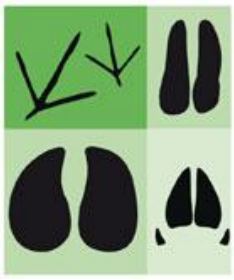

Perinatal Losses in Beef Herds in Orkney: Assessing Incidence and Associated Pathology from General Practice

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Abstract

There is a long history of high quality beef production in the Orkney Islands; however, perinatal losses (death of a full term calf from birth to 48 hours old) remain a major loss of potential income to producers. In an ideal situation, perinatal losses should occur in <2% of all calving cattle on British beef herds (Caldow et al., 2005). Local veterinary surgeons in Orkney perceived a high incidence of perinatal losses in beef herds which prompted further investigation of the incidence and aetiologies of these losses. A post mortem examination protocol and diagnostic algorithm were developed for the systematic investigation and categorisation of bovine perinatal losses in beef cattle, to allow the establishment of time of death, proximal cause of death and contributing factors to death.

The incidence of perinatal losses and association with specified calving-related factors were described in a convenience sample of beef suckler herds in Orkney (n=11 herds, 1101 cows) (targeted herds) for the 2016 calf crop (1st February to 10th June). The proximal cause of calf death and contributing risk factors to death were determined in beef calves presented to a veterinary practice in Orkney for the 2016 calf crop from both targeted and passive herds. Targeted herds were defined as recruited herds, which were required to submit all perinatal losses. Passive herds were defined as herds submitting calves ad-hoc according to farmer motivation, with no further perinatal loss submission requirements.

A total of 53 calves were submitted for gross post-mortem examination and further testing. Bovine perinatal mortality incidence varied from 1.6% to 12.4% across targeted herds, with an overall incidence of 5.1%, representing a higher incidence than the target for British beef herds. A proximal cause of death was reported for 89% of submissions. Diagnoses for perinatal losses included; anoxia, infection, congenital malformation and traumatocia. In submissions from targeted herds, death due to anoxia developing during stage two of parturition represented the largest cause of death (58%), with varying contributing factors. This was in comparison to submissions from passive herds, where death due to infection represented the largest cause of death (40%). Through application of a systematic diagnostic protocol, this study has indicated that perinatal losses in beef herds are a significant problem and require further industry attention to reduce losses.

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Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Rhona Norquay

Abbreviations

AI Artificial Insemination

BOHV Bovine Herpes Virus

BVDV Bovine Viral Diarrhoea Virus

DGR Dangerous Goods Regulations

DM Dry Matter

EBV Estimated Breeding Value

ELISA Enzyme Linked Immunosorbent Assay

FSC Foetal Stomach Contents

g Grams

IBR Infectious Bovine Rhinotracheitis

ID Identification

L Litre

PALS Periarteriolar Lymphoid Sheath

PCR Polymerase Chain Reaction

PM Post Mortem

PPE Personal Protective Equipment

PPS Premature Placental Separation

RT-PCR Reverse Transcriptase Polymerase Chain Reaction

SRUC Scotland's Rural College

TMR Total Mixed Ration

VAT Value Added Tax

VIDA Veterinary Investigation Diagnostic Analysis

ZST Zinc Sulphate Turbidity

1. Introduction

1.1 Parturition and Perinatal Mortality in Beef Herds

Production efficiency in beef herds can be measured by the number of calves produced per year, compared to the number of cows mated (Caldow et al., 2005). In an ideal situation, 95 calves per 100 cows mated should reach weaning; however, only around 25% of British beef herds actually achieve this (Caldow et al., 2005). Failure to achieve this target can result from infertility, abortion, perinatal losses or losses in pre-weaned calves. Parturition is a very complex process (Mortimer, 2009), and is one of the highest risk periods for morbidity and mortality of both the calf and the dam. As a consequence, compromise to the welfare of both the dam and/ or calf may result, with additional significant productivity implications for the farmer. Calving management is considered to be one of the most important associated factors in herds with high calf mortality (Vasseur et al., 2010), as it is thought that around 60% of dairy calf losses occur at birth (Spicer et al. 1994). In dairy herds, around 90% of all perinatal losses are alive at the start of parturition, 75% of all perinatal losses die within one hour of calving, and 15% of all losses die over one-hour post-partum (Mee, 2004a), so many of these deaths should be preventable through better management at parturition.

The vast majority of research into bovine parturition and perinatal mortality has been performed in dairy herds, with a lack of extensive data available from beef herds. It may be assumed that any previous study referred to in this chapter was performed in dairy herds, unless stated otherwise.

1.1.1 Normal Gestation and Parturition

Normal gestation length in cattle is around 280 days, but there is a natural variation of around ten days (Caldow et al., 2005). Initiation of parturition occurs in response to foetal stress, resulting from placental insufficiency due to foetal maturity and uterine enlargement, and other undefined stimuli (Mortimer, 2009). This leads to increased production of glucocorticoids by the foetus, which in turn leads to prostaglandin production from the foeto-maternal unit.

Prostaglandin will also be produced from the endometrium, leading to release of oxytocin in the dam. Cervical dilation will ensue, followed by contraction of uterine muscles. This process is known as stage one of parturition and can last between three and six hours. Stage one of parturition is typically displayed as restlessness in the dam and strings of mucous may be visible at the vulva (Stuttgen, 2011). The second stage of parturition involves expulsion of the calf and can vary in length between five minutes and several hours, characterised by abdominal contractions and straining (Stuttgen, 2011). The foetus is forced into the birth canal, stimulating the release of oxytocin, which in turn, leads to abdominal contractions. During delivery, the greatest force of abdominal contraction is seen when the foetal head is expulsed through the birth canal and vulva. Following delivery of the chest, the oxygen supply from dam to calf is closed off due to compression of the umbilical cord in the birth canal (Mortimer, 2009). It is at this point, that the calf should establish breathing independently. The most common presentation of the calf is with the spine in a dorsal position and the head and two front feet presented (Mortimer, 2009). Alternatively, the calf may be presented posteriorly, with two hind feet presented. All other presentations and positions are considered abnormal (Mortimer, 2009). Expulsion of the placenta is known as stage three of parturition and should occur within one to two hours of the birth of the calf (Stuttgen, 2011). Parturition occurs as part of a cascade and is irreversible when started (Mortimer, 2009). It is expected that under normal circumstances the neonatal calf should be standing within thirty minutes of birth (Sorge et al., 2009).

1.1.2 Perinatal Mortality Definition

Perinatal mortality may be defined as the death of the foetus or calf before, during or within 48 hours of calving, following a gestation period of at least 270 days, irrespective of the cause of death or the circumstances of the calving (Mee, 2015a). There is a natural variation of about 10 days for normal gestation (Caldow et al., 2005) so by convention, calves born <270 days gestation are defined as abortions and any calves born dead after this time are referred to as stillbirths (Mee, 2015b). Neonatal mortality may be defined as the death of a calf, which is born alive after a gestation period of at least 270 days, but dies within the first 48 hours of life, and is therefore considered a sub-category

within perinatal loss. Although definitions of perinatal mortality vary, for the purposes of this study, calves up to 48 hours old are included in this definition, as this was consistent with recent similar studies in dairy calves in Ireland (Mee, 2015b).

1.1.3 Reported Incidence

Almost all herds (dairy and beef) will experience perinatal mortality, although the incidence of such losses can vary dramatically between farms (Mee, 2004a). The majority of work investigating perinatal mortality has focussed on dairy herds and there is increasing concern over the welfare of the calf and the dam, with regards to perinatal mortality (Ortiz-Pelaez et al., 2008) and the concern that these losses are becoming normalised (Mee, 2015b). In an ideal situation, perinatal losses should account for <2% of all losses on beef farms (Caldow et al., 2005). However, in 1990, perinatal losses in Great Britain (including both beef and dairy calves) were estimated at 3.5-5% (Roy, 1990). It is difficult to compare perinatal mortality rates in other countries due to varying definitions, but they do appear to be similar (Table 1.1). Unpublished data collected by Northvet Veterinary Group, Orkney in 2014, from clients of the practice, estimated the bovine perinatal mortality incidence in beef herds to be 4.5%, from data from 4367 cattle on 88 farms.

Table 1.1; *Geographic comparison of perinatal mortality incidence in dairy herds (*Mee, 2013a*)*

Country	Perinatal Mortality Incidence (%)	Definition of Perinatal Mortality
Norway	2.0	Death within 24 hours of calving
Switzerland	2.4	Death within 24 hours of calving
Ireland	4.3	Death within 24 hours of calving
Israel	5.0	Death within 24 hours of calving
Poland	6.7	Death within 24 hours of calving
New Zealand	7.2	Death within 48 hours of calving
France	7.4	Death within 48 hours of calving
Hungary	7.7	Death within 24 hours of calving

1.1.4 Impact: Welfare and Costs

Animal mortality is considered one of the most important indicators of welfare on farm (Ortiz-Pelaez et al., 2008), but there is a concern that bovine perinatal losses have become normalised on farms in recent years (Mee, 2011a), potentially leading to a compromise in welfare. There are many welfare issues surrounding perinatal mortality, mainly associated with the cause of death. For example, trauma associated with parturition, severe enough to cause death obviously compromises calf welfare through pain and injury (Laven et al., 2009). Furthermore, calving trauma that is severe enough to cause death to the calf is likely to cause pain and suffering on the dam. On the contrary, death resulting from anoxia is a more controversial issue with regards to animal welfare, as the calf is not in a conscious state and may not be able to elicit a physiochemical stress response, resulting in a compromise of its welfare (Mee, 2013a). In these circumstances, the issue becomes more of an animal rights one; however, the welfare implications of bovine perinatal losses is an issue which requires awareness at an international level (Mee, 2011a).

There are numerous costs associated with perinatal losses, both directly and indirectly (Table 1.2). Indirect costs are associated with feeding and housing pregnant dams during housing (for spring calving cattle), which subsequently fail to produce a live calf, future genetic loss and reduced number of replacement heifers. Direct costs include the potential future value of the calf (sold for breeding, fattening or direct to slaughter), costs associated with disposal of the carcass and post-mortem investigations. Other indirect costs may be associated with time spent dealing with perinatal deaths (i.e. nursing sick calves which subsequently die, sourcing replacement calves), which could be used more efficiently for herd production.

Table 1.2; *financial cost of perinatal mortality (Agriculture and Horticulture Development Board, 2015) (Scotland's Rural College, 2016)*

	Cost
Loss of sale of calf	£530.03
Purchased feed including minerals	£32.44
Home-grown feed	£6.76
Purchased forage	£15.39
Home-grown forage	£44.46
Vet bills and medicine	£29.52
Bedding	£28.29
Other livestock expenses	£16.58
Carcass disposal	£15.00
Post mortem examination	£59.00
Total loss	£247.44
Loss including future sale of calf	£777.47

Other indirect costs associated with perinatal mortality in beef herds include farm labour, machinery costs, electricity, fuel, property maintenance and land rental. There may also be a psychological cost to perinatal losses, especially when incidence of losses is high or where management practices are perceived to have contributed to the loss.

1.2 Factors Associated with Perinatal Mortality

There are many factors which influence the occurrence of perinatal mortality, including maternal, foetal, environmental and management factors (Mee et al., 2014). Risk factors for perinatal mortality are variables, which are associated with an increased prevalence of losses (Mee, 2015b). These variables can be modifiable or non-modifiable at farm level with most variables for perinatal losses in dairy herds associated with calving, rather than pre-calving factors (Mee, 2015b).

Historically it has been estimated that over 50% of stillbirths were related to dystocia, with other significant risk factors including age at first calving, foetal gender, twinning, primiparity, gestation length and season of calving (Collery et al., 1996). Increasingly, in primiparous cattle, more perinatal losses are reported to be occurring, as a result of placental dysfunction and low calf birthweights (Berglund et al., 2003) rather than following dystocias.

1.2.1 Perinatal Mortality Risk: Pre-partum Factors

1.2.1.1 Breed and Sire Effects

Incidences of perinatal mortality can range from <3% to >20% for individual sires, suggesting a significant effect of breed and genetics of the sire on these losses (Mee et al., 2014). Sires have a significant role to play with regards to dystocia and when selecting sires, a negative estimated breeding value (EBV) for gestation length and positive EBV for calving ease should be selected (Caldow et al., 2005). This is particularly important when selecting sires for use on heifers. The sire will also influence effective pelvic area in dams when breeding

replacement heifers, which in turn can significantly influence perinatal mortality rates (Mee, 2012). When selecting sires, it is also important that inbreeding levels are kept to a minimum within the herd, as high levels have been associated with increased perinatal mortality incidence (Mee, 2013a). Some sires may be associated with increased incidences of genetic defects, due to the presence of sub-lethal genes, so this should also be considered during selection to reduce perinatal mortality incidence (Berglund et al., 2003).

Sire effects on birth weights in the calf are significant, with heritability estimated at 0.40-0.45. There are differences between breeds and between sires within the same breed (Holland and Odde, 1992). The use of continental breed sires is associated with increased risk of dystocias, and thus increased perinatal mortality, compared to traditional native British breeds as a sire (Casas et al., 2011), due to variations in muscle mass and conformation. The differences in birthweights in calves likely occurs during the first trimester as differences in bodyweights can be seen between breeds by 100 days gestation (Holland and Odde, 1992).

No association between breed of dam and increased rates of perinatal mortality has been shown; however, there is an association between the levels of assistance required at birth. British breeds are less likely to require assistance during parturition than continental breeds (Waldner, 2014). Additionally, there may be a breed association with regards to mothering ability (Prayaga et al., 2014), which may be a contributing factor to perinatal mortality.

Crossbreeding of traditional British breeds of cattle with continental breeds, may also lead to problems with parturition, especially crosses with Charolais or Simmental breeds (Philipsson, 1976). Crossing with the Aberdeen Angus breed have been shown to reduce calving problems (Philipsson, 1976). These differences in calving difficulties can in part be explained by differences in birth weights, which in turn may be partly explained by differences in gestation lengths between breeds (Philipsson, 1977).

1.2.1.2 Breeding Method

Natural service compared to artificial insemination and embryo transfer has been associated with increased risk of perinatal loss in cattle. Commonly with natural service, the service date is unknown so the increased risk may in part, be due to lack of supervision with unpredictable calving dates (Mee et al., 2014). On the contrary, it has been reported that *in-vitro* fertilisation and embryo transfer are associated with an increased rate of perinatal mortality, thought to be due to increased birth weights of calves and extended gestation periods (Christie, 2002). Additionally, *in-vitro* embryos have been associated with abnormal placental vasculature and development, musculoskeletal deformities and asynchronous organ development, leading to late foetal death. Assisted reproduction (embryo transfer, multiple ovulation and embryo transfer, *in-vitro* production and cloning) can also be associated with large offspring syndrome, which presents as weak contractions in the dam, increased incidences of hydroallantois and congenital defects which can lead to increased perinatal mortality (Schmidt, 2007). Where calves are born alive, heart failure and/ or pulmonary insufficiency may be exhibited, often resulting in death soon after birth (Schmidt, 2007). This is reported to be more common in clones, however, which are not common place in the farming industry.

1.2.1.3 Pre-calving Movement

Movement to calving units for parturition can have a major influence on perinatal mortality rates due to stress associated with isolation, new environment and potential new diet (Carrier et al., 2006). It is recommended that the optimum time for cattle movement to calving units is 1-2 days pre-partum, which allows adequate time for the dam to become familiar with the new surroundings and allows blood glucocorticoid levels to return to normal by the time of parturition (Heuwiser et al., 1987). In free-stall housing, around 36 hours pre-partum, cattle will isolate themselves from the rest of the herd naturally, so movement to maternity units 1-2 days pre-partum aids in this natural isolation process (Bao and Giller, 1991). In animals which are confined for parturition, vulval constriction and irregular labour may result in dystocia, so should be monitored closely (Meijering, 1984).

Cattle moved to calving units during stage two of parturition (post rupture of the chorioallantois), have a reduced risk of perinatal mortality, compared to cattle which are moved during stage one of parturition (pre rupture of the chorioallantois) (Carrier et al., 2006). The reason for this is that movement during stage two parturition is associated with reduced time to first lie down, decreased duration of parturition and decreased risk of dystocia or assistance (Carrier et al., 2006), compared to movement during stage one of parturition.

Any rapid or hurried movement during parturition will increase the risk of a sudden slip or fall, which is associated with a high risk of a stillborn calf (Mee, 2004a). Disturbance of nervous cows and heifers with farm activity can suspend calving behaviour, so it is vital that cattle should remain undisturbed after movement to the maternity unit. This is particularly important with heifers, as pre-partum stress in heifers can lead to incomplete dilation of the vulva or cervix at parturition, increasing the risk of perinatal mortality through dystocia (Dufty, 1981). Ideally, heifers should be calved away from older cows but should always maintain visual contact with other cattle to reduce stress associated with social isolation (Mee, 2008).

1.2.1.4 Gestational Nutrition

Nutrition of the dam during gestation has a significant role in the occurrence of perinatal mortality (Mee et al., 2014). Over-conditioning of the dam through *ad libitum* feed, or high levels of concentrate in the ration during gestation may lead to foetal oversize, and potentially foetal malposition or uterine torsion at parturition, which will increase perinatal mortality (Benjaminsson, 2007; Mee, 2004a). Over-conditioned cows at the time of drying off are also at an increased risk of developing dystocia at the subsequent calving (Chassagne et al., 1998) due to intra-pelvic fat depositions (Caldow et al., 2005). Over-fat dams are at high risk of hypocalcaemia due to mobilisation of fat reserves at calving and reduced magnesium availability. This may result in uterine inertia, which can be seen as ‘slow calving syndrome’ leading to foetal death (Mee, 2013a). For spring calving beef cows, the ideal body condition score at calving is 2.5/5 (Caldow et

al., 2005). However, in a study by Berry et al., 2007, there was little significant evidence to associate body condition score or body weight with increased risk of dystocia or stillbirth; however, this was partly due to a lack of variation of body condition scores in this study. This study was performed in a research herd where it is assumed nutrition and management were of a high standard. It is possible however, that within the ranges of peri-parturient body condition score ranges seen on most farms, body condition score will not influence dystocia and stillbirth rates (Berry et al., 2007).

There are few studies examining the effect of low body condition score on calf survivability (Waldner, 2014); however, thin cows may lack body reserves for rapid parturition and thus increase risk of perinatal mortality (Larson et al., 2004). If the dam loses body condition in the last trimester, there will be an increased risk of dystocia due to uterine inertia and failure of relaxation of the pelvic ligaments (Kroker and Cummins, 1979). Uterine inertia may be primary, due to hypocalcaemia, hypomagnesaemia, hyposelenosis, debilitation, increasing maternal age or premature parturition. It may also be secondary, seen in twin parturitions or with prolonged malposition of the foetus (Gundelach et al., 2009).

Severe nutritional deficiencies will influence the weight of a calf at birth; however, it has been reported that in most circumstances, the weight of the calf at birth is not influenced by deficiencies in the diet received by the cow, according to Eckles, 1916, as reported by Holland and Odde, 1992. Protein and energy restriction to the dam during gestation can increase the risk of perinatal mortality through poor colostrum quality (Lorenz et al., 2011), resulting in reduced vigour and poor health in the neonate (Mee, 2013b). Excessive or limited energy supplies during the last half to third of gestation can affect the calf birth weight by up to 8.2kg (Holland and Odde, 1992). Where this weight is gained by the calf, this increases the risk of dystocia and perinatal mortality.

Poor quality feed has been associated with increased risk of stillbirths (Waldner, 2014). The quality and quantity of stored feed can be affected by precipitation during the growing season, which will subsequently affect cow nutrition (Waldner, 2014). Dry matter intake will increase if, during the last trimester, air chill temperature is around -5°C. This will lead to increased risk of dystocia through increased calf birth weights, resulting from increased thyroid hormone concentration, increased blood and nutrient flow to the uterus, increased gestation length and reduced plasma oestradiol (McClintock, 2004). Pathogens associated with increased risk of perinatal mortality through consumption by the dam, may be present in feedstuffs associated with extremes in weather, poor feed storage, poor feeding conditions and low quality feedstuff (Whitlow and Hagler, 2016). Such pathogens include *Bacillus licheniformis, Aspergillus* spp., and *Candida* spp. Micro-mineral deficiencies are discussed in section 1.4.4.

1.2.2 Perinatal Mortality Risk: Factors Associated with Parturition

1.2.2.1 Age and Parity at Parturition

The risk of perinatal mortality is greater in primiparous cattle compared to multiparous cattle (Mee et al., 2014). In primiparous cattle, a younger age of calving is associated with an increased risk of perinatal mortality (Mee et al., 2014), with the highest risk seen in cattle calving at less than 24 months old, due to inadequate pelvic size (Mee et al., 2014). With calving at the age of 22 months for the first time, the probability of stillbirth for male and female calves is 0.29 and 0.21, respectively, whereas, at an age at first calving of 28 months, the corresponding values were 0.15 and 0.10 (Hansen et al., 2004). Therefore, to aid in reduction of perinatal mortality risk, to minimise feed costs and to allow an annual calving pattern, the recommended age at first calving in dairy cattle, is around 24 months (Ettema and Santos, 2004). Alongside age, body weight at first service should also be considered, with a target body weight of 60-65% of mature body weight at first service aimed for (Engelken, 2008). There are minimal effects of age of calving after second parturition on perinatal mortality risk, despite an increasing calf birth weight in pluriparous cattle (Holland and Odde, 1992). In dams calving over ten years old, the risk of perinatal mortality

increases, but the risk of producing a stillborn calf in these older cows still remains lower than in primiparous cattle (Waldner, 2014).

1.2.2.2 Calving Supervision

Larger herd sizes have led to a potential reduction in the amount of supervision at calving time due to the ratio of stock workers to cattle (Berglund et al., 2003). Poor supervision of parturition can lead to increased perinatal mortality due to prolonged parturition with resultant anoxia and acidosis (Lorenz et al., 2011). This could mean that many perinatal losses are recorded as stillbirths (Berglund et al., 2003), when they are actually born alive and die subsequently. There is no reported correlation between day of the week and increased risk of perinatal mortality (Mee et al., 2011). However, Mee et al. (2011) reported that the odds of an assisted calving increase if parturition occurs on a Sunday, which could potentially be due to more time available to assist at parturition or assisting when not required. Good calving supervision involves being present to assist when required but not assisting unnecessarily (Lorenz et al., 2011).

1.2.2.3 Calving Intervention

The vast majority of research with regards to parturition progression and intervention has been performed in dairy cattle, however, it may be assumed that the same principles apply to beef cattle. Extremely prolonged parturition is related to increased perinatal mortality (Mee, 2004a), through development of acidosis in the calf and subsequent decreased passive transfer of immunoglobulins (Lorenz et al., 2011). If no calf is produced after 6-12 hours of onset of stage one parturition, or noted onset, an internal examination of the dam should be performed (Mee, 2004a). Premature examination of the dam is not associated with increased risk of perinatal mortality, providing assistance is not provided prematurely (Mee, 2004a). In stage two labour, progress should be monitored, and timely intervention made, according to progress. The occurrence of stillbirths can be reduced by assisting cows with parturition if no progress is seen after 120 minutes after onset of stage two parturition (Scheunemann et al., 2011). Prolonged, but not necessarily difficult calvings (e.g. due to weak labour

resulting from poor abdominal and uterine contractions) may increase rates of stillborn calves (Berglund et al., 2003), with weak labour being the cause or the consequence of a difficult calving (Meijering, 1984).

1.2.2.4 Calving Assistance

Any level of assistance at parturition is associated with an increased risk of stillbirth (Waldner, 2014), even when this assistance is slight (Mee et al., 2011). Requirements for assistance with calving are increased with first parity dams, heavier weight of calf, male calves and previous calving assistance (McDermott et al., 1992). Twin pregnancies are also associated with increased risk for calving assistance and dystocia due to uterine inertia, the weight of calves born and malpresentation (Mee et al., 2011). Cattle requiring assistance during one calving season are more likely to require assistance in subsequent parturitions (Waldner, 2014). Although many of the risk factors for assistance at parturition are heritable, the heritability of malpresentation of the calf, as a trait, is low (Holland et al., 1993).

The risk of perinatal mortality increases with premature assistance at parturition (less than one hour after onset of stage two parturition), as this is associated with increased risk of use of mechanical aids, duration of assistance, recumbency in cows and dystocia, due to inadequate dilation of the pelvic canal (Mee, 2012). On the other hand, if assistance is delayed beyond two hours of foetal hooves appearing, perinatal mortality will also be increased through development of acidosis in the calf (Mee, 2012).

1.2.2.5 Calving Location

The location of calving plays a significant role in the prevalence of perinatal mortality. Calving at pasture has been associated with an increased risk of perinatal losses (Vernooy et al., 2007), thought to be related to reduced supervision at parturition (Hodge et al., 1982). On the contrary, dystocia risk is reduced in animals calving at pasture, 6.8% (McDermott, 1992) compared to animals that are confined (13.7%) (Mee et al., 2011). These differences in

dystocia rates may be due to differences in body condition score, nutrition, exercise, calving management, genotype and herd size (Mee et al., 2011).

1.2.2.6 Dystocia

Dystocia is defined as calving difficulty resulting from prolonged spontaneous calving or prolonged or severe assisted extraction (Mee, 2004a). This can result when there is a failure in one of three main components; expulsive forces, birth canal adequacy and foetal size and position (Mee, 2012). In an ideal situation <5% of adult cows and <15% of heifers should present with dystocia (Caldow et al., 2005); however, the incidence of dystocia and perinatal losses are population specific due to both genetic and non-genetic factors (Berry et al., 2007). Although dystocia is a major cause of perinatal mortality, death of the foetus pre-partum, presenting as stillbirth, can also lead to dystocia (Meijering, 1984).

The prevalence of dystocia in primiparae is significantly higher than in pluriparous cattle (Meyer et al., 2001), primarily due to foeto-pelvic incompatibility, which is commonly determined by the width of the shoulder and the thoracic cavity of the calf (Barrier et al., 2013). Dystocia can be reduced on farm by selecting appropriate heifers to breed from (i.e. no history of dystocia, twinning or perinatal mortality in the dam), calving heifers at 85% of their adult body weight, calving at body condition score 2.5/5 and good supervision during stage two parturition (Mee, 2004a). Pelvic measuring may be used in an attempt to reduce dystocia in primiparous cattle, through selection of heifers with appropriately sized pelvises, but may also aid in judgement at parturition as to whether per vaginum delivery is feasible, when the size of the calf is determined (Daly and Riese, 1992). Dystocias among mature cows can be seen when there are attempts at phenotypic alteration in the herd. This can occur because small framed cows are bred to breeds of bulls with greater growth potential (Larson et al., 2004) leading to an abnormally high rate of dystocia. If low birthweight is a trait that is selected for, cows with smaller pelvic areas and reduced mature size will be produced, which can lead to increased dystocias and thus increased

perinatal mortality, if homebred heifers are used as replacements (Larson et al., 2004).

Pharmacological induction of parturition can reduce rates of dystocia if oversized foetuses are predicted; however, there is an increased risk of perinatal mortality associated (Lorenz et al., 2011). The risk of foetal mortality following pharmacological induction decreases the closer to term the calf is at the time of induction (Mansell et al., 2006).

Foetal malposition is the most common cause of dystocia in older cattle; however it is not heritable and has low repeatability (Mee, 2012). Malpresentation is associated with a five times increased risk of stillbirth compared with normal presentation (Mee, 2012). Increased movement of the foetus, secondary to anoxia, may lead to malpresentation of the foetus, with a resulting dystocia (Dufty and Sloss, 1977).

Perinatal mortality following dystocia can result from anoxia, trauma or premature placental expulsion (Mee, 2004a). Prolonged parturition can lead to neonatal acidosis, resulting in tissue hypoxia and lactic acidosis which may depress the central nervous system, impairing the reflexes that initiate respiration (Nagy, 2009). The resulting hyperkalaemia may also cause cardiac arrest in the foetus (Norton and Munro, 1996).

1.2.2.7 Gender of Calf

The sex of the calf is thought to play a role in the incidence of perinatal mortality, with almost twice as many male losses compared to female losses (Meijering, 1984). Male calves tend to have longer gestations (over one day) than female calves (Mee et al., 2011) and this increases risks of dystocia and perinatal losses. Where perinatal mortality occurs following a normal parturition, there is very little difference between genders of calves (Meijering, 1984).

1.2.2.8 Weight of Calf

It has been estimated that greatest predictor of dystocia risk is calf birth weight (Mee, 2012) and the frequency of dystocia rises when the weight of the calf reaches a certain threshold (Meijering, 1984). The weight of the calf has been discussed in the nutrition section above. Male calves have a 9% greater birth weight compared to female calves Differences in weight between genders are relatively constant across breeds (Holland and Odde, 1992). In large calves, dystocia may result in anoxia and metabolic and respiratory acidosis, which may lead to reduced immunoglobulin absorption and increased disease susceptibility (Holland and Odde, 1992). The incidence of stillbirth also increases when the weight of a calf drops below a certain threshold (Meijering, 1984), which indicates an optimum weight range to prevent perinatal mortality. The birthweight of the calf will be approximately 7% of the dam's weight, with a range of 5-10% (Holland and Odde, 1992). At birth, if calves are too small, they may lack vigour, have reduced thermoregulation, and the ability to overcome parturitional stresses may also be reduced (Holland and Odde, 1992). Where calves have a low birthweight with a long crown-rump length, the risk of perinatal mortality is increased, potentially due to impaired foetal development (Barrier et al., 2013). Although mortality due to foetal oversize in comparison to foetal undersize is more common, the death of low birthweight calves should not be overlooked (Holland and Odde, 1992).

1.2.2.9 Length of Gestation

The length of gestation is easily measured where service dates are available and accurate. A short duration gestation (<267 days) will increase the risk of the birth of a dead calf (Meijering, 1984), however, this would be defined as abortion, rather than a perinatal loss (Mee, 2015a). Increasing gestational length is associated with increasing calf birth weight and thus an increased risk of dystocia (Holland and Odde, 1992). Although extended gestations allow for additional foetal growth, growth near term is estimated at only 100-250g/day so the extent of growth seen in calves in extended gestational periods will be low (Holland and Odde, 1992). The additional growth will however, increase the risk of dystocia by 13% for each one kilogram increase in calf birthweight (Johansen

and Berger, 2003) (weights above 42kg are considered foetal oversize (Holland and Odde, 1992).

1.2.2.10 Maternal Health

Pre-partum, the number of neutrophils circulating in the dam increases, and their function is enhanced (Chassagne et al., 1998). In one study by Chassagne et al. (1998), a high number of circulating neutrophils in the dam in late gestation favoured the parturition process and were associated with a decreased risk of stillbirth. However, a high circulating level of neutrophils may also indicate an early immune response to foetal infection, which is a major risk factor for perinatal mortality (Chassagne et al., 1998).

1.2.3 Perinatal Mortality Risk: Post-partum factors

1.2.3.1 Calf Resuscitation

Adequate calf resuscitation is essential to decrease perinatal losses occurring in the first few hours post-partum (Nagy, 2009). Following dystocia, resuscitation should involve establishment of normal respiration and correction of the acid-base balance (Nagy, 2009). Beef calves born following dystocia are up to six times more likely to become ill than calves born following eutocia; however appropriate resuscitation may decrease this risk (Nagy, 2009). Where no cardiac output is detected, resuscitation is generally not attempted in the field, as the calf is unlikely to be viable (Nagy, 2009).

Calves likely to require resuscitation can be identified both intra-partum and post-partum. During parturition, calves at high risk of dystocia from foetal oversize may be identified by the presence of large feet in the birth canal (Lorenz et al., 2011). A swollen tongue and cyanosis of the muzzle can also indicate prolonged parturition and the need for rapid intervention post-partum (Lorenz et al., 2011). Immediately post-partum, the viability of the calf should be assessed by muscle tone, sucking reflex, time to lift head, time to first standing and response to stimuli (Lorenz et al., 2011, Dwyer and Turner, 2012).

In a normal calf, sternal recumbency should be attained by 20 minutes and standing accomplished by 60-90 minutes post-partum (Lorenz et al., 2011). Prompt attention should be given to calves post-partum which remain in lateral recumbency, have poor pedal and suck reflexes, flaccid musculature and are dyspnoeic (Lorenz et al., 2011).

It is thought that acidosis is one of the main causes of post-partum death in neonatal calves (Mee, 2008b). Foetal acid-base balance may be altered by periodic reductions in blood flow through the umbilicus during stage two of parturition from uterine and abdominal contractions, and thus prolonged calving is associated with increased acidosis (Mee, 2008b). Inappropriate human traction technique can also contribute to acidosis if there are not regular breaks between applications of manual traction. All calves suffer some degree of respiratory-metabolic acidosis at birth, but the length of parturition and the duration and level of intervention during calving will affect foetal survival (Mee, 2008b). Sodium bicarbonate, administered intravenously, can be used for treatment of metabolic acidosis in bovine neonates to improve survivability (Bleul, 2009). Care must be taken with administration of sodium bicarbonate, as calves suffering from respiratory distress syndrome are often unable to exhale carbon dioxide adequately (Bleul, 2009).

Surfactant deficiency can be seen in calves delivered prematurely, by pharmacological induction or by caesarean section resulting in respiratory distress syndrome (Mee, 2008b). This is due to a lack of foetal catecholamine release, which inhibits lung liquid secretion and stimulates absorption of fluid and is triggered by passage through the birth canal (Mee, 2008b). Fluid in the airway is secreted from the lower respiratory tract and forced from the thorax during parturition. Excess fluid should be removed from the airway; however, it is not necessary to remove all fluid as is it will be resorbed by the lymphatic system within minutes of birth (Mee, 1994). In order to establish a patent airway, various methods can be applied, if assisted ventilation is required. In contrast to pre-partum administration, the use of glucocorticoids in calves post-

partum have not been shown to aid lung maturation and surfactant production (Bleul, 2009).

Primary apnoea can lead to increased perinatal mortality and attempts to stimulate respiration should be made as soon as the calf is born (Nagy, 2009). Blood acid-base balance can be improved through inducing gasp reflexes by common resuscitation methods such as hypothermal and nasal stimulation (Mee et al., 2013). Nasal stimulation with a digit or straw will elicit a gasping reflex, which may aid in maintaining patency in the upper respiratory tract (Mee, 1994). Acupuncture of the nasal philtrum may also be used for physical stimulation of respiration; however, results may be variable (Mee, 1994). Rubbing the calf with bedding or towels may induce respiration through stimulation of the phrenic nerve (Nagy, 2009). Positive pressure ventilation through the use of masks, (mouth-to-nose or mouth-to-mouth) may aid in resuscitation although there is risk of inflation of the abomasum which will further impede the calf's ability to breathe; the use of cuffed endotracheal tubes can overcome this issue (Nagy, 2009). Pharmacological resuscitation methods include the use of doxopram hydrochloride, which stimulates peripheral chemoreceptors and medullary respiratory centres of the brain (Nagy, 2009). Improved survivability has been seen in calves following the administration of doxopram; however, improvements are less likely where there is profound depression of the central nervous system (Nagy, 2009).

The position that the calf is placed in following birth can have a massive effect on respiration and lateral recumbency is detrimental to respiration (Mee, 2004a). Sternal recumbency and suspension from hind legs for less than ninety seconds is associated with improved respiratory effort following birth (Nagy, 2009). On the contrary, prolonged suspension by the hind-limbs will hinder respiration from pressure from abdominal organs on the thoracic cavity, and should be avoided (Nagy, 2009).

