difficulty. Additionally it is important to have national control programs for congenital genetic defects to avoid multiplication of deleterious genes. Moreover it is important that all malformations are reported.

*In conclusion*, the cause of a stillbirth of a non-infectious aetiology is likely to be multifactorial and difficult calving may explain only about half of the stillbirths from primiparous cows. As many as one third of the calves seemed to be clinically normal and born at full-term with no

obvious reason for death, which could be regarded as a surprisingly large proportion of the calves. Although a wide variation in birth weight was seen in all categories of death, these calves were, on average, lighter than the other calves. This is a target group of calves that warrants a more thorough investigation in further studies.

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## Sammanfattning

### Obduktionsstudie av dödfödda SLB-kalvar - dödsorsaker och dödstillpunkt.

Andelen dödfödda kalvar har ökat och den genomsnittliga frekvensen hos förstakalvande SLB är nu 10,3% (2002). Detta utgjorde bakgrunden till att denna studie initierades där 76 dödfödda SLB-kalvar från förstakalvare i 41 olika besättningar obducerades med syfte att fastställa dödsorsak samt tidpunkt i förhållande till födseln. Definitionen av en dödfödd kalv var att den efter fullgången dräktighet (minst 260 dagar) var död vid födseln eller inom 24 timmar efter födseln. Åtta kalvar (10,5%) bedömdes ha dött redan i livmodern. Knappt hälften av kalvarna (46,1%) bedömdes ha dött på grund av svår kalvning. Fyra kalvar (5,3%) hade olika typer av missbildningar (2 hjärtmissbildningar, 1 förstörd thymus, 1 urinblåsedefekt). Nära en tredjedel av kalvarna (31,6%) var fullt utvecklade och utan några tecken på missbildningar, kalvningssvårigheter eller andra or-

saker som kunde förklara dödfödelsen. Fördelningen av tjur- resp. kvigkalvar inom de olika kategorierna var i stort sett lika. Variationen i födelsevikter var stor inom alla kategorier, men den genomsnittliga födelsevikten var ca 6 kilo lägre för den tredjedel kalvar som föddes fullt utvecklade med oförklarad dödsorsak. Det konkluderades att orsaken till dödfödslar

(med en icke-infektiös sjukdomshistoria) är multifaktoriell och att svåra kalvningar bara förklarar cirka hälften av dödfödslarna. Att det hos en så stor andel som en tredjedel av kalvarna tycks finnas någon form av oförklarad vitalitetsproblem gör att orsakerna till dödsfallen för denna grupp av kalvar behöver studeras ytterligare i framtida studier.

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