INCREASING LAMB SURVIVAL IN PRINCE EDWARD ISLAND SHEEP FLOCKS

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ABSTRACT

The overall objective of this program of research was to describe patterns in lamb mortality in sheep flocks in Prince Edward Island (PEI), Canada, and to identify the most influential flock-level factors associated with lamb mortality risk. Data were collected retrospectively from 385 lamb post-mortem submissions (2005 to 2014), and prospectively over one year for 51 distinct lambing groups from 37 sheep flocks. The retrospective data revealed the importance of infectious causes of lamb mortality in PEI sheep flocks. The prospective data revealed that the mean group-level lamb mortality was 11.14% (± 6.64%), with 25 groups having lamb mortality greater than 10%, which is considered higher than acceptable. The following management factors most strongly contributed to decreased lamb mortality: using flock management goal-setting; seeking veterinary advice for medical treatment; using benzimidazole-derivative anthelminthics; feeding higher quality forage (higher crude protein, digestible energy, net energy for maintenance, and lower acid detergent fiber), applying visual lamb identification, feeding a coccidiostat to lambs, administering clostridial vaccination to lambs, and avoiding separation of hypothermic lambs from their dams. A history of neurological problems in ewes contributed to increased mortality in lambs. Gastrointestinal parasite infections, and selenium and vitamin E deficiencies, were widespread in the study flocks. These research findings suggest that, to improve lamb survival in PEI flocks, producers should work with their veterinarians to set flock performance goals, enhance the nutritional management of late-gestation ewes, and improve the preventative health management of ewes and lambs, especially related to gastrointestinal parasitism and infectious diseases.

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คุณงามความดีอันเกิดจากวิทยานิพนธ์นี้ขออุทิศแด่บิดา มารดา และบูรพาจารย์ทุกท่านของข้าพเจ้า

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AVC	Atlantic Veterinary College
BCS	Body Condition Score
BHBA	β-hydroxybutyrate
CI	Confidence Interval
epg	Eggs per gram
Ig	Immunoglobulin
NEFA	Non-esterified Fatty Acids
NMD	Nutritional Muscular Dystrophy
NRC	National Research Council
PCA	Principal Component Analysis
PEI	Prince Edward Island
ROC	Receiver Operating Characteristic
US	United States
USDA	United States Department of Agriculture

CHAPTER 1

GENERAL INTRODUCTION: LAMB MORTALITY

1.1 Lamb mortality

Various indicators are used to evaluate production efficiency in the sheep industry, including fertility (number of ewes delivering divided by ewes bred), prolificacy or lambing percentage (number of lambs born divided by ewes delivering), and productivity (number of lambs weaned or weight of lambs weaned divided by ewes bred).

The lambing system chosen by a producer is a significant factor affecting production efficiency (Boujenane et al., 1991). For example, fertility and prolificacy were 90% and 165%, respectively, in a one lambing per ewe per year system, while the rates were 70% and 155%, respectively, in an accelerated lambing system in which at least a proportion of ewes lambed more than once per year. These differences between systems were statistically significant. Productivity of ewes in a one lambing per ewe per year system and an accelerated lambing system were 151% and 161%, respectively (Benoit et al., 2009). A 4-year study in the Romeldale breed reported prolificacy between 128% and 182% (Vesely and Peters, 1965). The mean prolificacy reported by Dohoo et al. (1985) in Canada and Thomson et al. (2004) in New Zealand were also within this range (153% and 165%, respectively).

Lamb survival risk (number of lambs surviving divided by number of ewes delivering) and lamb mortality risk (number of dead lambs divided by the number of lambs born) are also common indices used to describe flock performance, as they measure phenomena that directly affect production efficiency. Survival risk by marking (docking and castration) in Australian flocks ranged from between 129% and 151% (Hamilton Prime Lamb Breeders, 2005) to between 60% and 105% (Elliott et al. 2011), depending on the study. Lamb mortality risks have been reported from various research groups in many areas around the world. However, these reports are derived from study populations that differ widely with respect to breed, period of follow up, and management system (Table 1.1).

A decrease in lamb mortality as lambs become older was noted in some studies (Southey et al., 2001; Mandal et al., 2007). Sawalha et al. (2007) also noticed that lamb mortality risk remained constant after 120 days of age. Most lamb losses have been found to occur during the first 3 to 21 days after birth (Green and Morgan, 1993; Nash et al., 1996; Khan et al., 2006; Mandal et al., 2007; Brien et al., 2010; Chniter et al., 2011). According to Dalton et al. (1980), 24%-40% of lamb deaths occurred at birth, whereas lambs dying within the first day accounted for 50% of lamb deaths. The proportion of lamb losses by 3 days of age was up to 21%-72% of lamb losses (Mandal et al., 2007; Brien et al., 2009), while between 22% and 91% of lamb deaths occurred within the first week of life (Dohoo et al., 1985; Green and Morgan, 1994; Nash et al., 1996; Hamilton Prime Lamb Breeders, 2005; Brien et al., 2009). The proportion of lamb mortality within the first 15 days was 64% (Mandal et al., 2007), and 44% of total lamb deaths occurred by 3 weeks of age (Huffman et al., 1985). Lambs dying during 1-3, during 4-7, and more than 7 days of age accounted for 30%, 11%, and 4% of lamb mortalities, respectively (Binns et al., 2002), and around 8% of pre-weaning lamb mortality occurred from day 8 to day 15 (Mandal et al., 2007). Yapi et al. (1990) found 72% of total lamb losses occurring by weaning.

High lamb mortality is an economic issue but also an animal welfare issue (Sawalha et al., 2007). Due to a positive relationship between ewe prolificacy and lamb mortality, a balance between the prolificacy and mortality is needed to maximize flock productivity while ensuring welfare issues are addressed (Demirören et al., 1995). Published targets for acceptable lamb mortality risk differ depending on the author, region, lambing system, breeds, and the post-natal period being considered. For instance, in one European study the authors suggest mortality within the first 28 days of age should not exceed 5% (Fragkou et al., 2010), while in Australia, 15% has been reported as a target of perinatal mortality (Hamilton Prime Lamb Breeders, 2005). According to a factsheet published in Canada, the acceptable limit of pre-weaning mortality should be 10% (Kennedy, 2012)

1.2 Risk factors of lamb mortality

Many factors have been reported associated with lamb mortality, and recognition of their associated risk is a fundamental step in diminishing lamb mortality.

1.2.1 Nutritional factors

Feeding management is one of the most influential factors determining animal well-being, including lamb mortality (Atashi et al., 2013). Nutritional management in late-gestation ewes has an obvious association with lamb survival. The lower lamb mortality observed in optimally fed ewes is in large part a result of birth weight and post-natal vitality (Russel et al., 1981; Bloomfield et al., 2003; Redmer et al., 2004).

To ensure optimal nutrition (neither over- nor under-nutrition) the number of fetuses carried by the ewe should be taken into account in grouping of pregnant ewes and balancing rations for these groups in the last trimester of pregnancy (Chniter et al., 2013). Determination of the number of fetuses by ultrasonography at 45 to 90 days of gestation has been recommended (Edmondson et al., 2012).

Optimal nutrition can be dependent on both the ration formulation and how it is fed. Depending on the quality of the forage fed, supplements may need to be provided to ewes, increasing over the last weeks prior to lambing, in order to improve colostrum production (Elliott et al., 2011). Lamb wastage from ewes fed more than once a day tends to be lower than that from ewes fed once a day. These findings might be attributed to the fact that feeding more than once a day might increase palatability of feed and the appetites of ewes. It might also reduce competition among ewes (Holmøy et al., 2012), or lead to closer observation of the ewes. Lamb mortality was greater in flocks in which trail feeding (delivering a trail of feed in a paddock from a vehicle) was used during the peri-partum period, compared to other flocks, likely as a result of mismothering; thus, trail feeding should be avoided during the early post-partum period (Thompson, 2011).

Restricted feeding in late-gestation ewes suppresses heat production-associated genes of their lambs; thus their lambs are more vulnerable to hypothermia than lambs born to non-restricted fed ewes (Ojha et al., 2013). Nutrition supply in late-gestation ewes influences the development of the brown adipose tissue (Ojha et al., 2013), a major factor in prevention of hypothermia in neonatal lambs. Inadequate nutrition has detrimental impacts on ewe mammary gland development, causing insufficient colostrum and milk production (Mellor and Murray, 1985). Consequently, lambs are less vigorous and unable to regulate their body temperature (Dwyer et al., 2016).

The body condition score of ewes in late gestation is related to lamb survival and should be maintained between 2.5 and 3.5, using a scale of 1 (emaciated) to 5 (overfat)

(Elliott et al., 2011). Chaarani et al. (1991) reported that pre-breeding ewes with proper body condition scores delivered larger lambs, and their survivability was greater than lambs of poor condition ewes, while poor condition ewes receiving proper supplements before breeding had lower postnatal mortality than non-supplemented ewes (Binns et al., 2002). Setting lambing periods based on availability of feed is another useful approach to improve lamb survival (Elliott et al., 2011).

Selenium and vitamin E are important nutrients that may influence lamb survival through their diverse roles in metabolism and antioxidant protection, including prevention of loss from nutritional muscular dystrophy. Transfer across the placenta provides the greater part of neonatal lamb selenium (Koller et al., 1984). Pre-partum selenium supplementation increases the blood selenium concentration of ewes and lambs, and colostrum and milk selenium concentration is also increased (Paulson et al., 1968; Cuesta et al., 1995). On the other hand, only a small amount of vitamin E is transmitted through the placenta, which supports a central role for colostrum quality and consumption (Njeru et al., 1994) in ensuring vitamin E adequacy in lambs. Selenium and vitamin E supplementation in pregnant ewes has been shown to decrease lamb mortality (Kott et al., 1983; Munoz et al., 2006). Further, fatty acids supplementation has improved the vigor of neonatal lambs; however, an over-supplementation negatively affected heat production of lambs and colostrum production of ewes (Capper et al., 2005; Chen et al., 2007). Low lamb vigor has been reported as a result of ewe cobalt deficiency (Fisher and MacPherson, 1991).

Lamb mortality can be reduced using an appropriate feeding management in the first few months (Mandal et al., 2007). Flocks providing both suckling assistance and tube feeding, as required, have greater lamb survival than flocks without these aids. However, routine tube feeding might interfere with learning to suckle, resulting in high neonatal losses (Holmøy et al., 2012). Suckling is not only beneficial to lambs through supplying nutrients for thermoregulation but is also important in the formation of the mother-offspring relationship (Nowak et al., 1997; Goursaud and Nowak, 1999). Mothering is delayed in lambs from large litters (Nowak and Lindsay, 1992); thus, this group of lambs is more susceptible to death from starvation than lambs born in smaller litters (Dwyer, 2008). Fostering, while widely used by producers, has not been shown to be very successful (Binn et al., 2002; Dwyer and Lawrence, 2005).