1.2.3.2 Umbilical Care

The umbilical cord should be allowed to separate from the calf naturally as premature rupture of the cord (from cutting, tearing or ligating) is often associated with decreased pulmonary gas exchange efficiency (Mee, 2004a), which may increase perinatal mortality. A long crown-rump length is associated with an increased risk of early umbilical rupture and compression of the cord, resulting in hypoxia (Barrier et al., 2013). In most situations, ligatures should not be applied to the umbilicus, unless there is excessive haemorrhage, following trauma from dystocia (Nagy, 2009). The umbilicus should be maintained in a clean, dry environment and appropriate colostral transfer should occur to minimise neonatal septicaemia (Nagy, 2009) and thus reduced perinatal mortality. When calving pens are dirty, calf residency time in such pens should be kept to a minimum to ensure optimal umbilical health (Mee, 2004a).

1.2.3.3 Colostral Intake

In cattle, no transfer of immunoglobulins occurs across the placenta, therefore, the calf is born agammaglobulinaemic and is entirely dependent on immunity provided though colostrum intake (Godden, 2008). Survival of the calf in the neonatal period is highly dependent on early and adequate intake of high quality colostrum (Godden, 2008). Adequate passive transfer is the result of colostral quality, volume ingested and the calf's ability to absorb immunoglobulins (Lorenz et al., 2011). As a basic goal, the aim should be to provide 10% of the calf's bodyweight in high quality colostrum in the first two to six hours of life, (Patel et al., 2014). The quality of colostrum is as important as the quantity of colostrum provided and a minimum quality of 50g of IgG/L is required for adequate passive transfer (Elizondo-Salazar and Heinrichs, 2009). The colostral immunoglobulin concentration decreases by 3.7% for each subsequent hour post-partum, therefore the time to first ingestion is vitally important (Lorenz et al., 2011). In cases where calves are in respiratory distress post-partum, frequent small volumes of colostrum should be administered to prevent abomasal over-dilation and subsequent pressure on the lungs. Plasma immunoglobulin concentration in calves appears to be greater when cows calve in individual pens compared to group housing (Lorenz *et al.,* 2011).

Low calf vitality, resulting from inflammation, pain or injury during parturition, may result in reduced ability to stand and thus reduced colostral intake (Murray and Leslie, 2013). If there is suspicion of failure of passive transfer, artificial colostrum replacers or supplements can be provided; however, with regards to preventing neonatal morbidity and mortality, they are poor in comparison to natural colostrum (Lorenz et al., 2011). Serum total proteins can be measured using refractometry to detect failure of passive transfer, with a serum protein measurement >52g/L thought to represent adequate colostral intake (Lorenz et al., 2011). Other laboratory-based tests to estimate IgG concentration in calves, include radial immunodiffusion, the sodium sulphite turbidity test and the zinc sulphate turbidity test (Godden, 2008).

1.3 Diagnostic Investigation of Perinatal Mortality

1.3.1 Aims of Investigation

The aim of any post mortem investigation in farm animals is to proximally provide a likely diagnosis of the cause of death and to determine the presence of contributory risk factors so that information can be relayed back to the client on how future losses may be prevented. Post mortem examinations may also be a useful tool in the detection of new and emerging diseases and provide an understanding of the pathogenesis of these diseases. In many situations, a singular cause of death can be difficult to establish, as many perinatal deaths are multifactorial. Risk factors which are not sufficient to cause death on their own, but require component causes (e.g. foetal oversize) should also be recorded. The presence of autolysis of the carcase and viscera from delays in post mortem examination also make diagnosis more difficult.

1.3.2 Components of the Investigation

Although various post mortem examination techniques exist for investigating bovine perinatal losses (Cabell, 2007; Mee, 2015b), there is currently no universally accepted diagnostic approach, or diagnostic criteria for interpretation of the findings of these investigations. In most cases, the presence of lesions suggestive of a cause of death, or the presence of a pathogenic micro-organism are accepted as the cause of death; however, in

some circumstances, presence does not necessarily mean causality. Investigations into perinatal losses should ideally include: 1) a clear case definition (i.e. full term stillborn calf or dead within 48 hours of birth); 2) collection of a herd and case history; 3) examination of the foetus and placenta; 3) collating laboratory results 4) examination of the dam and 5) examination of other pregnant and lactating animals (Mee, 2015b).

1.3.2.1 Farm and Case History

In order to assist in investigation of bovine perinatal losses, it is of vital importance to obtain a thorough history for both the herd and the individual case (Mee, 2015b). This will allow the investigator to identify potential risk factors associated with perinatal losses, but will also put the individual case into perspective with regards to incidence on the farm. It is best to use a standardised questionnaire so that important details are not missed, especially when multiple losses occur in a single calving season. Details of pre-partum management, nutrition, breeding, parturition and post-partum management should be included in all histories (Mee, 2015b). The incidence of dystocia may also be an important factor to assess as this can be a good indicator of herd management (Mee, 1999).

1.3.2.2 Gross Post Mortem Examination of Calf and Placenta

A standard post-mortem examination should be performed on all carcasses, regardless if there is an obvious cause of death (Mee, 2015a). This will ensure that important lesions are not missed whilst focussing on other, obvious lesions (Mee, 2015a). The purpose of the gross post-mortem examination is to estimate the time of death, sample collection and cause of death if possible (Mee, 2015a). External examination of the carcass will allow indicators of time of death to be detected; for example, corneal opacity (Mee, 2015b). Estimates of the stage of development of the foetus can be made from external examination using body weight, crown-rump length, teeth eruption, and hair coat (Mee, 2015b). Indicators of prolonged or traumatic parturitions may be seen through detailed external examination. Internal examination of the carcass can be

performed with the carcass in a cradle or in lateral recumbency, according to personal preference. Thorough examination of all internal organs, including the brain should be performed in all cases.

To aid in perinatal mortality investigation, placental tissue should be examined when available (Anderson, 2007). In many situations, the placenta is unavailable for examination due to expulsion of the placenta after delivery of the calf. Placental pathology can occur in the absence of foeto-pathology and care should be taken with interpretation (Mee, 2015a). For example, placental infection can lead to stillbirth through reduced blood flow to the foetus, without the presence of the organism in the foetus (Goldenberg and Thompson, 2003). Normally there are 75-100 cotyledons present and if any abnormal cotyledons are present, these should be submitted for microbiological examination and fixed in formalin for histopathological examination (Mee, 2015a).

In fresh placental tissue, red cotyledons are present with a clear, translucent inter-cotyledonary area (Anderson, 2007). When autolysis is present, the inter-cotyledonary placenta becomes less translucent and the cotyledons change to dull brown (Anderson, 2007). Inflammation in the chorio-allantois can be seen by opacity in the inter-cotyledonary area, thickening or exudate on the surface and depression of the cotyledons relative to the surrounding inter-cotyledonary area (Anderson, 2007).

1.3.2.3 Estimating Time of Death

Estimation of time of death allows a reduced list of differential diagnoses to be generated as many differentials only apply to a certain time of death. By narrowing down the differential list if will make establishing the cause of death much simpler and cost effective. Pre-partum death can be detected on post-mortem examination through generalised red staining of the tissues and sub-cutaneous oedema due to haemoglobin-stained fluid throughout the body cavities from haemoglobin breakdown (Wiske et al., 1994). Various degrees of autolysis can be seen with soft autolytic kidneys and liquefaction of the brain

(Holler, 2012), depending on how long the foetus has been dead pre-partum. Free-fluid in the chest and abdominal cavity is commonly seen in calves which die within one week of parturition (Berglund et al., 2003).

In calves that die from 12 hours pre-partum, the cornea will typically be clear, with the cornea of calves, which have died 12-24 hours pre-partum showing varying degrees of opacity (Mee, 2015b). Dehydration of the cornea will be seen in calves that die over two days pre-partum (Mee, 2015b). Calves that die close to parturition will have no red staining of tissues and there will be no thrombi present in umbilical arteries (Wiske et al., 1994). There is usually less autolysis present in these calves, than in calves that die pre-partum (Holler, 2012).

The presence of haemorrhages throughout the body, particularly in the spleen and heart, provide evidence of a functioning circulatory system during parturition. Other evidence of viability can be seen as partial aeration of the lungs and meconium staining of the skin and perineum (Holler, 2012). Calves that die shortly after parturition will have aerated lungs, minimal free fluid in the body cavities and blood clots may be present in the umbilical arteries (Holler, 2012).

Parturient losses can often be differentiated from post-parturient losses by history and the degree of pulmonary atelectasis present, representing post-natal survival (Mee, 2015b). The presence of umbilical thrombi indicates that there has been a functioning heart at the time of birth; however, small thrombi (<3mm diameter) may be present in calves, which die during or immediately after a prolonged parturition (Mee, 2015b). If the calf has stood, the palmar/plantar surface of the hooves will be worn off indicating survival post-partum (Mee, 2013a). The presence of milk or colostrum in the abomasum may be taken as definitive evidence that the calf was alive for a period of time post-partum.

1.3.2.4 Post Mortem Sampling: Detection of Micro-organisms

Collection of samples for microbiology should be obtained before contamination occurs through handling, and searing of the organ surface is recommended prior to sample collection (Mee, 2015a). Ideally the abomasum is used for collection of samples for microbiology (provided the calf has not fed or ingested environmental material), but lung or brain tissues are suitable alternatives (Mee, 2015a). In most laboratories, *Brucella* and *Campylobacter* cultures are performed routinely on foetal stomach contents (Anderson, 2007). Other bacteria are detected in the foetal stomach contents by gram staining and cultures (Anderson, 2007). Leptospiral species are typically examined by fluorescent antibody staining or microscopic agglutination tests, performed on kidney tissue (Anderson, 2007). Positive bacterial and mycotic cultures on placental tissue may be the result of environmental contamination. Due to their ubiquitous nature in the environment, in order to establish an aetiological diagnosis, opportunistic bacteria should be isolated in pure or nearly pure culture from foetal stomach contents or tissues (Anderson, 2007). Lesions consistent with bacterial infections should also be present in the foetus or the placenta (Anderson, 2007), including neutrophilic bronchopneumonia, as seen on histology. Routine screening for BVDV, using enzyme-linked immunosorbent assay (ELISA) antibody and antigen testing, and BHV-1 by real time polymerase chain reaction, is performed by most laboratories during perinatal mortality investigations (Anderson, 2007). Further details of each specific infectious agent are given in section 1.4.

1.3.2.5 Post Mortem Sampling: Histopathological Examination

Histopathology will usually provide useful information regarding infections, in particular *Neospora caninum (*Otter, 1999), and aids in establishing if microbiology and immunology results are significant (Anderson, 2007). The tissue samples preferred for histopathology vary between referral pathologists and should be clarified prior to commencement of perinatal mortality investigations and sampling (Anderson, 2007). Despite potential for liquefaction in the brain due to autolysis, fixation in formalin can provide useful histological results

(Anderson, 2007). Information regarding possible routes of infection can be determined through complete histological examination of a variety of tissues (Anderson, 2007). Hepatic changes are typically seen in infections entering via the placenta and umbilical vessels, whereas lung and digestive tract lesions are typically seen following infections in the placental fluid (Anderson, 2007). Occasionally, when severe placentitis is present, no significant lesions are seen in the foetus (Anderson, 2007).

1.3.2.6 Examination of Dam

Examination of the dam may be useful in investigation of bovine perinatal losses, in particular when no carcass is available for examination. A standard clinical examination should be performed on dams to detect presence of disease. Additionally, testing for the presence of antibodies against *Salmonella* spp., *Leptospira*, Bovine Herpes virus (BHV), Bovine Viral Diarrhoea Virus (BVDV), Schmallenberg virus, and *Neospora* should be performed. Although single serology can aid in diagnosis, given the widespread prevalence of antibodies to infectious agents, both through natural exposure and vaccination, this can often be of limited value (Paton et al., 1998). On the contrary, a negative result, depending on test specificity and sensitivity, can aid in excluding a diagnosis with some confidence. In most cases, the use of paired serology is not helpful due to the time delay between infection of the dam and the foetus (Mee, 2015b). Ideally a herd analysis of trace element and mineral status should be performed.

1.4 Aetiologies of Perinatal Losses

In previous studies on bovine calf losses (including abortions), diagnostic rates, where an aetiological cause of death have been reached, have ranged from 20-41% (Khodakaram-Tafti and Ikede, 2005; Kirkbride et al., 1973), but have been as high as 78% for stillbirths alone, depending on the degree of investigations performed and the types of animal investigated (Waldner et al., 2010). Where abortions are included in studies of bovine perinatal losses, diagnostic results may be reduced when compared to investigations into stillbirths and calves that

die in the neonatal period only (Waldner et al., 2010).

In a study performed by Waldner et al. (2010), diagnosis of the cause of death was established after gross post mortem examination, micro-organism culture and histopathological examination of tissues. In most circumstances, this level of testing and examination will improve diagnostic rates (Table 1.3).

Table 1.3; *Diagnosed causes of death in bovine perinatal loss studies*

Cause of Death	Berglund et al., 2003	Waldner et al., 2010*
Dystocia	46.1%	40.2%
Intrauterine death	10.5%	N/A
Unknown	35.5%	21.6%
Malformations	5.3%	4.3%
Unspecified infections	2.6	N/A
Thyroid gland lesions	N/A	8.9%
Myocardial myopathy	N/A	7.1%

**Other causes of death also diagnosed in this study not stated in table.*

1.4.1 Infectious Aetiologies as Causes of Perinatal Loss

Infectious aetiologies of perinatal mortality are difficult to definitively diagnose as direct detection, histological or serological evidence of infectious agents does not necessarily indicate causation (Goldenberg and Thompson, 2003). Inflammatory lesions suggestive of bacterial infection (e.g., bronchopneumonia, encephalitis) can be used as diagnostic criteria for infection as the cause of death in some cases of perinatal mortality (Mee, 2013a). The presence of infectious organisms in foetal tissues also does not prove causation; however, suspicion of an infectious aetiology is increased (Goldenberg and Thompson, 2003).

From studies conducted in dairy herds, infectious aetiologies represent a low proportion of the diagnoses made in perinatal mortalities (3-15%), unlike in abortions, where infectious aetiologies make up a high proportion of the positive diagnoses (Mee, 2013a). Prevention of perinatal mortality due to infectious aetiologies is based on limiting exposure to the causative organisms and maximizing the specific and nonspecific resistance of the calf and the cow (Townsend, 1994).

1.4.1.1 Bacterial Infections

1.4.1.1.1 Sporadic Bacterial Infections

In comparison to studies on infectious aetiologies in stillborn calves, a significantly larger number of studies have been performed in aborted foetuses. Ubiquitous bacteria present in the environment and mucosal surfaces such as *Bacillus* spp., *Escherichia coli, Haemophilus somnus, Pasteurella* spp., *Serratia* spp., *Staphylococcus* spp., *Trueperella pyogenes* and *Streptococcus* spp., can infect the placenta and the foetus opportunistically. Most bacteria are non-contagious so sporadic infections, rather than outbreaks are commonly seen (Anderson, 2007). From the opportunistic infection, it is assumed that a maternal bacteraemia results, leading to infection of the placenta (Anderson, 2007). Where abortion storms occur due to opportunistic pathogens, maternal health issues may be a problem (Anderson, 2007). Clinical signs in the dam are typically not seen with sporadic bacterial infections which cause abortions, although placentae may be retained (Anderson, 2007).

Bacterial infections may be ascending from the vagina or may be haematogenous in origin (Goldenberg and Thompson, 2003). Most bacterial infections ascend from the vagina to the uterus during early gestation; however, some bacteria may be resident in the uterus prior to gestation (Goldenberg and Thompson, 2003). The foetus becomes infected via the amniotic fluid if bacteria can cross the choriodecidual membranes or after the membranes rupture (Goldenberg and Thompson, 2003). Pneumonia is commonly seen following bacterial infection due

to the foetus breathing infected amniotic fluid (Goldenberg and Thompson, 2003). In women, infection with *Streptococcus, E.coli* and *Klebsiella* will result in infection of the amniotic fluid which will result in labour within hours to days of infection, due to a large inflammatory response (Goldenberg and Thompson, 2003). If the foetus cannot initiate an adequate inflammatory response, a stillbirth is the likely outcome of infection. In many cases, human foetuses are born with congenital pneumonia (Goldenberg and Thompson, 2003). In haematogenously acquired bacterial infections in humans, the liver and umbilical veins are targeted in the foetus (Goldenberg and Thompson, 2003). Although the majority of investigations into sporadic bacterial infections has been performed in aborted foetuses, the same principals would apply to stillborn calves.

1.4.1.1.2 Other Bacterial Infections

The following section provides detail on bacterial infections, which have been specifically associated with bovine perinatal losses.

Although the prevalence of *Leptospira hardjo* is low due to the extensive use of vaccination (McCoy et al., 1997), infection is typically associated with infertility, abortions and the birth of weak calves (Anderson, 2007) and is spread venereally, through milk, aborted material and urine (Palmer, 1994). When Leptospiral antigens are detected in the placenta, calves are generally 6-10kg lighter than when no antigens are detected (Smyth et al., 1999); however, few other clinical signs are seen in calves infected with *Leptospira*. No gross lesions are seen on post mortem examination of infected calves and foetal antibodies are not consistently found in infected foetuses (Murray, 1999). Microscopic agglutination tests can be useful on maternal serum to aid in diagnosis of *L. hardjo* infections in stillborn calves; however, care must be taken to distinguish between infection and previous vaccination (Anderson, 2007). Where both high maternal and foetal titres to *Leptospira hardjo* are present, it is highly likely that this is the primary pathogen associated with the abortion (Murray, 1999).

On the contrary, where other pathogens are present, *Leptospira* may be a secondary, opportunistic pathogen (Murray, 1999). Although culture is the gold standard test, it is very time consuming, meaning that fluorescent antibody testing and polymerase chain reaction (PCR) tests are more commonly used for diagnosing *Leptospira* (Cabell, 2007).

Brucella abortus is a potentially zoonotic pathogen, which typically causes abortions after the fifth month of gestation through ingestion of the bacteria (Givens and Marley, 2008). The birth of normal but infected calves is also possible, along with the birth of weak calves (Miller and Stack, 2012). Fortunately, infections with *B. abortus* are now rare due to eradication programmes (Anderson, 2007). Bacterial presence is required for diagnosis of infection with *B. abortus* and can be performed on foetal stomach contents, lung, uterine fluid and placental tissue (Anderson, 2007), using a Rose Bengal plate test or modified Ziehl-Neelsen stain (Millar and Stack, 2012). An antibody response is usually seen in the dam, detected by serology, but may not develop until two to three weeks after the birth of a dead calf (Miller and Stack, 2012). Infected placentae are typically thickened with necrotic cotyledons covered by brown exudate (Millar and Stack, 2012).

Although abortion is the typical reproductive presentation of *Salmonella* Dublin (Anderson, 2007), perinatal mortality may also be seen (Mee 2013a). Infections with *Salmonella* spp. occur sporadically, through infections of the digestive tract, however, abortion storms may often be seen (Anderson, 2007). Diagnosis of *Salmonella* Dublin is through culture of foetal samples or maternal serology (Sanchez-Miguel et al., 2014). Diagnosis is typically based on isolation and identification of the organism from the placenta or from the foetus (Djonne, 2007).

Campylobacter spp. infections typically result in abortion rather than stillborn calves (Anderson, 2007). Infections with *Campylobacter fetus* subspecies *venerealis* occur through venreal infection of the dam, however, infections with

Campylobacter fetus subspecies *fetus,* and *Campylobacter jejuni* occur sporadically (Anderson, 2007). Fibrinous exudation in the peritoneal and pleural cavity and peritoneal sac may be present in the foetus, along with splenomegaly (Anderson, 2007) on gross post mortem examination. Histopathology often reveals placentitis, with bronchopneumonia and abomasitis in the foetus (Anderson, 2007). Colonies of intra-cellular bacteria are normally seen on examination of the placenta, often associated with severe inflammation (Holler, 2012) and necrosuppurative placentitis (Kirkbride, 1993).

Tick born fever caused by *Anaplasma phagocytophilum* may result in the birth of a stillborn calf (Djonne, 2007). Infection in the dam is usually mild with signs of dullness, immunosuppression and pyrexia seen (Djonne, 2007). Abortion is the more typical presentation of Anaplasmosis, although active infections can be present in neonatal calves (Fowler and Swift, 1975). The main clinical finding seen in bovine perinatal losses is anaemia, combined with enlargement of the spleen and liver (Correa et al., 1978). To diagnose *Anaplasma* as a cause of bovine perinatal mortality, blood smears are required, with identification of the organism (Correa et al., 1978). Diagnosis can be confirmed through staining of blood smears with acridine orange and using immunofluorescence, or through PCR (Woldehiwet, 2017).

Q Fever, caused by *Coxiella burnetii,* has been recently diagnosed in Scotland in stillborn dairy calves, and is now considered endemic in 70% of dairy herds in the United Kingdom (Anon., 2017a). Most infections are asymptomatic, but abortions and stillbirths are a common finding, with recrudescence of infection occurring at parturition (Scotland's Rural College, 2017). Gross lesions are non-specific and diagnosis is based on culture, immunohistochemical and PCR testing on foetal tissues (Plummer, 2017). Immunofluorescence testing performed on paired sera from the dam, two weeks apart may aid in confirmation of diagnosis (Plummer, 2017).

Bacillus licheniformis is a known cause of abortion in cattle (Mitchell and Barton, 1986) but may also be a cause of perinatal losses. The bacterium is commonly found in soil and feed sources and in most cases, no clinical signs are seen in the dam (David, 1993). Post mortem examination of perinatal losses may reveal fibrinous pleurisy, pericarditis, suppurative bronchopneumonia and encephalitis (David, 1993). Placental thickening and necrosis of the cotyledons may also be present. Diagnosis is based on isolation of the organism in pure culture from foetal stomach contents or liver, combined with presence of typical lesions in the foetus and placenta (David, 1993).

Calf losses due to *Listeria* spp., may occur at any stage of gestation, and the birth of live but infected calves is possible (Osebold et al., 1960). Infected dams may show pyrexia and anorexia prior to parturition. Post mortem examination of perinatal losses may reveal a small, grey liver with indistinct spots. Additionally, necropurulent hepatitis and placentitis may be seen on histopathology. Diagnosis is confirmed by the presence of the organism cultured from the foetal stomach contents on blood agar (Kirkbride, 1993).

1.4.1.2 Mycotic Infections

Mycosis can cause perinatal losses, mostly in the last two months of gestation and normally occuring in winter and spring months when the concentration of fungi in the environment increases (Anderson, 2007). When exposed to mouldy hay or silage, spores are ingested and reach the placenta and foetus through the blood supply (Djonne, 2007). The causative agents include *Aspergillus, Mucor* and *Candida*, which are ubiquitous in the environment (Anderson, 2007). Clinical signs are not normally seen in the dam, with the exception of retained placenta (Anderson, 2007). Foetuses may be born with evidence of growth retardation, a pale, enlarged liver and dermatitis (Djonne, 2007). Circumscribed plaques may occasionally be present on the skin of the foetus (Anderson, 2007), but often lesions in the foetus are minimal or absent (Anderson, 2007). In such situations, examination of the placenta is critical to diagnosis (Anderson, 2007). Hyphae may be found in the liver, lungs, stomach content, skin or placenta, so culture is vital (Djonne, 2007). Due to the ubiquitous nature of mycotic organisms,

compatible lesions in the foetus or placenta should be combined with isolation or microscopic demonstration of the organisms (Anderson, 2007).

1.4.1.3 Viral Infections

Infections with viruses in early gestation may result in the development of congenital anomalies, which do not result in foetal death at the time of infection. Foetal death may however, occur later, secondary to the congenital anomaly, for example Schmallenberg virus causing hydrocephalus (Mee, 2014). Congenital malformations appear to be a common presentation of new and emerging viral diseases (Mee, 2014, Anon., 2016c), emphasising the need to have a system in place to record incidences of such pathologies, with further investigations implemented where appropriate.

Bovine herpes virus type one (BHV1) infection can result in abortion, but may also present as vulvo-vaginitis, conjunctivitis and encephalomyelitis in adult cattle and fatal neonatal infections (Anderson, 2007). In recent years, perinatal losses due to bovine Herpes virus have decreased due to the implementation of vaccination regimes (Holler, 2012). Outbreaks have occurred where vaccinations have been administered incorrectly (Holler, 2012). Examination of infected foetuses reveals autolysis with red-tinged fluid in the body cavities, with few gross lesions present (Anderson, 2007). A diagnosis of perinatal mortality due to bovine Herpes virus can be a difficult one, due to expulsion of the foetus prior to foetal infection, resulting from a rapidly progressing necrotising placentitis (Cabell, 2007). Focal necrotising lesions will often be present on histopathology of the liver and other tissues (Anderson, 2007). Fluorescent antibody tests (to detect antigen) and virus isolation can be used to diagnose the presence of bovine Herpes virus; however, the role in perinatal losses is questionable, due to the opportunistic nature of the virus (Holler, 2012). Virus isolation has more recently been replaced by PCR testing, which is faster and has high specificity and sensitivity (Holler, 2012). Both fluorescent antibody testing and virus isolation tests have reduced sensitivity where autolysis is present (Cabell, 2007).

Negative dam serology 3-4 weeks after an abortion or stillbirth can be useful in excluding a diagnosis of bovine Herpes virus.

Infection of the dam with non-cytopathic BVDV commonly results in abortion; however, infection between 100 and 150 days of gestation may result in the birth of a calf with congenital infection (Grooms, 2006). This may present as microencephalopathy, hydrocephalus, hydranencephaly, cerebellar hypoplasia, hypomyelination, micro-opthalmia, retinal degeneration, deranged osteogenesis and growth retardation (Grooms, 2006). Virus isolation can be used to detect the presence of BVDV, alternatively, virus or antibodies can often be detected on serology from perinatal losses (Paton, 1999). Where high degrees of autolysis are present in bovine perinatal losses, real time PCR or immunocytochemistry can be used to aid diagnosis (Paton, 1999).

Schmallenberg virus is an Orthobunyavirus, transmitted between cattle by *Culicoides* spp. midges, resulting in abortion, stillbirths and the birth of malformed calves due to its teratogenic nature (Garigliany et al., 2012; Mee, 2014). Calves infected with Schmallenberg Virus typically show severe malformations, including arthrogryposis, torticollis, scoliosis, hydrocephalus, hydranencephaly, brachygnathia and cerebellar hypoplasia (Mee, 2014). In addition to these deformities seen on gross post mortem examination, there may also be traumatic lesions present, due to dystocia caused by malpresentations (Mee, 2014). A diagnosis of Schmallenberg virus can be made by detection of the virus by real time PCR, performed on foetal brain tissue. Neutralisation tests and indirect immunofluorescence assays may also be used for diagnosis in the foetus (Beer et al., 2013). Serology of the dam and foetus will also aid in confirmation of the diagnosis, with antibody ELISAs available commercially (Beer et al., 2013).

1.4.1.4 Parasitic Infections

Neospora caninum is a protozoan parasite, which is transmitted both vertically in infected dams and through ingestion of oocysts shed in dog faeces, leading to both abortion and the birth of congenitally infected calves (Otter, 1993). Abortion is the main clinical sign seen with *N. caninum,* along with neurological abnormalities in calves born alive (Otter, 1999). Heart failure, due to inflammation of the myocardium, is the usual cause of death in neonatal calves (Otter, 1999). A diagnosis of *N. caninum* is based on the presence of non-suppurative inflammatory lesions in the organs, which can be confirmed by histopathological examination of fixed tissues, including brain, heart and placenta. Typical histopathological lesions include multifocal, non-suppurative encephalitis, myocardial necrosis, and a multifocal mononuclear placentitis (Otter, 1993). Immunocytochemistry can also be performed on heart and brain tissue to confirm the diagnosis (Dubey and Lindsay, 1996). Additionally, an ELISA test can be performed on the dam to aid in diagnosis of *N. caninum* as a cause of bovine perinatal mortality (Blanc et al., 2013), as most, if not all calves born to infected dams are likely to be infected (Thurmond and Hietala, 1996).

1.4.2 Malformations and Lethal Traits as Causes of Perinatal Loss

Congenital defects may be defined as any defect, which is present at birth (Mee, 2013a). They are generally considered rare disorders caused by genetic mutations or teratogens (Mee, 2015c), although they may be more common than previously thought, accounting for a significant proportion of perinatal losses in some herds (Whitlock et al., 2008). Sporadic congenital defects are present on most dairy herds, and will often result in abortions or stillbirths (Mee, 2015c), with most breeds (dairy and beef) susceptible to sporadic random genetic congenital defects. In cases of perinatal mortality, congenital defects seen include intestinal atresia, arthrogryposis, cerebellar hypoplasia, cleft palate, omphalocoele, ventricular septal defect, schistosomus reflexus and hydrocephalus, which can be readily detected at post-mortem examination (Mee, 2013a). Some traits may be lethal, whereas others may require

euthanasia of the animal on humane grounds, for example intestinal atresia (Whitlock et al., 2008).

Congenital defects can also occur as a result of genetic mutation, exposure to infectious agents, pharmaceutical teratogens and toxins (Whitlock et al., 2008). As a result ascertaining a definitive cause can be difficult. (Mee, 2013a). A hereditary basis to some congenital defects is being investigated (Mee, 2013a). Teratogens are not usually familial (Rousseaux, 1994), and include agents such as bovine viral diarrhoea virus, trauma during manual pregnancy diagnosis, copper deficiency, drugs (e.g. griseofulvin) Tennant, 1999) and the ingestion of certain plants (e.g. white hellebore) (Humphreys, 1988). The time of normal foetal development cessation may aid in diagnosing the cause of congenital abnormalities; however, often in herd problems, genetic analysis and investigations into field exposure to potential toxins are required (Rousseaux, 1994).

1.4.3 Nutritional Causes of Perinatal Loss

1.4.3.1 Micronutrient Deficiencies

Trace mineral and vitamin deficiencies contribute to a number of causes of foetal, neonatal, and postnatal losses in beef calves (Waldner and Blakley, 2014), with the maternal micronutrient status being the primary determinant of micronutrient status in the neonate (Mee, 2013b). In some cases, additional supplementation of micro-nutrients may be required in order to provide an adequate supply for foetal nutrition, where cattle are fed a forage diet (Lorenz et al., 2011). Micronutrient deficiencies associated with increased perinatal mortality in the UK include, vitamin E, selenium, and iodine (Mee, 2004b; Mee, 2011a).

Respiratory distress syndrome (RDS) is a specific condition which results in surfactant deficiency, commonly seen in premature calves (Mee, 2013a). It may

also be induced by micro-nutrient deficiencies, including selenium, copper, zinc and iodine (Mee, 2013a).

1.4.3.1.1 Selenium and Vitamin E

Selenium concentrations in cattle and the relationship to perinatal losses are unclear, with some studies finding no association with increased risks of stillbirths (Waldner and Van De Weyer, 2011). It may however, contribute to uterine inertia, resulting in prolonged parturition. A condition was described by Rice et al. (1986), whereby full term calves fail to breathe or stop breathing within ten minutes of birth. It was thought that this condition was due to hyposelenosis; however, in one study, supplementation of calves with similar clinical signs, with selenium, showed no improvement (McCoy et al., 1997). In another study by Davis and Myburgh (2016), one week after supplementation of the dam with selenium, an improvement was recorded with regards to perinatal weakness and death.

Perinatal mortality may be caused by nutritional muscular dystrophy, more commonly known as white muscle disease, caused by deficiency of vitamin E and or selenium in the dam. Both selenium and vitamin E are required for cellular membrane maintenance and when deficient, results in accumulation of calcium in and injury to the mitochondria (Abutarbush and Radostis, 2003). This will lead to cell death through unregulated homeostasis by the mitochondria. There are no differences on gross post mortem examination between hypovitaminosis E and hyposelenosis. Muscle pallor is the typical finding, in both cardiac and skeletal muscle, with streaks of pallor and mineralisation present in some cases (Van Metre and Callan, 2001). Histopathology of muscle tissue may be required for confirmation. Selenium levels can be measured both directly and indirectly (through glutathione peroxidase levels) in the liver and blood (Koller et al., 1985).

1.4.3.1.2 Iodine and Thyroid Insufficiency

Thyroid insufficiency is typically seen clinically as calves born with goitres, but other clinical signs may also be seen, including poor hair quality, foetal death (including abortions and stillbirths), and the birth of premature or weak calves with low birth weights (Cutler and Jones, 2003). Reproduction and foetal development, along with basal metabolic rate is largely influenced by thyroid hormones, which require adequate iodine concentrations for production. Development of thyroid insufficiency is usually linked to the diet. There are three main recognized causes of thyroid insufficiency; iodine deficiency, goitrogens and selenium deficiency.

Diagnosis of iodine deficiency as a cause of perinatal mortality can be difficult, particularly if the deficiency is marginal. The weight of the thyroid gland in perinatal losses should be used, combined with iodine content of the thyroid and histopathology, to aid in diagnosis. Is has been proposed that the fresh weight of the thyroid gland of a full term foetus should not exceed 0.03% of total body weight, or the bodyweight in kilograms divided by the thyroid weight in grams should not be less than 2.5 (Cutler and Jones, 2003). Thyroid iodine should exceed 1200mg/kg dry matter. Histopathology of the thyroid may reveal colloid depletion and hypertrophy of the cuboidal epithelium lining of the follicles, but hyperplasia may also be present (Cutler and Jones, 2003). Blood sampling can be performed on live animals to aid in diagnosis, with plasma inorganic iodine or serum thyroxine levels measured. These can be problematic and often diagnosis is based on a positive response to supplementation (Cutler and Jones, 2003). Goitrogens, which are present in brassicas and leguminous plants may affect the ability of the thyroid gland to concentrate iodine, proximally affecting thyroid hormone production (Cutler and Jones, 2003). Selenium deficiency leads to failure to convert thyroxine to its active form, triiodothyronine, and can subsequently cause thyroid insufficiency even when dietary iodine and thyroxine stores are adequate. Full description of selenium deficiency can be found in section 1.4.4.1.1.

1.4.4 Calving Associated Causes of Perinatal Loss

1.4.4.1 Anoxia

Anoxia (absence of oxygen reaching the tissues) can severely reduce the chance of survival in perinatal calves. It is often a sequel to dystocia; however, premature placental separation, foetal stress and umbilical occlusion resulting from prolonged parturition may also be a cause (Mee, 1999). The risk of anoxia development is greatest in posteriorly presented calves (Kasari, 1994). During dystocia, anoxia often results from occlusion or premature rupture of the umbilical cord (Kasari, 1994). Anoxia for four minutes or longer will result in poor viability or death of the calf (Dufty and Sloss, 1977). If the calf is born alive, there will be slower responses and in many cases, the calf will fail to achieve sternal recumbency (Dufty and Sloss, 1977). In some calves, death will follow a period of tetany and opisthotonus (Dufty and Sloss, 1977). Where complete occlusion of the umbilical cord occurs, for six minutes or longer, death from anoxia will occur in the foetus (Kasari, 1994).

Perinatal mortality due to anoxia can be difficult to detect on post-mortem examination in some cases: there may be no gross lesions on post mortem examination due to the short duration between acute anoxia and death (Mee, 1999). When conditions leading to chronic hypoxia are present, meconium staining and visceral haemorrhages may be seen (Mee, 1999). Meconium staining on the calf can be a sign of intra-uterine stress (Barrier et al., 2013), as meconium release is seen after compression of the umbilical cord. However, there does not appear to be a relationship between intensity of meconium staining and degree of anoxia (Dufty and Sloss, 1977). Post mortem examination following death due to anoxia often reveals haemorrhage and petechiae throughout the myocardium, spleen, thymus and abomasum (Dufty and Sloss, 1977), along with pulmonary atelectasis, organ congestion and meconium staining (Mee, 2013a). Impaired blood flow to the umbilical vessels may result in foetal attempts at respiration, leading to inhalation of amniotic fluid (Kasari, 1994) and subsequent meconium inhalation. Although anoxia does not always result in death it can contribute to weak calf syndrome, reducing viability (Dufty and Sloss, 1977).