1.2.2 Environmental factors

Lamb survival is affected by various environmental factors, such as temperature, wind, moisture, rainfall, and feed availability (Kleemann et al., 1990; Mandal et al., 2007; Hatcher et al., 2010). Environmental extremes experienced by the lamb and ewe after parturition can be important causes of neonatal mortality (Nowak et al., 2000), especially where ewes are not housed, and low temperature is one of the most common factors associated with such mortality (Dwyer, 2008). Chniter et al. (2011) found higher lamb losses during winter than any other seasons, and duration of cold weather has been reported as a determinant of lamb mortality in Australia (Hamilton Prime Lamb Breeders, 2005). The highest lamb mortality was found in flocks in which the most lambing occurred during rainy and windy periods. Lambs cannot appropriately regulate body temperature in prolonged wet and windy conditions (Alexander, 1962; McCutcheon et al., 1983), and because of the more suitable temperatures and weather conditions during spring, birth weights of lambs born in this season are higher than lambs born in other seasons. Moreover, lamb mortality risk is greater in lambs born during a wet season than in lambs born during a dry season (Berhan and Van Arendonk, 2006). The peri-partum mortality risk of spring-born lambs is low (Sawalha et al., 2007; Chniter et al., 2011). Reduced suckling is found when temperatures are very high and, consequently, dehydration from low milk consumption can lead to hyperthermia (Haughey, 1980; Stephenson et al., 1984).

The proportion of lamb losses due to infectious diseases is higher in lambs born indoors than lambs born outdoors (Binns et al., 2002). Contrarily, starvation and exposure are significant causes of death in outdoor lambing flocks (Fisher and Mellor, 2002). Proper housing management practices improve lamb survival, especially in case of lambing under poor environmental conditions (Dwyer, 2008; Thompson, 2011; Chniter et al., 2013). However, even within the same flock, environmental conditions and management practices may vary widely. Nash et al. (1996) reported greater mortality in lambs born in locations that received lower supervision from farmers. The significant financial investment required is a major limitation in providing appropriate housing and sufficient numbers of workers (Binns et al., 2002).

Supervision and intervention during the peri-partum period are important to avoid lamb losses, and decreasing mortality risk of lambs raised in appropriate housing may be attributed to regular monitoring, which lowers the risk of death from hypothermia and hypoglycemia (Rowland et al., 1992; Binns et al., 2002; Holmøy et al., 2012). The mean chill index in sheltered lambs was higher than non-sheltered lambs, and starvation-exposure was responsible for 58% of lamb mortality in non-sheltered lambs, while it was responsible for 21% of death in sheltered lambs (Upreti, 1989).

Due to the inevitability of several environmental conditions during autumn and winter, sufficient shelters have to be provided (Fisher, 2004). However, the hygiene of housing must be taken into account, specifically during the peri-partum period. Lambs in flocks with poor hygienic practices have a greater chance of exposure to pathogens (Chaarani et al., 1991; Rowland et al., 1992; Nash et al., 1996). A higher lamb mortality was found in flocks in which mothering pens were bedded down less frequently than once a day, compared to flocks with more frequent bedding down provided (Binns et al., 2002).

Many studies have been focused on effects of times, such as month, season, and year, on lamb mortality and factors associated with lamb mortality. In Canada, Demirören et al. (1995) found that June-lambing ewes (spring) had larger litter sizes than February-lambing ewes (winter), and lower lamb birth weights than February- and October-lambing ewes (autumn). Consequently, the mortality risk of June-born lambs was higher. The incidence of stillbirth in Finland was highest in spring (March-May) (Sormunen-Cristian and Suvela, 1999). In New Zealand, higher birth weights of spring born lambs and greater milk production of spring-lambing ewes, compared to other seasons, were reported; nevertheless, lamb mortality risks were not different among seasons (Fisher, 2004). Jenkinson et al. (1995) found that in New Zealand, the mammary glands of March-bred ewes had higher weights than December-bred ewes. The former group also had higher numbers of placentomes, which was partly responsible for greater fetal weight.

The incidence of losses from respiratory disorders in US sheep flocks was greatest in March and April (Nash et al., 1997). In contrast, season did not influence lamb mortality risk in the study of Notter and McClaugherty in the US (1991), while Maria and Ascaso (1999) reported that effects of season on lamb mortality in Spain depended on genotype of lambs. Year has some effects on ewe performances, such as fertility and prolificacy. However, the effects appeared to be random (Demirören et al., 1995). Year-to-year variation on lamb mortality has also been reported (Owens et al., 1985; Demirören et al., 1995; Sawalha et al., 2007; Piwczynski et al., 2012). The variation among months, seasons, and years might be the result of changing environmental and management factors during each period (Sormunen-Cristian and Suvela, 1999; Southey et al., 2001; Sawalha et al., 2007; Piwczynski et al., 2012). Because of lower pathogen accumulation and higher attention from farmers during the early lambing season, lambs born earlier in any given lambing period have higher survival than lambs born later (Nash et al., 1996).

Lamb losses are influenced by the number of sheep in a flock at lambing. Thompson (2011) found a linear association between this factor and lamb mortality. The average lamb survival until marking in flocks containing > 200 ewes was 14% lower than flocks containing ≤ 200 ewes. Another study (Binns et al., 2002) reported high postnatal mortality risk in flocks containing > 900 ewes lambing per year compared to smaller flocks. Mismothering is a major cause of lamb losses in large flocks (Thompson, 2011), and is attributed to high stocking density and trauma to lambs caused by inappropriate interventions provided to ewes with lambs at foot (Haughey, 1991). Split flock lambing is a useful approach to make better use of available labour during the peri-partum period (Andrewes and Taylor, 1986; Hawkins et al., 1989).

Predator attack is a common cause of lamb mortality in some areas, and guard animals, baiting, and shooting predators have been used to control the problem (Elliott et al., 2011). Young lambs are more vulnerable to predator attack than other groups of sheep, and are more likely to die from an attack (Bleich, 1999).

1.2.3 Genetic factors

Breed and genotype of lambs have been found to be associated with lamb mortality (Gama et al., 1991; Nash et al., 1996; Maria and Ascaso, 1999; Southey et al., 2001). Heritability for lamb vigor and mortality have been found to be low (Smith, 1977; Southey et al., 2001; Brien et al., 2009), however, these two factors are closely related to lamb weight, which can be controlled by genetic selection (Smith, 1977). Neonatal lamb vigor was associated with maternal genetic effects to a higher degree than with direct genetic effects. Additionally, lambs born to ewes having good maternal genetic traits had better direct genetic potential than lambs born to ewes having poor maternal genetic effects (Sawalha et al., 2007). Southey et al. (2001) reported a low heritability of maternal effect, although it was important for lamb survival during the first four months. An inverse correlation between maternal and direct effects was found in the same study.

Elliott et al. (2011) suggested culling ewes demonstrating poor maternal behavior, and preferentially selecting ewes with good temperament and rams from high survivability lines. Cold resistance is partly influenced by genetics; thus, selection based on the ability to resist cold environments is possible to improve lamb survival (Dwyer, 2008). Diameter of the pelvis and multiple-rearing ability of ewes can be selected genetically to maximize lamb survival (Haughey, 1991; Cloete et al., 2009). Breed selection can limit lambing difficulties such as large size and abnormal presentation of lambs during the birth process (Grommer et al., 1985; Carson et al., 2001). Litter size, which is a determinant of lamb mortality, also varies by breed (Demirören et al., 1995). Moreover, impacts of several factors on cause-specific lamb mortality depend on breed. For instance, lamb mortality from dystocia, trauma, and gastrointestinal problems in purebred lambs are greater than in crossbred lambs (Smith, 1977; Yapi et al., 1990). Death by exposure and starvation varies by breeds (Nash et al., 1996), which might be attributed to different metabolic rates, hormonal responses, and thermoregulation among breeds (Slee, 1978).

1.2.4 Ewe factors

Lower survival risk of lambs born to 2 year-old ewes compared to older ewes, which might be caused by smaller lambs and inexperienced, young ewes has been reported (Owens et al., 1985; Gama et al., 1991; Dwyer, 2003; Thomson et al., 2004). Maria and Ascaso (1999) reported greater lamb mortality risk in lambs born to first parity ewes than older ewes. Experienced ewes mother their lambs sooner than inexperienced ewes (Owens et al., 1985). Additionally, colostrum production and maternal behavior of multiparous ewes are greater than primiparous ewes (Gama et al., 1991). Two other studies reported higher lamb survival from ewes 2 and 6 years of age, compared to other age groups; thus, greater attention should be paid to lambs from both younger and older ewes (Sawalha et al., 2007; Piwczynski et al., 2012). The effect of ewe age on lamb mortality is even more apparent in the perinatal period (Sawalha et al., 2007). Southey et al. (2001) reported higher risk of lamb mortality in lambs born to two-year-old and younger ewes compared to older ewes. On the other hand, Demirören et al. (1995) reported 20% higher lamb mortality in 36 to 44 month-old ewes compared to younger ewes, and 13% higher in ewes 52 months and older, compared to younger ewes. The high mortality of lambs from older ewes might be a result of the high proportion of multiple births in this group. In crossbred lambs, the risk of dystocia depended on age of ewes, but this relationship was not found in purebreds. The main factors associated with dystocia in young and old ewes are immature size and old age, respectively (Smith, 1977). Impacts of ewe age on litter size and total lamb birth weight are also reported, as the proportion of non-singleton lambs and total lamb weight increases with ewe age (Demirören et al., 1995). In African sheep flocks, lambing of replacement ewes after 2.5 years of age will provide for maximal ewe pelvic cross-sectional dimensions, thus reducing dystocia due to maternal-fetal disproportion (Haughey, 1991).

Body conditions of ewes before and during gestation affect fetal growth rate, birth weight, and the vigor of neonatal lambs (Robinson et al., 1999; Dwyer, 2003; Dwyer et al., 2016). Undernourished ewes have inferior maternal behavior and form poorer mother-young relationships with their lambs (Dwyer et al., 2003). More undernourished ewes require lambing assistance than healthy ewes (Carson et al., 2001). Thin ewes are also more likely to suffer from pregnancy toxemia, and produce less colostrum; thus their lambs are more vulnerable to infection due to failure of passive transfer (Fragkou et al., 2010). Mandal et al. (2007) reported an inverse relationship between lamb mortality risk and body weight of ewes at lambing. The odds of lambs dying when born to very thin ewes (body condition score ≤ 1) was 2.5 times the odds of lambs from ewes with greater body condition (Christley et al., 2003). Survival of twin lambs was increased 13% by having a 0.6 greater condition score at lambing

(Thompson, 2011). The number of lambs surviving until weaning has been found to be influenced by ewes' body condition score at mating (Carson et al., 2001). Body condition score of late-gestation ewes should be maintained at 3.0 to guarantee enough energy reserves for the lambing process, bonding with their lambs, and producing appropriate sized lambs, all of which contribute to high lamb survival (Hatcher et al., 2010).

The health of the reproductive tract of ewes influences survivability of fetuses and lambs. Fetal blood supply can be blocked in ewes having vaginal prolapse, which can lead to prenatal mortality (Hinch et al., 1986). Pregnancy wastage (ovulation rate minus litter size) might reduce the area of the placental attachment site for fetuses (Wilkins et al., 1982), and fetal competition for nutrients is, therefore, higher in ewes having high pregnancy wastage; lambs from these ewes have lower chances of survival (Kleemann et al., 1990).

Various ewe health management practices to maximize lamb survival have been suggested. Health management practices for disease control are more effective if they are implemented before diseases occur (Nash et al., 1996). For example, campylobacteriosis vaccination has been employed in ewe lambs before and after breeding to minimize abortion, stillbirth and early lamb loss. Use of this vaccine has been shown to increase prolificacy by 7% (Thompson, 2011).

Colostrum and milk ingestion, both quality and quantity, are very important for the health and well-being of lambs. Availability of milk is a factor influencing lamb mortality (Nowak and Poindron, 2006), and the milk yield of ewes partly depends on number of lambs they have. Ewes having twins produce less milk than ewes with triplets (Ricordeau et al., 1990). The major underlying factor of death due to starvation is inadequate colostrum and milk (Hinch et al., 1986). Poor milk production of ewes contributes to the higher risk of death due to respiratory infection and total mortality from one day of age until weaning (Nash et al., 1997). Conformation of the udder and teat has some impacts on lamb mortality, as lambs of ewes with proper udder and teat conformation have greater survivability (Berger et al., 1989).

Maedi-visna virus infection can affect lamb survival through its effect on milk production (Pekelder et al., 1994). Lamb mortality risk of seropositive ewes was 2.8% higher than the risk of seronegative ewes. However, if the lymphocytic mastitis from the infection does not considerably suppress milk production, the risk might not be affected (Arsenault et al., 2003).

Rearing ability of ewes has considerable impacts on lamb survival (Everett-Hincks and Dodds, 2008). However, high lamb mortality within the first 24 hours might still be found in ewes having high maternal behavior scores (Sawalha et al., 2007). Rearing ability of ewes is affected by various factors, such as age, genetics, and diseases (Douglas and Leslie, 1986). A healthy mother-young relationship is formed with ewes having good maternal behavior, and survivability of lambs depends on the strength of this relationship. This may be due to a higher ability to learn from their dams in lambs having better bonds with their dams than other lambs (Dwyer, 2008).

1.2.5 Lamb factors

Lamb birth weight is one of the most important factors influencing lamb survival (Upreti, 1989), and is the most powerful predicting factor of neonatal survival (Huffman et al., 1985). Kleemann et al. (1990) introduced an equation for predicting lamb survival risk based on lambs' birth weight. Lambs with intermediate birth weights have a lower mortality risk than small and very large lambs (Piwczynski et al., 2012). However, the association between lamb birth weight and mortality was not detected in a study by Brien et al. (2009).

Thomson et al. (2004) found a birth weight range of 1.0-9.8 kg. Only 4% of lambs born had birth weights outside the range of 3.0-9.0 kg. However, death of this extreme group of lambs accounted for 23% of total lamb mortality risk. All lambs lighter than 1.5 kg in that study did not survive. Smith (1977) found that pre-weaning mortality risk of lambs having birth weights up to 5.5 kg was negatively correlated with birth weight, but the association between mortality risk and birth weight tended to be positive in heavier lambs.

Because of lower fat reserve and poorer vigor of low birth weight lambs, they are less likely to survive (Mellor and Murray, 1985). They also have higher surface area to body mass ratio, which leads to a greater risk of hypothermia due to excessive heat loss (McCutcheon et al., 1981; Thomson et al., 2004). These lambs are mostly born to ewes producing insufficient colostrum and milk (Mellor and Murray, 1985). Khan et al. (2006) reported that colostrum and milk consumption of low birth weight lambs was not sufficient, and their serum Ig concentration was lower than lambs having higher birth weights. Ultimately, the ability of small lambs to survive is lowered (Chniter et al., 2011). Low birth weight lambs are more likely to suffer from starvation and hypothermia soon after birth, and infectious diseases later on (Conje, 2003). On the contrary, large lambs spend less time standing and suckling (Owens et al., 1985), and normally consume enough colostrum and receive proper maternal care from their ewes (Mandal et al., 2007).

Starvation-hypothermia and dystocia are common causes of mortality in small and large lambs, respectively (Yapi et al., 1990; Christley et al., 2003; Thomson et al., 2004; Sawalha et al., 2007). Yapi et al. (1990) reported that losses attributed to starvation, respiratory problems, trauma, septicemia, tetanus, navel ill, and congenital defects were greater in small lambs than heavier lambs. Only gastrointestinal problems were more common in heavier lambs than small lambs. The risk of non-infectious mortality is higher in lambs having low birth weight (MacLeod, 1983). Smith (1977) found a quadratic relationship between birth weight and risk of dystocia, with the incidence of dystocia lowest in intermediate weight lambs.

Birth weight is influenced by several factors, including year, type of birth (litter size), sex, weight of ewes at mating, age of ewe, parity of ewe, genetic, ewe health status, pregnancy wastage, and flock size (Smith, 1977; Huffman et al., 1985; Sormunen-Cristian and Suvela, 1999; Christley et al., 2003). Lambs in small litters have greater birth weights. Older ewes mostly give birth to larger lambs, while small lambs are found when pregnancy wastage is high (Owens et al., 1985; Kleemann et al., 1990; Christley et al., 2003; Thomson et al., 2004; Sawalha et al., 2007; Simensen et al., 2010).

Feeding systems and feed availability also affect birth weight of lambs (Huffman et al., 1985; Sawalha et al., 2007). Feed competition is high in large flocks, which, consequently, causes low birth weight lambs (Simensen et al., 2010). Very thin ewes (body condition score ≤ 1) have been found to produce lambs weighing 200 g less than ewes with higher body condition scores, and generally, the birth weights of female lambs are lower than that of male lambs (Christley at al., 2003). Lambs born later in a season have lower birth weights than lambs born earlier, which might be a result of different environmental conditions. Furthermore, weak ewes usually conceive later in the season than healthy ewes (Christley et al., 2003). There are some interaction effects among factors influencing lamb birth weight, such as interactions between breed, age of ewe, and type of birth (Smith, 1977).

Birth coat also has some impacts on lamb survivability. Lambs born with thick birth coats are more likely to survive than lambs born with fine birth coats (Upreti, 1989). On the contrary, the effect of birth coat on lamb survival was not detected in a study by Brien et al. (2009).

Based on Benoit et al. (2009), proportions of singleton, twin, and triplet lambs were 67%, 27%, and 6% of total lambs born, respectively. Notter and McClaugherty (1991) reported the lowest mortality risk in twins, compared to lambs from other birth types. However, several studies have found that lamb mortality risk increases with litter size (Berger et al., 1989; Upreti, 1989; Demirören et al., 1995; Southey et al., 2001; Holmøy et al., 2012). Anoxia during parturition might partly lead to high mortality risk in lambs from large litters (Kleemann et al., 1990). It is easier for singleton lambs to access the udders (Piwczynski et al., 2012), and they receive better attention from their dams (Gama et al., 1991). In addition, different supervision and management practices implemented in singleton and multiple birth lambs might also be responsible for different survivability of these lambs (Huffma et al., 1985; Sormunen-Cristian and Suvela, 1999; Sawalha et al., 2007). Triplets having the same birth weight as singletons have a lower risk of mortality, which might be due to small singleton lambs usually resulting from some pathological problems (Green and Morgan, 1994). Yapi et al. (1992) found no effect of litter size on lamb mortality, while Smith (1997) and Berger et al. (1989) both found that the effect of litter size on lamb mortality varies by breed and age of ewes.

Due to the limited capacity of the uterus, multiple birth lambs are lighter than singleton lambs (Atashi et al., 2013). Birth weight of the lamb was found to be decreased by one kilogram on average with each additional lamb in the litter (Thomson et al., 2004). However, the extent of the reduction in birth weight depended on the age of the ewes, and was slightly lower in young ewes (Smith, 1977).

Effects of birth order on lamb survival-related factors have been noted. First-born non-singleton lambs spend more time at the vulva during the birth process than other lambs (Owens et al., 1985). Time of grooming is negatively correlated with the birth order, as first-born lambs receive more grooming than lambs born later (Atroshi and Osterberg, 1979). Singleton and first-born lambs in sets of twins can find udders and suckle earlier than first-born lambs in larger litters (Owens et al., 1985).

Losses from starvation, hypothermia, and mismothering are lower in singleton lambs than in multiple birth lambs (Hinch et al., 1986; Gama et al., 1991; Green and Morgan, 1994). Singleton lamb death due to starvation mostly occurs within the first day, and there is often some evidence of complications from parturition (Haughey, 1993). Sawalha et al. (2007) reported higher stillbirth risk in singleton lambs, but lower mortality between 1 and 14 days of age in this group of lambs, compared to non-singleton lambs. In contrast, Nash et al. (1996) reported that the negative effect of large litter sizes on lamb mortality was limited to the first 24 hours of life. Due to negative effects of large litters on lamb survival, close supervision should be provided to non-singleton lambs (Chniter et al., 2011).

Litter size is influenced by several factors, and is low in ewes younger than 2 years of age and ewes having low weight at mating (Atashi et al., 2013). The chance of having triplets increases with parity (Maria and Ascaso, 1999), and the number of lambs per ewe per lambing is lower in flocks using accelerated lambing systems, compared to flocks lambing once a year (Sormunen-Cristian and Suvela, 1999). Litter size is negatively associated with flock size and the proportion of barren ewes (Simensen et al., 2010). Breed also has some impact on litter size (Simensen et al., 2010). Season affects the number of lambs, with the largest litter size found in ewes lambing during the natural lambing season (Sormunen-Cristian and Suvela, 1999). Large litters are also found in flocks in which grass silage is used as the main forage (Simensen et al., 2010).

Mortality in female lambs is lower than in male lambs (Smith, 1977; Binns et al., 2002; Mandal et al., 2007; Brien et al., 2009). Nash et al. (1996) found that intact male lambs had greater risk of mortality from 1 day of age until weaning than female lambs, while no effect of gender on mortality within the first day was found. Wether lambs have a lower risk of pre-weaning mortality than female lambs, while the overall risk of mortality in male lambs was 23% greater than female lambs (Southey et al., 2001). The incidence of systemic diseases was also greater in male lambs (Mandal et al., 2007). The higher mortality risk in male lambs is probably related to sex-linked genes and a lower ability to access to colostrum and get attention from their dams (Nash et al., 1997; Mandal et al., 2007). Different hormonal profiles between genders might result in higher birth weights of male lambs than female lambs (Thomson et al., 2004; Atashi et al., 2013). The high birth weight of male lambs leads to greater chance of dystocia (Binns et al., 2002), and, even after birth weight was adjusted, Smith (1977) still found a greater mortality risk in male lambs. However, the difference in mortality risks between male and female lambs was not found in some other studies (Berger et al. 1989; Upreti, 1989).

The interaction between ewes and lambs is important for lamb survival (Nowak and Poindron, 2006). Lambs have to compete with their herd mates for survival, so lambs with high competitiveness have a lower risk of mortality (Nowak and Poindron, 2006). Attention from ewes increases when lambs are more active (Owens et al., 1985). The ability of lambs in searching for colostrum is also important to their survivability (Dwyer, 2008), and there is an inverse correlation between birth weight and interval from lambing to first attempt to stand, first attempt to suckle, standing and suckling. Lambs taking longer to make their first attempt to stand and initiate udder seeking are more likely to die (Owens et al., 1985). Newborn lambs suffering from brain injury from prolonged lambing are mostly unable to suckle appropriately (Holmøy et al., 2012), as suckling and locomotor activities of lambs are impaired by the brain injury from prolonged lambing.