Anoxia following premature separation of the placenta is considered a minor cause of perinatal mortality, thought to be associated with excess selenium supplementation, induction of parturition and subclinical hypocalcaemia (Mee, 2013a). Large quantities of mucous in the respiratory tract, may be suggestive of suffocation of the calf during parturition, often secondary to premature cessation of placental function (Berglund et al., 2003).

1.4.4.2 Trauma

Traumatic lesions occurring during or post-partum, are reported to occur in 4.1% of perinatal mortality cases in beef herds (Waldner et al., 2010). The most common traumatic lesions seen in calves include rib fractures, rupture of the femoral nerve, spinal fractures, rupture of the liver, diaphragmatic tears, collapsed tracheas and fractures of the limbs. Fractured ribs are commonly seen following excessive pressure during passage through the pelvis, from both foetal oversize and excessive traction from the herdsman, or excessive expiratory movements secondary to anoxia (Schuijt, 1990). Artificial respiration and resuscitation may also lead to fractured ribs due to excessive force on the thorax by the herdsman (Bellows et al., 1986). Fractured ribs are typically seen as separation of the ribs from the sternum at the costo-chondral junction (Bellows et al., 1986) and liver lacerations and abdominal haemorrhage may be a sequel to vertebral or rib fractures (Murray and Leslie, 2013). Fractured ribs seriously compromise the ability of a calf to survive (McCoy et al., 1997) and when combined with other traumatic lesions, often deem the calf incompatible with life (Schuijt, 1990).

Sub-cutaneous oedema, bruising and fractures of the distal limbs are typically seen in perinatal calves due to excessive pressure from calving aids or improper placement of obstetrical chains and ropes (Bellows et al., 1986). Additionally, dystocia can cause both trauma and anoxia, which can be seen as localised subcutaneous oedema in the limbs, neck, and forehead (Wiske et al., 1994). Haemorrhage of the forehead, forelimbs, hind limbs or perineum may be seen

along with subdural and epidural haemorrhages of the brain and meninges (Wiske et al., 1994), following dystocia. Such haemorrhages have been detected in calves born following both dystocia and eutocia; however, bruising is more extensive after a difficult calving (Barrier et al., 2013). Lesions seen in the vertebrae following a traumatic birth include myelomalacia, spinal cord compression or severed spinal cord (Murray and Leslie, 2013). Perinatal mortality may also result from trauma to the calf from the dam post-partum, typically seen as fractured ribs, subcutaneous haemorrhage and fractures to the limbs (Wiske et al., 1994).

1.4.5 Other Causes of Perinatal Loss

1.4.5.1 Omphalorrhagia

The prevalence of omphalorrhagia as a cause of perinatal mortality is unknown (Mee, 2013a). The severity of cases can range from a small amount of perivascular haemorrhage surrounding the umbilical vessels to extensive haemoperitoneum with no other source of haemorrhage (Mee, 2013a). Calves are generally found dead within 1-48 hours of birth and may show signs of conjunctival pallor (Mee, 2013a). The prevalence of this condition in neonatal calves is unknown, as is the aetiology to this condition (Mee, 2013a). Possible aetiologies include; rapid rupture of the umbilical vessels, mycotoxicosis, prematurity, maternal injury and thrombocytopenia in the calf (Mee, 2013a). Post-mortem examination will reveal minor perivascular haematomas, and, if the blood loss is intra-abdominal, haemoperitoneum and intra-abdominal blood coagulation. No other sources of haemorrhage are usually detected (Mee, 2013a).

1.4.5.2 Hypothermia

There are two types of hypothermia, exposure and immersion (Torell et al., 1998). Immersion hypothermia is more commonly seen in neonatal calves due to saturation of the hair with uterine fluid (Torell et al., 1998). Hypothermia can result in a decreased venous outflow from the small intestine and a reduction in the transport of substances from the intestines to blood, which in a neonatal

calf, may predispose to disease from lack of immunity (Olsen et al., 1980). Diagnosis of hypothermia may be based on an appropriate history, combined with gross post mortem finding. Cold stress can be identified on post-mortem examination by subcutaneous oedema and haemorrhage of the distal forelegs and hind legs. Although this is similar to oedema seen with dystocia, it occurs to some extent in all four limbs, combined with subcutaneous oedema in the ventral sternum (Wiske et al., 1994). On the contrary, oedema seen with dystocia is usually seen in either the forelimbs or the hind limbs, depending on presentation.

1.4.5.3 Twin to Twin Transfusion Syndrome

When twin foetuses originate from a single zygote (monochorionic), a single chorion will develop, leading to unequal distribution of the blood supply due to vascular anastomoses (Peter, 2013). This typically presents as asymmetrical foetal growth and even foetal mortality (Peter, 2013). This condition is common in human identical twin pregnancies; however, it has not been reported in cattle, although the occurrence is possible (Peter, 2013). In humans the syndrome is characterised by intense congestion in one twin and severe anaemia in the other twin, but is an uncommon finding in live born twins (Becker and Glass, 1963).

1.4.5.4 Placental Dysfunction

Premature separation during parturition of the placental cotyledons from the caruncles is thought to occur in between 5-10% of bovine perinatal losses (Peter, 2013). An infectious aetiology is thought to be the cause of most premature placental separation cases, although abnormal covering of the internal os of the cervix may also account for some separation (Peter, 2013). Premature placental separation will lead to anoxia in the foetus, which proximally results in death (Peter, 2013). Impaired placental function, poor maternal nutrition and maternal stress can lead to impaired foetal organ development, through insufficient nutrient transfer to the foetus, resulting in reduced chances of survival around parturition (Barrier et al., 2013). Diagnosis of premature

placental separation is based on an appropriate history by the client (placenta expulsed along with or prior to delivery of the calf), combined with gross signs of anoxia in the foetus.

1.4.5.5 Infanticide

Although not commonly reported or studied in cattle, infanticide among animals is a widespread phenomenon with no definite explanation (Blaffer, 1979), which leads to perinatal mortality. One possible explanation to infanticide is the elimination of a handicapped or supernumerary infant (Blaffer, 1979). In pigs, infanticide is characterised by the pig showing aggressive and restless behaviour towards their offspring and making attempts to kill them within the first 24 hours post-partum (Quilter et al., 2007). It is considered that this behaviour may be due to genetic predisposition, previous experience, metabolic and endocrine status and local environmental factors and is seen more often when sows are confined at birth. The incidence of infanticide in pigs varies at farm level, which suggests it is due to a genetic predisposition or previous experience. A genetic predisposition to infanticide does exist (Quilter et al., 2007) and may be a potential source of perinatal mortality in calves. Bovine perinatal losses due to infanticide may be based on appropriate history and traumatic lesions in the calf, which do not fit with trauma occurring during parturition. Infanticide may be both intentional and unintentional.

1.4.5.6 Unexplained

Causes of stillbirth of non-infectious aetiologies are usually multifactorial. In up to 50%, (Mee 2013a), despite a thorough post-mortem examination, no aetiology is found for stillborn calves (Mee, 2015b). In these situations, although no cause of death is reached, in most cases, the time of death is usually established and many aetiologies can be eliminated (Mee, 2015b), which can be reassuring to the client.

1.5 Conclusions

A large number of studies have been performed investigating perinatal mortality in dairy herds in Great Britain and Ireland; however, work in the British beef industry is lacking. In Canadian beef herds, progress is being made as to the issues surrounding perinatal losses, but work in this field in Great Britain is lagging behind.

In order to further our understanding of perinatal mortality in beef herds in Great Britain, a standardised definition of these losses should be created. From here, investigations into incidence and aetiologies can proceed. Once the incidence and aetiologies have been better defined, focus needs to be placed on modifiable risk factors such as age at first calving, gestational nutrition, body condition score at parturition, calving management and sire selection. If control is aimed at less modifiable factors such as parity, season of calving and foetal gender, little improvements will be seen with regards to perinatal mortality rates (Mee, 2011a).

The lack of motivation of farmers in investigating perinatal losses, has led to a so-called 'farm blindness' with perinatal losses becoming normalised and the incidences under estimated (Mee, 2011a). Perinatal losses represent a huge loss to the beef industry both in terms of finance and welfare standards (Brickell et al., 2009) and are an issue that needs addressing before action is forced upon the industry (Mee, 2011a).

In general practice in the United Kingdom, gross pathological examination and sampling techniques often prove undiagnostic, resulting in submission of the entire carcass to local laboratories. Due to the geographical location of local laboratories, this may lead to reduced diagnostic rates due to time delay and temperature variations during submission of the carcass. Furthermore time and fuel costs associated with taking a carcase to the laboratory may put farmers off. High costs and diagnostic rates of between 5-50%, as reported by Mee, 2013a, have discouraged farmers from submitting neonatal losses for

investigation. Additionally, during gross pathological examination, if there is an obvious cause of death, examination may cease and additional examination or sampling may not occur.

This study was initiated due to a perceived increasing and high level of neonatal losses in beef herds in Orkney, by local Veterinary surgeons. The study aimed to propose a standardised system for investigation of bovine perinatal losses, which could be used both in practice and in referral situations. Additionally, it was aimed to reveal some of the common aetiologies and to quantify the incidence of perinatal losses on local farms, as this knowledge is lacking.

2. Development of a Protocol for the Systematic Investigation and Categorisation of Bovine Perinatal Losses in Beef Cattle

Perinatal mortality (defined as a full term stillborn calf or death within 48 hours post-partum) has a significant impact on animal welfare and high associated costs to the livestock owner (Anon., 2015a). The associated aetiologies are poorly understood in British beef herds and this is a significant barrier to reducing these losses. Investigation of bovine neonatal pathology can include clinical history, gross post mortem examination and a wide range of laboratory testing on various samples, but there is currently no accepted standard investigation protocol. Additionally, there is no established consensus on the appropriate interpretation of the results generated. Interpretation is particularly complex, as abnormal findings may have originated in the pre-, intra-, or post-partum period. In addition, while there may be a single identifiable proximal cause of death (factor which causes death directly), there can be multiple significant contributory risk factors, which must also be identified and recorded to allow a full understanding of the case. For example arthrogryposis developing pre-partum does not cause death directly, but may lead to prolonged parturition and the development of anoxia. While anoxia is the proximal cause of death, arthrogryposis is a major contributory factor, which in turn has multiple potential aetiologies (infectious, inherited, nutritional and toxic). It is therefore of vital importance that the investigator is able to establish a timeline of pathological processes and a comprehensive description of all contributing risk factors. The absence of an established protocol for investigation and categorisation of bovine perinatal deaths means that the process can be subjective and variable between individual operators, may be incomplete in some cases and makes comparison between reported findings difficult. A comprehensive standard protocol would allow users to generate a greater understanding of aetiologies and contributing risk factors, allow comparison between studies that use the protocol and ultimately facilitate effective identification of appropriate preventative measures.

2.1 Aim

The aim of this part of the study was to develop a protocol for the comprehensive investigation and categorisation of bovine perinatal losses.

2.2 Materials and Methods

Ethical approval for clinical research involving animal subjects, material or data was granted by the University of Glasgow, Ethics and Welfare Committee. The protocol was developed over four distinct stages as shown in Table 2.1.

Table 2.1; *An overview of the four stages used during the development of a protocol for the investigation and categorisation of bovine perinatal deaths*

Stage	Detail	Timeline
Literature review	Extensive review of literature to determine known causes of perinatal death, diagnostic criteria and associated risk factors	August-December 2015
Development of standard investigation protocols	Clinical history, gross post mortem, sampling and laboratory testing protocols developed in consultation with expert pathologists and SRUC	January 2016
Collection of case material	Submission of cases from beef herds to a post-mortem facility in Orkney, with each case investigated using the established investigation protocols. Preliminary findings reported to the submitting farmer.	1st February 2016-10th June 2016
Development of a standard categorisation algorithm	Based on a process established during review of all submitted cases by a panel including expert pathologists, SRUC, University clinicians and veterinary surgeons.	October 2016

2.2.1 Literature Review to Establish Definitive List of Causes of Death

An extensive on-line review was performed using the search engine, Google Scholar (as it was most easily accessible search engine) to establish the definitive causes of death and diagnostic criteria for bovine perinatal losses. Additional specific searches were also performed on PubMed, Cattle Practice and Web of Science. Search phrases included bovine perinatal mortality, bovine stillbirths, aetiologies of bovine perinatal mortality, causes of death in beef

calves and perinatal mortality in beef cattle. Existing Veterinary Investigation Diagnostic Analysis codes, 2016 (VIDA, case definitions used by the current UK veterinary surveillance network) were also included in the review. Based on the results of the extensive literature review, a definitive list of the causes of bovine perinatal death, diagnostic criteria and associated risk factors was established.

2.2.2. Development of Standard Investigation Protocols

2.2.2.1 Development of History Questionnaire

A standard clinical history questionnaire for bovine perinatal mortality was developed. The questionnaire collected relevant information from the pre-, intra- and post-partum management of the dam, based on the known risk factors for perinatal mortality. The questionnaire was reviewed and refined by two expert pathologists, three veterinary surgeons and staff at SRUC and was pre-tested by an Orkney beef farmer (Appendix 1).

2.2.2.2 Development of Gross Post Mortem, Sample Collection and Laboratory Test Protocol

Initial one-to-one training of the author in bovine neonatal gross post mortem examination and sample collection was provided by John Mee MVB MVM PhD DipECBHM, Teagasc, Fermoy, for one week duration. Training allowed the author to develop a standard written gross post mortem protocol for the approach to examination of perinatal dead calves.

The tissue collection and diagnostic test protocol were discussed prior to commencement of the study, with three trained specialists (John Mee (Teagasc), Sandra Scholes and Timothy Geraghty (Scotland's Rural College (SRUC)). The definitive tissue collection and sampling protocol drew extensively from established sampling and laboratory testing protocols at SRUC Disease Surveillance centres, modified following consideration of the output of the literature review (2.2.1 above). Consideration was also given to the geographical distance between the post mortem facility and the laboratory with regards to

tissue collection, handling and delivery. The tissue collection and test protocol was developed to be easily followed and used by veterinary surgeons in general practice and pathologists at veterinary laboratories.

2.2.3 Submission and Investigation of Cases

Beef clients of Northvet Veterinary Group, on Orkney, were made aware of the opportunity to submit suitable calves for post-mortem examination at a farmers meeting held in 2015. Eligible calves (stillborn or dead within 48 hours of birth) were accepted between 1st February and 10th June 2016 (corresponding to the spring calving season and the availability of the author). Further details of the post mortem examination and testing outcomes can be found in Chapter 3.

2.2.3.1 Post Mortem Facility

A basic post-mortem examination facility was already available at Northvet Veterinary Group, Lamaquoy, Orkney. The room was dual purpose and also used as an examination room for farm animals. Following the author's training in post mortem examination and experience of facilities at Teagasc, the facility at Northvet was modified and equipped to allow post mortem investigations to be conducted in a similar manner and to the same standard. On arrival, specimens were introduced directly to the facility through an external door, preventing contamination of other facilities within the building.

A stainless steel table (75cm x 115cm), with a central drainage hole, was used for examination of calves, which was manually cleaned, using a wire brush and mains water only, on completion of each post-mortem examination. A hosepipe, connected to the mains water system was available for use in the post-mortem facility. A double check valve was present on this hose pipework, in compliance with The Water Supply (Water Fittings) Scotland) Byelaws, 2014. No specific drainage requirements were necessary in the post-mortem facility as a private septic tank was in place. Sharps and clinical waste bins were available within the post mortem facility and all material was disposed of according to regulations. A homemade hoist (pulley system) was installed from the roof of the post mortem facility, suspended on overhead steel cables, to allow elevation of calves on to

the examination table, single-handed. The pulley system also allowed suspension of the carcass to aid packaging in plastic bags after completion of the gross post mortem examination, for collection by the client. Scales (Maxi Walk-on Pet Scales) were available for weighing carcasses (range 0-150kg). Due to logistics, these were placed immediately outside of the post-mortem facility, but within the practice building. Scales were periodically (at minimum weekly) calibrated using Hogboxes (weighing 10kg). All post-mortem examinations were performed wearing full-length, disposable plastic gowns and disposable plastic gloves. Gloves were changed between submissions. Waterproof trousers and wellington boots were worn at all times in the post-mortem room and were to be disinfected at the end of each post-mortem examination. Hand wash facilities were available within the post mortem facility.

2.2.3.2 Carcass Submission, Disposal and Provisional Results

Farmers were asked to contact Northvet Veterinary Group prior to delivery of a perinatal loss to the post mortem facility. The standard clinical history questionnaire (2.2.2.1) was completed by the farmer before submission, and the standard post-mortem, sample collection and laboratory test protocols followed for every case (2.2.2.2). All submissions were delivered in either a leak-proof container (Hogbox 190L) or sealed in double plastic bags. Placentae were to be submitted with all calves where possible. A unique identification number was given to each submission, linking it to the submitting herd. All carcasses were collected for disposal by burial by the client after completion of the investigation, according to Animal By-Product (Scotland) Regulations 2003 (Anon., 2003) (on farm burial of carcases is a derogation applicable within the Orkney Islands). Carcasses and exteriorised viscera were sealed in double plastic bags for collection.

Provisional results (including provisional diagnoses when possible) were reported to submitting farmers in all cases during the period of case submission. Provisional diagnoses were reached by the author, after consideration of clinical history, gross pathological examination and laboratory test results and refined during ad hoc dialogue between the author, SRUC and expert pathologists

(including periodic case discussion via telephone and review of photographic material).

2.2.4 Development of a Standard Algorithm for Catergorisation of Time and Cause of Death

2.2.4.1 Case Review Conference

At the end of the data collection period, submissions were discussed in detail at a case review consensus conference with a group of trained specialists (John Mee, Teagasc; Timothy Geraghty and Sandra Scholes, SRUC) and veterinary Surgeons (Robert Norquay, Northvet Veterinary Group; Jayne Orr and Kathryn Ellis, University of Glasgow). The group reviewed each case in turn by semi-structured discussion by reviewing the history, gross post mortem findings (including photographs), results of laboratory testing and histopathology. The aim of the discussion was to reach a consensus view on the time of death, proximal cause of death, and to identify all significant contributory factors.

2.2.4.2 Creation of the Interpretation Algorithm for Time and Cause of Death

As the group worked through each case a refined systematic process emerged that was subsequently used by the author to create a written interpretation algorithm for the proximal cause of death and contributing factors. The final algorithm was then reviewed a final time by one expert pathologist (John Mee, Teagasc).

2.3 Results

2.3.1 Case Definitions of Proximal Causes of Death

The determined lists of the causes of bovine perinatal death established following review of the literature are shown in Tables 2.2, 2.3 and 2.4 for pre-, intra-, and post-partum death, respectively.

Table 2.2; *Case definitions and associated risk factors of the proximal causes of death* ***pre-partum*** *in bovine perinatal losses*

Cause of Death	Case Definition	Risk factors

Congenital defect incompatible with life	• Major congenital malformation identified by gross pathology as cause of death (lesions incompatible with life). Examples include;
	  ○ Schistosomus reflexus
	• Note - Non-lethal congenital malformations may be present that contribute to cause of death but are not proximal cause of death. Examples include;
	  ○ Arthrogryposis
		• Random genetic mutation
		• Exposure to infectious agents
		  ○ BVDV
		  ○ Schmallenberg virus
		• Pharmaceutical teratogens
		• Toxic plants

Hyposelenosis/ hypovitaminosis E	• Identified by gross pathological lesions ○ Pale cardiac/ other muscle • Plus either histopathology of muscle tissue or Foetal liver selenium / vitamin E below reference range.	• Low selenium/ vitamin E diet in the dam

Infection	**Sporadic ascending bacterial infection**
	• Gross lesions indicative of infection
	  ○ E.g. enlarged and/or congested lymph nodes
	• Plus either positive micro-organism culture and/ or Histopathology suggestive of *in-utero* inflammatory insult
		• Presence of ubiquitous pathogens in environment or vagina
		  ○ *Escherichia coli*
		  ○ *Haemophilus somnus*
		  ○ *Pasteurella* spp.
		  ○ *Serratia* spp.
		  ○ *Staphylococcus* spp.
		  ○ *Trueperella pyogenes*
		  ○ *Streptococcus* spp.

	Specific bacterial infection • Gross lesions indicative of infection ○ E.g. enlarged and/or congested lymph nodes • Positive micro-organism culture • Histopathology suggestive of *in-utero* insult	E.g. infection with; • *Leptospira* spp. • *Listeria* spp. • *Brucella* spp. • *Campylobacter* spp. • *Salmonella* spp. • *Anaplasma phagocytosis* • *Coxiella burnetti* • *Bacillus licheniformis*

	Viral infection
	• Gross lesions suggestive of infection
	  ○ Presence of congenital defect
	  ○ Focal necrotising lesions
	• Further testing required for confirmation
	  ○ PCR, virus isolation, serology
		E.g. infection with;
		• BVDV
		• Bovine Herpes virus
		• Schmallenberg virus

Table 2.2 continued; *Case definitions and associated risk factors of the proximal causes of death* ***pre-partum*** *in bovine perinatal losses*

Cause of Death	Case Definition	Risk factors

Infection	**Parasitic infection**
	• Gross lesions suggestive of infection
	  ○ Multifocal, non-suppurative encephalitis
	  ○ Myocardial necrosis
	  ○ Multifocal mononuclear placentitis
	• Further testing required for confirmation
	ELISA, histopathology of brain or myocardium
		Neospora caninum infection in the dam via horizontal or vertical transmission

	Mycotic infection
	• Gross lesions indicative of infection
	  ○ Enlarged and/or congested lymph nodes
	  ○ Growth retardation
	  ○ Pale, enlarged liver
	  ○ Dermatitis
	  ○ Placentitis
	Plus either positive micro-organism culture and/ or Histopathology suggestive
		• *Aspergillus* spp.
		• *Mucor* spp.
		• *Candida* spp.

Iodine Insufficiency	• Identified by gross pathological examination
	  ○ Thyroid gland weight over 30g
	  ○ Poor hair quality
	• Histopathology of thyroid gland
	Thyroid iodine concentration <1200mg/kg DM is supportive
		• Iodine deficiency in diet of dam
		• Goitrogens
		  ○ Brassicas
		  ○ Leguminous plants
		• Selenium deficiency in diet of dam

Table 2.3; *Case definitions and associated risk factors of the proximal causes of death* ***intra-partum*** *in bovine perinatal losses*

Cause of Death	Case Definition	Risk Factors
Anoxia	• Evidence of anoxia on gross post mortem examination regardless of other pathological findings (excluding acute fatal trauma) o Sub-serosal, conjunctival, splenic, epicardial and pleural haemorrages o Organ congestion o Meconium staining	• Prolonged parturition o Foetal oversize o Congenial malformation o Uterine inertia o Infection o Diagnosed on history and presence of lesions such as oedema of the limbs, neck, head or tongue • Premature placental separation • history of delivering placenta prior to, or along with calf
Traumatocia	• History of observed or assisted difficult calving consistent • Gross pathological evidence of acute trauma likely sufficient to have caused death o Liver rupture o Fractured ribs o Fractured vertebrae o Limb fracture with haemorrhage	• Foetal oversize o Absolute o Relative • Excessive traction during parturition • Presentation and position
Twin to Twin Transfusion Syndrome	• History of multiparous birth • Gross pathological findings Pale carcass	• Multiparous birth

Table 2.4; *Case definitions and associated risk factors of the proximal causes of death* ***post-partum*** *in bovine perinatal losses*

Cause of Death	Case Definition	Risk Factors
Acute bacterial infection	See Table 2.2	• Inadequate colostral intake • Poor quality colostrum

Colostrum aspiration	• History of assisted feeding
	• Gross pathological findings
	  ○ Marked congestion and/ or consolidation of cranio-ventral lung lobes
	  ○ Colostrum present in trachea and/ or lower bronchioles.
		• Oesophageal tubing or bottle feeding with colostrum
		• Cleft palate

External omphalorrhagia	• History of bleeding from navel
	• Blood in pen
	• Gross pathological findings
	  ○ Pale carcass
	• No evidence of other pathology from standard follow-up testing
		• Unknown risk factors but potentially include
		  ○ Rapid rupture of umbilical vessels
		  ○ Mycotoxicosis
		  ○ Prematurity
		  ○ Maternal injury
		  ○ Thrombocytopenia in the calf

Hypothermia	• History of low rectal temperature
	• Gross pathological findings
	  ○ Subcutaneous oedema in all four limbs and ventral sternum
		• Exposure hypothermia
		  ○ Low environmental temperature
		  ○ Poor management at parturition
		• Immersion hypothermia
		  ○ Saturation of hair coat with fluid (usually uterine)

Internal omphalorrhagia	• Gross pathology indicative
	  ○ Haemoabdomen
	  ○ No obvious source of haemorrhage
	  ○ Pale carcass
		• Unknown risk factors but potentially include
		  ○ Rapid rupture of umbilical vessels
		  ○ Mycotoxicosis
		  ○ Prematurity
		  ○ Maternal injury
		  ○ Thrombocytopenia in the calf

Non-parturient trauma	• History of non-parturient trauma
	  ○ Trampling or attack by dam
	  ○ Trauma from other source (stockworker, machinery etc.)
	• Gross pathological findings of trauma
	  ○ Haemorrhages
	  ○ Fractures
		• Infanticide
		• Trampling in crowded pen
		• Accidental trauma in environment

2.3.2 Development of Standard Investigation Protocols

2.3.2.1 Development of History Questionnaire

The developed history questionnaire can be found in Appendix 1.

2.3.2.2 Development of Gross Post Mortem Protocol

During training at Teagasc, Fermoy, 13 post mortem examinations were observed, and seven post mortem examinations were performed by the author under direct supervision, allowing development of the standardised protocol used during the study (Appendix 2). Full detailed description of post mortem examination technique in Chapter 3. A standard post mortem examination report was completed by the author on completion of the post mortem examination (Appendix 3) detailing relevant gross post-mortem findings.

2.3.2.3 Development of Tissue Collection and Test Protocol

The definitive tissue collection and test protocol developed in this study can be found in Appendix 4.

2.3.3 Submission of Cases

A total of 53 of bovine perinatal losses were submitted for investigation from 1st February 2016- 10th June 2016. All calves submitted fulfilled the study criteria (full-term stillborn or death within 48 hours post-partum). All cases were subject to standard investigation processes, and all cases were then discussed at the case review conference.

2.3.4 Standard Categorisation of Time and Cause of Death Algorithm

The final categorisation algorithm uses a four-step process;

1. Categorise the time of death of the calf
2. Use flow charts to identify all applicable diagnoses
3. Identify a single most likely proximal cause of death and record other diagnoses as contributory factors
4. Prepare a concise summary statement of the case

2.3.4.1 Categorising Time of Death

Three categories for time of death are used:

- Dead at the beginning of parturition (pre-partum)
- Alive at the beginning of parturition but dead on delivery (intra-partum)
- Alive on delivery but dead within 48 hours of delivery (post-partum)

For categorisation of death pre-partum, the following criteria must be met;

- Presence of haemoglobin staining of tissues, and/ or
- Serosanginous fluid in thorax and/ or abdominal cavity, and/ or
- Opaque cornea

Combined with presence of the following supportive findings;

- Flaccid muscle tone
- Foetal hoof presence (not predated)
- Absence of blood clot in umbilical artery
- Atelectic lungs
- Consistent history (no signs of life during assisted parturition; absence of swallow reflex, withdrawal reflex or anal tone)

For categorisation of death intra-partum, the following criteria must be met;

- Consistent history (alive on examination during parturition but dead on delivery) and/ or presence of all of the following;
- Absence of haemoglobin staining in tissues
- Foetal hoof presence (not predated)
- Absence of blood clot in umbilical artery
- Absence of milk in abomasum
- Absence of serosanginous fluid in thorax or abdominal cavity

For categorisation of death post-partum, the following criteria must be met;

- Presence of milk in abomasum, and/ or
- Presence of blood clot in umbilical artery, and/ or
- Consistent history (alive on delivery)

Combined with presence of all of the following;

- At least partial inflation of lungs
- Rigid muscle tone (death to post mortem interval dependent)
- Foetal hooves absent (not predated)

2.3.4.2 Identifying all Applicable Diagnoses for Pathology

Three flow charts (one for each time of death category) are then used for the systematic interpretation of the investigation findings (history, gross pathology, laboratory test results and histopathology). The three flow charts are shown in Figures 2.1, 2.2 and 2.3. For calves categorised as dead pre-partum, only Figure 2.1 is used. For calves categorised as dead intra-partum, both Figures 2.1 and 2.2 are used. For calves categorised as dead post-partum, all three Figures are worked through. This therefore ensures the detection and recording of significant pathology present from a time-period prior to the time of death. For example; For a calf categorised as dead post-partum with arthrogryposis, signs of anoxia and infection, Figures 2.3 should be worked through for the congenital malformation, Figure 2.2 for the signs of anoxia, and Figure 2.1 for the signs of infection.

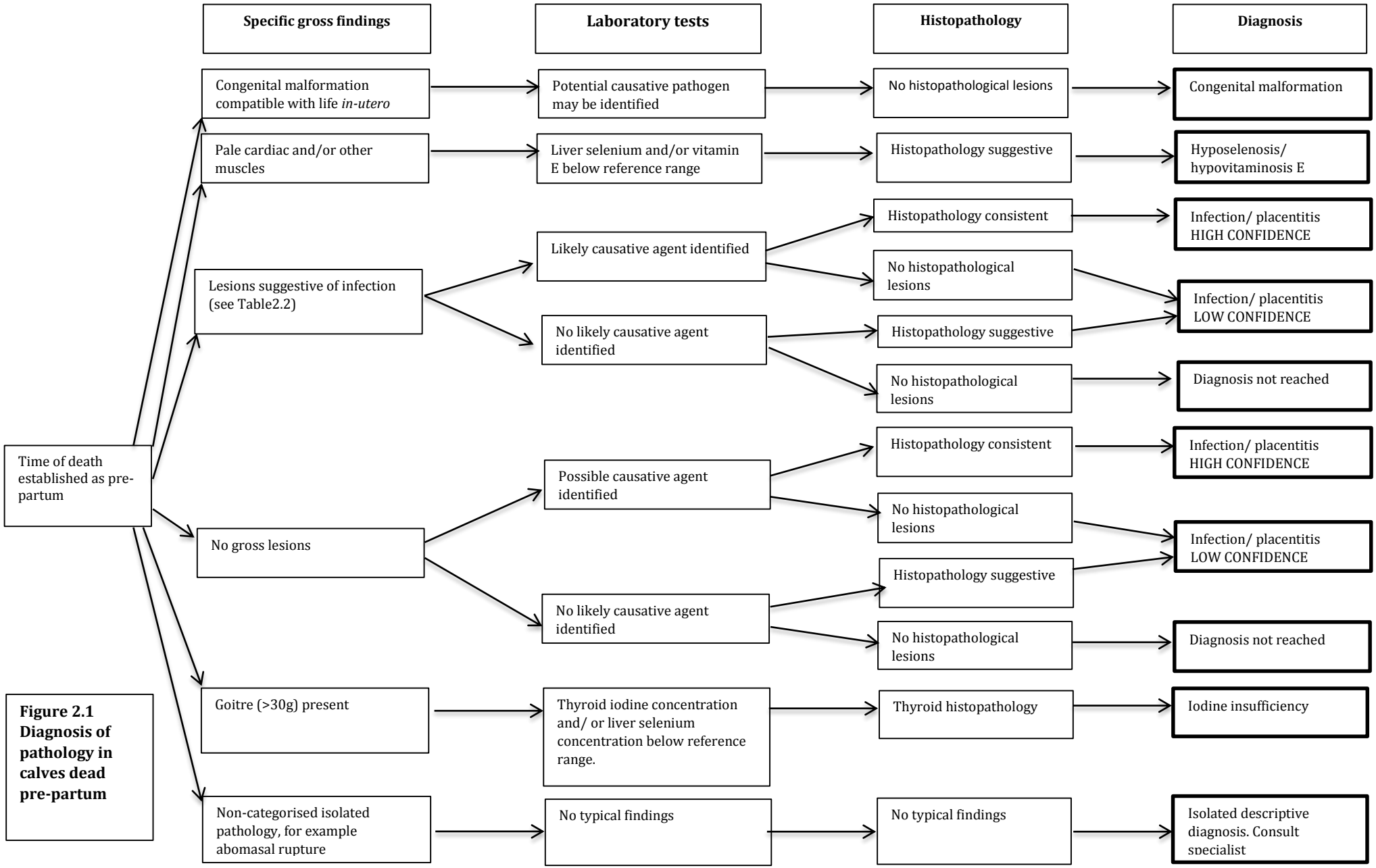


Figure 2.1 Diagnosis of pathology in calves dead pre-partum

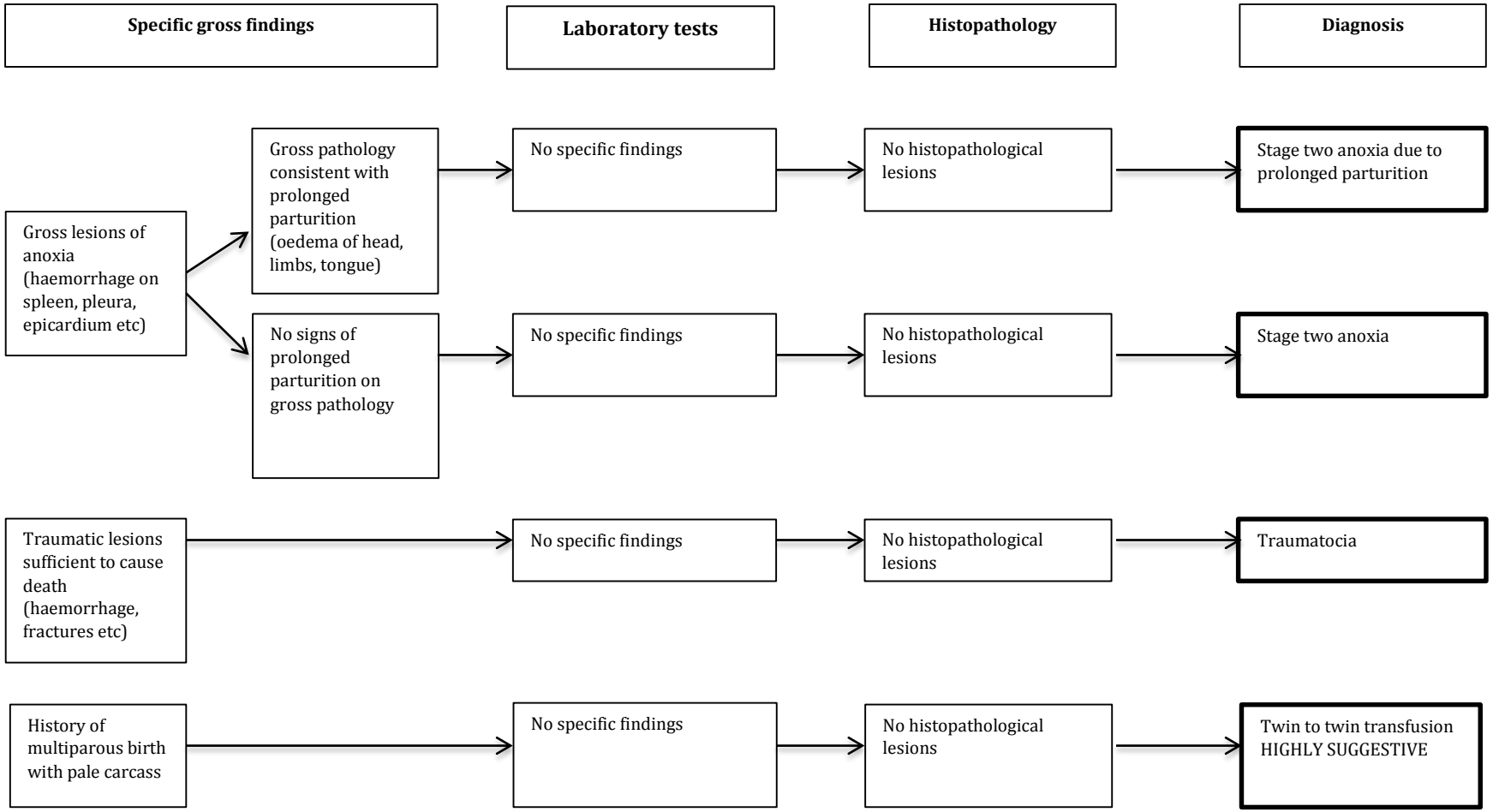


Figure 2.2 Diagnosis of pathology in calves dead intra-partum flow chart

(To be used in conjunction with Figure 2.1 for calves categorised as dead intra-partum)