Lamb vigor can be measured by several parameters, such as time to stand, time to suckle and rectal temperature (Hergenhan et al., 2014). Effects of vigor on lamb survival are stronger than the effects of birth weight (Nash et al., 1996). Lamb vigor varies by birth weight, breed, gender, type of birth, age of ewe, and year of birth (Smith, 1977). Lambs injured at birth have lower vigor than uninjured lambs (Haughey, 1991; Dwyer, 2003), and the incidence of injury after birth is greater in lambs with poor vigor and lambs born to ewes with poor maternal behavior (Dwyer, 2008). Strong vigor lambs are more likely to receive sufficient colostrum and maternal care; thus, the risk of infection is lower in these lambs than in weak lambs (Nash et al., 1996; Nash et al., 1997). The mortality risk from 1 day of age until weaning of lambs with average vigor will be high if they are born from ewes producing inadequate milk. The mortality risk of strong lambs is lower than average vigor lambs, regardless of the available milk supply. On the contrary, weak lambs, regardless of the volume of the milk supply, have a higher risk of mortality. Thus, special attention is required for weak lambs (Nash et al., 1996).

1.2.6 Producer characteristics

Attitude of owners affects lamb mortality through management practices implemented in the flocks. For instance, breeding stock producers worried about high lamb losses and had positive attitudes toward improving lamb survival by making changes to their management practices. Profit gain from increasing lamb survival is the major concern of sheep producers (Elliott et al., 2011). Experience of owners also affected incidence of neonatal death. Farmers having more than 15 years of sheep farming experience had lower lamb mortality risk. However, the mortality risk did not relate to agricultural education of farmers (Holmøy et al., 2012). To accept a new strategy, sheep producers were more likely to comply with opinions and experiences of other producers than research outcomes (Elliott et al., 2011).

1.2.7 Management practices

There are two main lambing systems used in sheep production: accelerated lambing and annual lambing. Sormunen-Cristian and Suvela (1999) reported that flocks employing accelerated lambing systems had lower litter sizes, and greater birth weights and litter weight than annual lambing systems. More labor is required in accelerated lambing systems, and predicted profits from accelerated lambing systems over annual lambing systems are not always realized. Sormunen-Cristian and Suvela (1999) reported that lamb mortality risk during the first three months in annual lambing systems was higher than in accelerated lambing systems. On the contrary, Benoit et al. (2009) found that the risk of stillbirth and mortality from birth up to 10 days of age in annual lambing systems was lower.

Management practices that increase litter sizes lead to a higher mortality risk (Gama et al., 1991; Christley et al., 2003), thus lamb mortality risk can be reduced by breeding management to moderate litter size (Mandal et al., 2007).

Routine ewe management practices can influence lambing success, and young ewes may require greater attention than experienced ewes (Kleemann et al., 1990). Shearing ewes prior to parturition is recommended because of its advantages for lamb survival, which might be due to an increased dry matter intake leading to greater lamb birth weight and milk production of sheared ewes, and an increased frequency of seeking shelter by sheared ewes (Corner et al., 2006; Nowak and Poindron, 2006; Keady and Hanrahan, 2009; Elliott et al., 2011; Sphor et al., 2011).

According to Elliott et al. (2011), sheep producers believed that using teasers for estrus synchronization, minimizing handling and interrupting ewes during the peri-partum period, and limiting duration of the breeding period would be beneficial to lamb survival. Binns et al. (2002) found higher stillbirth risk in flocks that routinely used coccidiostats and lambed outdoors. This might be a confounding relationship, because use of coccidiostats is more frequent in flocks that used intensive management systems (Berriatua et al., 1994).

Postnatal morality risk was higher in flocks that replaced more than 20% of the flock per year. High replacement rates may be due to a higher prevalence of diseases in ewes, leading to high early culling rate, and these diseases may also affect lamb survival directly or indirectly. Further, young replacement animals have a higher risk of lamb loss, and may not have full immunity against pathogens that circulate in the flocks, so they are more susceptible to diseases (Binns et al., 2002).

Lambing interventions have to be provided promptly and properly, if required, to minimize mortality (Rowland et al., 1992; Cloete et al., 1993). Appropriate interventions on sick lambs, such as offering commercial electrolytes, can prevent death (Binns et al., 2002). Hypothermic lambs should be detected and treated as soon as possible to avoid losses (Eales et al., 1983).

1.3 Common causes of lamb mortality

Lamb mortality results from both infectious and non-infectious causes. Each location and flock has a different pattern of lamb mortality, including time of death, pathological findings, and cause of death (McFarlane, 1966; Nash et al., 1996). In their review of causes of neonatal mortality, Fragkou et al. (2010) found that stillbirths were mostly result from non-infectious causes. During the neonatal period, most deaths are attributed to dystocia, hypothermia, starvation-exposure, and mismothering (Woolliams et al., 1983a; Mellor and Stafford, 2004); however, the majority of studies have been conducted in flocks lambing outdoors; causes of lamb loss may differ under confinement systems.

Common causes of lamb losses reported in Australian sheep flocks were exposure (56%), starvation (16%), dystocia (16%), and predators (9%) (Hamilton Prime Lamb Breeders, 2005). Dwyer (2008) suggested roughly classifying causes of lamb mortality into four categories: parturition process-related problems, adaptation to external environment, functional disorders, and infectious diseases.

Starvation has been found to be the most common cause of mortality in many studies, especially during the early neonatal period (Huffman et al., 1985; Berger et al., 1989; Green and Morgan, 1993). Starvation could be a result of poor quality colostrum, inadequate colostrum and milk production, poor maternal behavior, and perinatal asphyxia (Woolliams et al., 1983b; Hamilton Prime Lamb Breeders, 2005; Mandal et al., 2007). It could also be a consequence of lack of appetite due to subclinical diseases (Green and Morgan, 1993). Lambs suffering from starvation lose the ability to generate heat from metabolism, which may lead to death from hypothermia (Eales et al., 1983). In one study, almost 50% of mortality was attributed to hypothermia (Houston and Maddox, 1974). Death by hypothermia can be avoided by drying and providing sufficient colostrum to newborn lambs (McCutcheon et al., 1983).

The main cause of death in singletons is lambing difficulty (Dalton et al., 1980). Malpresentation, disproportionate size of ewes and lambs, and uterine inertia are the primary causes of dystocia (Hamilton Prime Lamb Breeders, 2005). Hypoxia from prolonged parturition causes lesions in the nervous system of lambs, and the lesions impair the lamb's ability to control movement and body temperature (Haughey, 1973). Hypoxia and trauma associated with the lambing process can be a primary or secondary cause of mortality (Haughey, 1980; Dwyer, 2003). More than 80% of lambs dying within the first 3 hours had evidence of birth injuries. The proportion of lambs having birth injuries was also high in lambs dying due to starvation, mismothering, and exposure (Haughey, 1993), while Dwyer (2008) found that only one percent of trauma was caused by ewes after birth.

Death attributed to congenital defects and functional disorders are usually rare (Haughey, 1991; Green and Morgan, 1993). Viral infection, teratogen exposure, high environmental temperature during organogenesis, and chromosomal anomalies are possible causes of congenital defects (Haughey, 1991). Some areas have certain mineral deficiencies, such as copper, iodine, and selenium. Lambs born in these areas may have congenital abnormalities, and lamb survival risk in these areas is probably lower because of this (Haughey, 1991). Atresia ani, lower jaw deformities, heart defects, and central nervous system defects are common congenital defects in lambs (McFarlane, 1965; Haughey, 1991). Premature lambs, lambs suffering from dystocia, and lambs having congenital defects cannot properly adapt to environmental changes after birth, and mortality is high during the first few days (Khalaf et al., 1979; Weiner et al., 1983).

Infectious diseases were the most frequent causes of lamb losses in a study in France (Benoit et al., 2009). The prevalence of mortality due to infectious diseases ranged from 10% to 30% (Wiener et al., 1983; Yapi et al., 1990; Haughey, 1993). The incidence of death attributed to infectious diseases is more common in older lambs, but differs by flock (Wiener et al., 1983; Haughey, 1991; Rowland et al., 1992; Green and Morgan, 1994). Infectious deaths seem to be more common in the tropical region (Mukasa-Mugerwa et al., 2000; Turkson and Sualisu, 2005). This may be mediated by poor ewe nutrition leading to failure of passive transfer; lambs consuming insufficient colostrum are more susceptible to infectious diseases (Mandal et al., 2007).

The respiratory system was a major body system associated with lamb mortality in Nash et al. (1997) and Mandal et al. (2007). These findings are not consistent with results from Matthews and Ogden (1957), who reported that the proportion of death attributed to pneumonia was only 10%. Nevertheless, evidence of pneumonia is commonly found in dead lambs, regardless of the primary cause of death. Pneumonia was the primary cause of 14% of all lamb losses in Canadian flocks, and more than half of participant flocks lost their lambs due to this problem (Dohoo et al., 1985). A large proportion of deaths attributed to abnormalities in the respiratory system occurred within the first three months (Nash et al., 1997; Mandal et al., 2007). Pneumonia-causing pathogens are often found to be normal respiratory flora (Kimberling, 1988), but stresses from environmental or management changes can impair lamb immunity, and contribute to the pathogenesis of respiratory diseases (Rook et al., 1990).

Flock health and management practices are factors related to severity and dissemination of respiratory diseases (Nash et al., 1997). According to Dohoo et al.

(1985), *Pasteurella* spp. was the most frequent pathogen responsible for lamb pneumonia. Deaths caused by respiratory disorders were low in lambs born to multiparous ewes, in ewes producing adequate milk, and in lambs with average birth weight and strong vigor (Nash et al., 1997). The risk of respiratory-related mortality varies by breed of lambs (Yapi et al, 1990; Nash et al., 1997).

Diseases affecting the gastrointestinal system are often associated with lamb mortality (Khan et al., 2006). Major pathogens found in the gastrointestinal system, in a study by Mandal et al. (2007), were *Haemonchus* spp. and *Eimeria* spp. The extent of shedding of internal parasites was higher in accelerated lambing management flocks (Benoit et al., 2009). Intestinal parasites and coccidia infection are common problems in Canadian sheep flocks. However, individual resilience to these infections varies widely, and in most cases the proportion of sheep dying from these diseases is limited; most sheep dying from parasitic causes are lambs (Dohoo et al., 1985).

Thirteen percent of flocks reported experiencing enterotoxemia causing by *Clostridium perfringens* type D. Although the proportion of sheep affected was only 0.3%, all of affected animals died (Dohoo et al., 1985). Death caused by respiratory, gastrointestinal problems and unknown causes were greater in male than in female lambs (Mandal et al., 2007).

Infection of the umbilicus can lead to abscessation and to inflammation in other tissues, such as the meninges, which can become the ultimate cause of death (Purvis et al., 1985; Chaarani et al., 1991; Green and Morgan, 1993). Coliform bacteria, especially

E. coli, were reported as the most common cause of septicemia in Canadian lambs, and mostly caused death within the first week of life (Dohoo et al., 1985).

Some vertically-transmitted pathogens can bring about fetal loss, abortion, or weak lambs (Haughey, 1991). Most abortion diseases in sheep affect only one pregnancy as a result of an effective immune response to the infection (Haughey, 1991). Several infectious diseases influencing lamb survival can be prevented by vaccination, such as campylobacteriosis and enterotoxaemia. However, vaccination is mostly used in flocks after experiencing problems (Rook et al., 1990).

Common causes of lamb morbidity in a survey study in Canadian sheep flocks were contagious ecthyma, starvation, pneumonia, coccidiosis, and non-coccidiosis diarrhea, while common ewe health problems were foot problems, external parasites, internal parasites, mastitis, pneumonia, predator attack, and vaginal/uterine prolapse. The major causes of death in ewes were pneumonia, predator attack, and vaginal/uterine prolapse (Dohoo et al., 1985).

Although management systems and environmental conditions of flocks are different, causes of lamb mortality can be similar. Identification of underlying factors of common causes of lamb mortality is required in order to provide appropriate management practices to minimize lamb losses (Rowland et al., 1992; Nash et al., 1996). Health and nutritional status of ewes, flock hygiene, and colostrum consumption are main factors related to lamb mortality (Chaarani et al., 1991). One risk factor can be associated with various causes of death; thus, improved management of a risk factor may have effects on several causes of death (Nash et al., 1996). However, there is no strategy for improving lamb survival that is appropriate for all flocks (Elliott et al., 2011). Green and Morgan (1994) found that causes of mortality in lambs 43 to 180 days of age seemed to vary by flock and were endemic in those flocks.

1.4 Lamb immunity

Fetal lambs are sustained through an epitheliochorial placenta, which limits the transfer of immunoglobulins in utero; thus, neonatal lambs require immunological substances to be obtained from colostrum in the first few hours post-partum. The concentration of total protein in the serum of post-suckling lambs can be used as an indicator to monitor colostrum production, ingestion, and absorption. Circulating immunoglobulin and total protein concentrations of dead lambs were found to be lower than in surviving lambs (Khan et al., 2006; Chniter et al., 2013); concentrations of immunoglobulin and total proteins were positively correlated with birth weight (Christley et al., 2003; Khan et al., 2006). Lamb circulating immunoglobulin concentration and litter size are negatively correlated (Christley et al., 2003). Lambs born later in any given lambing period tend to experience greater microbial challenge, poorer supervision, and higher competition with earlier born lambs. Thus, their serum immunoglobulin concentration is lower than lambs born earlier in the season (Christley et al., 2003).

The concentration of immunoglobulin in colostrum was associated with survivability of lambs during the first 15 days of age. Lambs of ewes with high colostral immunoglobulin concentrations had higher serum immunoglobulin concentrations, and were more likely to survive (Khan et al., 2006), due to enhanced protection of lambs from infectious diseases (Christley et al., 2003). Low serum immunoglobulin can be a result of insufficient colostrum production, poor quality colostrum, inability of the lamb to suckle the required colostrum, or to absorb immunological substances (Christley et al., 2003). Colostrum production is diminished by chronic mastitis, which in turn reduces lamb serum immunoglobulin concentration (Christley et al., 2003).

1.5 Post-mortem examination

Emphasizing the value of post mortem examination, Fragkou et al. (2010) suggested examining the first 10 dead lambs of every lambing season, and 1 of every 10 lambs after that. According to McFarlane (1965), patterns of lesions found in dead lambs vary from area to area. Age at death can be roughly estimated from post-mortem examination, and the pattern of age at death is helpful in identifying certain problems within a flock (Green and Morgan, 1994; Fragkou et al., 2010).

Mummification, severe autolysis, and eyeball collapse indicate that the fetus died before parturition. Death related to intra-uterine infection can cause sub-epithelial plaques at the feet or accessory digits (McFarlane, 1965). In the case of ante-partum death, the severity of organ autolysis varies by duration of time between death and beginning of delivery process (McFarlane, 1965). The different shapes of the umbilical artery can give clues about the time of death. A lamb is classified as a post-partum death if a thrombus is present in the umbilical arteries, which indicates a functional heart (McFarlane, 1965). A pointed-end umbilical artery without a thrombus indicates ante-parturient deaths. Lambs dying just before parturition are fresh and the end of the umbilical artery is square without a thrombus. Lambs that die at birth have the taperedended umbilical artery with no blood clot (McFarlane, 1965; Fragkou et al., 2010). The different shapes of the artery end may be associated with changing elastic tissue properties as time progresses (McFarlane, 1965).

Renal autolysis is found in lambs dying during the early stages of lambing. The lesion is not evident if lambs die at a late stage, but subcutaneous edema can be found in these cases. Lambs dying very soon after birth have wet navels and non-inflated lungs. The navels of lambs that survived for hours are still wet, the lungs are inflated, and separation of the hoof membrane has just started. Lambs dying a few days after birth have shriveled navels and hardened hooves (McFarlane, 1965; Fragkou et al., 2010).

An abomasum empty of colostrum and a small intestine indicates that the lamb had not received any food prior to death. Meconium in the large intestine of lambs dying after 2 days of age indicates abnormal intestinal function (McFarlane, 1965; Haughey, 1991).

According to Green and Morgan (1994), lesions presented in lambs dying from 7 to 42 days of age were mostly associated with inappropriate management. Congenital defects found in lambs dying during this period were mainly heart defects. Chronic abnormalities, acute infections, and lesions from nutrition-related diseases were common in lambs dying from 43 to 180 days of age.

Subcapsular liver rupture was the most common lesion found by Green and Morgan (1993), and all lambs having this lesion were stillbirths or died no later than a few hours after birth. Large sized lambs and lambing difficulty were related to this abnormality (Woolliams et al., 1983a, b). Edema of the neck was the second most common abnormality found, and a wrong head position was probably a cause of this lesion (Green and Morgan, 1993). Other abnormalities associated with death during parturition were tracheal congestion, oxygen deprivation, vessel rupture, and internal organ rupture (Green and Morgan, 1993).

The color of perirenal and pericardial fats is normally cream-pink at birth but is changed to red-brown by catabolism. Severity of cold stress and the amount of energy reserves can be determined from color and texture of the fats at time of death as it will be darker and softer as it is metabolized. The pericardial fat is metabolized before the perirenal fat (McFarlane, 1965; Haughey, 1991). Lambs dying from starvation had little or no fat tissue around the kidney and heart, and no stomach content in the abomasum (Green and Morgan, 1993).

Subcutaneous edema at the distal limbs, tail, face, muzzle, and ears is another abnormality related to cold stress. The degree of edema depends on the severity of cold stress. The thermoneutral temperature of neonatal lambs is around 28 $^{\circ}$C (Haughey, 1991). Subcutaneous edema is also an abnormality caused by venous constriction due to prolonged parturition, and is commonly presented in lambs that die during the birth process. Severity of the edema and autolysis of internal organs may indicate the degree and duration of the pressure (McFarlane, 1965).

Injuries in the central nervous system are commonly associated with perinatal death, and the system must be examined in all dead lambs (Haughey, 1991). Predation can be a primary or secondary cause of death; if it is a primary cause, pathological abnormalities other than hemorrhage and contusion will not be found (Haughey, 1991).

1.6 Summary

The profit from sheep farming depends in large part on lamb production per ewe per year; thus, minimizing lamb mortality risk is a fundamental goal of sheep producers. Many sheep producers in different areas around the world have been confronted with unsatisfactorily high lamb mortality risks, including some PEI sheep producers. Common causes of lamb mortality vary from flock to flock due to a multitude of factors, including environmental conditions and management practices of the flock. In order to improve lamb survival risk, the underlying factors of lamb mortality have to be identified for each flock. Determination of common causes of lamb mortality is a first step to revealing underlying factors, thus post-mortem examination of dead lambs is a key technique to be employed. Hypothermia, hypoglycemia, dystocia, and infection have been reported as common causes of lamb mortality; however, a primary cause of death cannot be identified in a considerable proportion of lambs because the lack of a specific characteristic is not unusual. Several underlying factors of lamb mortality have been identified, such as lamb birth weight, lamb gender, and ewe colostrum production. Some underlying factors are difficult to manipulate; nevertheless, most factors can be controlled by appropriate management practices. The nutritional and health status of ewes and lambs are major factors associated with lamb mortality. Thus, appropriate feeding and health management practices are required to minimize lamb mortality. For instance, sufficient nutrients to support higher nutrient requirements must be provided to late-gestation ewes. Adequate colostrum intake is required as a source of nutrients and immunity for lambs. Disease status within the flock has to be evaluated in order to identify common health problems, and protective measures must be provided for any current problems. Proper management practices in other aspects, such as housing, breeding, lambing, and lamb management, are also warranted to ensure high lamb survival.

1.7 Objectives of the thesis and thesis outline

The general objective of this series of studies will be to identify the most influential factors associated with lamb mortality in PEI sheep flocks. The ultimate goal of this program of research is to provide evidence to the PEI sheep industry to inform appropriate management practices and interventions for maximizing lamb survival.

The distinct objectives of the six research chapters are to:

1. Determine common causes of lamb mortality based on post-mortem records, from 2005 to 2014, from the Atlantic Veterinary College Diagnostic Services, University of PEI. This objective will be addressed in Chapter 2.

2. Determine flock characteristics, management practices, and late-gestation ewe health statuses in PEI sheep flocks from September 2014 to August 2015. This objective will be addressed in Chapter 3.

3. Identify factors associated with liver selenium and vitamin E concentrations in dead lambs. This objective will be addressed in Chapter 4.

4. Evaluate efficacy of the handheld Precision Xtra® device for measuring the blood β-hydroxybutyrate concentrations in late-gestation ewes. This objective will be addressed in Chapter 5.

5. Identify factors affecting the serum β-hydroxybutyrate concentrations in late-gestation ewes. This objective will be addressed in Chapter 6.

6. Identify flock-level factors associated with lamb mortality risk. This objective will be achieved in Chapter 7.

7. The general discussion and conclusion are in Chapter 8.

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Table 1.1 Lamb mortality risks reported from previous studies

Age of lambs at death	Breed of lambs	Country of study	Percent mortality	Management system	Reference
Pre-partum	N/A	Australia	3.4	N/A	Hamilton Prime Lamb Breeders, 2005
Birth process (stillbirth)	N/A	Canada	4.9	N/A	Dohoo et al., 1985
Birth process (stillbirth)	N/A	UK	4.0	N/A	Binns et al., 2002
Birth process (stillbirth)	N/A	France	4.7	Organic, accelerate lambing	Benoit et al., 2009
Birth process (stillbirth)	N/A	France	2.7	Organic, annual lambing	Benoit et al., 2009
By 24 hours	Targhee	USA	7.9	N/A	Huffman et al., 1985
By 24 hours	N/A	USA	8	N/A	Gama et al., 1991
By 24 hours	N/A	USA	8	N/A	Nash et al., 1996
By 24 hours	N/A	UK	3.3	N/A	Binns et al., 2002
1-3 days	N/A	UK	2.1	N/A	Binns et al., 2002
4-7 days	N/A	UK	0.6	N/A	Binns et al., 2002
7-28 days	N/A	UK	0.25	N/A	Binns et al., 2002
By 5 days	N/A	Norway	3	N/A	Holmøy et al., 2012
By 7 days	N/A	UK	7.7-11.7	N/A	Green and Morgan, 1993
By 10 days	N/A	USA	10	N/A	Southey et al., 2001

Age of lambs at death	Breed of lambs	Country of study	Percent mortality	Management system	Reference
2-14 days	N/A	UK	2.4	N/A	Christley et al., 2003
By 21 days	Muzaffarnagari	India	7.5	N/A	Mandal et al., 2007
By 21 days	Targhee	USA	14.3	N/A	Huffman et al., 1985
By 1 month	D'man	Tunisia	13.6	N/A	Chniter et al., 2013
By 50 days	N/A	USA	15	N/A	Southey et al., 2001
By 56 days	N/A	USA	25	N/A	Nash et al., 1996
2-60 days	N/A	USA	14	N/A	Gama et al., 1991
By 70 days	D'man	Tunisia	13.4	N/A	Chniter et al., 2011
By tagging (between 18 hours and 84 days)	N/A	New Zealand	5	N/A	Thomson et al., 2004
By 84 days	N/A	New Zealand	11	N/A	Thomson et al., 2004
By 150 days	Finnish Landrace	Finland	15.2	Accelerated lambing	Sormunen-Cristian and Suvela, 1999
By 150 days	Finnish Landrace	Finland	16.4	Annual lambing	Sormunen-Cristian and Suvela, 1999
Pre-weaning	Ghezel	Iran	7.1	N/A	Atashi et al., 2013
Pre-weaning	Mehraban	Iran	4.1	N/A	Atashi et al., 2013
Pre-weaning	Muzaffarnagari	India	6.6	N/A	Mandal et al., 2007
Pre-weaning	Merino	Australia	21	N/A	Brien et al., 2009