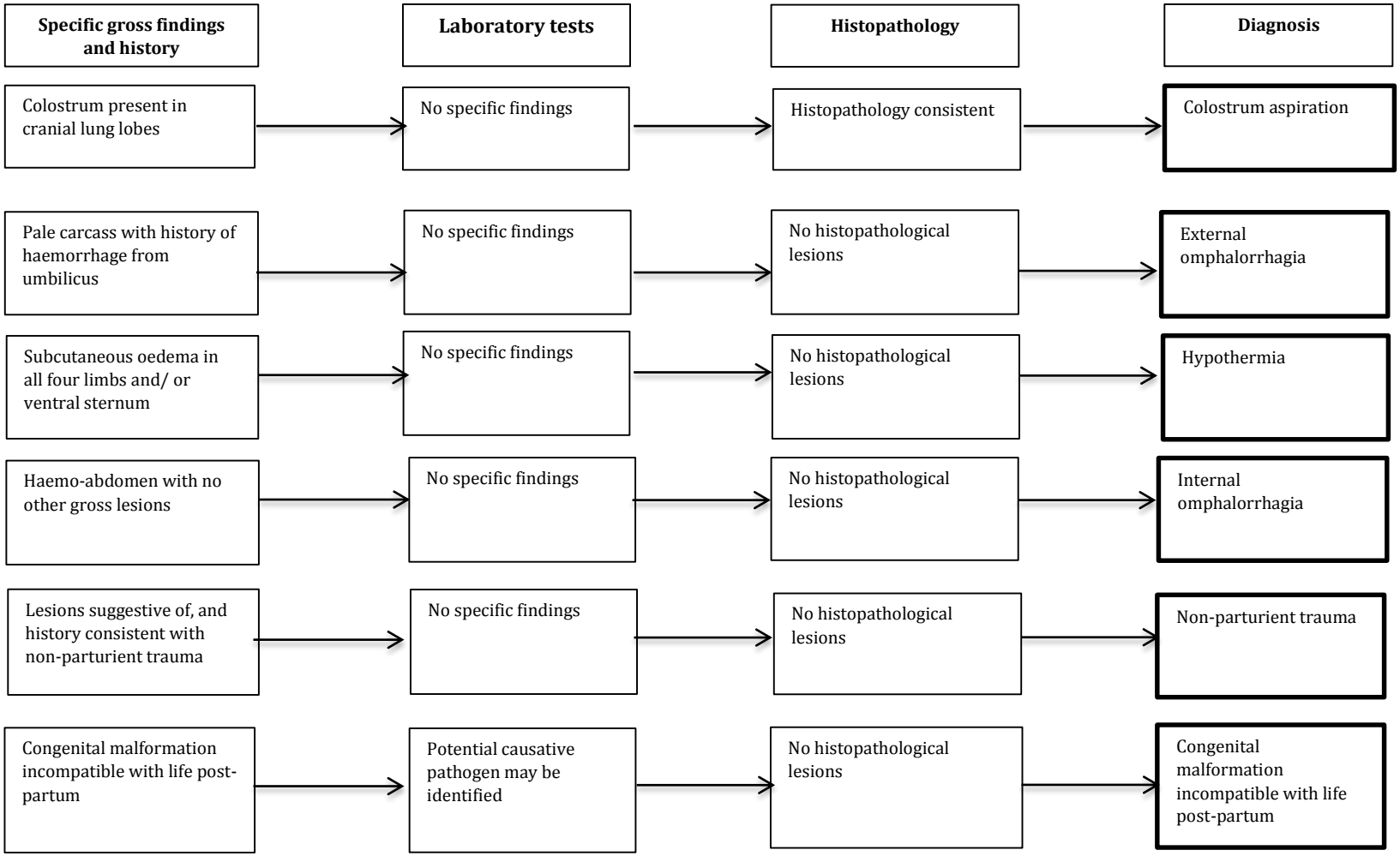


Figure 2.3 Diagnosis of pathology in calves dead post-partum flow chart

(To be used in conjunction with Figures 2.1 and 2.2 for calves categorised as dead post-partum)

2.3.4.3 Selecting a Single Proximal Cause of Death

When diagnostic criteria are met for only a single diagnosis, this is taken as the proximal cause of death. When multiple diagnoses are identified, a single most-likely proximal cause of death is identified, with all other diagnoses then considered as contributory factors. The selection of a single proximal diagnosis in these cases remains a subjective clinical decision made on a case by case basis. To minimize the subjective nature of this process, two tables were produced to give a guide as to what would normally be considered the proximal cause (tables 2.5 and 2.6). After consideration of the time of death, the appropriate table should be worked through for all diagnoses. For example; where congenital malformation is present along with anoxia and infection, congenital malformation versus infection initially shows infection to be the most likely proximal cause of death. Subsequently infection versus anoxia shows that anoxia is the most probable cause of death. The resulting proximal cause of death will be the same, regardless of the order of diagnoses worked through.

Table 2.5; *Table used to establish probable proximal cause of death in calves categorised as dead **pre-, or intra-partum***

Diagnosis	Congenital malformation	Infection	HypoSe/Hypo vitamin E	Iodine insufficiency	Anoxia	Traumatocia	Twin to twin transfusion	Non-categorised isolated pathology
Congenital malformation	Congenital malformation	Infection	HypoSe/Hypo vit E	Iodine insufficiency	Anoxia	Traumatocia	Twin to twin transfusion	*Non-categorised isolated pathology
Infection	Infection	Infection	*Infection	*Infection	Anoxia	Traumatocia	Twin to twin transfusion	*Non-categorised isolated pathology
HypoSe/Hypo vitamin E	HypoSe/Hypo vit E	*Infection	HypoSe/Hypo vit E	Iodine insufficiency	Anoxia	Traumatocia	Twin to twin transfusion	*Non-categorised isolated pathology
Iodine insufficiency	Iodine insufficiency	*Infection	Iodine insufficiency	Iodine insufficiency	Anoxia	Traumatocia	Twin to twin transfusion	*Non-categorised isolated pathology
Anoxia	Anoxia	Anoxia	Anoxia	Anoxia	Anoxia	Traumatocia	Anoxia	Anoxia
Traumatocia	Traumatocia	Traumatocia	Traumatocia	Traumatocia	Traumatocia	Traumatocia	Traumatocia	Traumatocia
Twin to twin transfusion	Twin to twin transfusion	Twin to twin transfusion	Twin to twin transfusion	Twin to twin transfusion	Anoxia	Traumatocia	Twin to twin transfusion	*Non-categorised isolated pathology
Non-catergorised isolated pathology	*Non-categorised isolated pathology	*Non-categorised isolated pathology	*Non-categorised isolated pathology	*Non-categorised isolated pathology	Anoxia	Traumatocia	*Non-categorised isolated pathology	*Non-categorised isolated pathology

** Clinical judgement must be used to determine the severity of the both pathologies to determine which pathology would be more likely to be the proximal cause of death.*

Table 2.6; *Table used to establish probable cause of death in calves categorised as dead* ***post-partum***

Diagnosis	Congenital malformation	Infection	Anoxia	Colostrum aspiration	External omphalorrhagia	Hypothermia	Internal omphalorrhagia	Non-parturient trauma	HypoSe/Hypo vitamin E	Iodine insufficiency	Non-categorised isolated pathology
Congenital malformation	Congenital malformation	*Infection	Anoxia	Colostrum aspiration	External omphalorrhagia	Hypothermia	Internal omphalorrhagia	Non-parturient trauma	*Congenital malformation	*Congenital malformation	*Non-categorised isolated pathology
Infection	*Infection	Infection	Anoxia	*Colostrum aspiration	External omphalorrhagia	Hypothermia	Internal omphalorrhagia	Non-parturient trauma	*Infection	*Infection	Non-categorised isolated pathology
Anoxia	Anoxia	Anoxia	Anoxia	Anoxia	Anoxia	Anoxia	Anoxia	Anoxia	Anoxia	Anoxia	Anoxia
Colostrum aspiration	Colostrum aspiration	*Colostrum aspiration	Anoxia	Colostrum aspiration	N/A	Hypothermia	N/A	Non-parturient trauma	*Colostrum aspiration	*Colostrum aspiration	*Non-categorised isolated pathology
External omphalorrhagia	External omphalorrhagia	External omphalorrhagia	Anoxia	N/A	External omphalorrhagia	N/A	N/A	N/A	External omphalorrhagia	External omphalorrhagia	*External omphalorrhagia
Hypothermia	Hypothermia	Hypothermia	Anoxia	*Hypothermia	N/A	Hypothermia	N/A	N/A	Hypothermia	Hypothermia	*Hypothermia
Internal omphalorrhagia	Internal omphalorrhagia	Internal omphalorrhagia	Anoxia	N/A	N/A	N/A	Internal omphalorrhagia	N/A	Internal omphalorrhagia	Internal omphalorrhagia	Internal omphalorrhagia
Non-parturient trauma	Non-parturient trauma	Non-parturient trauma	Anoxia	Non-parturient trauma	N/A	N/A	N/A	Non-parturient trauma	Non-parturient trauma	Non-parturient trauma	Non-parturient trauma
HypoSe/Hypo vitamin E	*Congenital malformation	*Infection	Anoxia	*Colostrum aspiration	External omphalorrhagia	Hypothermia	Internal omphalorrhagia	Non-parturient trauma	HypoSe/Hypo vit E	Iodine insufficiency	*Non-categorised isolated pathology
Iodine insufficiency	*Congenital malformation	*Infection	Anoxia	*Colostrum aspiration	External omphalorrhagia	Hypothermia	Internal omphalorrhagia	Non-parturient trauma	Iodine insufficiency	Iodine insufficiency	*Non-categorised isolated pathology
Non-categorised isolated pathology	*Non-categorised isolated pathology	*Non-categorised isolated pathology	Anoxia	*Non-categorised isolated pathology	*External omphalorrhagia	*Hypothermia	Internal omphalorhhagia	Non-parturient trauma	*Non-categorised isolated pathology	*Non-categorised isolated pathology	*Non-categorised isolated pathology

** Clinical judgement must be used to determine the severity of the both pathologies to determine which pathology would be more likely to be the proximal cause of death.*

2.3.4.4 Preparation of Single Summary Statement

A single summary statement is then produced to a standard format. For example;

'*The findings of this investigation are consistent with death of this calf occurring post-partum. The proximal cause of death is considered most likely to be external omphalorrhagia. Risk factors associated with this are unknown but may include; rapid rupture of umbilical vessels, mycotoxicosis, prematurity, maternal injury or thrombocytopenia in the calf.*'

'The findings of this investigation are consistent with death of this calf occurring intra-partum. The proximal cause of death is considered most likely to be anoxia, with one other contributory factor identified (congenital malformation). Risk factors associated with anoxia in this case, include prolonged parturition secondary to presentation. Risk factors associated with congenital malformation include, infection, random genetic mutation and exposure to teratogens.'

2.3.5 Overview of Complete Process

To summarise, an overview of the systematic process for investigation of bovine perinatal losses is outlined below.

- Collect detailed history from client regarding pre-, intra- and post-partum management of calf and dam (see Appendix 1).
- Perform gross post mortem examination (as per Appendix 3) with collection of appropriate samples and laboratory tests (see Appendix 4).
- Establish time of death using criteria in Tables 2.2-2.4.
- Establish all applicable diagnoses for gross post mortem findings, laboratory testing and histopathology using Figures 2.1-2.3.
- Establish most likely proximal cause of death on a case by case basis referring to tables 2.4-2.5 as a guide. Record all other diagnoses not considered probable proximal cause of death are contributing factors to death.
- Prepare summary statement for farmer.

2.4 Discussion

The protocol developed during this study attempts to provide a comprehensive, step-wise guide to investigating and categorising bovine perinatal mortality. Although systems have been developed for classification of the causes of death for bovine perinatal losses (Mee, 2013c), and protocols developed for examination and sampling of such losses (Berglund et al., 2003), no specific protocol has previously been reported in the literature which covers the full investigation process. By covering all aspects of the investigation, it is hoped that this protocol would facilitate comprehensive investigation, reduce inter-operator variation and provide a standard format for interpreting, recording and reporting results. Use of the protocol may improve diagnostic rates of bovine perinatal losses.

Discussion of cases, with trained specialists, ensured some level of consistency and agreement in the categorisation of the proximal cause of death, and contributing factors to death. Using expert panel discussions is novel in terms of deciding the time and cause of death of bovine perinatal losses. In previous studies into bovine perinatal mortality, the cause of death and time of death have been established based on the decisions of a single person (Berglund et al., 2003). The case review conference was used in this study for convenience purposes, however, future research may involve investigation of alternative options for capturing expert consensus opinion.

Investigations into the cause of death in bovine perinatal losses can be a complex process due to multifactorial contributing factors and the need to establish the sequence of pathological changes through time. One of the main issues in the development of an algorithm designed for use in medical situations is the complexity of the design (Svirbely, 2016). Where algorithms are too complex, it may be off putting to the operator, resulting in avoidance of its use for investigations. The algorithm developed in this study was designed to be as simple as possible to use, without over simplification, though this is a difficult balance. Although the protocol developed in this study is specific for perinatal loss investigations, it is hoped that through future research, there is potential to

develop similar protocols for investigation of all ages of bovine deaths, allowing a uniform approach to these investigations. Algorithms only work well in each specific target population (Svirbely, 2016), so separate algorithms would need to be developed for each separate age group and modified for each production type.

One of the issues with development of a medical algorithm is, that there may be temptation to over-fit the clinical findings to suit a suspected diagnosis (Svirbely, 2016). The author is of the opinion that subjectivity with regards to interpretation of establishment of time of death should be relatively low due to the clear definitions and discrete nature of the criteria used for its establishment. However, the interpretation of other findings may still be subject to a high degree of variability, depending on operator. This variability of interpretation may be reduced through the development of a photograph database, to work in conjunction with this protocol, demonstrating specific pathologies related to each cause of death. This is an area for future research development.

The protocol (including the algorithms) for investigations of bovine perinatal losses was only finalised after completion of the study. This ensured that all diagnoses fulfilled the criteria of the algorithms, to ensure that errors in future use were kept to a minimum. Review by specialists working in this field was limited but aided in this error reduction. The algorithms developed were based on a small number of prospective cases to ensure consistency in the investigations performed, rather than using retrospective cases to increase case numbers. In the development of a medical algorithm and testing protocol, it is of vital importance, that all pathways are repeatedly tested at all stages of development and altered accordingly (Sverbily, 2016). Future development of this algorithm would involve further peer review, testing in the field and on-going review and alterations as and when required.

Establishing when the calf dies may aid in future prevention of similar losses, as preventative efforts can be concentrated to a particular time period. An important criteria used for establishing time of death in this study was based on farmer history, which, to be meaningful, required a high level of observation at parturition. This was similar to methods used by Waldner et al., 2010. Such detailed history may not be available in all perinatal loss investigations, which is where gross pathology alone will be sufficient to determine time of death. The tables produced for establishing time of death in this study will permit this (Tables 2.2-2.4). The gross pathologies used to establish time of death in this study corresponded to the pathological processes, which occur at each time of death. This method was similar to other studies into bovine perinatal losses (Berglund et al., 2003). One issue with the time of death categorisation is the time interval between death and post mortem examination. If examination is performed within two hours of death (Stærkeby, 2007), rigor mortis may not have occurred, which may be misleading to the inexperienced operator. Due to the nature of the time of death categorisation, multiple pathological and history observations are taken into account for example in the aforementioned scenario; inflation of lungs or a history suggestive of a calf born alive, should be present, aiding in establishment of the correct time of death.

Establishing the cause of death may not be as simple as establishing the time of death, but by adhering to the above examination and sampling protocol, and causes of death algorithm, in most cases a proximal cause of death and contributing factors to death can be concluded. In many cases, the established proximal cause of death may not be the most important finding from the investigation, but the contributory factors may provide more information on future prevention and reasons for development of the proximal cause of death. This in turn may aid in reduction of these losses through focused attention on risk factors associated with the contributing factors.

All post-mortem examinations in this study were performed by the same individual after training was received prior to commencement of the study. In order for maximal results to be obtained, it would be recommended that

practitioners and pathologists interested in pursuing this subject area, were trained in bovine neonatal pathology. This may decrease the likelihood of practitioners and pathologists performing such investigations, due to time and financial constraints associated with training. To the untrained individual, the differences between proximal cause of death and contributing factors to death may appear daunting. It should, however, be emphasised that, a conclusion as to the proximal cause and contributing factors to death may be determined through the reporting of and discussion of the findings with a trained specialist, providing all pathologies are detected and recorded. The use of a high quality camera would aid in the process of determination of the cause of death, allowing pictures to be shared with a trained specialist. During this study, the use of an online sharing system proved to be the most efficient method for sharing of photographs between multiple persons. This technique of information sharing has been established in other medical industries, with successful outcomes (Martinez et al., 2008). It is hypothesised by the author that there may be a correlation between bodyweight and weight of specific organs (e.g. liver), where no pathology is present. Where there is no correlation, it is suspected that pathology will be present, for example congestion. This is an area for future research and if proven correct, would provide a more objective approach to the detection of pathology, reducing the amount of training required in this subject area. The protocols and algorithms developed in this study have not been tested on persons untrained in bovine perinatal pathology. This is an area for future work.

In the current study, all post mortem examinations were performed within 48 hours of birth/death. Although occasionally, this meant that examinations were performed at weekends or out with normal working hours, the benefit was seen through the production of meaningful result seen in this study (see Chapter 3). In order for improved diagnostic results to be seen in other investigation centres performing similar examinations, dedication to performing examinations within the 48 hours post-mortem is essential. This may however, come at a trade-off between providing an out of hours service (and associated costs) and prompt post mortem examination to obtain meaningful diagnostic results. The longer a foetus is dead, the less likely it will be of diagnostic value (Mee, 2015).

Many challenges were faced during post mortem examinations in this study, due to the absence of custom-built facilities. The installation of the hoist was not essential for performing post mortem examinations in this study; however, it did allow easy manipulation of large carcasses single-handed. In rural veterinary practices, work in isolation is a common occurrence, so installation of a suitable lifting aid for post mortem examinations would be of benefit in a similar practice facility. The table height in this study was not adjustable, which potentially could lead to back pain, resulting from poor posture. For permanent post-mortem facilities, and conforming with the Manual Handling Operations Regulations 1992, a table which had adjustable height, or which prevented poor posture would need to be purchased. In an ideal situation weighing scales should be attached to the lifting aid, to prevent contamination of the environment outside of the post-mortem facility. This is of particular risk with infectious or zoonotic material, especially where pregnant women are on site (Lovatt and Mitchell, 2008). On completion of the gross post-mortem examination, carcasses and loose viscera were sealed in plastic disposal bags, doubled where necessary. Containment within the plastic bags proved difficult, due to the weight of the carcass and muscle rigidity in multiple cases. An alternative would be to place the carcass and loose viscera in the box used for delivery of the calf to the investigation centre; however, this is aesthetically unacceptable and may discourage submissions for examination. Biohazard risks may also be increased where farmers have immediate contact with the carcass and viscera.

Bovine neonatal pathology is a very complex subject, which does not always follow a standardised format, making development of an algorithm very difficult. The author is aware that the protocols and algorithms developed in this study are subject to future testing and will be adjusted accordingly. They do, however, provide a baseline reference to reduce variation in investigations and diagnoses, which, to the author's knowledge, has not been previously available. Additionally, through the use of a standardised gross post mortem examination protocol and tissue collection and sampling protocol, it is hoped that improvements can be made into the diagnostic rate of bovine perinatal loss investigations. It is hoped that through the use of the systematic time of death tables and cause of death algorithms, and appropriate dissemination of results

and training of both veterinary practitioners and pathologists individuals will gain the confidence to perform more bovine perinatal mortality investigations. This in turn will allow prevention strategies to be put in place on farms, with the ultimate goal of reducing bovine perinatal losses and improving welfare on British beef farms.

3. Cause of Death and Associated Contributing Factors in Perinatal Losses in Beef Herds in Orkney

Bovine neonatal pathology is a specialised subject which has been investigated in considerable detail in the dairy sector (Mee, 2013a). Common pathologies found in perinatal dead dairy calves include lesions associated with anoxia, traumatic lesions from parturition and congenital malformations (Mee, 2013a). Although studies have been performed worldwide on perinatal losses in beef herds, with causes of death attributed to dystocia, thyroid gland lesions and developmental abnormalities (Wiske et al., 1994; Waldner et al., 2010), they have not been conducted extensively in the British beef industry and therefore the aetiology of perinatal mortalities on British beef farms requires investigation. As perinatal losses have a significant impact on the number of calves reared per cow per year, identification of these aetiologies has the potential to allow targeted preventative measures to be implemented. In addition, through the understanding of the causes of death in perinatal losses, a more evidence-based approach may be used in the treatment of sick neonatal calves showing similar disease processes.

3.1 Aim

The aim of this part of the study was to determine the cause of death in perinatal beef calves presented to a veterinary practice in Orkney over one spring calving season.

3.2 Materials and Methods

3.2.1 Definitions and Study Period

The following definitions were used throughout the study;

- Stillbirth- full term calf (with a full hair coat and erupted or partially erupted teeth) born dead
- Intra-partum death- alive at start of parturition but dead on delivery
- Neonatal loss- born alive but death within 48 hours of birth
- Perinatal loss-the birth of a full term stillborn calf, or calf which dies within 48 hours of birth
- Proximal cause of death- the factor which is the direct cause of death
- Contributing factor to death- factor which has contributed to death, but is not the cause of death directly

The study period was defined as 1st February 2016 to 10th June 2016 and any perinatal losses or data collected outside of this time period were excluded from the study.

3.2.2 Study Populations

Calves were submitted from two populations; targeted and passive. All perinatal losses from both populations were eligible for investigation, which was performed within 48 hours of death. Placentae were requested to be submitted with all calves where available.

3.2.2.1 Targeted Submissions

Targeted herds (n=11) were recruited based on a willingness to participate after discussion of the requirements of the study at an open meeting for Northvet Veterinary Group clients held in December 2015. All beef clients were invited to attend. To be eligible for the study, herds were required to be beef suckler, have cattle calving in the months February to June, 2016, and be present on mainland Orkney. A request was made to submit all perinatal losses to Northvet

Veterinary Group for investigation. In addition, targeted herds were requested to complete a calving data record sheet for the defined study period, which was used in the incidence study (see Chapter 4 and Appendix 5). This provided calving data for the entire herd including all calvings (successful and those resulting in a perinatal death). Each targeted herd was provided with a leak-proof container (Hogbox 190L) for transportation of the calf to the post mortem facility (Lamaquoy, Orkney). A flat rate of £100 plus VAT was charged for investigation of perinatal losses, regardless of the number of calves submitted over the total study period (additional costs were subsidised by Glasgow University, School of Veterinary Medicine (GUVS), Northvet Veterinary Group and SRUC). As an additional incentive to participate in the study, a veterinary health plan review was offered free of charge, to discuss all aspects of herd management, health issues and prevention on farm. Demographic herd health data were also collected from targeted farms and are presented in Chapter 4.

3.2.2.2 Passive Submissions

Passive herds were clients of Northvet Veterinary Group that had not signed up to the full study, but could submit beef perinatal losses on an ad-hoc basis during the defined study period. There was no requirement to submit all perinatal losses from these herds; this was the fundamental difference between these two calf populations. Calves were required to be sealed in two plastic bags and delivered to the post mortem facility by the farmer (Lamaquoy, Orkney). All post-mortem examinations for passive herds were charged at a standard rate of £34.24 plus VAT, with an additional cost of £54 plus VAT for culture and histopathology. No further incentive was provided for submission of calves

3.2.3 Case Identification

All calves were delivered to the post mortem facility in labelled bags or boxes, allowing identification of the herd of origin. Each herd was assigned a unique identifier, which consisted of the type of herd: targeted (T) or passive (P), as well as an individual farm number. On submission, each individual calf was given

a unique identification number, corresponding to the type of herd, for example T1 for targeted submissions and P1 for passive submissions. Official identification ear tags were not required to be in place.

3.2.4 Systematic Pathological Investigation of Individual Cases

For all calf submissions (targeted and passive) farmers were asked to complete a history questionnaire, which asked questions about the pre-, intra and post-partum period (Appendix 1). The information in this history questionnaire was used to help establish the time and cause of death where appropriate.

A standard post-mortem examination was performed by the same trained Veterinary surgeon (author) (training provided by John Mee MVB MVM PhD DipECBHM, Teagasc, Fermoy, 2016) on all submitted calves. The gross post mortem exam protocol was developed through discussion with Scotland's Rural College (Timothy Geraghty, Sandra Scholes) and Teagasc, Fermoy (John Mee). Details of the construction of this protocol can be found in Chapter 2 and the gross post mortem examination protocol itself can be found in Appendix 2. A standard set of tissue samples was collected from all cases for microbiological testing and histological examination as per the tissue collection and sampling protocol (see Chapter 2 and Appendix 4). The tissue collection and sampling protocol permitted submission of additional tissues as deemed appropriate based on necropsy findings. All samples (excluding the fixed brains) were sent to SRUC Aberdeen via first class Royal Mail post (to ensure next day delivery) as soon as possible after the post mortem examination and were stored at 4°C until posting. Samples collected on Thursday afternoon, Friday, Saturday and Sunday were stored at 4°C by Northvet Veterinary Group until posting on Monday. Brains were stored for at least two weeks to allow suitable fixation before dispatch to the lab. Samples which were collected, but not tested (swabs of the left kidney and left cerebrum), were frozen at -20°C and stored by Scotland's Rural College (SRUC), Aberdeen, for future use.

3.2.5 Disposal of Carcasses

All carcasses were sealed in double plastic bags and were collected by the farmer for disposal by burial, following post mortem examination, according to Animal By-Product (Scotland) Regulations 2003 (Anon., 2003).

3.2.6 Data Storage and Analysis

Data were recorded according to the unique identifiers given to each herd and each individual submission. Gross post mortem findings were recorded on a post mortem examination report form (Appendix 3) and subsequently entered manually into a spreadsheet (Microsoft Excel 2010), for analysis. Results of further testing (microbiological culture and histopathological examination) were also recorded in a spreadsheet for analysis. Photographs taken throughout the post mortem where appropriate were also identified and stored for subsequent review.

3.2.7 Establishing Time and Cause of Death

Results from the history questionnaire, gross post mortem examination and laboratory testing, were used to initially categorise each submission by time of death. Chapter 2 describes and presents the criteria used to define the time of death as either pre-, intra- or post-partum. Once the time of death was established, the proximal cause of death and any factors contributing to the proximal cause of death were also identified using the algorithms discussed in Chapter 2. A case review meeting of the author, veterinary surgeons (Robert Norquay, Kathryn Ellis and Jayne Orr) and pathologists (John Mee, Teagasc, and Timothy Geraghty and Sandra Scholes, SRUC) reviewed all the results from each calf along with the stored photographs of the gross post mortem and came to a final conclusion for the time of death, proximal cause of death and contributing factors to death. It was this process of logical clinical reasoning of each individual case that highlighted the need for a defined protocol and algorithm that could be used to make the process of assigning a proximal cause of death

and contributing factors to death more transparent and repeatable (see Chapter 2).

3.2.8 Feedback to Farmers

Preliminary results were discussed with the client after completion of the gross post mortem examination of each calf. Following completion of laboratory testing, and the establishment of the preliminary proximal cause of death and contributing factors, a further discussion with the client occurred. A meeting was held in October 2016 for all clients who participated in the study (both targeted and passive), where final results and herd level conclusions of the study were discussed and presented to all participating farms. In January 2017, overall herd results were discussed with each individual targeted herd.

3.2 Results

3.3.1 Calf Submissions

The total number of calves submitted from both targeted and passive herds for investigation was 53. Thirty-nine calves were submitted for investigation from targeted herds. Analysis of the calving data record sheet recorded by targeted herds (n=11) showed that 56 calves were actually eligible for submission for post-mortem examination i.e. 17 calves suffering perinatal mortality were not submitted. The submission rate from targeted herds was 69.6%. The 17 calves not submitted for investigation were all born on herds T8 and T9. All other targeted herds submitted all perinatal losses for investigation. Due to the low submission rate from herds T8 and T9, the data from the calves that were submitted from these herds (n=6 submissions) were categorised as passive rather than targeted. This left a total of 33 calves from 9 farms as targeted submissions and the submission rate from targeted herds re-calculated to be 100%

Table 3.1 *Submission rates of perinatal losses for investigation for targeted herds*

Herd	Number of perinatal losses	Number of submissions	Submission Rate (%)
T1	3	3	100
T2	2	2	100
T3	1	1	100
T4	4	4	100
T5	9	9	100
T6	6	6	100
T7	3	3	100
T8	16	2	13
T9	7	4	57
T10	3	3	100
T11	2	2	100
Totals	56	39	88

A total of 20 calves were submitted from passive herds. This was made up of two calves from herd T8 and four calves from herd T9, as described above, plus fourteen calves from passive farms. A total of 12 passive herds (not including targeted herds moved to this group) submitted calves. All but one passive herd submitted one calf each. Herd P8 submitted three calves.

3.3.2 Time of Death

After consideration of farmer history, gross post mortem examination, laboratory testing and histopathology results, a time of death was established for all submissions (Table 3.2). The majority of calves died in the intra-partum period.

Table 3.2; *Time of death of all submissions (targeted and passive) (n=53)*

Time of death	Number of calves	Percentage of calves (%)
Pre-partum	7	13.2
Intra-partum	29	54.7
Post-partum	17	32.1

3.3.3 Proximal Cause of Death

Overall, a proximal cause of death was diagnosed in 89% of all submissions (n=47/53). Results of investigations for each calf submission can be found in Appendices 10-13.

3.3.3.1 Proximal Cause of Death in Calves Dead Pre-partum

Of the seven calves categorised as dead pre-partum, two calves died from an *in utero* infection, one calf had a congenital malformation and in the other four calves a diagnosis could not be reached. The results are shown in Table 3.3.

Table 3.3; *Proximal cause of death and contributing factors in all calves diagnosed as dead pre-partum*

Herd	Calf	Proximal cause of death	Contributing factors to death		
T4	T7	Mesenteric Torsion	Congenital malformation (intestinal atresia)		
P1	P7	*In utero* infection	Positive *Leptospira hardjo* serology		
T9	P4	*In utero* infection	Non-specific *in utero* inflammatory reaction detected on histopathology		
T4	T10	Not reached	Twin	*Lichtheimia corymbifera* (placenta) and *E.coli* (placenta and FSC)	Premature placental separation
T7	T26	Not reached	Thyroid iodine concentration below reference range		
T11	T33	Not reached	Non-specific *in utero* inflammatory reaction detected on histopathology		
P7	P13	Not reached	N/A		

T= targeted herd/submission, P= passive herd/submission, FSC= foetal stomach contents

3.3.3.2 Proximal Cause of Death in Calves Dead Intra-partum

Of the 29 calves categorised as dead intra-partum, 21 calves died from anoxia developing during stage two of parturition, three calves died from *in utero* infection, one calf died from traumatocia and one calf died from congenital malformation. In three cases a diagnosis could not be reached. The results are shown in Table 3.4.

Table 3.4; *Proximal cause of death and contributing factors in all calves diagnosed as dead intra-partum*

Herd	Calf	Proximal Cause of Death	Contributing factors to death		
T1	T1	Stage 2 anoxia	Posterior presentation, congenital malformation (arthrogryposis)	Premature placental separation	*Bacillus licheniformis*, *Lichtheimia corymbifera* cultured from placenta
T1	T2	Stage 2 anoxia	Foetal oversize*	*E.coli* (FSC)	
T2	T4	Stage 2 anoxia	Foetal oversize*	*Aspergillus niger* cultured from FSC	
T2	T5	Stage 2 anoxia	Foetal oversize*		
T4	T8	Stage 2 anoxia	Unobserved calving		
T4	T9	Not reached	Twin		
T5	T12	Stage 2 anoxia	Posterior presentation	Twin	
T5	T13	Stage 2 anoxia	Foetal oversize*	*Bacillus licheniformis* cultured from FSC	
T5	T14	Stage 2 anoxia	Foetal oversize*	*Bacillus licheniformis*, *Aspergillus Fumigatus*, *Candida* spp. cultured from placenta	Unspecified pre-partum illness in dam (characterised by severe loss of body condition)
T5	T16	Stage 2 anoxia	Non-specific *in utero* inflammatory reaction detected on histopathology		
T5	T17	Stage 2 anoxia	*Candida* spp. cultured from placenta and FSC		
T5	T18	Not reached	Dam had stillborn calves in past		
T5	T19	Stage 2 anoxia	Presentation due to congenital malformation (contracted tendons in hindlimbs)	*Serratia liquifaciens* cultured from placenta	
T6	T20	Stage 2 anoxia	Non-specific *in utero* inflammatory reaction detected on histopathology		
T6	T23	Stage 2 anoxia	N/A		
T6	T24	Stage 2 anoxia	Foetal oversize*	Non-specific *in utero* inflammatory reaction detected on histopathology	
T6	T25	Traumatocia	Foetal oversize*		
T7	T27	*In utero* infection	*Aspergillus fumigatus* cultured from FSC		
T10	T31	Stage 2 anoxia	Twin	Unobserved calving	
T10	T32	Congenital malformation (schistosomas reflexus)	*E.coli* cultured from FSC		
T10	T33	Stage 2 anoxia	Foetal oversize*		
P2	P8	Stage 2 anoxia	Foetal oversize*		
P12	P20	*In utero* infection	Non-specific *in utero* insult detected on histopathology		
P3	P9	Stage 2 anoxia	*Lichtheimia corymbifera* cultured from placenta	Premature placental separation	
T8	P1**	*In utero* infection	*Bacillus licheniformis* cultured from FSC		
T8	P2**	Not reached	N/A		
T9	P3**	Stage 2 anoxia	Foetal oversize*	Posterior presentation	*E.coli* cultured from FSC
T9	P5**	Stage 2 anoxia	*E.coli* cultured from FSC		
T9	P6**	Stage 2 anoxia	N/A		

* foetus over 42kg (Holland and Odde, 1992)

** Submissions from targeted herds categorised as passive submissions due to low submission rates from these herds.

T= targeted herd/submission, P= passive herd/submission, FSC= foetal stomach contents

3.3.3.3 Proximal Cause of Death in Calves Dead Post-partum

Of the 17 calves categorised as dead post-partum, seven calves died from infection, four calves died from anoxia developing during stage two of parturition and two calves died from congenital malformations. One calf died from each of the following conditions - post-partum trauma, tubing accident, thrombus in the heart and abomasal rupture. The results are shown Table 3.5.

Table 3.5; *Proximal cause of death and contributing factors in all calves diagnosed as dead post-partum*

Herd	Calf	Proximal Cause of Death	Contributing factors to death	
T1	T3	Infection	*Staphylococcus* spp. and *E.coli* cultured from lung and liver	Pre-partum illness in dam (Johnes disease)
T3	T6	Infection	*Aeromonas hydrophila* cultured from liver and lung	
T5	T11	Tubing accident	Hypothermia	
T5	T15	Infection	*Bacillus licheniformis* cultured from liver	
T6	T21	Stage 2 anoxia	Foetal oversize*	*E.coli* cultured from liver and lung
T6	T22	Stage 2 anoxia	Foetal oversize*	*E.coli* cultured from liver and lung
T7	T28	Abomasal rupture	*E.coli* cultured from liver and lung	
T11	T39	Congenital malformation (intestinal atresia)	*Klebsiella oxytoca* cultured from liver and lung	
P4	P10	Infection	*E.coli* cultured from liver and lung	
P10	P18	Stage 2 anoxia	N/A	
P8	P14	Infection	*E.coli* cultured from brain, liver and lung	
P8	P15	Infection	*E.coli* cultured from brain, liver and lung	
P8	P16	Infection	*E.coli* cultured from brain, liver and lung	
P9	P17	Thrombus in heart	*E.coli* cultured from liver and lung	
P11	P19	Post-partum trauma	*Bacillus licheniformis* cultured from FSC	
P6	P12	Stage 2 anoxia	*E.coli* cultured from liver and lung	
P5	P11	Congenital malformation (hypotrichosis)	Pre-partum illness in dam (hydro-allantois)	

* foetus over 42kg (Holland and Odde, 1992)

T= targeted herd/submission, P= passive herd/submission

3.3.4 Herd Level Results from Targeted Herds

In the majority of herds, no pattern was seen with regards to the time of death or the proximal cause of death (Table 3.6). On two farms, all submissions died during parturition (T2 and T10). On one farm (T2), all submissions (n=2) were diagnosed as death due to stage two anoxia, secondary to foetal oversize. On one farm (T6), the 3/6 submissions died due to stage two anoxia, secondary to foetal oversize. Two other submissions from this farm also died from stage two anoxia but due to reasons other than foetal oversize. The incidence of perinatal mortality on each individual herd and the targeted population as a whole is described in Chapter 4.

The most common cause of death in targeted submissions was stage two anoxia due to foetal oversize (9/33 calves). Nine other submission from targeted herds also had a proximal cause of death of stage two anoxia but with other contributing factors (infection, malpresentation etc.).

Table 3.6; *Herd level results for time and proximal cause of death in targeted submissions*

	Time of death			Proximal cause of death*									
Herd	Pre-partum	Intra-partum	Post-partum	Stage 2 anoxia due to malpresentation	Stage 2 anoxia (foetal oversize)	Stage 2 anoxia (unexplained)	Stage 2 anoxia (infectious)	Infection	Not reached	Congenital malformation (infectious cause)**	Milk/colostrum aspiration	Abomasal rupture	Traumatocia
T1		2	1	1	1			1					
T2		2			2								
T3			1					1					
T4	2	2				1			2	1			
T5		7	2	2	2		2	1	1		1		
T6		4	2		3	1	1						1
T7	1	1	1					1	1			1	
T10		3			1	1				1			
T11	1		1						1	1			
Total	4	21	8	3	9	3	3	4	5	3	1	1	1

* Only identified causes of death diuagnosed in this study are presented in table. Other causes of death do occur. ******Likely causative micro-organism cultured from foetus

3.3.5 Herd Level Results from Passive Submissions

On one of the farms (T9), the majority of submissions (3/4) died intra-partum due to anoxia, although with varying contributing factors (Table 3.7). In herd P8, all submissions died post-partum of infection. No other patterns with regards to time of death and cause of death were seen at a herd level. The most common proximal cause of death in passive submissions was infection (8/20 calves). In an additional three calves, infection was also a contributing factor to the proximal cause of death (stage two anoxia).

Table 3.7; *Herd level results for time and cause of death in passive submissions*

	Time of death			Proximal cause of death**							
Herd	**Pre-partum**	**Intra-partum**	**Post-partum**	**Stage 2 anoxia (foetal oversize)**	**Stage 2 anoxia (unexplained)**	**Stage 2 anoxia (infectious)**	**Infection**	**Not reached**	**Congenital malformation (infectious cause)**	**Thrombus in heart**	**Post-partum trauma**
T8*		2					1	1			
T9*	1	3		1	1	1	1				
P1	1						1				
P2		1		1							
P3		1				1					
P4			1				1				
P5			1						1		
P6			1			1					
P7	1							1			
P8			3				3				
P9			1							1	
P10			1		1						
P11			1								1
P12		1					1				
Total	3	8	9	2	2	3	8	2	1	1	1

***** *Targeted herd with low submission rates. Data were categorised as passive rather than targeted.*

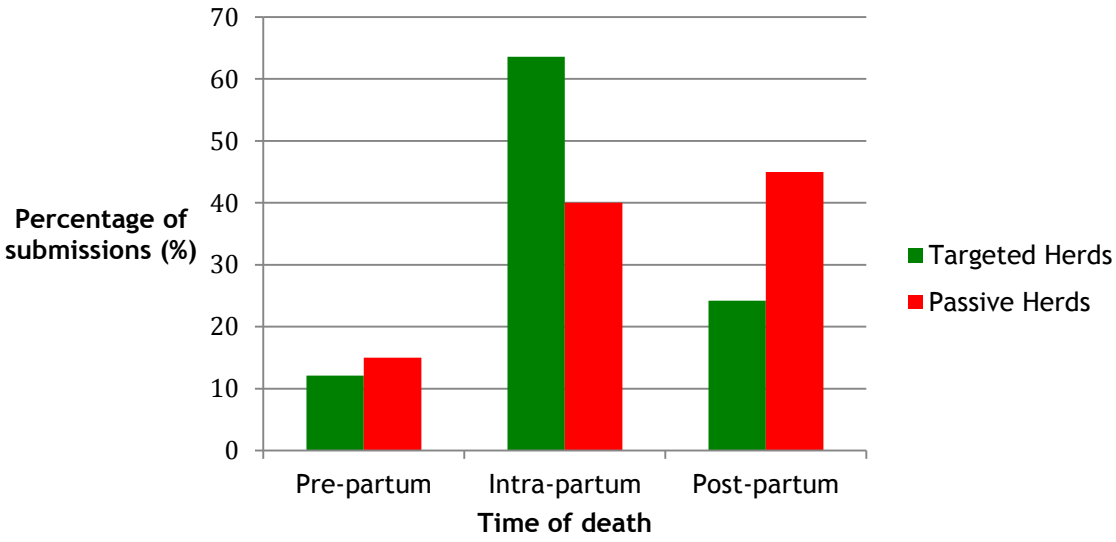
***Only identified causes of death in this study. Other causes of death do occur.*

3.3.6 Comparison of Targeted and Passive Herd Results

3.3.6.1 Comparison of Time of Death Between Targeted and Passive Herds

A proximal cause of death was diagnosed in 88% of targeted submissions and 90% of passive submissions, meaning diagnostic rates from both populations were very similar. The greatest number of submissions from targeted herds died during parturition (63.6%). This was in comparison to submissions from passive herds, where the greatest number of submissions died post-partum (45%). Deaths pre-partum were similar in both populations (Figure 3.1).

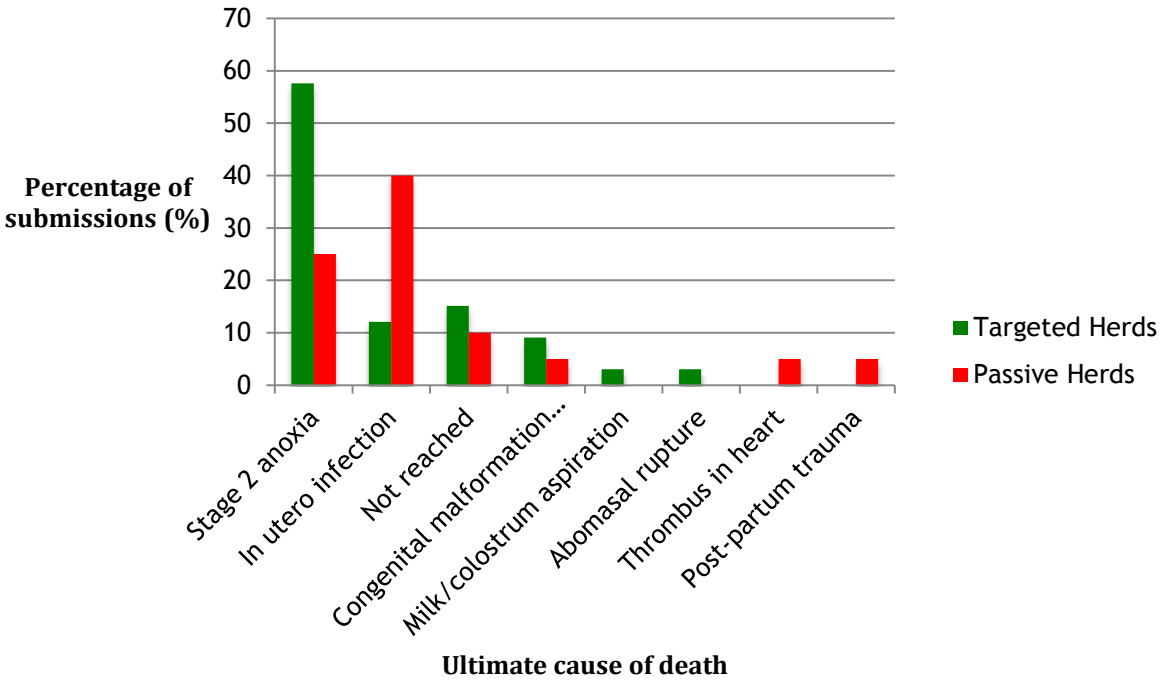
Figure 3.1; *Comparison of time of death between targeted and passive submissions*



3.3.6.2 Comparison of Proximal Causes of Death Between Targeted and Passive Herds

The most common proximal cause of death in targeted herds was anoxia developing during stage two of parturition (57.6%). This was in comparison to submissions from passive herds, where 25% of submissions had anoxia developing during stage two of parturition as the proximal cause of death (Figure 3.2). In submissions from passive herds, the most common cause of death was infection (40%) which was only diagnosed in 12 % of targeted herds (Figure 3.2).

Figure 3.2; *Comparison of proximal cause of death between targeted and passive submissions*



3.4 Discussion

The proportion of calves with a diagnosed cause of death in this study (89%) was higher than in other studies into bovine perinatal losses, which ranged from 5-50% (Berglund et al., 2003; Mee, 2013a). The standardised post mortem protocol

(including sampling for laboratory testing) that was used for all submissions, potentially increased the diagnostic rate reported in this study, which may not have been the case in previous studies, where laboratory testing was not performed routinely (Berglund et al., 2003). This allowed detection of micro-organisms and lesions which may not have been detected by gross examination only. The diagnostic rate was consistent with other work done on beef calves by Waldner et al., 2010 (78% in stillborn calves, 88% in neonatal calves), where histopathology was performed on all cases; increasing the number of submissions where a definitive diagnosis was reached. Although collected from all submissions in this study, brain histopathology was not performed routinely, due to time constraints of the histopathologists and financial constraints of the study. All samples are in storage and would be available for future examination, allowing confirmation of some of the ultimate causes of death (e.g. stage two anoxia). In addition to the standard set of samples collected from all submissions, the protocol produced encouraged the collection of additional samples, guided by the pathology seen.

The numbers of submissions from both herd groups (targeted and passive) were relatively low overall, potentially leading to bias in the results seen in this study. In an ideal situation, the study should be repeated on a larger scale to increase the power of the study.

The highest proportion of deaths in this study in targeted submissions was death due to anoxia (n=18/33). This was consistent with findings from other studies (both targeted and passive) on dairy calves internationally, which ranged from 5-41% as reported by Mee, 2011. In this study, the cause of death was classified as anoxia, developing during stage two of parturition, even if the calf was born alive, as the effects of anoxia during stage two of parturition can prevail into the neonatal period. It may be possible that anoxia developed post-partum in a small number of cases; however, in this study, all cases diagnosed with anoxia as the proximal cause of death had a history suggestive of development during

parturition, for example, prolonged parturition. In multiple cases, anoxia developed due to a slow calving or 'bradytocia', which may have been the result of congenital malformations, presentation, foetal oversize or maternal health. Bradytocia itself, is not a cause of death (Mee, 2013c), but is a major factor which needs consideration when discussing prevention of perinatal mortality. The high prevalence of anoxia is an area of concern in this study, although there were various contributing factors to anoxia. In an industry aiming to achieve high welfare standards, stage two anoxia due to foetal oversize or prolonged parturition, is an area which has the potential to be improved through basic farmer training in assistance at parturition and breeding management. Other contributing factors to anoxia developing during stage two of parturition may be harder to improve.

Micro-organism culture was positive in 49% of targeted submissions and 60% of passive submissions (overall 52.8% of submissions). In 17 submissions from both targeted and passive herds, the presence of micro-organisms were not considered significant, as histopathological lesions were not consistent with infection. It may be possible, that the time between invasion of micro-organisms and death due to the effects of the micro-organisms, was too short to cause changes in the organs examined by histopathology, suggesting that infectious causes of death may be even higher than reported in this study. This is however, only speculative. Alternatively, it may be possible that calves are born with a bacteraemia, but do not succumb to disease. This may be an area for future research. Additionally, it may be possible that cross contamination from the environment and handling of tissues may have resulted in positive micro-organism culture, although all attempts were made to keep this to a minimum.

The reason for the higher proportion of infectious causes of death in passive submissions in this study is unknown, 40% in this study compared to 5-33%, Mee, 2013a), although the small sample size in this study needs to be considered. One

possible theory is due to the prolonged period of time the cattle on the Orkney Islands are housed (October-May). It is possible that during the housing period, dams are exposed to a significant bacterial load, which may lead to ascending infections during cervical relaxation pre-partum. The majority of cattle in this study were housed on slatted accommodation, which when faeces are of a thick consistency, may lead to a high environmental load of micro-organisms as the faeces do not fall through the slats to the slurry pit below. In women, ascending bacterial infections can lead to chorioamnion infection and an inflammatory response in the foetus. The infection is often limited to the chorioamnion and only infects the foetus occasionally (Goldenberg and Thompson, 2003), but despite this, an inflammatory response is seen in the foetus. This pathogenesis may account for the signs of inflammation and infection seen in multiple calves (n=6), where no infectious organisms were isolated from the carcass, for example calf P20, Table 3.4. Further work may involve comparing histology from placentae from both healthy calves and perinatal losses, to detect signs of chorioamnionitis. In previous studies, the presence of inflammatory lesions in perinatal dead calves, were used to confirm the cause of death as a bacterial infection alone (Mee, 2013a). However, in this study, confirmation of such infections was provided through micro-organism culture and histopathology performed on all submissions. The author is of the opinion that this method provides more reliability and a less subjective approach to the diagnosis of an infectious cause of death.

Immune-compromise in the dams may be seen, through poor quality nutrition and housing environment. In one study, a comparison was made between immune function in dairy cows housed on rubber mats compared to on concrete (O'Driscoll et al., 2009). This study showed that cows housed on concrete had greater physiological stress and activated immunity compared to those housed on rubber flooring. Concrete slatted accommodation is a common floor type for beef herds in Orkney, and this may be a contributing factor to the infectious causes of bovine perinatal loss, resulting from compromised immune systems in the dam. This is only speculative but would be an area for future research. No

nutritional assessment was performed on farm as it was beyond the scope of this study, however, this may warrant further investigation in future studies.

Bacteraemias in the dam, with pathogens such as *Escherichia coli* have been seen following rectal examination in cattle particularly when the mucosa is damaged and in compromised dams (Stem et al., 1984). It is unknown whether these bacteraemias are transient or persistent, (Stem et al., 1984), however, this may be another potential route of infection to foetuses. Routine pregnancy diagnosis via trans-rectal ultrasonography is performed in many of the herds in this study, which in compromised dams, may increase foetal infection risk leading to perinatal deaths. This route of infection may represent a very minor potential route of infection to the foetus, more so around the time of trans-rectal examination (leading to abortion rather than stillbirth) and further work would be required to assess its significance.

In 15 submissions from both passive and targeted herds, positive *Escherichia coli* cultures were found. Bacterial cultures performed more than 24 hours post-mortem may be associated with increased detection of *Enterobacteriacae*, potentially due to bacterial transmigration (Riedel, 2014). The risk of bacterial transmigration is reduced when the carcass is cooled to 4°C and there is minimal movement of the carcass post-mortem, and samples are collected within 24 hours of death (Riedel, 2014). Unfortunately cooling of the carcass post-mortem was not feasible due to the cost and logistics involved; however weather conditions during the spring in Orkney may limit bacterial growth. The degree of movement of the carcass is unknown as it was the responsibility of the farmer to transport the carcass to the post-mortem facility. It is suspected that movement of the carcass was as minimal as it could be, with regards to delivery of the carcass to the post mortem facility.

In five of the submissions where *E.coli* was cultured, the calf was categorised as stillborn. There are few published data on *Escherichia coli* as a pathogen of bovine stillbirth, although it is an acknowledged cause of bovine abortion

(Givens and Marley, 2008) and pure growths from aborted bovine foetuses are deemed pathologically significant (Anderson, 2007). *Escherichia coli* have been recognised to be present in foetuses born alive full term and may lead to neonatal death (Mickelsen and Evermann, 1994). Bacterial infections of the neonate which are contracted *in utero* are uncommon compared to infections contracted in the post-natal period (Mickelsen and Evermann, 1994). Although septicaemias, pneumonia and enteritis can develop in the early post-partum period in humans, they can be contracted *in utero* or during parturition in the birth canal (Mickelsen and Evermann, 1994; Goldenberg and Thompson, 2003). During this study, multiple pure growths of *Escherichia coli* were cultured from foetal stomach contents and foetal tissues, along with mixed growths also. These cultures were seen in combination with gross lesions and histopathology suggestive of inflammation and infection. Although a ubiquitous organism in the environment and a possible contaminant, it would need to be suspected that *Escherichia coli* cultured from these cases was indeed a significant finding and was the main pathogen associated with the infectious cause of death category.

In humans, many of the pathogenic bacteria leading to foetal death are present in the uterus early in gestation but only infect the foetus when foetal respiration begins (Goldenberg and Thompson, 2003). In one study on human foetal respiration, only some foetuses made respiratory efforts of such force as to cause amniotic fluid aspiration (Schaffer, 1956). Although similar studies have not been performed on bovine foetuses, this may account for the varying number of calves with positive lung cultures in this study and the multiple cases of congenital pneumonia seen. After immuno-competence has developed at around 125-150 days of gestation (Brock, 2003), a time period of 10-14 days is required in order for the foetus to form an immune response against bacterial infections (Conner et al., 1976). It may be possible that the foetus becomes infected *in utero*, allowing time for an immune response to be produced by the time of parturition. In these calves the bacterial culture may be negative due to the immune response, but signs of infection may remain.

In this study, some of the causes of death recorded have previously not been considered as significant pathogens in relation to bovine perinatal mortality, in particular *Lichtheimia corymbifera*. *Lichtheimia corymbifera* (formerly known as *Absidia),* was cultured from three placental tissues in this study and to the author's knowledge, there are few published data on this micro-organism as a cause of stillbirths in calves. *Lichtheimia corymbifera* is a recognised cause of bovine abortions and dams typically do not show signs of illness during gestation (Piancastelli et al., 2009). Initially it was assumed that the micro-organisms cultured from placental submissions were contaminants due to their ubiquitous nature in the environment (Anderson, 2007); however, given the similarity to abortions caused by *Lichtheimia corymbifera,* where no clinical signs are seen, it would need to be suspected that this organism may indeed be involved in bovine stillbirths. In this study, all identified cases were classed as contributing factors to death, and it is not suspected that this organism is unique to perinatal losses in the Orkney Islands. Further work is required to establish a true case definition, to identify if this organism is truly involved bovine perinatal mortality.

In light of the high number of bacterial and mycotic organisms detected in this study, the sample collection technique should be considered, although no obvious source of contamination was identified in this study. In order to reduce environmental contamination of tissues, samples should be collected prior to evisceration and within 24-48 hours of death (Riedel, 2014). Evisceration prior to collection of samples may have been a potential source of contamination of the samples, however, great care was taken by gentle tissue handling and ensuring surfaces were visibly clean, to avoid gross contamination of organs prior to sampling. Furthermore all lung tissue samples were collected from the caudal lung fields, which reduced contamination from multiplying bacteria present in the upper respiratory tract (Riedel, 2014) and foetal stomach content samples were collected after searing of the abomasal surface, which is a proven technique to reduce specimen contamination (Carpenter and Wilkins, 1964).

The proximal causes of death clearly varied between the two populations of calves submitted. This may reflect motivations for farmers to submit calves for investigation. Passive herds were not required to submit all perinatal losses, but submitted calves on an ad hoc basis. It is speculated that this may have led to bias in the results with farmers only submitting calves when, for example, they did not have an obvious cause of death, or they had time to bring calves to Northvet Veterinary Group. Passive herds had a higher number of infectious causes of death in the study (proximal cause of death in 40% of submissions from passive herds, compared to 12.1% of submissions from targeted herds). It is speculated that infectious disease may be difficult for the farmer to detect and so submission of a calf for investigation may be more likely if cause of death is unknown. On the contrary, passive herds would be less likely to submit calves for post-mortem examination if the cause of death was suspected, for example death due to dystocia. In addition, there may also be a psychological aspect to submission of calves, with passive herds less likely to submit calves for investigation if the cause of death was related to poor farm management or practice (e.g. traumatic birth or delayed intervention at parturition). What motivates farmers to submit calves for further investigation is an interesting avenue for further investigation. Submissions from passive herds may not be truly representative of disease within the individual herd or the Orkney beef population due to the lack of requirement for all perinatal dead calves to be submitted. On the contrary, results from the targeted herds were less prone to bias with regards to cause of death, as producers were required to submit all losses regardless of perinatal mortality incidence or suspected cause of death. This is an important point to remember for disease surveillance purposes as trends in endemic diseases can only be established when representative clinical material is examined (Watson *et al.*, 2008), which was the case from submissions from targeted herds.

Bias may also have been seen with regards to time of death from passive submissions, with a higher proportion of calves submitted from passive herds which died post-partum and pre-partum, compared to intra-partum. This again

may reflect on motivation for submission of calves. Farmers may be of the opinion that death during calving is 'one of those things' and is almost expected, while in contrast if a calf is born alive and subsequently dies, farmers are more keen to pursue a cause of death as they have more control over the calf post-partum. The same holds true for calves which die pre-partum, as this may be seen as an 'abnormal' occurrence which requires investigation. This is however, only speculative.

Two targeted herds (T8 and T9) did not submit all perinatal calf losses for investigation. The low submission rate seen in these herds may have been due to lack of man-power or time constraints associated with delivery of the carcass to the post-mortem facility during a busy time period (Watson et al., 2008). This reason for low submission rates was confirmed on discussion with the farmers after completion of the study. A collection service may have improved submission rates; however, in the current study this was not feasible. Misunderstanding of the requirements of the study may also have been an issue associated with low submission rates, however, all farm managers were individually visited prior to commencement to ensure full understanding. In the herds with low submission rates, perinatal loss incidences were high (see Chapter 4), which may have been a contributing factor to the reduced submission rate. Farmers may have been embarrassed to admit that perinatal mortality was a large problem on the farm and may be fearful of blame when a high number or cases were submitted. Prior to commencement of the study, it was explained to all participants that no blame or judgement would be made; however, nervous or fearful personalities need to be considered in such studies. Improvements with submission rates may have been seen with anonymous submission of calves but this would not allow an overview of cases in each herd or the reporting of results back to the farmer.

Placental submission rates were low in this study (n=6/53), despite a requirement to submit the placenta with all calf submissions. Submission rates

may have been low due to the low numbers of placentae delivered by the dam at the time of parturition (Berglund et al., 2003). Additionally, the majority of cattle calved in slatted accommodation or at pasture, meaning that the placenta may have been difficult or impractical to locate. Manual handling of the placentae by the farmer may also have been an issue, due to the slimy nature of placentae, as reported to the author by participating farmers. Due to the nature of delivery of placentae by the dam, high rates of contamination are likely. This may be an issue with micro-organism culture of the placenta, leading to falsely high rates of infectious organisms cultured. Collection methods and storage of placentae are issues that should be addressed in future studies.

Premature placental separation (PPS) can be associated with weak calf syndrome (Mee, 2013a). Mycotic micro-organisms were cultured from all placentae submitted from cases of premature placental separation (n=3), as reported by the farmer and it may be possible that premature placental separation does occur in many cases of placental mycoses and may account for more perinatal losses than previously thought. Consistent with other studies (Mee, 2013a), calves with PPS which were alive at the start of parturition, showed signs suggestive of death due to anoxia (epicardial and pleural haemorrhages).

In an English study on Holstein calves by Simpson, 1990, quoted by Berglund et al., 2003, there was often evidence of trauma, with severe rib fractures in the majority of dead calves and in other studies internationally, trauma at parturition was one of the most common causes of death in calves (25-46.1%) (Mee, 2011). However, to the author's surprise, traumatic injuries resulting from traumatocia were present in only a few calves, and death due to trauma was diagnosed on only one submission. Calves with traumatic lesions had an average weight of 55.4kg compared to an average weight of 39.7kg in calves that did not have traumatic lesions. This is consistent with results from previous studies as the incidence of dystocia increases with increasing calf birthweight (Meijering, 1984). It may be possible that this figure has been biased by the presence of

pathologically small calves included in this comparison and is an area for further research. Consistent with studies on Holstein calves (Steinbock et al., 2003), male calves accounted for the majority of perinatal submissions with traumatic lesions. This again is as expected, considering the higher average weight of male calves compared to female calves in this study and the resulting dystocia risk. From first-hand experience, the majority of beef farmers in the Orkney Islands are very aware of foetal oversize (both relative and absolute) and prefer to opt for caesarean section rather than manual traction for delivery. This may account for the reduced number of traumatic injuries seen in this study compared to others; however, this is only speculative and would not apply to all herds.

Neospora caninum was not diagnosed as a cause of bovine perinatal mortality in this study; however, extensive testing for this organism was not performed. Histopathological examination was performed in most cases on both myocardium and liver and when no degenerative or inflammatory lesions suggestive of the presence of *Neospora caninum* were present, no further testing was undertaken due to restriction in funding and time commitments of the histopathologist. It is possible that *Neospora caninum* was not present in any of the submissions, although, diagnosis may have been improved through examination of brain tissue by histopathology (Dubey and Schares, 2006), or by PCR testing. In comparison to other studies (Waldner et al., 2010), the prevalence of BVDV in this study was particularly low (n=1 antibody positive). This was unsurprising due to the implementation of the Orkney BVDV Eradication scheme, launched in 2001 (Anon., 2017b), and the Scottish BVDV Eradication Scheme, launched in 2010 (Anon., 2016a).

In the current study, congenital defects were present in 11.3% of total perinatal losses (both targeted and passive). This Figure is higher than in many passive studies (5%) (Mee, 2013a), but lower than studies on perinatal mortality using active surveillance (20%) (Mee and Keneally, 2010). Submissions from passive herds may have biased this Figure as again there was no obligation to submit

calves if there was an obvious cause of death. This was reflected in the absence of congenital malformations present in calves submitted from passive herds. Hence the prevalence of congenial defects in beef suckler herds in the Orkney Islands may be higher than this Figure as congenital malformations will occur on passive herds as well. The prevalence of cardiac defects was low in this study, potentially due to examiner inexperience and the over-looking of subtle cardiac pathology. However, despite the low prevalence of cardiac defects in this study, results were comparable with other studies in beef herds (Walder et al., 2010).

Micronutrient deficiencies were not extensively investigated in this study, with only selenium and vitamin E concentrations measured in the liver, where thyroid weight exceeded 30g or a request was made by the farmer. Although bradytocia resulting from uterine inertia can be associated with hyposelenosis or hypovitaminosis E in dams, it was beyond the scope of this study to measure such concentrations in every submission. Symptoms indicative of respiratory distress syndrome were seen in a few submissions; however, no micro-nutrient levels were measured in these cases as funding was limited.

No Figure was available in the literature as a target weight to predict relative foetal oversize in beef cattle; however, a foetal weight of 42kg was used in this study based on previous work by Holland and Odde, 1992. An average bodyweight of 600kg for the dam was used in all cases, although there may have been a wide variation in this. The consistent Figure of 42kg used throughout this study for all calves, reduced the reliability of the data involving foetal oversize. In future studies, to calculate an accurate weight for relative foetal oversize, dams should be weighed and an estimate made for the target weight of the foetus (7% of dam bodyweight) (Holland and Odde, 1992). This was not feasible in this study.

No insemination dates were available during this study as natural service was used in the majority of herds. However, a calf was considered full term if the incisor teeth were partially or fully erupted and the hair coat was full. Congenital abnormalities in the dentition and hair coat were accounted for and a reasonable estimate as to whether the calf was full-term or not was made. If service dates were available, then a more accurate gestation lengths could be established. This would be more feasible for future studies on dairy herds.

The findings associated with the pathology of bovine perinatal losses in this study may have occurred sporadically in the 2016 calving period, so to improve reliability in the results, the study should ideally be repeated in future calving periods. Logistics and funding will not permit this in the current study, but this would be an area for further work.

Overall, the results of this study do not differ drastically from results from previous studies into the causes of bovine perinatal losses, although the high prevalence of death due to anoxia and infectious causes, are an area of concern. Furthermore, the difference between the cause and time of death between targeted and passive herds highlights issues with using laboratory submissions as a source of national surveillance (as they rely on passive data). This study has generated many questions, with regards to the reason for such high numbers of losses due to infection and this is an area of work, which should be targeted in future research. Additionally, more work is required to educate farmers on the contributing factors to anoxia, in an attempt to reduce these losses through appropriate breeding and management at parturition.

4. Bovine Perinatal Mortality Incidence in Beef Herds in Orkney

Production efficiency in the beef herd can be directly measured by the calf output per cow mated per year (Caldow et al., 2005) with perinatal losses having a significant financial and emotional cost (Anon., 2016b). In addition to these costs, incidences of perinatal mortality on farm have recently been used as an indicator of welfare and may reflect the quality of stockmanship and herd management (Nyman et al., 2011). In recent years, local veterinary surgeons on Orkney have perceived there to be a high incidence of perinatal loss in beef suckler herds (4.5%) (Northvet Veterinary Group benchmarking data, 2014, unpublished). No previous investigations have been undertaken to fully quantify such losses and it was expected that the incidence may be even higher than suspected due to the lack of veterinary consultation obtained by farmers regarding such losses and the normalisation of these losses on farm (Mee, 2015a).

4.1 Aim

The aim of this part of the study was to describe the incidence of perinatal mortality in a convenience sample of beef herds in Orkney over one spring calving season.

4.2 Materials and Methods

4.2.1 Recruitment of Farms

Targeted herds were (n=11) were recruited as previously described in Chapter 3. In brief to re-iterate, this was a convenience sample based on willingness to participate, location on mainland Orkney, with beef cattle calving between 1st February and 10th June 2016. Previous perinatal loss incidence was not relevant for participation.

4.2.2 Data Collection

4.2.2.1 Herd Background Data

Participating herds were required to complete a short questionnaire providing background information on herd management and husbandry prior to commencement of the study (Appendix 6).

4.2.2.2 Parturition Data for All Calves

From 1st February to 10th June 2016, basic details were recorded by the farmer, including breed of sire and dam, time and date of calving, gender of calf and body condition score of dam (5 point scale) (Appendix 5), for all parturitions on farm (cows and heifers). Body condition score was assessed by palpation of the tail head, lumbar vertebrae and ribs for fat coverage. In addition, singleton or multiparous births were recorded as well as calving ease. Calving ease was recorded on a scale of 0-5 with descriptions of each score provided; 0- unobserved, 1- observed but not assisted, 2- mild assistance by farmer, 3- hard assistance by farmer (requiring use of mechanical aid), 4- veterinary delivery per vaginum, 5- caesarean section (Mee et al., 2008). Farmers were also asked to record if the calf was dead or alive at birth and if the calf was alive at 48 hours post-partum. It was requested that all perinatal losses during the study period were submitted for investigation (see Chapter 3). Each farm manager was visited prior to commencement of the study to ensure full understanding of the requirements. Training in body condition scoring was provided by the author, where required.

4.2.3 Data Analysis

Data were analysed using Microsoft Excel 2010. Where entries were missing in submitted data, no presumptive entries were made, but all other available entries were used for analysis. Descriptive data and temporal trends were analysed. Three separate metrics for perinatal calf loss were calculated. These were:

1. Incidence of stillbirth (calves born dead) = number of calves born dead/ total number of calves born;

2. Incidence of neonatal loss (died between 0-48 hours) = number of calves born alive but died within 48 hours of birth / total number of calves born alive;

3. Incidence of overall perinatal loss (calves born dead or died with 48 hours) = number of calves born dead or died with 48 hours / total number of calves born.

Each metric was applied to every individual herd and the entire study group combined. Temporal trends were analysed by evaluating incidence by month, day of the week and time of parturition. The combined dataset of all targeted herds, was utilised to evaluate relationships between calving associated factors and incidence of perinatal mortality by means of a series of chi-squared analyses. Significance was determined where $P<0.05$. Perinatal losses were analysed as opposed to separate analyses of stillbirth and neonatal loss incidence, in relation to individual calving factors were not analysed due to size of the data sets. Herd level factors were collected for background descriptive purposes but were not analysed due to design of the study.

4.2.4 Reporting of Results

Individual meetings were held with each individual farmer to report herd level results on completion of the study. A follow up meeting was held in October 2016 for all participating farmers, where results were presented and discussed. A questionnaire was completed by all attending farmers at the meeting in October 2016, which asked questions regarding farmer perception of perinatal loss incidence and motivations and deterrents for investigating perinatal losses (Appendix 7).

4.3 Results

During the study period 1st February- 10th June 2016, 1101 calves were born across all targeted herds. Data were recorded for every individual parturition (see Appendix 5) by nine herds (899 calves). Data sets were not complete for every individual parameter recorded on these nine herds. However, for analysis of the effect of each parameter on perinatal loss, only complete parameter data sets were used in analysis. Where there were less than 1101 entries, the number of parturitions used in analysis for each parameter is stated in each section below. Data were only recorded for perinatal losses (not all parturitions) on the other two herds. For these two herds, basic data regarding calving date, calf gender and twin births for all other parturitions, (where the calf was alive at 48 hours) were available from the British Cattle Movement Society (BCMS). Therefore, data from a further 202 parturitions were included for analyses where appropriate.

4.3.1 Herd Background Description

Approximately 30 farmers attended the open meeting for farmers, held by Northvet Veterinary Group in December 2015. From this, 11 herds were recruited for participation in the study representing approximately 7% of beef cattle in Orkney (Northvet Veterinary Group benchmarking data, 2014 unpublished). Targeted herds were widely geographically distributed throughout Orkney mainland.

4.3.1.1 Herd Size

Herd sizes ranged from 49 to 130 females calving in the period 1st February to 10th June 2016. The mean number of females calving in each herd was 101.

4.3.1.2 Accommodation

On most targeted herds (n=10), the majority of calvings occurred indoors in both group and individual pens. In one herd, all calving occurred at pasture unless assistance was required. Calving in group pens (both slatted courts and straw bedded pens) occurred in four herds and calving in individual pens (straw bedded) occurred in six herds (Table 4.1). In nine herds, dams and calves were managed in individual pens for the 48 hours post-partum if the calf was alive.