Age of lambs at death	Breed of lambs	Country of study	Percent mortality	Management system	Reference
Pre-weaning	N/A	US	25.9	N/A	Yapi et al., 1990
Pre-weaning (60 days)	Pak-Karakul	Pakistan	9	N/A	Khan et al., 2006
Pre-weaning (60 days)	Thalli	Pakistan	12	N/A	Khan et al., 2006
Pre-weaning (100 days)	Merino	Poland	5.3	N/A	Piwczynski et al., 2012
N/A	N/A	Canada	12.1	N/A	Dohoo et al., 1985
N/A	N/A	UK	7-10	N/A	Binns et al., 2002

CHAPTER 2

CAUSES OF DEATH IN LAMBS UP TO 90 DAYS OF AGE SUBMITTED FOR POST-MORTEM EXAMINATION TO THE ATLANTIC VETERINARY COLLEGE DIAGNOSTIC SERVICES (2005-2014)

2.1 Abstract

A retrospective analysis of data from 385 lamb post-mortem examinations, based on submissions to the Atlantic Veterinary College Diagnostic Services from 2005 to 2014, was performed to identify patterns of lamb mortality in PEI flocks. Overall, 71% of submissions resulted in identification of a primary cause of death, with the likelihood of a diagnosis being higher in older lambs ($p < 0.001$). Of 367 submitted lambs that were born alive, respiratory lesions were present in almost 70%. The most frequent primary cause of death was infection (41%), with the most frequent sites of infection being the gastrointestinal (39%) and respiratory (24%) systems. The likelihood of infection being identified as the cause of death increased significantly with age ($p < 0.001$). Together, these results suggest management changes to reduce pathogen exposure and increase disease resistance, including passive transfer of immunoglobulins, are required in early growing lambs.

2.2 Introduction

Lamb production in the Maritime Provinces (New Brunswick, Nova Scotia and Prince Edward Island) has grown significantly over the past few years. The estimated sheep inventory of Prince Edward Island (PEI) increased from 3901 head in December 2006 to 7959 head in December 2011 (The Government of PEI, 2013). Lamb survival is a fundamental determinant of flock performance in sheep production and becomes more important as producers use more intensive management practices with high economic inputs such as total confinement, concentrate feeding, out-of-season breeding, accelerated lambing, and use of higher genetic value rams and ewes.

According to Fragkou et al. (2010), neonatal lamb loss during the first 28 days of life should be less than 5%. Nevertheless, average lamb mortality risks ranging from 4% to 26% have been reported from studies of sheep populations in various countries (Yapi et al., 1990; Green and Morgan, 1993; Nash et al., 1996; Kennedy and Johnston, 2011; Piwczynski et al., 2012; Atashi et al., 2013). Risk of death varies with age, with most deaths occurring within the first week of life (Green and Morgan, 1993).

Perinatal deaths are commonly categorized as stillbirths and neonatal death. A stillbirth is the delivery of a full-term lamb that either has no heartbeat on arrival or dies before taking a breath, whereas a lamb born alive that dies during the first 4 weeks is classified as a neonatal death. Non-infectious disorders, especially dystocia-related problems, have been reported as the most important causes of stillbirth (Fragkou et al., 2010). For instance, liver laceration is one of the most frequent traumatic lesions found in stillborn lambs. The lesion is also commonly found in early neonatal deaths. It is thought to occur most likely as a result of dystocia (Green and Morgan, 1993). Starvation was the major cause of lamb mortality during the first 7 days in a UK study of housed lambs (Green and Morgan, 1993). On the other hand, trauma and infectious diseases, including navel ill, enteritis, pneumonias, and internal parasites, have been reported as common causes of death in older lambs (Purvis et al., 1985; Chaarani et al., 1991; Green and Morgan, 1994). Pneumonia-related lesions of various types have been commonly found in post-mortem examinations; however, in many cases, these lesions have not been the primary cause of death (Matthews and Ogden, 1957). Higher mortality risks pre-weaning were found in ram lambs when compared to ewe lambs (Brien et al., 2009), and males were found to be more likely to die from respiratory and gastrointestinal disorders when compared to female lambs (Mandal et al., 2007). These latter two studies followed lambs until weaning.

Post-mortem examination of lambs is an essential tool for lamb mortality investigations (McFarlane, 1966). Retrospective analysis of cause of death provides valuable information to guide interventions and management changes which can improve lamb survival in individual flocks, regionally, and for the entire industry. However, recent Canadian data on laboratory-confirmed causes of lamb mortality has not been published. There is only one previously published Canadian study (Dohoo et al., 1985) that provided analysis of the cause of death in lambs submitted to diagnostic laboratories; this study was based on diagnostic data from 1978-1982.

The objective of this study was to describe the common causes of lamb mortality in PEI flocks using diagnoses obtained from post-mortem reports of lambs up to 90 days of age submitted to the Atlantic Veterinary College Diagnostic Services from 2005 to 2014.

2.3 Materials and Methods

Data for this study were retrieved from the electronic case records of the Atlantic Veterinary College Diagnostic Services, University of PEI. The following search criteria were used to interrogate the database: ovine, whole carcass post-mortem submissions, less than or equal to 90 days of age at time of death (including those submissions classified as abortions), where the date of accession fell within the range of January 2005 and May 2014. An age range extending to 90 days was chosen to permit examination of causes of death that, although not strictly neonatal, may have their origin in disease processes originating in the neonatal period. Standard information gleaned from the diagnostic record included flock of origin, month and year of death, sex, birth type, age and weight of lambs.

Abnormal findings were categorized by body system using reported gross and histopathological results. Results from further diagnostic tests, including bacteriology, virology, parasitology and toxicology were also examined. The reported primary cause of death was classified into one of eight categories: infection, trauma, congenital defect, dystocia, milk aspiration, starvation (including hypothermia/hypoglycemia), nutritionally-related diseases and others. Polioencephalomalacia, intestinal/mesenteric torsion, ruminal acidosis, nutritional muscular dystrophy (NMD), urinary calculi and bloat were included in the nutritionally-related diseases category. The body system associated with the primary cause of death was recorded: gastrointestinal, respiratory, neurological, musculoskeletal, cardiovascular, hepatic, urinary and multiple systems. Age at death of lambs was categorized into 5 groups: less than 1 day, 1-2 days, 3-7 days, 8-30 days and more than 30 days. A lamb born at term but dead was classified as a stillbirth or, if the lamb was considered pre-term due to its appearance and/or historical information provided by the owner, it was classified as an abortion.

Data were recorded in a spreadsheet and analyzed using descriptive statistics. Stata 13 (StataCorp LP, College Station Tx, US) was used for statistical analyses. Proportional mortality risks were calculated for common causes of death and body system associated with cause of death. Lambs with a missing value in a variable were not included in calculation of that variable. Chi-square testing was performed to compare proportions between groups, where appropriate. P-values < 0.05 were considered statistically significant.

2.4 Results

2.4.1 Demographic and health data

A total of 385 records were obtained using the search criteria (Fig. 2.1). These lambs were submitted from 40 sheep flocks in PEI. Mean number of lambs submitted per year was 38 (95% CI 26, 50) with a median of 32 lambs and a range of 18 to 64 lambs. The submission pattern was highly seasonal, with 94% of submissions occurring during the period frrom January to July (Fig. 2.2).

Stillborn and aborted lambs accounted for 3.4% and 1.3% of the total submissions, respectively; data from lambs in these categories (n = 18) were considered separately for the purposes of the analyses below, unless otherwise indicated.

Gender of lamb was recorded for 297 of 385 lambs (including aborted fetuses and stillbirths); there were 50.2% and 49.8% ewe and ram lambs, respectively. Proportions of lambs born as singletons, twins, triplets, quadruplets, quintuplets and sextuplets lambs comprised 9.3%, 45.6%, 34.2%, 7.8%, 2.6% and 0.5% of submissions (including aborted fetuses and stillbirths), respectively, where this was recorded (n = 193 of 385). Multiple lambs from the same litter were submitted on the same day 12 times (28 lambs), but this may underestimate the number of litter mates submitted because litter mates submitted on different days would not be identified as such. Age at death was recorded for 343 of 385 lambs; mean and median age were 25 (95% CI 22,

28) and 19 days, respectively. For lambs born alive (n = 367 of 385) where body condition was recorded (n = 304), 67.4% had acceptable body condition, whereas 32.6% were in poor condition. Adipose tissue depletion was found in 52.4% of cases in which adipose tissue status was examined (n = 233).

The mean weight of the lambs submitted as stillborn or dying in the first 24 hours (n = 40) was 3.13 kg, ranging from 1.39 to 6.00 kg. Proportions of lambs born as singletons, twins, triplets, quadruplets and quintuplets in this group were 9.1% (3/33), 39.4% (13/33), 36.4% (12/33), 9.1% (3/33) and 6.1% (2/33), respectively (n = 33). The mean weights of singletons, twins, triplets, quadruplets and quintuplets in this group were 3.84, 3.65, 3.22, 3.13 and 1.50 kg, respectively.

2.4.2 Body systems affected

Lung lesions were found in four out of five examined aborted fetuses; however, the bacterial respiratory lesions were considered responsible for two abortions. Protozoal encephalitis and protozoal placentitis were responsible for one and two abortions, respectively.

The proportions of stillbirth lambs having lesions in the respiratory, hepatic, cardiovascular, urinary and neurological systems were 76.9%, 15.4%, 30.8%, 30.8% and 23.1%, respectively (n = 13). Lesions of the gastrointestinal system were not found in this group of lambs, and one lamb had an umbilical abscess. The system associated with death could not be identified in most stillborn lambs (61.5%). Abnormalities of the musculoskeletal system were responsible for 2 stillbirths (15.4%). One stillbirth was caused by a combination of abnormalities of the cardiovascular and neurological systems, and another had generalized lesions. Placentitis was found as a primary cause of one stillborn case where the placenta was submitted with the stillbirth.

For lambs born alive (n = 367), the proportions of lambs having lesions in the respiratory, gastrointestinal, hepatic, cardiovascular, urinary and neurological systems were 69.2%, 43.0%, 31.1%, 24.8%, 19.9% and 15.2%, respectively. Lesions associated with the umbilicus were found in 2.2% of lambs born alive.

The gastrointestinal system was the most common abnormal system responsible for death (25.1%). The respiratory system was responsible for death in 11.4%. Seventeen percent of deaths were caused by major abnormalities in more than one body system. Classification of body system as "open diagnoses" was more common in lambs < 3 days of age when compared to older lambs (48/84 vs. 49/259, respectively; $p < 0.001$). A comparison of age-stratified proportions of lambs classified by system affected by the primary cause of death is shown in Table 2.1 for the 343 born-alive lambs where age at death was recorded. For lambs dying during the first 2 days of life, multiple system failure was the most common diagnosis, followed by musculoskeletal lesions. Conversely, all of the born-alive lambs with cardiovascular lesions died after the first 2 days of life. From the Chi-squared testing, involvement of the gastrointestinal system was less apparent in lambs dying at < 3 days of age when compared to older lambs ($p < 0.001$), with gastrointestinal deaths peaking within the 31-90 days lamb group. Parasitological and histopathological evidence of gastrointestinal helminths, *Eimeria* spp., abomasal lesions, and meconium aspiration were found in 6.8%, 15.8%, 8.2% and 6.5%, respectively, in 367 born-alive lambs.

2.4.3 Primary cause of death

The primary cause of death as determined by the pathologists was not apparent in 61.5% of stillborn lambs. Non-infectious (2 dystocia and 1 congenital defect) and infectious causes contributed to 23.1% and 15.4% of stillbirths, respectively. Infection was determined as the primary cause of abortion in all five aborted fetuses. The causative agents in three of these abortions were protozoa (two toxoplasmosis and one undetermined), while the others were twin lambs from the same litter dying due to pneumonic bacteria (*Staphylococcus chromogenes*) from their ewe in utero.

Over all groups and years, a cause of death could not be identified in 27.8% of lambs born alive. Of those lambs with an identified cause of death, infection was by far the most common cause (41.1%). The next most common cause of death was the category of nutritionally-related diseases (7.9%), such as NMD, intestinal volvulus, ruminal acidosis and urinary calculi. Trauma, congenital defects, starvation, dystocia and milk aspiration were responsible for 5.7%, 3.8%, 2.7%, 2.2% and 0.8% of death, respectively. Other causes were responsible for 7.9%. The “other” category (n = 29) could be further categorized into lambs dying as a result of euthanasia and non-euthanasia causes (4.6% and 3.3%, respectively). Principal causes leading to the need for euthanasia were infection (29.4%), trauma (17.6%), congenital defects (17.6%) and nutritionally-related diseases (17.6%), while 17.6% were open diagnoses. Of the 12 non-euthanized lambs in this category, there was 1 loss due to predator attack. Deaths of 11 lambs resulted from a variety of pathologies including enteritis, abomasal ulcer, abomasal rupture, heart failure, blood loss, pleural effusion and urinary outflow obstruction.

Proportions of lambs attributed to each cause of death are shown stratified by year (Table 2.2). There was variation from year to year, with the following serving as examples: the proportion of lambs with an open cause of death ranged from 0% to 55.6%; the proportion of lambs with an infection as the primary cause of death ranged from 18.2% to 58.6%; and the proportion of lambs with nutritionally-related diseases ranged from 0% to 19.1%.

The proportion of deaths attributed to infection varied by month (Fig. 2.2), increasing as the winter-spring lambing season progressed. The non-infectious causes of death and open diagnoses did not appear to have a seasonal pattern, exhibiting substantial variability from month to month.

Proportions of lambs attributed to each cause of death are shown, stratified by age group (Table 2.3). From the Chi-square testing, the proportion of lambs with no identified cause of death was higher for young lambs dying from birth up to 2 days of age when compared to older lambs (57.1% vs. 19.7%, respectively; $p < 0.001$). The likelihood of infection being identified as the primary cause of death increased with age, with infection comprising over half (54.2%) of the documented causes in lambs dying after 7 days, compared to 16.5% in lambs < 7 days ($p < 0.001$). Trauma was more common in the first week compared to later in life (11.8% vs. 1.8%, respectively; $p < 0.001$). Congenital defects were not identified in lambs dying in the first 24 hours after birth, but were identified in 4.6% of older lambs. Starvation was not responsible for death within the first 2 days, but accounted for 3.9% of deaths in older lambs. Almost all deaths due to nutritionally-related disorders occurred in lambs 3 days or older, accounting for 10.0% of death in this age group, whereas one 1-day old lamb died from this cause. Seventeen percent of lambs dying in the first day had dystocia as the primary cause. Death from milk aspiration was found only in lambs 1 to 5 days of age, and was responsible for 4.1% of deaths in this age group.

The relationships between age of the lamb (in day) and primary cause of death were further examined through summary descriptive statistics (Table 2.4). While variation was great, deaths attributed to infection and nutritional disorders which were the 2 most commonly diagnosed primary causes of death overall, mostly occurred around 1 month of age. Deaths due to starvation, congenital defects and other causes were identified earlier, on average, at approximately 3 weeks of age, while other causes, such as trauma, milk aspiration and dystocia, had mean and median values < 1 week of age.

2.4.4 Further examination of infection as a cause of death

With a high proportion (41.1%) of lambs dying from infectious causes, this category was examined more closely. The proportion of deaths attributed to infection varied by calendar month (Fig. 2.2), increasing as the typical winter-spring lambing season progressed. The absolute number of lambs dying of infectious causes peaked in January and February, while the median age of cases of infectious death peaked in July and August (Table 2.5).

Out of 151 lambs dying from infectious diseases, bacterial infections were most common, found in 62.2% of cases. Other causative agents including protozoa, viruses, intestinal parasites, and fungi, were identified in 19.9%, 6.0%, 4.0% and 0.7% of lambs, respectively. A causative pathogen could not be identified in 11.3% of cases in this category. In 3.3% of lambs dying from infection, more than one pathogen was identified, and all of these lambs had significant lesions in the gastrointestinal system.

The most common systems associated with causative pathogens were the gastrointestinal system (39.1%) and the respiratory system (23.8%). The proportion of identified infections involving the neurological, cardiovascular, musculoskeletal, urinary and hepatic systems were 5.3%, 4.0%, 2.0%, 0.7% and 0.7%, respectively. Most infected lambs had 1 system involved, but 2 body systems were listed as affected in 1.3% of lambs dying of infectious causes. Common infectious agents identified in lambs dying due to infection of the gastrointestinal system were *Clostridium* spp., *E. coli*, *Sarcina*-like bacteria, corona virus, *Eimeria* spp., *Cryptosporidium* spp., and gastrointestinal helminths. Of 367 submitted born-alive lambs, gastrointestinal helminths were noted in 6.8%, but were deemed responsible for death in 1.1%. The most common helminths found were nematodes, such as *Strongyloides* spp., *Haemonchus contortus*, *Nematodirus* spp. and *Trichuris* spp. Other parasites included *Moniezia* spp. and *Giardia* spp. *Eimeria* spp. was found in 15.8%, but was deemed the principle cause of mortality in 6.5% of 367 born-alive submitted lambs. Infectious pneumonia was the primary cause of death in 9.8% of 367 laboratory reports. *Pasteurella* spp. and *Mannheimia haemolytica* were the most frequent pathogens found in pneumonia cases, accounting for more than half of pneumonia submissions. Other pathogens identified as the cause of pneumonia were *Trueperella pyogenes, Staphylococcus* spp., respiratory syncytial virus and fungi. Less than 1.0% of infectious deaths were attributed to aspiration pneumonia.

Septicaemia was identified in 23.8% of 151 lambs where infection was the main cause of death or 9.8% of all laboratory reports. The principle body systems affected were the respiratory system, umbilicus, gastrointestinal system, neurological system, urinary system, cardiovascular system and hepatic system in 41.7%, 22.2%, 16.7%, 5.6%, 2.8%, 2.8% and 2.8%, respectively, of lambs with septicaemia. A principle system of infection could not be identified in 5.6% of cases of infectious death. Infectious placentitis was responsible for three cases of lambs with infectious deaths, one of which resulted in stillbirth, and in the other two, in abortion.

Antimicrobial sensitivity test results were available from 21 lambs submitted. Twenty-two isolates were identified; and 68.2%, 9.1%, 9.1%, 4.6%, 4.6% and 4.6% were pathogens isolated from the respiratory system, brain, abdomen, intestine, liver and pericardium swab, respectively. Fourteen of 22 isolates (63.6%) were identified as *Mannheimia haemolytica;* their sensitivity results are shown in Table 2.6. There were three isolates of *Listeria monocytogenes*; one of them was tested against tulathromycin and found to be intermediately susceptible. Another two *Listeria monocytogenes* isolates were tested against seven antimicrobial drugs; both were susceptible to erythromycin, oxytetracycline, penicillin, trimethoprim-sulfa and florfenicol but resistant to ceftiofur. One isolate was susceptible to streptomycin, but another isolate was intermediately susceptible. Two *Bibersteinia trehalosi* isolates were tested against tulathromycin, and they were susceptible. An isolate of *Pasteurella multocida* was susceptible to ceftiofur, penicillin, trimethoprim-sulfa and florfenicol, intermediately susceptible to erythromycin, and resistant to oxytetracycline and streptomycin. A sample of *E.coli* from the intestine was susceptible to erythromycin, oxytetracycline and florfenicol, and intermediately susceptible to penicillin and tilmicosin. A gram-positive bacillus from a pericardial swab was susceptible to ceftiofur, penicillin, trimethoprim-sulfa and florfenicol, and intermediately susceptible to erythromycin but resistant to oxytetracycline and streptomycin.

2.5 Discussion

2.5.1 Demographic and health data

While the results of this study of laboratory submitted lambs cannot be considered representative of the proportional mortality causes among sheep flocks in PEI, our study population was large, including 385 lambs submitted for post-mortem examination during January 2005 to May 2014, from 40 flocks in PEI. This study is providing some useful data where previously there were none. Half of the 40 study flocks submitted only 1 or 2 lambs over the study period, likely from producers with an apparent outbreak looking for the cause. In each year, lambs were submitted from between 6 and 18 flocks; therefore, the study results would reflect proportional mortality causes on a portion of PEI flocks. There are no published year-over-year data indicating the number of flocks in PEI over the study period, but in 2015 there were 100 flocks registered with the PEI Department of Agriculture and Fisheries (C. Wood, pers. Comm.). Extrapolated from this, we might speculate that submissions were made from about 10% of PEI flocks each year. Total numbers of sheep in PEI increased from 4000 in 2005 to 8200 in 2012 (C. Wood, pers. comm.).

There are a number of biases inherent in laboratory-based data. Clearly not all sheep producers in PEI submitted lambs, and not all dead lambs for each contributing flock were submitted. Almost 43% of lambs were submitted by a single large flock, and thus the data are weighted to reflect the mortality patterns of this flock. Further, the lambs submitted were not a random sample of all deaths in any given flock. It was likely that, for some lambs, the cause of death was obvious to the producer (e.g., laid on by their mothers, predator attack, hypothermia), and, therefore, the lamb was not submitted for diagnosis while lambs with unfamiliar or ambiguous signs may be over-represented. Thus, the study population cannot give an unbiased representation of the population of lambs dying in PEI flocks over this period.

Despite these limitations, patterns in proportional mortality risks from laboratory-based data can be useful to broadly rank diseases in order of importance for a geographical region (Dohoo et al., 1985) to guide interventions and control efforts, and to generate hypotheses related to important causes of lamb wastage to be tested in prospective and/or controlled studies. Further, these data can offer the opportunity for passive surveillance (Dorea et al., 2013) of trends in mortality over time, aiding in the identification of conditions that might be emerging as more significant problems for the industry.

From a sheep producer survey in PEI in 2013 (Ratanapob et al., unpublished), half of 22 respondent flocks had > 1 lambing period in the calendar year. All but one of these flocks had a group of ewes that lambed during the first four months of the year, reflecting the natural pattern of sheep estrous activity that is governed primarily by photoperiod. In contrast, only 7 of 22 flocks had a ewe group which lambed during the period considered out-of-season for most breeds of sheep (July-December). These results may partially explain the small numbers of lambs submitted in the second half of each year in the present study. High submissions rate between January and March might also partly result from unfavorable environmental conditions leading to higher mortality, especially starvation-hypothermia (Chniter et al., 2011).