Table 4.1; *Overview of dam accommodation at parturition and post-partum*

Number of Farms	Group Pen	Individual Pen	Pasture
Accommodation at parturition	4	6	1
Accommodation for 48 hours post-partum	1	9	1

4.3.1.3 Nutrition

During housing, grass silage formed the basis of the ration on all targeted farms with pit grass silage fed to cows and heifers on nine farms, and baled grass silage fed to cows and heifers on two farms. Six farms provided silage as part of a total mixed ration, as mixed and weighed by a feed wagon. Barley was fed as a component of the total mixed ration on two farms and barley straw fed as a component of the total mixed ration on five farms. On the farms where no total mixed ration was supplied, grass silage without additional feed supplementation was fed. The diet remained constant in the 48 hours post-partum on all farms. Pre-calving mineral supplementation, added to the silage both as part of the total mixed ration and top dressed, was provided on 10 farms.

4.3.1.4 Service Age and Methods

Heifers calved for the first time at two years old on six farms and at three years old on three herds. In two herds, calving occurred for the first time in heifers at both two and three years old depending on growth (Table 4.2). Natural service was used for the majority of service but artificial insemination was performed on a small proportion of cows on three farms. No sexed semen was used on any of the targeted herds. A variety of bull breeds were used, with Aberdeen Angus being the most commonly used sire (Table 4.2). Only one breed of sire was used in herd T3 (Aberdeen Angus). Herd T1 used the highest number of different breeds of sire (n=5).

Table 4.2; *Overview of herd breeding management*

Herd	Heifer age at first calving (years)			AI used		Breed of sire (% of calves born per herd)						
	2	2&3	3	Yes	No	Lim	Sal	AA	Si	BS	CH	St
T1	✓			✓		19	0	6	0	15	41	19
T2		✓			✓	0	0	24	59	0	17	0
T3	✓				✓	0	0	100	0	0	0	0
T4	✓			✓		17	0	30	28	0	25	0
T5			✓		✓	0	0	65	0	0	35	0
T6			✓		✓	NR	NR	NR	NR	NR	NR	NR
T7	✓			✓		14	41	0	45	0	0	0
T8		✓			✓	0	0	16	0	84	0	0
T9			✓		✓	25	4	45	26	0	0	0
T10	✓				✓	NR	NR	NR	NR	NR	NR	NR
T11	✓				✓	30	70	0	0	0	0	0

Lim- Limousin, Sal- Saler, AA- Aberdeen Angus, Si- Simmental, BS- Beef Shorthorn, CH- Charolais, St- Stabiliser

NR- not recorded

4.3.1.5 Disease Prevention

No vaccinations were used on two herds. All other herds had various vaccination protocols in place (Table 4.3).

Table 4.3 *Herd level vaccination status*

Herd	BVDV	*Leptospira hardjo*	Scour pathogens*	Clostridial spp.	*Bovine Herpes* virus
T1	✓	✓	✓	✓	✓
T2	✓	✓			
T3			✓		
T4	✓	✓			
T5	✓	✓	✓		
T6	✓	✓	✓		
T7		✓			
T8	✓	✓		✓	
T9					
T10					
T11	✓	✓			

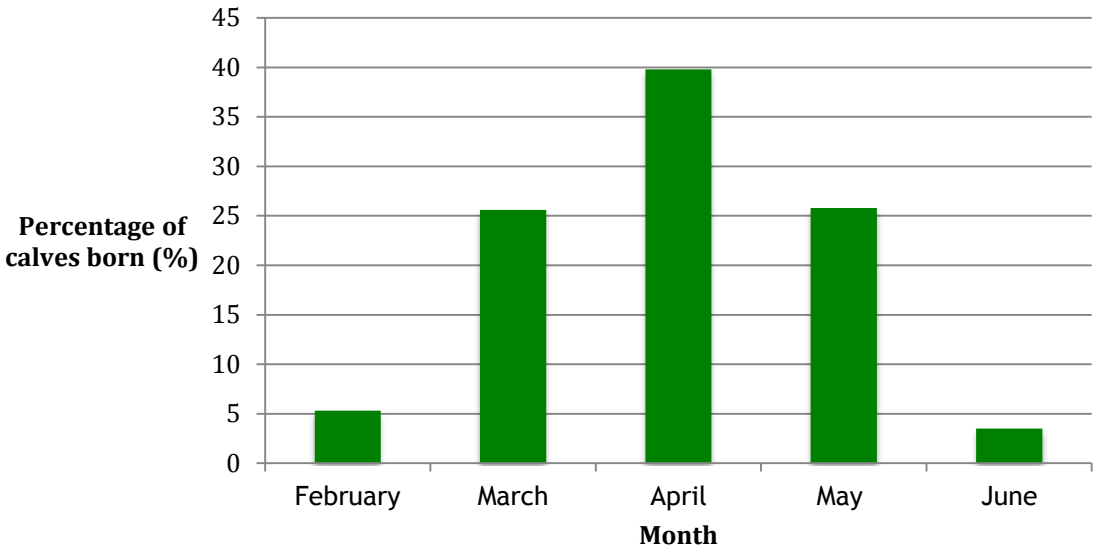
***** *Rota virus, Corona virus, E.coli*

4.3.2 Parturition Data for All Calves

4.3.2.1 Month of Parturition

The month of parturitions were recorded for all parturitions across all 11 herds (n=1101). The greatest number of calves were born in April (n= 438) (Figure 4.1). The least number of calves were born in June (n=39); however, data were not collected for the entire month of June. The median number of calves born per month was 282 calves.

Figure 4.1; *Percentage of calves born per month*



4.3.2.2 Day of Week of Parturition

The day of the week that parturition occurred was recorded for all parturitions across all herds (n=1101). The greatest percentage of parturitions were recorded as born on a Saturday (15.7%).

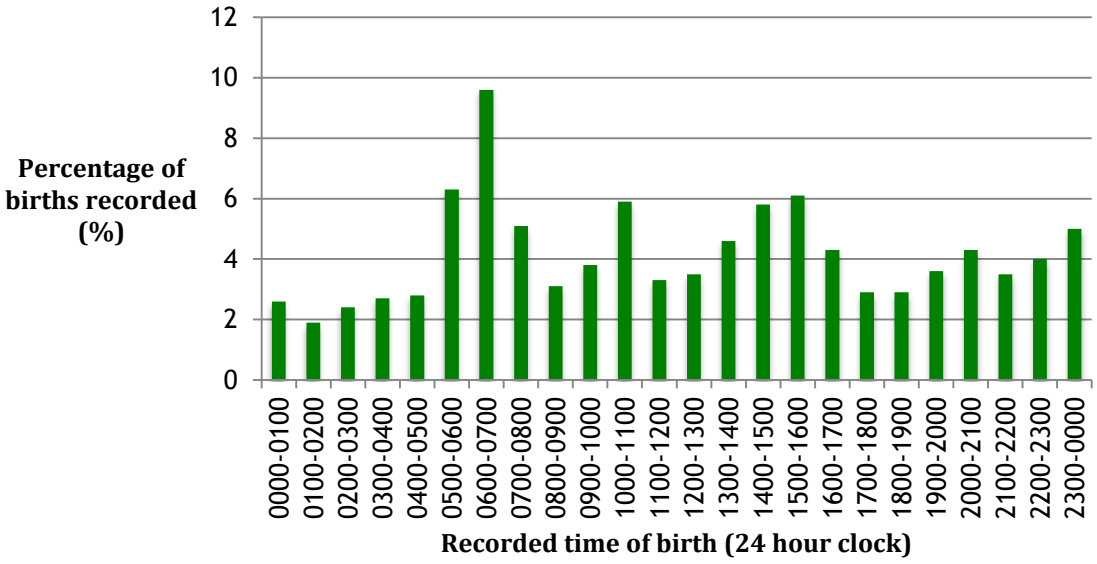
Table 4.4; *Distribution of parturitions by day of the week*

Day of Week	Percentage of Parturitions (%)
Monday	14.5
Tuesday	12.8
Wednesday	13.3
Thursday	14.0
Friday	15.3
Saturday	15.7
Sunday	14.4

4.3.2.3 Time of Parturition

The time of parturition was recorded for 782 calves during the study. The greatest percentage of calves were recorded as born between 0600 and 0700. The least number of calves were recorded as born between one and two o'clock in the morning (Figure 4.2).

Figure 4.2; *Distribution of the time of all parturitions*



4.3.2.4 Breed of sire

The breed of sire was recorded for all parturitions across all herds (n=1101). The greatest percentage of calves were born with Aberdeen Angus as the sire (Table 4.5).

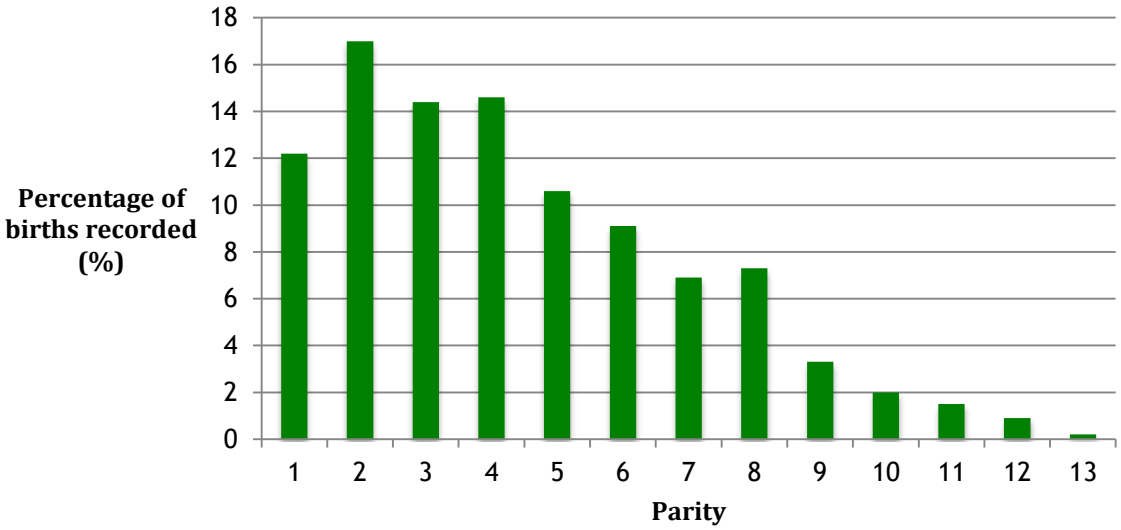
Table 4.5; *Distribution of parturitions by breed of sire*

Breed of Sire	Percentage of Parturitions (%)
Aberdeen Angus	31.1
Charolais	17.3
Limousin	8.9
Saler	9.1
Shorthorn	12.9
Simmental	18.4
Stabiliser	2.3

4.3.2.5 Dam Parity

Dam parity was recorded for 565 calf births, with a range of 1st to 13th parity (Figure 4.3). The median parity in this study was 4th.

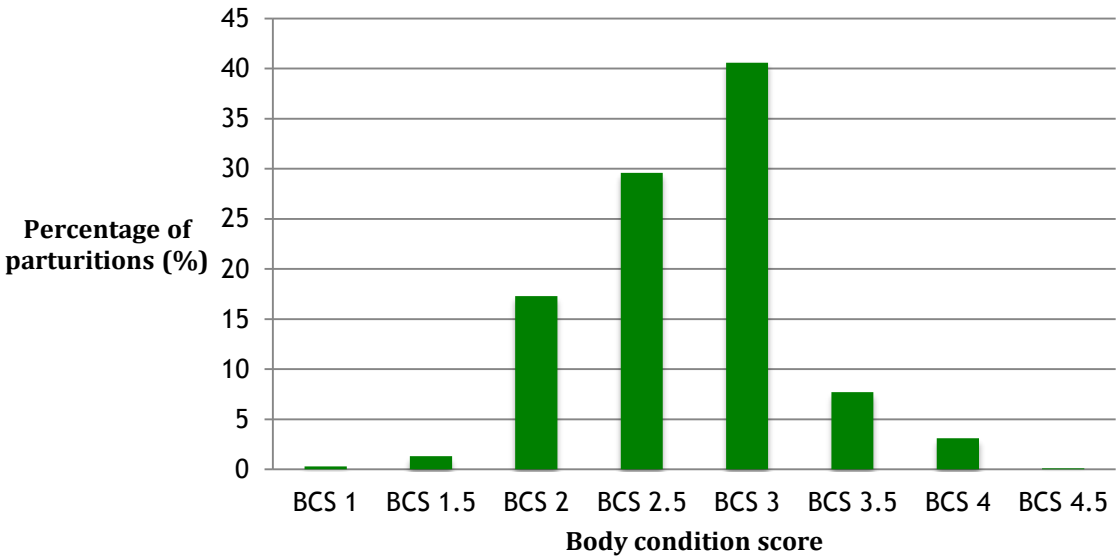
Figure 4.3; *Parity of dams for all births*



4.3.2.6 Body Condition Score of Dams

Body condition score of the dam at parturition was recorded for 869 calves born. Body condition score ranged from 1/5 to 4.5/5, with a median body condition score of 3/5.

Figure 4.4; *Body condition score of dams*



4.3.2.7 Calf Gender

Calf sex was recorded for all births across all herds for the duration of the study (n=1101). A total of 564 female calves were born, compared to 537 male calves.

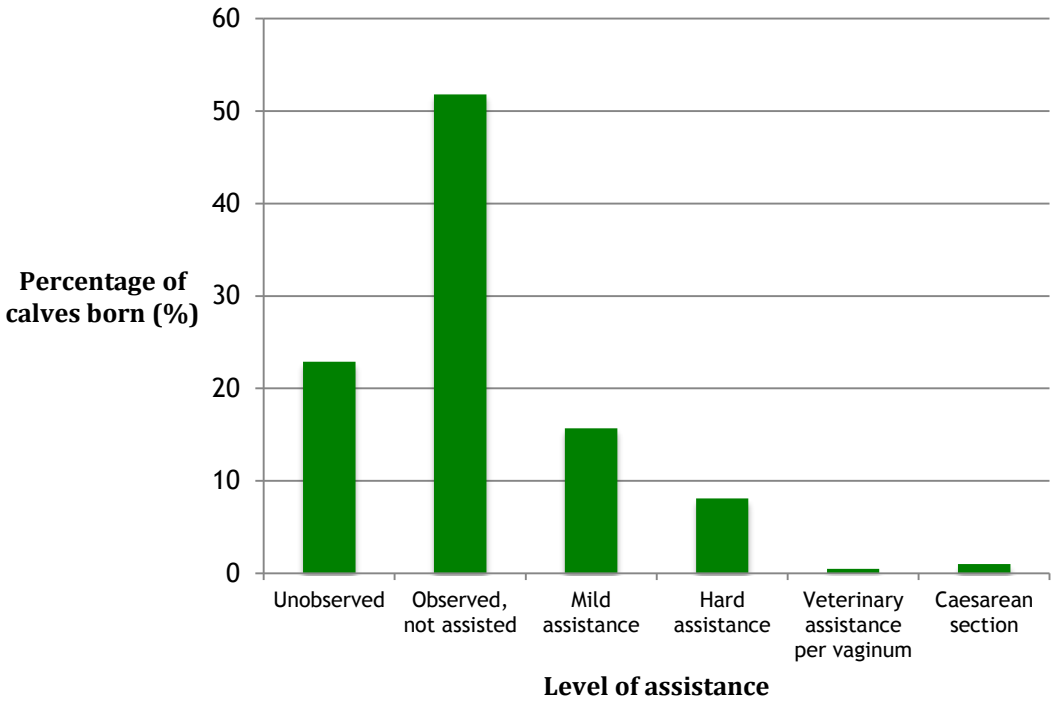
4.3.2.8 Multiparous Births

The number of calves born to each dam was recorded for all calf births across all targeted herds (n=1101). Eighty-six calves (7.7%) were born as multiparous births, with the remainder of calves born as singleton births (n= 1015).

4.3.2.9 Assistance at Parturition

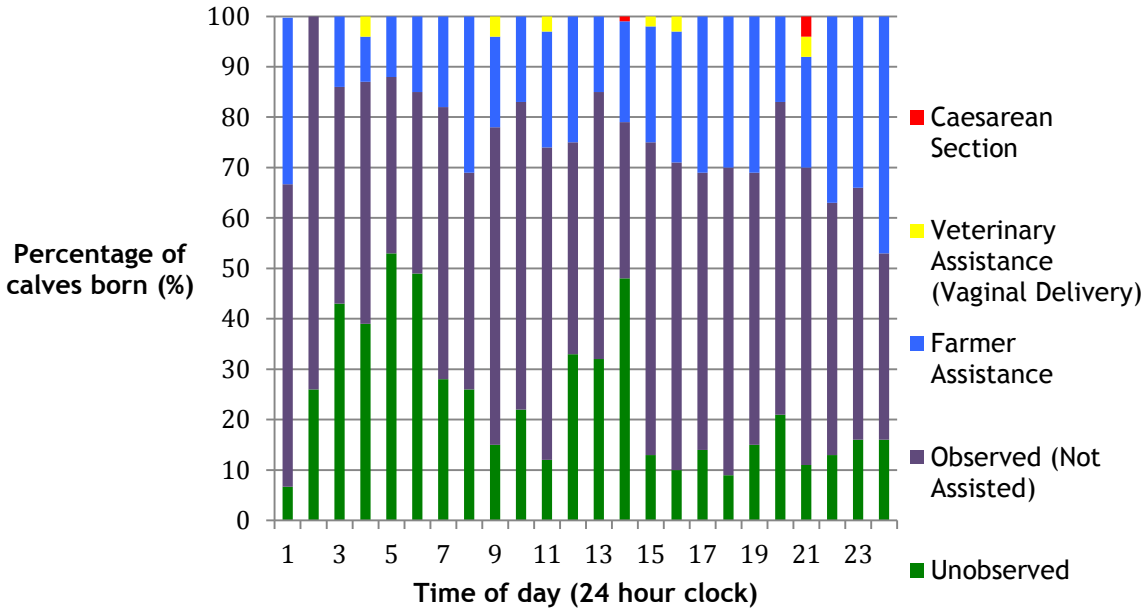
The level of assistance was recorded for 878 births during the study period (Figure 4.5). The greatest percentage (51.8%) of parturitions were observed but not assisted.

Figure 4.5; *Level of assistance given to cows at parturition*



The greatest number of unobserved births were recorded as occurring between six and seven o'clock in the morning (time when first discovered). The least number of unobserved births were recorded as occurring between one and two o'clock in the morning (time when first discovered). The greatest number of observed but unassisted parturitions occurred between six and seven o'clock, 10 and eleven o'clock in the morning and three and four o'clock in the afternoon. The least number of observed but unassisted parturitions were recorded between 12 and one o'clock in the morning. The greatest number of assisted births occurred between two and three o'clock in the afternoon and eleven o'clock and midnight at night. No assisted births occurred between two and three o'clock in the morning (Figure 4.6).

Figure 4.6; *Time of assistance at parturition*



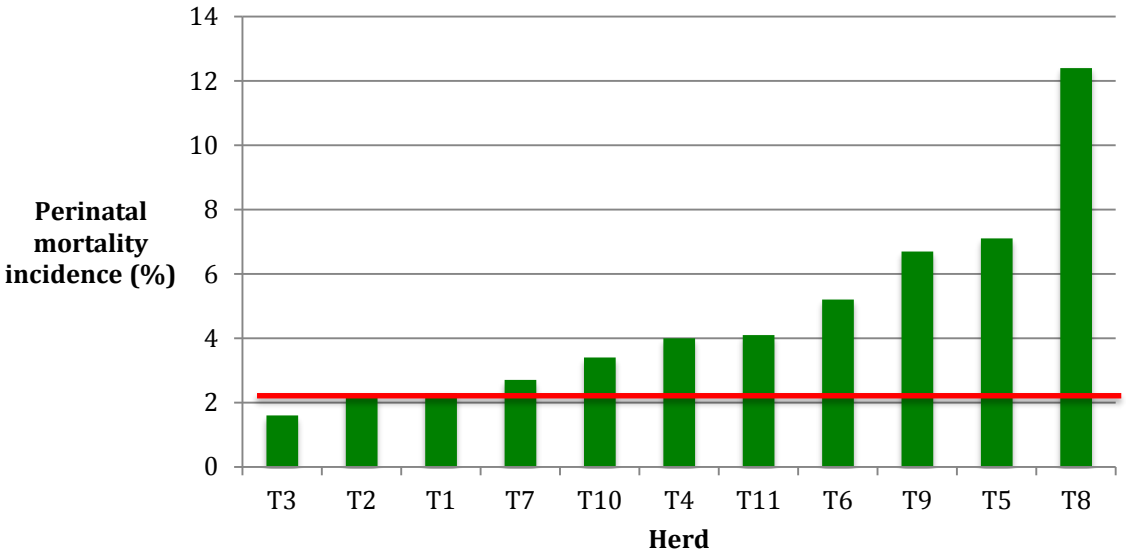
4.3.3 Perinatal Mortality Incidence

During the study period 1st February- 10th June 2016, 1101 calves were born across all targeted herds. A total of 56 calves died in the perinatal period on targeted herds, with 1045 calves alive at 48 hours post-partum. The highest number of perinatal losses on an individual farm was 16, and the lowest number was one (Table 4.6). The median number of perinatal losses on an individual farm was three calves. The highest incidence of perinatal loss in an individual herd was 12.4% and the lowest incidence for perinatal loss in an individual herd was 1.6% (Figure 4.7). The mean incidence of perinatal loss at an individual herd level was 4.7% with an overall incidence of 5.1%, across all herds.

Table 4.6; *Overview of perinatal losses and incidence by herd*

Herd	Number of Calves Born	Number of Stillbirths	Number of Neonatal Losses	Number of Calves alive at 48 Hours	Stillbirth Incidence (%)	Neonatal Loss Incidence (%)	Perinatal Loss Incidence
T1	130	2	1	127	1.5	0.8	2.3
T2	90	2	0	88	2.2	0.0	2.2
T3	61	0	1	60	0	1.6	1.6
T4	100	4	0	96	4.0	2.1	4
T5	126	7	2	117	5.6	1.7	7.1
T6	115	4	2	109	3.5	1.8	5.2
T7	109	2	1	106	1.8	0.9	2.7
T8	129	13	3	113	10.1	2.6	12.4
T9	105	6	1	98	5.7	1.0	6.7
T10	87	2	1	84	2.9	1.2	3.4
T11	49	1	1	47	2.0	2.1	4.1
Total	1101	43	13	1045	3.9%	1.2%	5.1%

Figure 4.7; *Perinatal loss incidence by herd. The red line shows the target level (2%) for perinatal mortality on beef farms (Caldow et al., 2005).*



4.3.3.1 Stillbirth and Neonatal Mortality Incidence

A total of 43 calves were stillborn across the 11 herds. The highest number of stillbirths on an individual farm was 13 and the lowest number was zero (Table 4.6). The median number of stillbirths per herd was two calves. The highest incidence of stillbirths per herd was 10.1% and the lowest incidence was 0%. The mean stillbirth incidence at an individual herd level was 3.5% with an overall incidence of 3.9% (43/1101), across all herds.

A total of 13 calves died in the neonatal period (0-48 hours) (1058 calves at risk) (Table 4.6). The median number of neonatal losses on an individual farm was one calf. The highest incidence of neonatal loss in an individual herd was 2.6% and 0% was the lowest incidence for neonatal loss in an individual herd. The mean incidence of neonatal loss at an individual herd level was 1.4% with an overall incidence of 1.2%, across all herds.

4.3.4 Perinatal Loss Incidence by Calving Factors

4.3.4.1 Month of Parturition

The greatest perinatal mortality incidence occurred in the month of May (7%). No difference was seen in perinatal mortality in relation to month of parturition (P >0.05).

Table 4.7; *Perinatal mortality incidence in relation to month of birth*

Month	Number of parturitions	Perinatal mortality incidence (%)
February	58	3.4
March	282	3.5
April	438	5.0
May	284	7.0
June	39	5.1
Not recorded	0	N/A

4.3.4.2 Day of Week of Parturition

The greatest perinatal mortality incidence was seen in calves born on a Sunday (8.2%, Table 4.8). No difference was seen in perinatal mortality in relation to day of the week of parturition (P >0.05). Additionally, no difference was seen in perinatal mortality in relation to parturition occurring at the weekend or through the week (P >0.05).

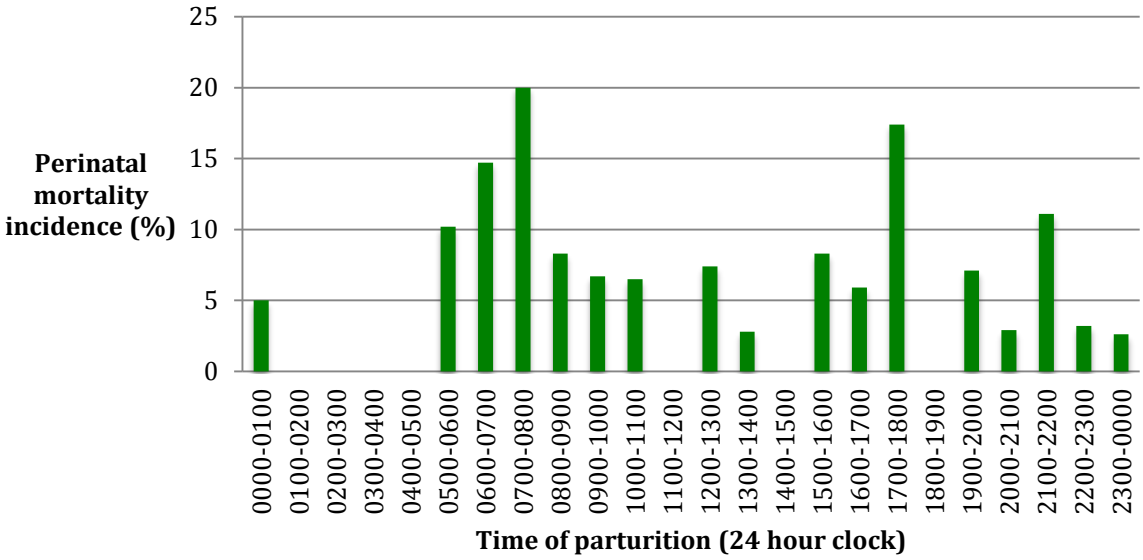
Table 4.8; *Perinatal mortality incidence in relation to day of week of parturition*

Day of week of parturition	Number of parturitions	Perinatal mortality incidence (%)
Monday	159	3.8
Tuesday	141	5.7
Wednesday	146	2.1
Thursday	154	5.8
Friday	169	4.1
Saturday	173	5.8
Sunday	159	8.2
Not recorded	0	N/A

4.3.4.3 Time of Parturition

The greatest perinatal mortality incidence was recorded between 0700 and 0800 (Figure 4.8). A difference in perinatal mortality incidence in relation to time of parturition was observed ($P < 0.05$).

Figure 4.8; *Perinatal mortality incidence in relation to time of parturition*



4.3.4.4 Breed of sire

The greatest perinatal mortality incidence occurred in calves with a Shorthorn bull as the sire (Table 4.9). No difference was seen in perinatal mortality incidence in relation to breed of sire (P >0.05).

Table 4.9; *Perinatal mortality incidence in relation to breed of sire*

Breed of sire	Number of parturitions	Perinatal mortality incidence (%)
Saler	100	2.0
Charolais	191	2.6
Limousin	98	3.1
Stabiliser	25	4.0
Simmental	203	5.1
Aberdeen angus	342	5.8
Shorthorn	142	10.6
Not recorded	0	N/A

4.3.4.5 Dam Parity

Dam parity was recorded 565 parturitions, but was unfortunately only recorded for 17 perinatal losses. Of 17 the perinatal losses where dam parity was recorded, a greater mortality was seen in primiparous dams (Table 4.10).

Table 4.10; *Perinatal mortality in relation to dam parity*

Parity	Number of parturitions	Perinatal mortality incidence (%)
1st parity	70	4.3
2nd-10th parity	481	2.9
>10th parity	14	0
Not recorded	536	N/A

4.3.4.6 Body Condition Score of Dams

Body condition score was recorded for the dam in 45 perinatal losses. The median body condition score in perinatal losses was 2.5/5, compared to 3/5 where calves were alive at 48 hours. The greatest perinatal mortality incidence was seen in under-condition dams at parturition (11.0%, Table 4.11). A difference in perinatal mortality incidence was seen between the ranges of body condition scores of the dams ($P < 0.05$).

Table 4.11; *Perinatal mortality incidence in relation to body condition of dam at parturition*

Condition of dam	Number of parturitions	Perinatal mortality incidence (%)
Under-conditioned*	164	11.0
Target condition**	677	4.0
Over-conditioned***	28	0
Not recorded	232	N/A

**body condition score <2.5/5 (Caldow et al., 2005)*

***body condition score 2.5-3.5/5 (Caldow et al., 2005)*

****body condition score >3.5/5 (Caldow et al., 2005)*

4.3.4.7 Gender of Calf

There was a difference in perinatal mortality incidence between calf sexes, with a higher incidence in male calves (7.4%; $P<0.01$, Table 4.12).

Table 4.12; *Perinatal mortality in relation to calf gender*

Gender	Number of parturitions	Perinatal mortality incidence (%)
Female	564	2.8
Male	537	7.4
Not recorded	0	N/A

A higher proportion of male perinatal losses were born stillborn compared to death during the neonatal period (75% compared to 25%). This was not statistically significant (P value >0.05).

4.3.4.8 Multiparous Births

A higher perinatal mortality incidence was seen in multiparous births compared to singleton births (16.5% compared to 4.1%, Table 4.13), although this was not significant (P value >0.05).

Table 4.13; *Perinatal mortality incidence in relation to number of calves born at each parturition*

	Number of calves	Perinatal mortality incidence (%)
Single birth	1015	4.1
Twin birth	86	16.5
Not recorded	0	N/A

4.3.4.9 Assistance at Parturition

The greatest perinatal mortality incidence was seen in calves born with veterinary assistance (25.0% of veterinary assisted parturitions) (Table 4.14). The lowest perinatal mortality incidence was seen in calves born observed but unassisted (1.3%). No significant difference was seen in perinatal mortality incidence in relation to the level of assistance provided at parturition (P >0.05).

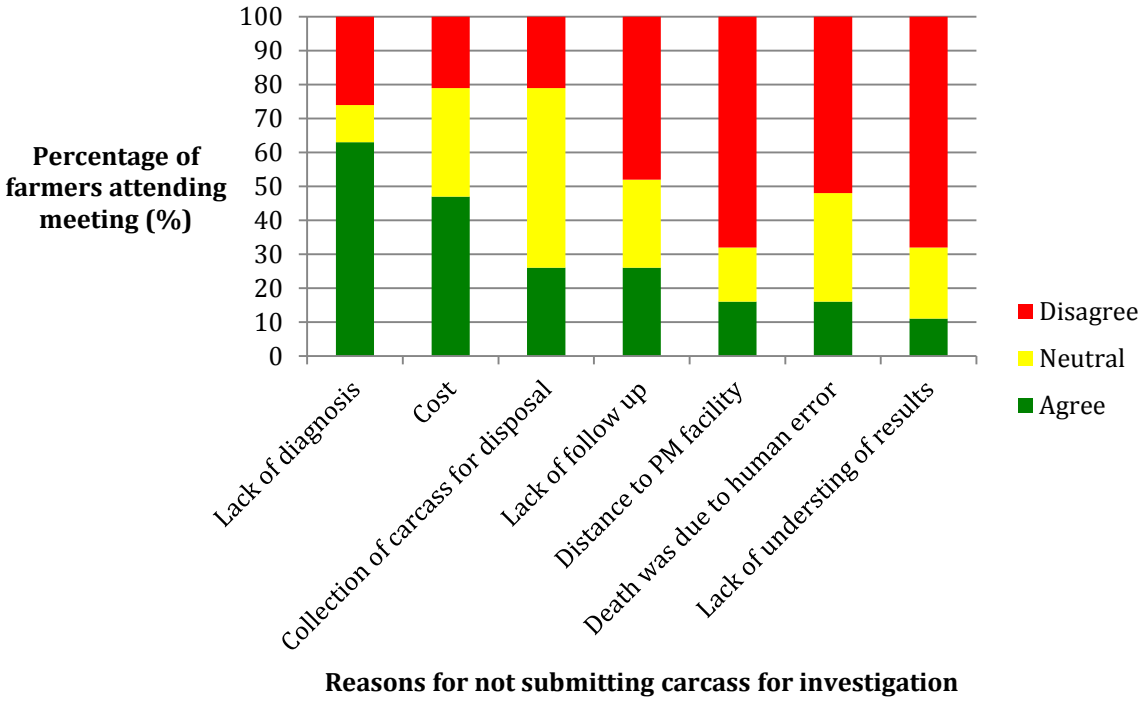
Table 4.14; *Perinatal mortality incidence in relation to calving score*

Level of assistance	Number of parturitions	Perinatal mortality incidence (%)
Unobserved	**201**	**10.9**
Observed, not assisted	**455**	**1.3**
Mild assistance	**138**	**7.2**
Hard assistance	**71**	**21.1**
Veterinary assistance per vaginum	**4**	**25**
Caesarean section	**9**	**22.2**
Not recorded	**223**	**N/A**

4.3.5 End of Study Questionnaire

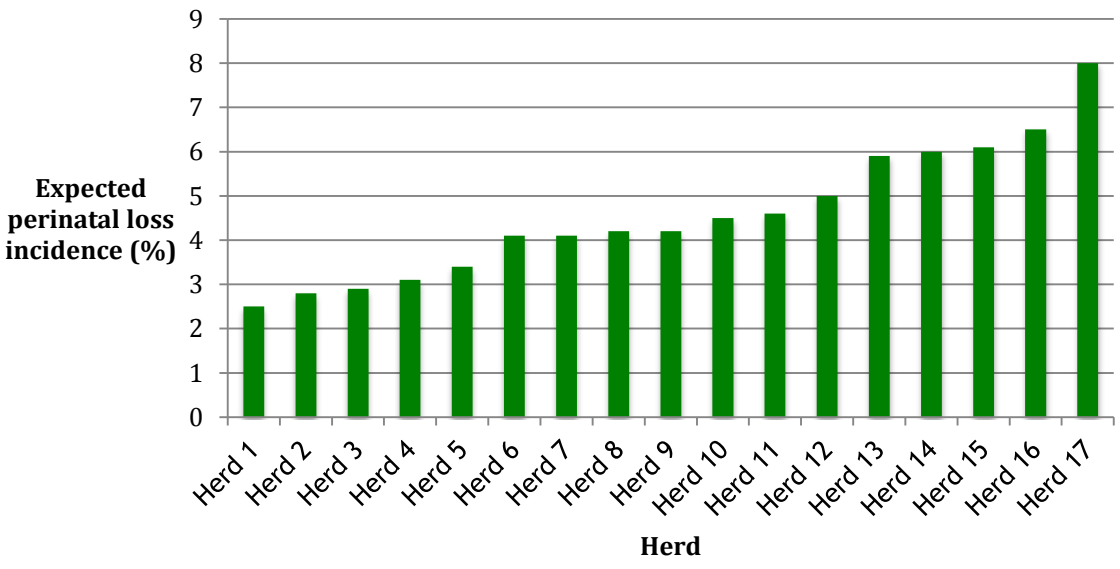
Nineteen farmers attended the post-study meeting held in October 2016. The most common reason for lack of submission of calves for investigation of perinatal deaths was due to lack of diagnosis, according to the end of study questionnaire (Figure 4.9). Distance to the post mortem facility from the farm was not a common reason for lack of submission of carcasses for investigation of perinatal loss.

Figure 4.9; *Reasons for lack of submission of carcasses for investigation*



An expected perinatal loss incidence on each herd was completed by 17 farmers. The expected incidence ranged from 2.5% to 8%, with a mean incidence of 4.6% (Figure 4.10).

Figure 4.10; *expected perinatal loss incidence by herd according to farmer*



4.4 Discussion

One of the most important indicators of animal health and welfare on farm, is the number of deaths per year, and in particular, the number of perinatal losses (Mee, 2013a). Perinatal mortality incidence in beef herds in the Orkney Islands has been perceived to be high by local veterinary surgeons and farmers; however, no detailed investigation of the incidence has been performed in the past. Although it was perceived that perinatal loss incidence in Orkney was high, it is suggested that such losses are often under-estimated. This is due to the lack of requirement to register such losses, providing calves die before they are legally required to be tagged and registered under The Cattle Identifications Regulations 1998 (Ortiz-Pelaez et al., 2008). A true Figure of perinatal mortality in British beef herds is hard to estimate due to the lack of active surveillance nationally, but also due to the variation in the definitions of perinatal mortality (Mee, 2013a).

In 2015, it was estimated that the average incidence of perinatal mortality in beef suckler cows in Scotland was 4%, according to data collected from 11,138 cows over 111 herds (Anon., 2015b). In consideration of this figure, the perinatal mortality incidence of 5.1% found in this study was higher than expected in the Orkney Islands, and higher than is acceptable in British beef herds, especially with a target of 95 calves weaned per 100 cows mated (Caldow et al., 2005). This Figure was also significantly higher than studies internationally on beef perinatal mortality incidence (2.4%); however, comparison is difficult due to the variation in definitions of perinatal mortality (Bleul, 2011).

Excluding one herd, all other herds in this study had a perinatal mortality incidence above the target Figure of 2% (Caldow et al., 2005) and all herds had at least one bovine perinatal loss. Although perinatal mortality incidence may be reducible in beef cattle in Orkney, it is also possible that some losses are unavoidable (for example due to congenital malformations) and the prevalence of unavoidable losses is an area for further study, allowing a more accurate

target level to be set. This also applies at a national level. No information is provided on how the previous target level of perinatal mortality incidence was calculated (Caldow et al., 2005) and it may be appropriate to reconsider this, taking in to account both avoidable and unavoidable perinatal mortalities.

Bias in this study may have been seen, due to some herds participating due to a previous high incidence of perinatal mortality on farm and a desire to investigate further. On the contrary, participation from farmers who did not perceive perinatal mortality to have a high incidence on farm, but were pro-active in disease control and surveillance may also have been over-presented. In the author's opinion, a combination of reasons for participation was seen, although bias towards participation from herds with a previously high perinatal mortality incidence was observed. To reduce bias in future studies, a larger population size and random selection of herds would be required, although farmer compliance may be reduced with random selection. Further bias may have been seen in this study with regards to actions taken to try to prevent perinatal losses. With participation in a study analysing the incidence of perinatal losses at an individual herd level, if incidences appeared high to the farmer, corrective actions may have been made to try to reduce these losses. In some circumstances these remedial actions may have been taken under normal circumstances, but participation in the study may have furthered these actions. This is however, only speculative.

It has been reported that in some herds perinatal mortality is an issue year after year; however, on other farms perinatal mortality incidence can be sporadically high in one particular calving period (Mee, 2013a). Discussion with participating farmers and completion of a questionnaire regarding annual losses, completed at the end of the study (Appendix 7), suggest that the average perinatal losses seen during the study period were as expected in an average calving period and in particular, were as expected for the calving period studied. However, in order to

reduce bias, incidences should be calculated over multiple calving periods, allowing yearly trends to be considered.

It has previously been speculated that increasing herd sizes (Berglund et al., 2003) and increased number of calves born (Uetake, 2013) can lead to increased perinatal mortality incidences due to reduced supervision at parturition. This may be suggestive of a lack of focused attention on individual animals by each stock worker, and more attention of the herd as a whole. This data were not analysed in this study, but is an area for future research, where more information regarding stock worker to cow ratio and experience of the stock worker would need to be taken into account.

A numerically higher incidence of perinatal mortality was seen in the month of May compared to April, despite a reduced number of calves born in May. The incidence of perinatal mortality decreased again in June. One possible theory behind these findings is that silage quality reduces throughout the season, allowing growth of mycotic organisms. This may lead to foetal exposure to mycotic organisms through the blood supply (Djonne, 2007), which may result in the birth of a stillborn calf. A reduction in perinatal mortality incidence is seen in the month of June, which differs from results seen in other studies on pasture-based dairy herds, which found that there was a peak in perinatal mortality in June (Auran, 1972; Philipsson, 1976; Mee, et al., 2008). In the 2016 calving period, the majority of cattle in this study were turned out to pasture in the last week of May. In previous studies, it was postulated that the stress of turnout led to increased perinatal mortality; however, this was not the case in this study. In the current study, the reduced perinatal mortality seen in June may be related to the reduced number of cattle calving in this month or that data were not collected for the entire month. Alternatively, many cattle are turned out to pasture in June, meaning that cattle have less exposure to pathogens, which build-up during the housing period. No analyses with regards

to temporal trends in aetiology were performed in this study, but would be an area of work, which would warrant further investigation.

A second theory to explain the apparent increase in the perinatal mortality between April and May is, with the reduced number of cattle calving in May compared to April, less attention is given to pre-partum cows and detection of parturition. This in theory leads to more unobserved parturitions, which may increase perinatal losses due to prolonged parturition with resultant anoxia and acidosis (Lorenz et al., 2011). Fatigue in stock workers after a prolonged calving period, resulting in reduced observation of parturition may also account for the increased incidence of perinatal mortality in May compared to April. Employee performance has been reported to significantly increase perinatal mortality rates (Uetake, 2013). A true comparison of data collected from February- May and data collected in June cannot be made due to the incomplete data set from the month of June. Logistics and other commitments prevented a full data set collection.

Although no distinct pattern was seen with the time of births, consistent with previous studies (Edwards, 1979; Stevenson, 1989), the greatest numbers of births were recorded between the hours of six and seven in the morning. The greatest numbers of unobserved births were also recorded between the hours of six and seven in the morning. It is possible that this reflects the time that stock workers observe the cattle for the first time each morning and may not be an accurate recording for time of birth. For example, the farmer may do a final check at 12am, a cow could calve at 3am but the farmer doesn't check the cows again until 6am the following morning and so this calf is recorded as calving at 6am. Additionally, this may also reflect pre-occupation by the stock worker. At arrival on the farm in the morning, multiple tasks require attention, potentially leading to an increase in the number of unobserved births if cattle are truly calving at this time of day. A similar situation may explain the high number of

unobserved births occurring between two and three o'clock in the afternoon, when the stock worker returns from lunch. This is however, only speculative.

The greatest numbers of assisted births were recorded between the hours of six and seven in the morning, two and three in the afternoon and 11 and 12 at night. One theory for this is that these times reflect when the stock worker returns to observe the animals after periods of time doing other tasks or periods of rest. It is possible that these animals have been observed to have started stage two parturition many hours prior, but return to observation at these times, reveals that parturition has not progressed as expected, hence the assistance. In addition, it is possible that a high level of assistance occurs at 11pm (approximate time of final check of cattle) to prevent further assistance during the night, despite possible premature assistance. This conclusion is only speculative in this study; however, it is consistent with findings by Gleeson et al. (2007).

A low number of assisted births were recorded between midnight and five in the morning, despite a reasonably high number of observed but unassisted births during this time period. This finding is consistent with work by Gleeson et al. (2007), who found that unrestricted access to silage led to an even distribution of parturitions throughout the day and night, but was associated with a reduced number of dystocias, compared to restricted access to silage. All cows in this study had *ad libitum* access to silage. Additionally, stock worker fatigue may have a role in this pattern of assistance, with less enthusiasm or energy to assist cattle during the night. It may also be possible that there are less stock workers available to assist outside of normal working hours if multiple persons are required for assistance, leading to a reduced number of assisted births during the night (Gleeson et al., 2007). This is only speculative as no data were collected with regards to working hours or number of stockpersons available during the night.

It must be highlighted that the scale used for scoring of level of assistance at parturition was not intended to be a linear scale with regards to difficulty of delivery, but rather the simplest method of categorisation of the parturition for the farmer to record. In many situations, veterinary assistance is often less traumatic than delivery by the farmer due to the skills of veterinary surgeons in comparison to farmers. In an ideal situation, each parturition should be given a score as to the difficulty of delivery, however, this may add further subjectivity to the scoring.

Although similar trends were seen with regards to various calving factors and perinatal mortality incidence, compared to previous studies on perinatal mortality, it may also be possible that some of these associations may have occurred by chance. In order to investigate these apparent associations and to the improve reliability of these results, the study should be repeated on a larger scale, over multiple calving periods.

The husbandry, management and calving patterns seen in the farms in this study were representative of the majority of beef farms in Orkney, as observed by local veterinary surgeons at Northvet Veterinary Group, and as recorded through benchmarking data, 2014, collected by Northvet Veterinary Group (unpublished). The geographical distribution of targeted herds provides a more reliable assessment of the perinatal mortality incidences seen throughout the Orkney mainland, when compared to herds within one parish.

Due to the nature of the data collection method used in this study (questionnaire), the quality of the data collected with regards to each calving or herd background, may be of variable reliability as it relied on farmers recording the information accurately. This was observed in particular with some of the parameters where data were missing. Recall bias can be influenced by multiple factors; however, the time interval between the event and recording of data is a

massively important factor (Coughlin, 1990). Prior to commencement of the study, it was requested that all data were recorded at the time of occurrence of each event (i.e. data recorded at the time of each parturition) but due to other commitments, it was suspected that this was not a feasible option in this study. Variations in perceptions between individuals, for example, with calving ease or body condition scoring, are very subjective, leading to a degree of bias between stockpersons within herds and between herds. Improvements in reliability may have been seen if body condition scores and calving assistance scoring were performed by the same trained individual on all animals. This was not feasible due to the fact cows were calving at any time of the day and night and having a trained individual present on all farms at all times was not practical. Alternatively, data could have been recorded by the same stockperson on each farm to improve reliability, but again this was not feasible due to working hours and other commitments on a commercial farm. Data were recorded at a time of year where farmer's attention and concentration on accurate data recording may be reduced due to heavy workload. This was unavoidable due to the nature of data recorded. Much of the univariate data analysed in this study was biased, due to the size of the data set collected. This could be improved through increasing the size of the data set in future studies. Two herds were unable to submit data from parturitions where the calf was alive at 48 hours post-partum. Although this was disappointing, it was anticipated due to the heavy workload of farmers at the time of study, with the additional possibility of stockworkers being unaware of the full requirements of the study. In order to prevent similar results in future studies, discussions should be held with all workers on the farm prior to commencement of the study.

The majority of dam body condition scores did not vary greatly and fell within the target body condition score for cattle at the point of calving. This is concurrent with other studies, which did not see a wide range of body condition scores, preventing comparisons of risk factors (Berry et al., 2007). In the current study, a higher perinatal mortality incidence was seen in cows which were under-conditioned at parturition, which may reflect lack of body reserves for

parturition, as discovered by Larson et al., 2004. There may also be additional factors relating to under-conditioned cows, contributing increased risk of perinatal mortality; for example, multiparous births or chronic disease. Over-conditioning at parturition was not associated with increased perinatal mortality incidence in the current study, but again, this may reflect the small study size and small range of body condition scores recorded.

The number of different breeds of sires used in this study was relatively small, but would be fairly representative of the breeds used on beef suckler farms in Orkney (as observed by local veterinary surgeons at Northvet Veterinary Group), with the addition of a few other breeds (British Blue and British Blonde). No benchmarking data are available on breeds present in Orkney. The Aberdeen Angus as a breed has been traditionally used on beef farms in Orkney, due to historical ease of calving and good growth rates associated with this breed. This tradition has changed over recent years with the introduction of a variety of different continental breeds. The reasons for these changes are due to the ever-increasing demand to for more efficient beef production and larger, leaner carcasses produced by continental breeds. There may also be an aspect of crossbred vigour associated with the use of multiple sires on multiple farms. Results from this study suggest that perinatal mortality incidence is higher in calves sired by Aberdeen Angus or Shorthorn bulls, compared to other popular breeds of bull, which is in contrast to results of previous studies (O'Shaughnessy et al., 2013). This result was not statistically significant, due to the low sample size in this study.

Although this study provides an insight into the perinatal mortality incidence in the Orkney Islands, to achieve a true value, an increased sample size, ideally across all beef holdings on the Orkney Islands, is required. To account for year to year variation, data should also be collected over multiple spring calving periods. This would also reduce the possibility of chance associations between various factors and perinatal mortality.

5. General Discussion

Bovine perinatal mortality is a common occurrence on British beef farms and little has been done to fully investigate the true incidence or aetiologies of these losses, or to indicate areas for improvement to prevent such losses. Perinatal mortality on British farms, including both stillbirths and neonatal losses, have become normalised and almost expected both by farmers and veterinarians, due to the high numbers of cases experienced by some herds (Mee, 2011a). These deaths represent a huge loss to the industry, and in a market which is becoming increasingly focused on improved animal welfare and in which profit margins are already tight (McLaughlin, 2011), significant improvements need to be made to reduce perinatal mortality on British beef herds. This is a role to be undertaken both by farmers and veterinary surgeons.

Until now, no protocol and standard categorisation of results has been available for the investigation of bovine perinatal losses. This study has produced a robust diagnostic approach to these losses, allowing a standardised investigative approach. The information captured in the protocols and algorithms developed in this study is inherently often difficult to categorise into neat diagnoses due to the complex nature of the subject; for example, multiple pathologies present and the differentiation between cause of death per se and contributing factors to death. All attempts have been made to reduce possible errors in the diagnostic approach, through review of existing literature, specialist training and consultation with experts in the field. Continued testing is required at all stages of development of a medical algorithm, and this includes further testing in the field. Despite this, all attempts have been made to reduce possible errors in the algorithm, although testing was limited due to the small study size. The algorithms designed in this study have been developed to be used by veterinary surgeons in general practice, but also to be used in veterinary referral laboratories. The algorithms have not however, been tested on persons untrained in bovine perinatal pathology, and this would be an area for future research. Although the diagnostic approach has been specifically designed for use in bovine perinatal loss investigation, it is hoped that it may provide a basis

for the development of similar protocols and algorithms in other bovine age groups, and also in other species. This would again allow a standardised investigative approach to all post mortem investigations, with the aim of improving diagnostic outcomes.

The investigative approach used in this study was consistent for all submissions, resulting in a high diagnostic rate (89%). The results of this study allowed high quality feedback to be given to the farmer, not only on the time of death and proximal cause of death, but also on any contributing factors to death. In many circumstances, it was these contributing factors which were of higher significance in future prevention of losses, than the proximal cause of death, for example; foetal oversize resulting in death due to stage two anoxia. One of the hindrances to farmers for submission of perinatal losses, according to the end of study questionnaire, was the low rate of diagnostic results. This study has shown that high diagnostic rates can be achieved, which may improve submission rates in the future. This would however, depend on the quality of feedback given to the farmer, which may be improved by suggestions on prevention of similar losses in the future and not just a statement as to why the calf died.

This study has highlighted issues with reliance on passive submissions for disease surveillance, as bias may be seen in the results. There were major differences in the time and proximal causes of death between targeted and passive submissions in this study. Where the cause of death is suspected by a farmer, submissions would be less likely. However, to gain a better understanding of the aetiologies of bovine perinatal losses, and to target areas for improvement, a targeted approach should be encouraged.

Financial constraints may be a hurdle in future bovine perinatal loss investigations, if the diagnostic approach outlined in this study is used. Government funding subsidises farm animal gross post mortem examination and

further investigations (at the discretion of the pathologist) and allows testing of specific diseases to be performed at no additional cost to the farmer. Funding does not, however, allow extensive routine testing (for example histopathological examination) of all submissions, which may often result in a diagnosis of 'not reached'. In order for high diagnostic results to be seen in future investigations, it may be necessary to transfer these costs to the farmer, but would risk reduced submission rates, based on cost.

It was speculated by local veterinary surgeons, that the bovine perinatal mortality incidence in Orkney was high, and this was confirmed in this study. The study was limited by a small study population, over one spring calving period and should ideally be repeated on a larger scale, over multiple years. Despite these limitations, the bovine perinatal loss incidence in Orkney was higher than is acceptable in Scottish beef herds. A target perinatal incidence of 2% was set by Caldow et al. (2005), although no description as to how this target was calculated is given. From results of this study, it is likely that this incidence level should be re-calculated to account for unavoidable losses (e.g. congenital malformations). Although the perinatal loss incidence was high in this study, it was as expected by the farmers, according to the end of study questionnaire (expected mean incidence of 4.6%). This was an interesting result and may emphasise the normalisation of these losses. On many farms, bovine perinatal losses are an issue every year and are not considered a problem; however, if the same number of losses occurred in older animals, questions would be asked. It is hoped that if an appropriate target level is set for bovine perinatal loss incidence, this can be conveyed to farmers in an attempt to reduce the normalisation of these losses.

A higher than expected number of calf deaths due to infection were found in this study. These results may have been biased by submissions from passive herds, although the results are slightly worrying and may warrant investigation on a larger scale over multiple calving periods. Many of the pathogens cultured in this

study have previously been considered insignificant or contaminants; however, more research is required in this field to assess this. No issues were raised with regards to sample technique or storage and transportation of the samples. A cause of death due to infection has implications with regards to feedback to the farmer, as the significance of these results and advice on future prevention is unknown. This may lower the confidence of the farmer in these investigations.

Anoxia developing during stage two of parturition was a common proximal cause of death in this study, but despite this, there was not one standard contributing factor. The contributing factors to the development of anoxia during parturition varied greatly, many of which may not be easily preventable in future cases. On the other hand, other contributing factors to anoxia, such as prolonged parturition due to presentation of the calf, or foetal oversize, should be reducible through appropriate breeding and parturition management.

It was hoped that outcome of this study would improve productive efficiency on beef farms in Orkney by increasing the number of calves born alive through the discovery of the aetiologies of bovine perinatal losses. Although many of the perinatal losses were unavoidable, many others have risk factors which should be modifiable and aid in reduction of these losses. Future work is required to assess the significance of dam health in perinatal losses, but also to assess farm management and attitude towards breeding and parturition protocols. More effort is required with regards to knowledge exchange to farmers on the best practices for parturition, breeding and on neonatal calf care, but this needs to be formed on a strong evidence base. This study has provided some insight in to these, but further work is required both in Orkney and at a national level. Additionally, encouragement should be given to farmers to engage in honest benchmarking recording, to allow establishment of a true bovine perinatal loss incidence and to identify areas for improvement.

Although many farmers are highly motivated and are keen to investigate any loss on the farm, others are harder to engage with and motivate. This was seen in herd T8 in this study, who, despite a high perinatal loss incidence, was not keen to investigate these losses. To some a perinatal loss incidence of 12.4% may appear high, but to this farm, this may be normal, or even an improvement on previous years. In such situations, discussions should be held regarding target levels, with an aim of discovering areas for improvement.

In summary, the development of a robust protocol and algorithm for bovine perinatal losses should allow these deaths to be investigated effectively, resulting in high diagnostic rates. The variation in results between passive and targeted herds has highlighted the need to approach these losses in a targeted manner. Additionally, the results of the targeted submissions have provided a broader understanding of the aetiologies of bovine perinatal losses, many of which should be potentially avoidable. This study has confirmed that the bovine perinatal mortality incidence in Orkney is higher than the current target perinatal loss incidence, although this may need to be re-calculated to account for unavoidable losses on the farm. The overall outcome of this study is to reduce further bovine perinatal losses on beef herd, although more work is required to expand this research to provide an evidence-based approach to prevention. This in turn should improve knowledge exchange between veterinary surgeons and farmers and ultimately reduce perinatal losses on beef herds.

Appendices

Appendix 1. History Questionnaire

Unique herd identification number.....

Full cow ear tag number.....

Date and time of calving			
Body condition score of dam			
Breed of Sire			
Date and time of death			
	Yes	**No**	**Comments**
Did you see calf being born?			
Did the cow show visible straining effort during calving?			
Could the cow see other cows during calving?			
Was there meconium staining on calf?			
Did cow show signs of illness before calving?			
Was cow down before calving? (For reasons other than calving)			
Was cow down after calving?			
	Yes	No	Comments
Was calf alive at first examination? (When first put hand in cow)			
Was calf born alive?			
Was assistance required with this calving?			Grade 0-5 (see calving data record)
Were there signs of the placenta coming away before/during calving?			
Was there anything abnormal noticed with regards to the calving?			
Has she had a stillborn calf in past?			
Did the calf require resuscitation at birth?			
Were any drugs administered to the calf at birth?			
Was the calf separated from the cow after birth?			
Was the cow aggressive towards her calf?			
Did calf show any signs of illness before death?			

	Over 2 days before	1 day before	12-24 hours before	6-12 hours before	At calving	After calving	Not Moved
When was cow moved to calving pen?							
Where did she calve?	Individual pen		Group pen		At grass		

	Less than 1hour	1-2 hours	2-4 hours	4-6 hours	6-12 hours	Over 12hours	Not observed
How long was she left after water bag or calf appeared before intervention?							

1. **What time did she show signs of starting calving? .....**
2. **What time did she begin stage 2 calving (water bag present)? .....**

Calf Details

3. **Was calf position;** **Normal (two front feet and head) ☐**
   Abnormal ☐
   Please specify….....
4. **Single ☐ Twin ☐ Triplet ☐**
5. **How were the cow and calf managed for 48 hours after calving?**
   Single pen ☐ Group housed ☐
6. **How was colostrum given to the calf?**
   Suckling cow ☐ Stomach tubed ☐ Bottle fed ☐ Combination ☐
7. **If colostrum was given that was not from the cow, what was the source?**
   From another cow on farm ☐ Artificial powdered ☐ Frozen ☐
   Sourced from other farm ☐ Paste ☐
8. **What time was first feed of colostrum given?**
   .....
9. **What volume of colostrum was given at first feed?.....**

Please complete and return with all stillborn or calves that die within 48 hours of birth

Appendix 2. Gross Post Mortem Examination Protocol

The following section outlines the systematic approach taken for gross post mortem examination of each calf submitted to the study. Where the protocol states 'assess', the organs or tissues were examined for gross lesions and changes in size, shape, colour or consistency in comparison to 'normal'.

- Collect history regarding dam, sire, parturition and the post-partum period from farmer (see Appendix 1).
- Weigh calf.
- Attach hoist to calf and lift calf on to examination table.
- Place calf in right lateral recumbency (Figure 5.1).

Figure 5.1; *Calf placed on examination table in right lateral recumbency*

- Assess muscle tone through flexion and extension of the limbs (Figure 5.2).

Figure 5.2; *Manipulation of limbs to assess muscle tone*

- Assess foetal hoof covering presence (Figure 5.3).

Figure 5.3; *Assessment of foetal hoof covering (present in photograph)*

- Confirm gender.
- Assess anus for patency.
- Assess for presence of meconium staining.
- Assess eyes- cornea, lens and conjunctiva (Figures 5.4 and 5.5).

Figure 5.4; *Assessment of cornea and lens*

Figure 5.5; *Assessment of conjunctiva for congestion and pathology*

- Assess mouth- gingiva colour (Appendix 8) (Figure 5.6), tongue and hard palate (Figure 5.7).

Figure 5.6; *Assessment of congestion in gingiva*

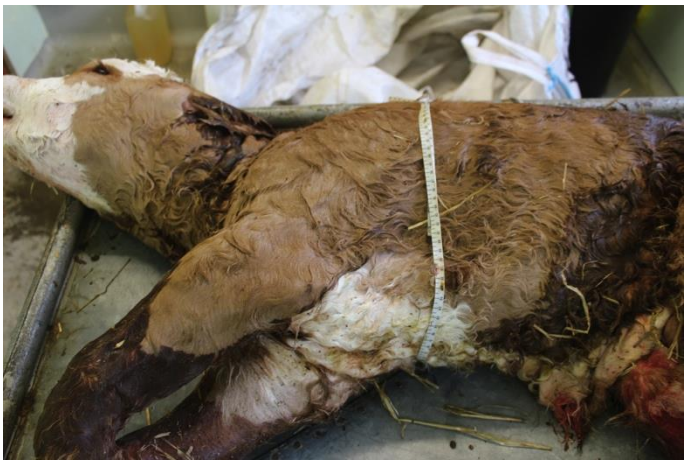
Figure 5.7; *Assessment of tongue and hard palate*

- Measure crown-rump length (occiput to sacro-coccygeal junction) (Figure 5.8).

Figure 5.8; *Measurement of crown-rump length*

- Measure girth (circumference of chest at point caudal to the point of the elbow) (Figure 5.9).

Figure 5.9; *Measurement of girth*



- Lift left hind-limb and cut through left coxo-femoral joint to allow dorsal reflection of the limb (Figure 5.10).

Figure 5.10; *Incision through coxo-femoral joint of left hind-limb*

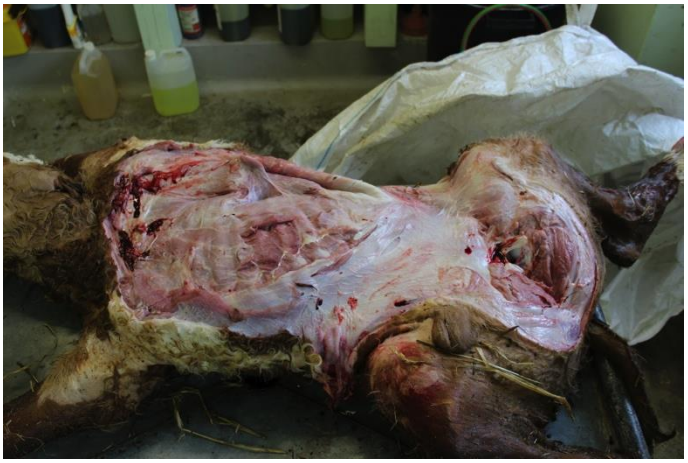
- If possible, collect 5ml blood from femoral blood vessels (compression on chest may aid collection) using syringe. Decant sample into labelled plain Vacutainer (Figure 5.11).

Figure 5.11; *Collection of blood sample from femoral blood vessels*

- Incise skin over ventral midline and through left axilla to allow dorsal reflection of skin and left forelimb (Figure 5.12 and 5.13).

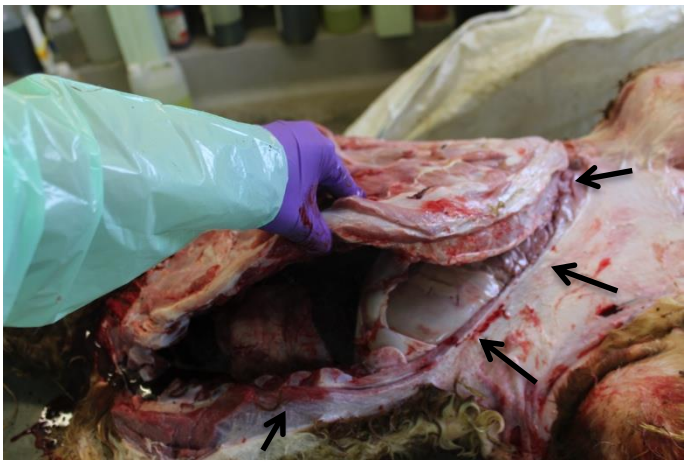
Figure 5.12; *Incision through skin over ventral midline*

Figure 5.13; *Incision through skin with reflection of left forelimb*



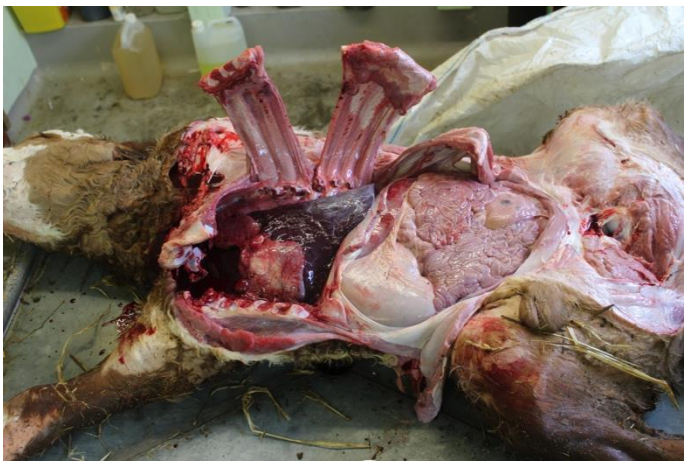
- If no blood sample collected from femoral blood vessels, collect from radial blood vessels by method described as above.
- Incise at the caudal edge of the caudal rib and extend incision ventrally to the xiphoid. Extend incision through left costo-chondral junction, allowing dorsal reflection of left thoracic wall (Figure 5.14).

Figure 5.14; *Incision at caudal ribs to open thoracic cavity*



- Incise left abdominal wall in the cranio-dorsal aspect and continue incision in caudo-ventral curve, allowing ventral reflection of the left abdominal wall.
- Examine abdominal and thoracic organs grossly (Figure 5.15).

Figure 5.15; *Inspection of thoracic and abdominal cavities*



- If no colostrum has been given to calf, sear ventral aspect of abomasum with blow torch (Figure 5.16). Collect 5ml abomasal fluid sample using plain vacutainer and 1 inch 18 gauge needle over seared abomasal surface (Figure 5.17). Assess appearance of abomasal fluid.

Figure 5.16; *Blow torch searing of abomasal surface*

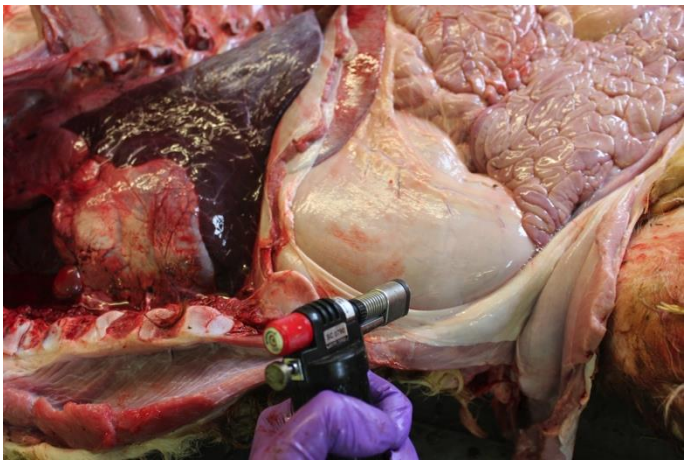
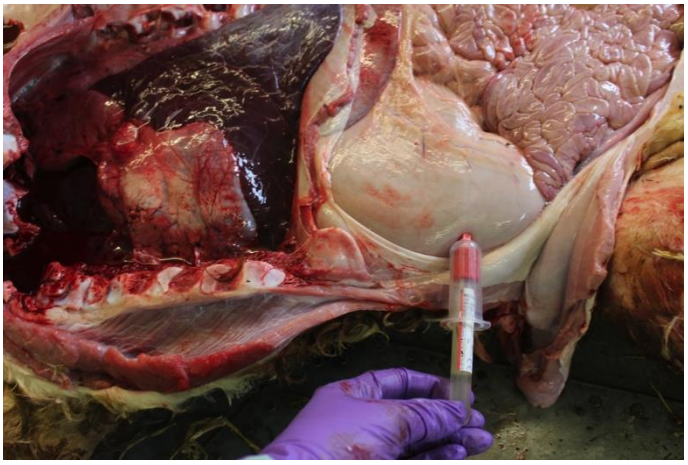
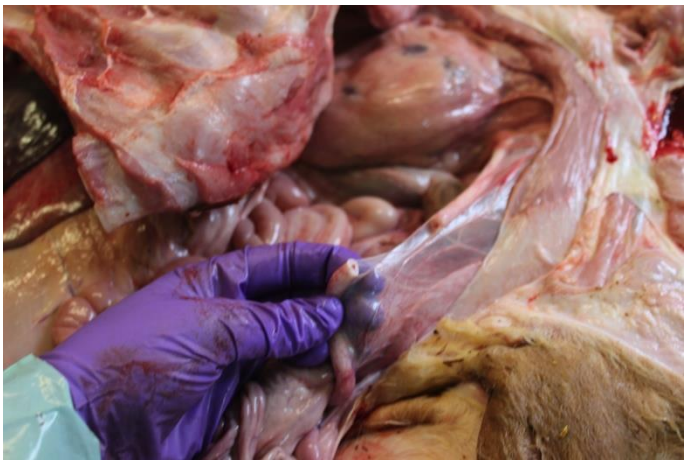


Figure 5.17; *Collection of abomasal fluid sample*



- Examine intestines and mesenteric lymph nodes
- Transect umbilical arteries approximately five centimetres from umbilicus to determine presence of blood clot. If present, measure diameter of blood clot (Figure 5.18).

Figure 5.18; *Assessment for presence of blood clot in umbilical artery*



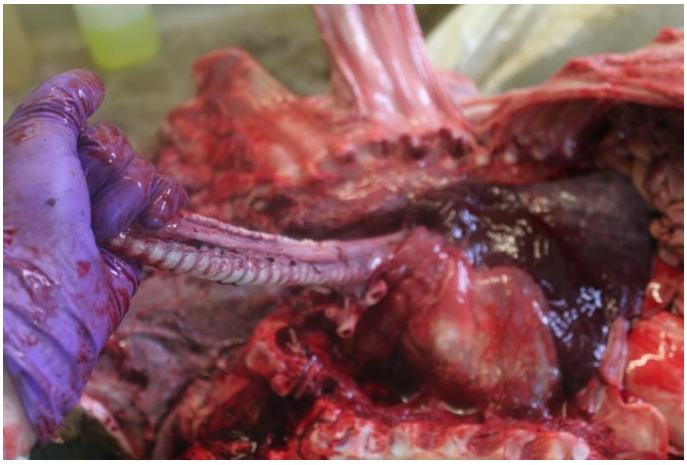
- Remove liver, weigh (0.1g accuracy) and examine grossly. Collect two 1cm^3 samples of liver. Place one in 10% formal saline and one into sterile sample pot.
- Grossly examine spleen, collect 1cm^3 sample and place in 10% formal saline.
- Incise the skin on midline on the ventral neck from larynx to thoracic inlet and reflect skin laterally (Figure 5.19).

Figure 5.19; *Incision and reflection of skin in ventral neck*

- Examine thymus grossly.

- Remove thyroid gland and weigh (0.1g accuracy). Fix one lobe of thyroid in 10% formal saline. If thyroid weight over 30 grams, retain second lobe for further testing of iodine levels.
- Transect trachea caudal to larynx and remove pluck. Maintain incised trachea in upright position to ensure luminal contents remain in place (Figure 5.20).

Figure 5.20; *Removal of pluck*

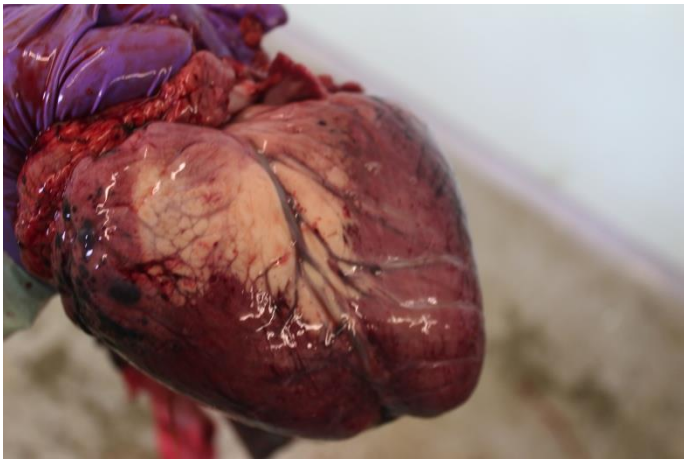


- Incise full length of trachea into bronchi and examine contents and mucosal surface.
- Assess lungs for inflation by gross appearance or submersion in water, where there is doubt (Figure 5.21). Collect two 1cm^3 samples from caudal lung field. Fix one sample in 10% formal saline.

Figure 5.21; *Assessment of pluck*

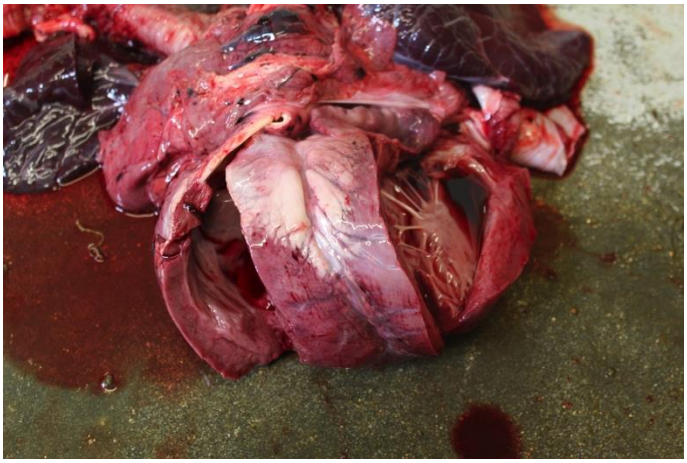
- Incise pericardium and assess epicardium (Figure 5.22).

Figure 5.22; *Assessment of epicardium*



- Incise both ventricles to assess valves, septum, endocardium and atria (Figure 5.23).

Figure 5.23; *Assessment of internal structure of heart*



- Incise skin on medial aspect of forelimbs and assess limbs.
- Incise skin on lateral aspect of hind limbs and assess limbs.
- Assess spine.
- Incise between occipital condyles and the atlas bone to disarticulate head from neck. Place head in vice (Figure 5.24).

Figure 5.24; *Head removed and placed in vice*

- Incise skin at right angles to midline, caudal to the lateral canthus and cranial to the supra-orbital foramen and bilaterally from the occipital condyles to the frontal bones. Reflect skin caudally.
- Using saw, incise the skull at right angles to midline, caudal to the lateral canthus and cranial to the supra-orbital foramen, approximately 1cm

deep. Two incisions (approximately 1cm deep) can then be made extending from the occipital condyles to the frontal bones, bilaterally. Using a blunt object, the loose flap on the skull can be elevated and reflected caudally, allowing visualisation of the brain (Figure 5.25 and 5.26).

Figure 5.25; *Incisions in skull to allow examination of brain*

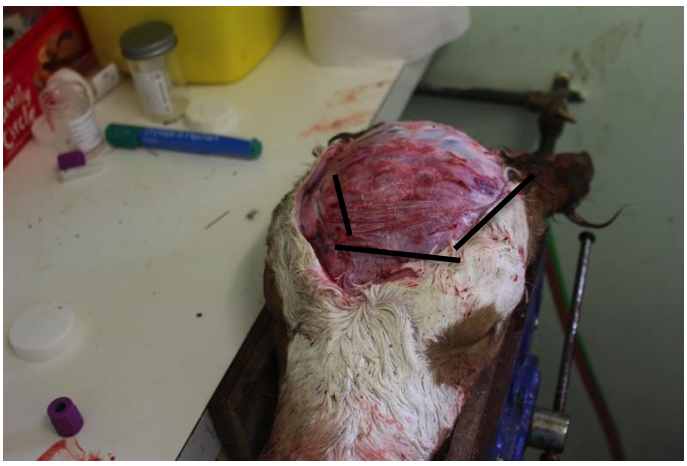


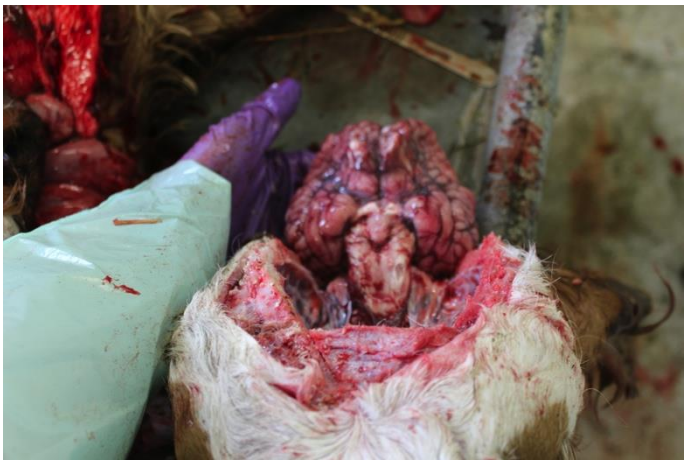
Figure 5.26; *Reflection of skull to allow examination of brain*

- Take swab of left cerebral hemisphere prior to contamination from handling.
- Visually assess degree of congestion in meninges (see Appendix 9) (Figure 5.27).

Figure 5.27; *Assessment of meningeal congestion*

- Holding the head vertically, the frontal cerebrum can be elevated to expose the optic nerves and infundibulum, both of which can be severed (Figure 5.28). The entire brain should be fixed in 10% formal saline.

Figure 5.28; *Removal of brain from skull*



- Examine placenta grossly if available. Collect two 1cm^3 samples of placenta and place one sample in 10% formal saline.
- Store samples at 4°C until next day delivery to testing laboratory can be ensured.
- Package and seal carcass and viscera for disposal.
- Clean and disinfect post-mortem facility using suitable disinfectant.

- Collate results from history, gross post mortem examination, culture and histopathology and report results to farmer.

Appendix 3. Post Mortem Examination Report

Diagnosis	
Animal ID	
Farm ID	
Laboratory ID	
Time and date of death	
Time and date of PM	
Weight	
Girth	
Crown-rump length	
Sex	
Meconium staining	
Foetal hooves	
Umbilicus	
Muscle tone	
Anus	
Cornea	
Conjunctiva	
Cyanosis	
Teeth	
Mouth	
Tongue	
Fore limbs	
Hind limbs	
External ribs	
Abomasal dilation	
Abomasal fluid	

Kidney	
Liver	
Umbilical clot	
Bladder	
Intestines	
Spleen	
Abdominal fluid	
Pleural fluid	
Pleura	
Lungs	
Heart	
Cervical thymus	
Thoracic thymus	
Trachea	
Thyroid	
Neck	
Brain	
Microscopy	
Fungal	
Thyroid iodine	
ZST	
Histopathology	
Comments	

Completed example of post mortem examination report;

Diagnosis	Stage two anoxia
Animal ID	Calf 1
Farm ID	
Laboratory ID	
Time and date of death	12pm, 4th March, 2016
Time and date of PM	1pm, 5th March, 2016
Weight	45kg
Girth	52cm
Crown-rump length	61cm
Sex	Male
Meconium staining	Present
Foetal hooves	Present
Umbilicus	Normal- no iodine staining
Muscle tone	Flaccid
Anus	Normal
Cornea	Normal
Conjunctiva	Congested
Cyanosis	Grade two
Teeth	Erupted
Mouth	Normal
Tongue	Swollen
Fore limbs	Haemorrhages from calving ropes. Oedema present
Hind limbs	Normal
External ribs	Normal
Abomasal dilation	Absent

Abomasal fluid	Normal
Kidney	Normal
Liver	Normal
Umbilical clot	Absent
Bladder	Empty
Intestines	Normal
Spleen	Haemorrhages present
Abdominal fluid	Absent
Pleural fluid	Absent
Pleura	Haemorrhages present
Lungs	Atelectic
Heart	Epicardial haemorrhages present
Cervical thymus	Haemorrhages present
Thoracic thymus	Normal
Trachea	Normal
Thyroid	14g
Neck	Oedema present
Brain	Grade two congestion
Microscopy	No abnormalities detected
Fungal	No abnormalities detected
Thyroid iodine	Not tested
ZST	Not tested
Histopathology	No abnormalities detected
Comments	Stage two anoxia due to foetal oversize

Appendix 4. Tissue Collection and Sampling Protocol

Table 5.1; *Tissue samples for collection and tests performed*

Tissue	Quantity	Factor Investigated	Test	Criteria for testing	Comments
Blood	5ml whole	Serum immunoglobulin level	Zinc sulphate turbidity test	Only performed on calves over 24 hours old	Collect into plain Vacutainer tube
		BVDV antigen and antibody	ELISA	All calves	
		Leptospira hardjo	Microscopic agglutination test	All calves	
Abomasal Fluid	5ml	Bacteria	Culture on blood agar and MacConkey agar	Collect only if no ingestion of material post-partum e.g. colostrum	Collect into plain Vacutainer tube
		Brucella spp., *Coxiella* spp.	Modified zeihl neelsen		
		Gram positive and gram negative bacteria, including spirochaetes and *Campylobacter* spp. and *Salmonella* spp.	Gram stain		
		Fungal organisms	Direct microscopy and culture on Sabouraud's medium		Addition of potash-ink mounting fluid may aid identification.

Table 5.1 continued; *Tissue samples for collection and tests performed*

Tissue	Quantity	Factor Investigated	Test	Criteria for testing	Comments
Spleen	1x1cm^3	Examination of tissue	Histopathological examination	All calves	Fix in 10% formal saline
Liver	1x1cm^3	Examination of tissue	Histopathological examination	All calves	Fix in sample in 10% formal saline
	1x1cm^3	General culture	Culture on aerobic, non-selective plates and examination of smear	Cultured only if no abomasal fluid	
	1x1cm^3	Bovine *Herpes* Virus	RT-PCR	All calves	
Myocardium	1x1cm^3	Examination of tissue	Histopathological examination	All calves	Fix in 10% formal saline
Lung	1x1cm^3	General culture	Culture on aerobic, non-selective plates and examination of smear	All calves Cultured only if no abomasal fluid	Collect from caudal lung field
	1x1cm^3	Examination of tissue	Histopathological examination	All calves	Collect from caudal lung field. Fix in sample in 10% formal saline
Thyroid	1 lobe	Examination of tissue	Histopathological examination	All calves	Fix one lobe in 10% formal saline
	1 lobe	Iodine level		Performed if thyroid >30g	

Table 5.1 continued; *Tissue samples for collection and tests performed*

Tissue	Quantity	Factor Investigated	Test	Criteria for testing	Comments
Brain	Entire	Examination of tissue	Histopathological examination	All calves	Fix entire in 10% formal saline
Placental cotyledon	1x1cm^3	Examination of tissue	Histopathological examination	When available	Fix one sample in 10% formal saline
	1x1cm^3	General culture	Culture on aerobic, non-selective plates and examination of smear	When available	
		Brucella spp., *Coxiella* spp.	Modified zeihl neelsen		
		Gram positive and gram negative bacteria, including spirochaetes and *Campylobacter* spp.	Gram stain		
		Fungal organisms	Direct microscopy and culture on Sabouraud's medium		

Appendix 5. Calving Data Record

Date	Ear tag	Breed of sire	Breed of dam	Calf alive at birth	Calf alive at 48 hours	Male/ female	Time	Twins	BCS	Calving score	Comments
Calving score 0 unobserved **1** observed but not assisted **2** easy pull **3** hard pull **4** vet assistance **5** caesarean section											
BCS= body condition score of cow (1-5)											

Appendix 6. Herd Level Questionnaire

	2015	2016
Number of cows calving in spring (1st February- 31st May)?		
Number of heifers calving in spring?		
Number of stillborn calves? (full term calves dead at birth or died with 48 hours)?		

	No	Product	When
Do cows receive any supplementary vitamins or minerals pre-calving?			
Do heifers receive any supplementary vitamins or minerals pre-calving?			

	BVDV	Leptospirosis	Rota/Corona Virus	IBR	Other
Are cows vaccinated?					
Date given?					
Are heifers vaccinated?					
Date given?					

	Bull	AI	Embryo Transfer	
Breeding method used on farm (number of cows)				

	TMR	Pit silage	Hay	Barley	Straw
What are cows fed in the last month of gestation?					
What are heifers fed in the last month of gestation?					

1. **If TMR fed, what are the components? Silage, barley etc.**

.....

2. **Where do cows calve?**

   Individual pen ☐ Group Pen ☐

3. **Where do heifers calve?**

   Individual pen ☐ Group Pen ☐

4. **When are cows moved to calving pen:**
   2 days before ☐ 1 day before ☐ 12-24 hours before ☐ 6-12 hours before ☐ point of calving ☐ not moved ☐

5. **When are heifers moved to calving pen:**
   2 days before ☐ 1 day before ☐ 12-24 hours before ☐ 6-12 hours before ☐ point of calving ☐ not moved ☐

6. **How often are cows observed during calving**
   Hourly ☐ 2-4 hours ☐ 4-6 hours ☐ 6-12 hours ☐ over 12hours ☐

7. **How often are heifers observed during calving:**
   Hourly ☐ 2-4 hours ☐ 4-6 hours ☐ 6-12 hours ☐ over 12hours ☐

Appendix 7. End of Study Questionnaire

Please answer the following questions based on your own farm. The questions all relate to calves that are stillborn or die within 48 hours of birth.

- How significant do you consider neonatal losses to be on your farm?
    - Minor loss of production each year
    - Significant loss of production each year
    - Worrying loss of production each year

- How many breeding females (cows and heifers) do you have?

- On your farm how many calves per year would you normally expect to be born dead or die within 48 hours?

- What is most likely reason to motivate you to submit calves for post mortem?
    - More dead calves than I would expect normally
    - More dead calves than benchmark Figures
    - Cluster of dead calves within calving season
    - Dead calves at the start of calving period
- How much would you be prepared to pay for a post -mortem and lab fees combined i.e. total cost per calf?
    - up to £50
    - up to £80
    - up to £100
    - up to £120

- The deterrents to me to submitting calves for post mortem are;

	Strongly Disagree	**Disagree**	**Neural**	**Agree**	**Strongly Agree**
Distance to post mortem facility					
Collecting carcass afterwards					
Lack of diagnosis					
Guilt that death was my fault					
I don't understand the results					
Lack of follow up					

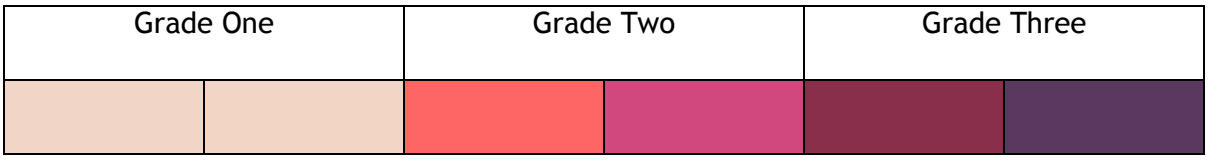
- What would you estimate to be the total cash cost to you of losing a calf at this stage assuming it has had no extra costs such as drugs/ vet /milk replacement?

- What would you estimate the total losses associated with a calf dying at this stage? Consider loss of future revenue, culling cow, replacement cost where applicable etc.
- Please add any further comments or suggestions

Appendix 8. Colour Chart of Grades of Cyanosis in the Gingiva

The following colour colour chart was developed by the author after initial training in gross post mortem technique to aid in assessment of the gingiva. The gingiva should be assessed distal to the incisors, overlying the mandibular symphysis, and graded according to the following colour chart.

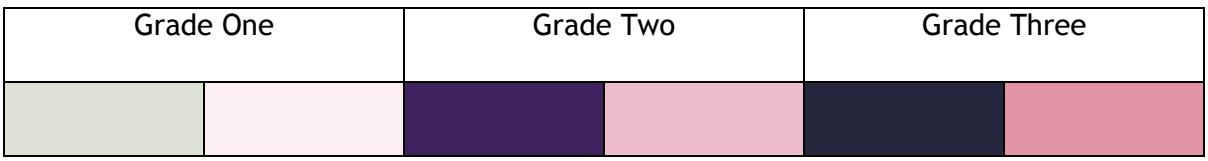
Figure 5.29; *Colour chart showing grades of cyanosis in the gingiva*



Appendix 9. Colour Chart of Grades of Brain Congestion

The following colour chart was developed by the author after initial training in gross post mortem technique to aid in assessment of the level of meningeal congestion. The degree of congestion in the meningeal blood vessels should be assessed on the cerebral surface, and graded according to the following colour chart.

Figure 5.30; *Colour chart showing grades of congestion in the brain*



Appendix 10. Calf Measurements from Gross Post Mortem Examination

Table 5.2; *Calf measurements from gross post mortem examination*

	Measurement			
Calf	**Sex**	**Weight (kg)**	**Crown Rump Length (cm)**	**Girth (cm)**
T1	M	43.5	73.0	74.0
T2	M	55.7	100.0	82.0
T3	M	35.6	85.0	71.0
T4	M	52.1	87.0	80.5
T5	M	56.7	NR	NR
T6	M	31.2	81.0	66.0
T7	M	42.6	81.0	73.0
T8	M	43.7	86.0	78.0
T9	M	28.5	83.0	68.5
T10	F	21.8	69.0	55.0
T11	M	33.0	NR	NR
T12	M	35.6	81.5	71.5
T13	F	46.5	90.0	77.0
T14	M	50.9	94.0	78.0
T15	F	36.0	85.0	75.0
T16	M	39.0	83.0	73.0
T17	F	51.5	85.0	73.0
T18	M	38.4	81.0	73.0
T19	M	41.3	85.0	70.0
T20	M	35.9	82.0	NR
T21	M	62.5	99.0	91.0

Table 5.2 continued; *Calf measurements from gross post mortem examination*

Measurement				
Calf	**Sex**	**Weight (kg)**	**Crown Rump Length (cm)**	**Girth (cm)**
T22	F	54.3	94.0	87.5
T23	M	43.3	85.0	75.0
T24	M	63.5	84.0	88.0
T25	M	65.0	98.0	88.0
T26	M	36.1	76.0	69.5
T27	F	27.6	77.0	63.0
T28	F	27.0	70.0	63.5
P1	M	43.8	86.0	59.0
P2	F	38.7	94.0	74.0
P3	F	52.0	91.0	84.0
T4	M	49.2	90.0	78.0
P5	M	45.9	88.0	80.0
P6	F	41.3	88.0	74.0
T29	F	31.9	82.0	69.0
T30	M	33.6	NR	NR
T31	M	63.0	99.0	84.0
T32	M	31.6	74.0	66.5
T33	M	52.4	75.0	80.0
P7	M	27.4	80.0	62.0
P8	F	49.6	93.0	94.0
P9	M	41.5	88.0	75.0
P10	M	57.9	88.0	88.0
P11	F	21.2	76.0	69.0
P12	F	37.2	79.5	77.5

Table 5.2 continued; *Calf measurements from gross post mortem examination*

Measurement				
Calf	**Sex**	**Weight (kg)**	**Crown Rump Length (cm)**	**Girth (cm)**
P13	M	45.5	96.0	73.0
P14	F	31.4	72.0	67.0
P15	M	57.0	89.0	84.0
P16	M	54.6	76.0	85.0
P17	F	30.2	72.0	69.0
P18	F	43.5	88.0	77.5
P19	F	29.0	75.0	65.0
P20	F	24.2	78.0	63.0

Appendix 11. Gross Pathology Present in Calves on Examination

Table 5.3; *Gross pathology present in calves on examination*

Calf	Cornea	Conjunctiva CG	Conjunctiva	Tongue	Spleen	Lungs	Pleura	Heart	Trachea	Thyroid WT (g)	Neck	Brain CG
T1	Opaque	3		Swollen		ATX and OE		EP HX	Blood	20.0		2
T2		2		Swollen		ATX, OE and CNG		EP HX		17.0	OE and CG	2
T3		1				Very thickened, focal area in left lung		EP HX	Bloody froth	14.0		3
T4	Lens opaque	2	HX	Swollen		ATX and OE		EP HX		14.0		3
T5	Lens opaque, right eye ruptured	2			HX	PI		EP HX		24.0		2
T6	Lens opaque, HX in anterior chamber	2	HX			PI		Flaccid, pale		27.5	OE	2
T7	Cloudy	1	Pale	Swollen		ATX		Flaccid		18.1		1
T8		1	Pale			PI		EP HX	Frothy mucous	14.0		1
T9	Lens opaque	1	Pale			PI				20.0		2
T10	Globe sunk in orbit,HX in cornea, cornea cloudy	1	Pale	Swollen		ATX, OE, EMP				5.0		1
T11		1			HX	I		Flaccid	Colostrum	12.0		1
T12		1	Pale			ATX		EP HX		11.7		2
T13	Cloudy	3		Swollen		ATX	HX	EP HX		26.4	CG	3
T14	Lens opaque	3		Swollen		ATX, OE and CNG		EP HX		16.0	OE and CG	2
T15		2	HX		HX	EMP, bulla both lungs	EMP	EP HX	HX	18.0	HX	3

Table 5.3 continued; *Gross pathology present in calves on examination*

Calf	Cornea	Conjunctiva CG	Conjunctiva	Tongue	Spleen	Lungs	Pleura	Heart	Trachea	Thyroid WT (g)	Neck	Brain CG
T16	Lens opaque	1				PI		EP HX		20.0		3
T17		2				ATX		EP HX	Yellow fluid	17.0		3
T18	Cloudy	1	Pale			ATX		Abnormal BV RV		14.0		3
T19		2				ATX		VSD, RV dilated				2
T20	Cloudy	3		Swollen		Bruising with rib marks, partially inflated	Fibrin	Pericarditis		16.6	HX	1
T21		2			HX	PI	HX	EP HX	Bloody froth	18.0		2
T22	Lens opaque	2			HX	PI	HX	EP HX	PTX	20.0	OE	2
T23	Lens opaque	2	HX			PI				14.0		3
T24	Lens opaque	1	Pale	Swollen		PI	HX	EP HX	Frothy mucous	22.0	OE	2
T25		3		Swollen		ATX and OE	HX	EP HX	Bloody froth	17.0	OE and CG	3
T26	Cloudy	1	Pale			ATX		V dilated, epicardial striations		4.0	OE	1
T27	Cloudy	1	Pale			ATX				7.0		1
T28	Lens opaque	2				PI, EMP		EP HX	Colostrum	18.0		1
P1		1	Pale	Swollen		PI			Bloody mucous	11.0	OE	1
P2		2				PI			Frothy mucous	15.0		2
P3	Lens opaque	2				ATX and CNG		EP HX	Meconium inhalation	32.0		3
T4	Cloudy, dehydrated	1	Pale			ATX and OE				25.0		1
P5	Lens opaque	1				PI	HX	EP HX		15.0		3
P6	Lens opaque	2		Swollen		ATX and OE	HX	EP HX		24.0	OE	3
T29	Lens opaque	1	Pale			PI	HX	EP HX	Meconium inhalation	14.0	OE	2
T30	Lens opaque	1	Pale		Swollen	Vestigial, ATX	No DFM			7.0		2

Table 5.3 continued; *Gross pathology present in calves on examination*

Calf	Cornea	Conjunctiva CG	Conjunctiva	Tongue	Spleen	Lungs	Pleura	Heart	Trachea	Thyroid WT (g)	Neck	Brain CG
T31		2				PI		EP HX		36.0	OE and CG	3
T32	Cloudy	1	Pale	Swollen		PI		Flaccid		10.0		1
T33		1	Pale			PI			Frothy mucous	14.0		2
P7	Cloudy	1	Pale			ATX				7.0	OE	1
P8		2				ATX	HX	EP HX		18.0	OE	2
P9	Lens opaque	3				ATX	HX	EP HX		15.9		3
P10		2			HX	CS	EMP	EP HX	Bloody froth	19.0		3
P11	Cloudy	1	Pale	Swollen		ATX				13.1		1
P12	Lens opaque	2			HX	EMP, bulla left lung		EP HX		20.6		3
P13	Cloudy	1	Pale			ATX		Pale		15.0		1
P14	Lens opaque	1				PI		Flaccid	White mucous	9.6		3
P15	Cloudy	3		Swollen		PI	HX	EP HX	Frothy mucous	34.0	CG	3
P16		1	Pale			PI		EP HX	Frothy mucous	16.0		1
P17	Lens opaque	2				PI		Abnormal BV RV		19.0		1
P18		3			HX	PI			Bloody froth	17.0		2
P19	Lens opaque	1	Pale		Pale	PI		Flaccid		11.1		1
P20	Lens opaque	2				PI		CG		14.7	LN LRG	3

Where no entry is made, no gross abnormal pathology was present. ATX- atelectic, BV- blood vessel, CG- congestion, CS- consolidated, DFM- diaphragm, EMP- emphysema, I- inflated, HX- haemorrhage, LRG- large, LN- lymph node, NSL- no significant lesion, OE- oedema, PI- partially inflated, PTX- petechiae, RV- right ventricle, V- ventricles, WT- weight

Appendix 12. Results of Laboratory Testing on Submissions

Table 5.4; *Results of laboratory testing on submissions*

	Laboratory Test					
Calf	**Microscopy**	**Fungal**	**Leptospira**	**BVDV**	**IBR**	**ZST**
T1	Negative	*Bacillus licheniformis, Lichtheimia corymbifera*	Negative	Negative	Negative	N/A
T2	Negative	*Aspergillus niger*	Negative	Negative	Negative	N/A
T3	*Aeromonas hydrophila*	Negative	Negative	Negative	Negative	N/A
T4	Negative	Negative	Negative	Negative	Negative	N/A
T5	*E.coli*	Negative	Negative	Negative	Negative	N/A
T6	Mixed growth	Negative	Negative	Negative	Negative	N/A
T7	Negative	*Bacillus licheniformis*	Negative	Negative	Negative	N/A
T8	Negative	Negative	Negative	Negative	Negative	N/A
T9	*E.coli*	Negative	Negative	Negative	Negative	N/A
T10	Negative	Negative	Negative	Negative	Negative	N/A
T11	Negative	Negative	Negative	Negative	Negative	N/A
T12	*E.coli*	Negative	Negative	Negative	Negative	N/A
T13	Negative	Negative	Negative	Negative	Negative	N/A
T14	Negative	Negative	Negative	Negative	Negative	2
T15	Negative	Negative	Negative	Negative	Negative	N/A
T16	*E.coli*	Negative	N/A	Positive	Negative	6
T17	Negative	*Aspergillus fumigatus*	Negative	Negative	Negative	N/A
T18	Negative	Negative	Negative	Negative	Negative	N/A
T19	Negative	Negative	Negative	Negative	Negative	N/A
T20	*E.coli*	Negative	Negative	Negative	Negative	N/A
T21	*Klebsiella oxytoca*	Negative	Positive	Positive	Negative	6

Table 5.4 continued; *Results of laboratory testing on submissions*

	Laboratory Test					
Calf	**Microscopy**	**Fungal**	**Leptospira**	**BVDV**	**IBR**	**ZST**
T22	*Staphlococcus and E.coli*	Negative	Negative	Inconclusive	Negative	0
T23	Negative	Negative	Negative	Negative	Negative	N/A
T24	Negative	*Bacillus licheniformis*	Negative	Negative	Negative	N/A
T25	*E.coli*	Negative	Negative	Negative	Negative	N/A
T26	*E.coli*	Negative	Negative	Negative	Negative	6
T27	*E.coli*	Negative	Negative	Negative	Negative	N/A
T28	Negative	Negative	Negative	N/A	Negative	N/A
P1	*E.coli*	*Lichtheimia, Aspergillus, Candida*	Negative	Negative	Negative	N/A
P2	Negative	Negative	Negative	Negative	Negative	N/A
P3	Negative	Negative	Negative	Negative	Negative	N/A
T4	Negative	Negative	Negative	Negative	Negative	N/A
P5	Negative	*Bacillus licheniformis*	Negative	N/A	Negative	22
P6	Negative	Negative	Negative	Negative	Negative	N/A
T29	Negative	Negative	Negative	Negative	Negative	N/A
T30	Negative	Negative	Negative	Negative	Negative	N/A
T31	Negative	*Candida*	Negative	N/A	Negative	N/A
T32	*Serratia liquifaciens*	Negative	Negative	Negative	Negative	N/A
T33	Negative	Negative	Negative	Negative	Negative	N/A
P7	Negative	Negative	Positive	Negative	N/A	N/A
P8	Negative	Negative	Negative	Negative	Negative	N/A
P9	*E.coli*	Negative	Negative	Negative	Negative	3
P10	Negative	Negative	Negative	Negative	Negative	N/A
P11	*E.coli*	*Lichtheimia corymbifera*	N/A	N/A	N/A	10
P12	Negative	Negative	Negative	Negative	Negative	N/A
P13	Mixed growth	Negative	Negative	N/A	Negative	N/A

Table 5.4 continued; *Results of laboratory testing on submissions*

	Laboratory Test					
Calf	**Microscopy**	**Fungal**	**Leptospira**	**BVDV**	**IBR**	**ZST**
P14	*E.coli*	Negative	Negative	Negative	Negative	2
P15	*E.coli*	Negative	Negative	Inconclusive	N/A	9
P16	*E.coli*	Negative	Negative	Negative	Negative	3
P17	*E.coli*	Negative	Negative	Negative	Negative	1
P18	Mixed growth	Negative	Negative	Negative	Negative	N/A
P19	Negative	*Bacillus licheniformis*	N/A	N/A	Negative	N/A
P20	Negative	Negative	Negative	Negative	Negative	N/A

N/A- not applicable

Appendix 13. Histopathology Results from Submissions

Table 5.5; *Histopathology results from submissions*

Calf	Lung	Liver	Spleen	Heart	Thyroid
T1	N/A	N/A	N/A	N/A	N/A
T2	N/A	N/A	N/A	N/A	N/A
T3	N/A	N/A	N/A	N/A	N/A
T4	N/A	N/A	N/A	N/A	N/A
T5	N/A	N/A	N/A	N/A	N/A
T6	Bronchiolitis, squames, macrophages, partially expanded alveoli.	Portal hepatitis	Prominent lymphoid population in PALS, plasma cells in red pulp	Epicarditis, neutrophils, plasma cells, swollen mesothelial cells	abundant colloid
T7	PM change	PM change	PM change	PM change	no colloid, clusters of smaller follicles
T8	N/A	N/A	N/A	N/A	N/A
T9	Slightly expanded alveoli, squames	Leucocyte population, congestion	Lymphoid population in PALS, follicle formation	NSL	abundant colloid
T10	Partially expanded alveoli, squames,	NSL	Narrow PALS	NSL	abundant colloid
T11	Pleomorphic cells in lumina	Basophillic aggregates in portal area	Lymphoid population in PALS, cellular red pulp	NSL	abundant colloid
T12	RBC extravasation squames meconium, partially expanded alveoli	NSL	NSL	Perinuclear spaces in myocytes	abundant colloid
T13	Partially expanded alveoli	NSL	Extra medullary haematopoiesis, macrophages,	NSL	vascularised papillary in growths
T14	N/A	N/A	N/A	N/A	N/A
T15	Partially expanded alveoli, meconium, squames	NSL	N/A	Variation in stainable myofibrillar component of myocytes	N/A
T16	RBC extravasation, fibrinoid exudation, meconium, prominent leucocytoclastic membrane	Congestion	Narrow PALS	NSL	abundant colloid
T17	PM change	PM change	PM change	PM change	PM change
T18	Expanded alveoli, squames	Rarefaction of hepatocellular cytoplasm	Contracted red pulp, wide PALS, early germinal formation	NSL	abundant colloid

Table 5.5 continued; *Histopathology results from submissions*

Calf	Lung	Liver	Spleen	Heart	Thyroid
T19	Round cells in lumina	PM change	Narrow PALS	Variation in stainable myofibrillar component of myocytes	abundant colloid
T20	N/A	N/A	N/A	N/A	N/A
T21	N/A	N/A	N/A	N/A	N/A
T22	Leucocytoclastic pneumonia pulmonary necrosis, haemorrhage, intralesional bacteria	Lymphohistiocytic portal hepatitis	Plasma cells in red pulp, narrow PALS	Purulent epicarditis	abundant colloid
T23	Partially expanded alveoli, squames	Sinusoidal erythrophagocytosis	Erythrophagocytosis, contracted red pulp, narrow PALS	N/A	abundant colloid
T24	N/A	N/A	N/A	N/A	N/A
T25	Variable expansion of alveoli, multifocal red blood cell extravasation	Vacuolation of hepatocytes	Narrow PALS	Variable perinuclear spaces in cardiomyocytes	abundant colloid
T26	Squames, meconium, bacteria, haemorrhage	Vacuolation of hepatocytes	PALS without germinal centre formation	N/A	abundant colloid
T27	Slightly expanded alveoli	Lymphoid population,	Wide PALS, early germinal centre formation	NSL	abundant colloid
T28	N/A	N/A	N/A	N/A	N/A
P1	PM change	PM change	PM change	PM change	PM change
P2	Slightly expanded alveoli, squames	Prominent connective tissue	NSL	Red blood cell extravasation	abundant colloid
P3	Slightly expanded alveoli, squames	Sparse lymphocytes	Narrow PALS without germinal centre formation	NSL	abundant colloid
T4	Alveoli expanded, squames, interlobular oedema, lymphohistiocytic infiltrate	Vacuolation of hepatocytes, lymphocytes	Moderate lymphoid population in PALS	Variable perinuclear spaces in cardiomyocytes	abundant colloid
P5	Bronchointerstitial pneumonia, hyaline and leucocytoclastic membranes, meconium, leucocytes, emphysema	Vacuolation of hepatocytes	Narrow PALS, congested red pulp	Sparse neutrophils, macrophages	vascularised papillary in growths
P6	Alveoli partially inflated, distension of lymphatic vessels	Rarefaction of hepatocellular cytoplasm	Wide PALS, early germinal centre formation	N/A	abundant colloid
Calf	**Lung**	**Liver**	**Spleen**	**Heart**	**Thyroid**
T29	Partial expansion of alveoli, squames, meconium	Lymphoid population,	Sparse nuclear debris	Variation in stainable myofibrillar component of myocytes	abundant colloid
T30	N/A	N/A	N/A	N/A	N/A
T31	Unexpanded	NSL	Moderate lymphoid population in PALS	NSL	abundant colloid
T32	Slightly expanded alveoli, squames	PM change	PM change	Variation in stainable myofibrillar component of myocytes	abundant colloid

Table 5.5 continued; *Histopathology results from submissions*

Calf	Lung	Liver	Spleen	Heart	Thyroid
T33	N/A	N/A	N/A	N/A	N/A
P7	N/A	N/A	N/A	N/A	N/A
P8	N/A	Congestion	Prominent lymphoid tissue, contracted red pulp	RBC extravasation	abundant colloid
P9	RBC extravasation, fibrinoid exudation, leucocytes in airway, cytoclastic membrane formation, purulent bronchopneumonia	Leucocyte population	Dense colonies of bacteria in red pulp	Small dense bacterial colonies in interstitium	abundant colloid
P10	Expanded alveoli	Vacuolation of hepatocytes	N/A	Perinuclear spaces in cardiomyocytes	abundant colloid
P11	N/A	N/A	N/A	N/A	N/A
P12	Squames meconium alveoli unexpanded	Portal and perivenous connective tissue	NSL	NSl	N/A
P13	PM change	PM change	PM change	NSL	PM change
P14	N/A	Mononuclear population	Neutrophils around PALS	Perinuclear halo in cardiomyocytes	abundant colloid
P15	N/A	N/A	N/A	N/A	N/A
P16	N/A	N/A	N/A	N/A	N/A
P17	N/A	N/A	N/A	N/A	N/A
P18	Meconium, hyaline shock bodies in vascular lumina, rbc extravasation in air spaces	Connective tissue in portal area	PM change	NSL	abundant colloid
P19	N/A	N/A	N/A	N/A	N/A
P20	Purulent bronchopneumonia with fibrinous exudation, multinucleated macrophages	Lymphohistiocytic portal hepatitis, extramedullary haematopoiesis	N/A	Perinuclear paucity of myofibrils	N/A

N/A – not applicable, NSL- no significant lesions, PALS- peri-arteriolar lymphoid space, PM- post mortem

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