In the present study, more than half of lambs (54.0%) died during the period up to 21 days after birth (including aborted fetuses and stillbirths). In Quebec, where management systems are quite similar to PEI, Arsenault et al. (2003) conducted a prospective study on 29 commercial flocks which included risk factors for lamb mortality. They found that 59.5% of pre-weaning losses (includes aborted fetuses and stillbirths) occurred during the first 2 days of life. In comparison, our study of laboratory-submitted lambs revealed 26.5% lamb wastage occurring in the first 2 days of life, although this is likely an underestimation of true lamb wastage, given that many lambs that died may not have been submitted for necropsy.

Binns et al. (2002) and Brien et al. (2009) found higher mortality in male than female lambs. On the other hand, another study reported a higher survival risk in male lambs (Atashi et al., 2013), while mortality risks for gender were not statistically different in a study in Quebec (Arsenault et al., 2003). Proportions of ram and ewe lambs identified in the present study were very similar; however, these laboratory-submitted proportions cannot be inferred as the true mortality risk for each gender.

The mean body weight of stillborns and lambs born alive but dying within 24 hours after birth was 3.13 kg. This is lower than the average birth weights reported in other studies from various breeds, which ranged from 3.5 to 5.3 kg (Christley et al., 2003; Thomson et al., 2004; Atashi et al., 2013). Our population was biased in comparison to these studies because only dead lambs were included. Birth weight is an important determinant of lamb survival (Berger et al., 1989; Chniter et al., 2011; Atashi et al., 2013). In smaller lambs, weakness, less adipose tissue reserve, and low milk availability (low milk production ewes) can lead to higher mortality (Mellor and Murray, 1985). One study demonstrated that 40% of lambs with a birth weight between 1.0-2.5 kg survived, lower than survival risks of 80%, 84% and 85% for lambs with birth weights of 2.6-3.5 kg, 3.6-4.5 kg and 4.6-6.0 kg, respectively (Upreti, 1989). Arsenault et al. (2003) also reported significantly higher mortality risks in lambs less than 3.9 kg compared to 5.0 kg and heavier lambs.

Singleton, twin and triplet-born lambs have accounted for 67%, 27% and 6% of total lambs born in a French study (Benoit et al., 2009). From a longitudinal study, mortality risks were reported to be 6%, 19%, 34% and 49% for singleton, twin, triplet and quadruplet plus quintuplet lambs, respectively (Upreti, 1989). Inadequate supervision during the perinatal period was responsible for the high mortality risks of non-singleton lambs (Sormunen-Cristian and Suvela, 1999). These findings provide some explanation for our finding that twins comprised the highest proportion of the diagnostic laboratory submissions, since mortality increases with litter size and twins are less frequent than singletons but more common than triplets and higher-order multiple birth.

Of lambs born alive where their body condition was evaluated in the present study (n = 304), 32.6% were considered inacceptable. Huffman et al. (1985) reported that 58% of lamb deaths were due to starvation. However, that prospective study was conducted on a randomly selected population of paddock-lambs born over January to March, a time of low temperatures in southwest Idaho, and included lambs up to only 21 days of age. In the present study, for comparison to the data of Huffman et al., 42% of laboratory-submitted lambs had too thin body condition, when considering the subgroup of lambs born alive from January to March, up to 21 days of age. Overall, starvation was not a major cause of death in the present study; it was identified as a primary cause of death in only 2.7% of born-alive lambs submitted. Based on published research and empirical knowledge, it is likely that our proportion underestimates the true risk of starvation-hypothermia deaths in flocks in PEI. This low risk may reflect the small flock size and more intensive lamb management of PEI flocks compared to sheep populations studied by other researchers. Alternatively, the differences in starvation mortality percentages may reflect a producer bias against submission of lambs where starvation-hypothermia was the most likely apparent diagnosis. This question could be resolved in a prospective study of lamb mortalities in PEI.

Stillbirth accounted for 3.4% of lambs examined in the present study. A survey by Dohoo et al. (1985) and Binns et al. (2002) found 4.9% and 4.0% stillbirths in Canadian and UK sheep flocks, respectively. According to Fact Sheets, the Ontario GenOvis program annual report 2011 and the Canadian genetic evaluation annual reports (Kennedy, 2012; Kennedy and Johnston, 2012; Kennedy and Robertson, 2013), average stillbirth risk in Ontario sheep flocks, as reported by producers enrolled on a genetic improvement program ranged between 0.7% and 11.8%. The proportion of stillbirths found in the present study was low compared to studies conducted in US and Canadian flocks (Berger, 1997; Kennedy, 2010), where they found that stillbirth comprised 30% and 22% of total lamb losses. Differences between studies could reflect differences in management or lambing systems used in different parts of North America or the UK, and/or regional prevalences of endemic infectious diseases resulting in higher stillbirth risks, such as *Toxoplasma* spp., *Campylobacter* spp. and *Chlamydophila* spp. Alternatively, the difference may be due, in part, to a low submission rate of stillbirths by producers because they perceive a high rate of diagnostic failure as found in the present study. A prospective study of prenatal lamb wastage in PEI could help resolve this question.

Abortions accounted for 1.3% of submitted lambs examined in the present study. Dohoo et al. (1985) reported 1.4% of ewes experienced abortion in Canada 35 years ago. Similarly, 1.2%-1.7% of ewes in Peru experienced abortion (Ameghino et al., 1984). In a sheep producer survey conducted in PEI in 2013 (Ratanapob et al., unpublished), producers reported an observed abortion risk of just 0.7% of pregnant ewes.

2.5.2 Body systems affected

Overall, the most common system associated with the primary cause of death in the present study was the gastrointestinal system, but this depended on the age of lamb at death. In lambs older than 7 days of age, disease of the gastrointestinal system was responsible for > 1/3 of deaths (Table 2.1). By contrast, respiratory lesions were the most frequently noted lesions in dead lambs submitted. These findings indicate prevailing but less severe respiratory problems, which agree with Matthews and Ogden (1957) who reported that lesions associated with pneumonia were commonly seen in lambs dying due to other causes. Both the gastrointestinal and respiratory systems have been reported as main causes of death in lambs elsewhere (Khan et al., 2006).

Lesions in the abomasum were found in 8.2% of submitted lambs. In 60% of lambs having abomasal lesions, the principle cause of death was related to these lesions.

No comparable data from other studies could be found, but these results appear to reflect a high risk of abomasal lesions and their importance as primary causes of death in this study population. Abomasal bloat and abomasitis have been reported as major causes of perinatal death in some sheep flocks (Vatn, 1995), and lesions in the abomasum are frequently found in post-mortem examination of lambs (Overas et al., 1990). In the present study, almost all lambs having abomasal lesions were older than 21 days of age. Only 1 younger lamb (10 days) had this lesion. This finding was consistent with a previous article reporting these lesions primarily in lambs 2 to 6 weeks old (Overas et al., 1990). Various *Clostridium* spp. (*C. sordellii, C. perfringens, C. septicum*) and *Sarcina*-like bacteria were isolated from most lambs with these lesions, in agreement with the findings of Vatn et al. (2000). Other pathogens identified from abomasal tissues of affected lambs were *Mannheimia haemolytica, E. coli* and *Streptococcus* spp., suggesting that these abomasal lesions are not associated with any one etiologic agent, but instead may be reflective of a disease process in 3 to 5 weeks old lambs where bacterial pathogens are opportunistic invaders.

The proportion of carcasses (including aborted fetuses and stillbirths) having meconium aspiration was 8.0% in the present study. More than half (54.8%) of lambs with meconium in lung tissues were aborted fetuses, stillbirths and lambs born alive but dying soon after birth, and all had died by 5 days of age. For lambs younger than 5 days of age, cause of death could not be identified in 64.5%; but dystocia was also determined as a cause of death in only 6.4%. Birth weights of lambs were not available, but average weight of lambs with meconium aspiration and dying pre-partum to a few hours after birth was 3.0 kg. However, this might not be representative of birth weight in meconium-aspirated lambs because it was calculated from only eight lambs. Meconium aspiration syndrome is a well-documented cause of death in neonatal animals - it is an abnormal consequence of prolonged parturition with a consequent release of meconium into the amniotic sac that is triggered by prenatal hypoxia (Gooding et al., 1971; Amir et al., 1999). Induced meconium-aspirated lambs have been used as a model for the development of human medicine for treating meconium aspiration (Cuesta et al., 1998; Lakshminrusimha et al., 2015), but population-based incidence has not been reported for sheep. A retrospective study reported that 34.6% of calves dying within the first 14 days after birth had aspirated meconium in their lung tissues (Lopez and Bildfell, 1992). Meconium aspiration was found in 38.9% of aborted fetuses and stillbirths in the present study.

2.5.3 Primary cause of death

Infection was the most common cause of mortality identified in the present study (41.1% of the lambs born alive). Infection was also the sole cause of death in submitted abortions. Huffman et al. (1985) reported 28.3% of lamb deaths were caused by infection, and analysis of Canadian diagnostic laboratory data (Dohoo et al., 1985) also reported that overall, the main cause of death in lambs < 6 months of age was infectious diseases. When age at death was taken into account, non-infectious causes were more frequent than infectious causes in lambs dying pre-partum until 7 days after birth. In contrast, infectious causes were more frequent in lambs older than 7 days (Table 2.3). The former finding does not concur with Fragkou et al. (2010) who state that infectious and non-infectious causes play an equal role in deaths of lambs born alive but dying during the first 3 days of life. In the present study, trauma, dystocia and congenital defects were responsible for most non-infectious losses during the first 3 days. The differences in study findings probably resulted from different breeds and peri-parturient management affecting the frequency of dystocia-related deaths in the two study populations.

The gastrointestinal system was the most common system associated with infectious death, responsible for death in 15.3% (59/385) of total lambs submitted or 16.1% (59/367) of lambs born alive. Dohoo et al. (1985) reported that 8.1% and 9.8% of lambs died due to coccidiosis and gastrointestinal nematodes, respectively. Some producers attempt to diagnose these problems themselves, and better control measures are currently available. These may explain, in part, the lower proportions of diagnoses in these categories in the present study (6.5% and 1.1%, respectively). *Clostridium* spp. was another pathogen causing gastrointestinal infections leading to mortality; it appeared to be the primary cause of death in 4.6% of born-alive lambs included in the present study.

Pneumonia was identified in 13.9% of lambs sent to laboratories in Canada (Dohoo et al., 1985). Similarly, in the present study, pneumonia was the primary cause of death in 11.4% of the submissions. *Pasteurella* spp. and *Mannheimia haemolytica* were the most frequent pathogens found in pneumonia cases, accounting for more than half of pneumonia submissions in both studies.

Starvation was responsible for 6.6% of lamb carcasses submitted for post-mortem examination in the study of Dohoo et al. (1985), while only 2.7% of submitted deaths in the present study were attributed to this cause. According to Green and

Morgan (1993), starvation was the main cause of death during the first week of life in lambs. However, in the present study, 6 out of 10 lambs that died from starvation were older than 10 days of age. All losses due to starvation were found between January and June. Possible explanations are many, but might include a high incidence of mismothering due to large litter size, and/or inadequate nutrition of ewes in winter months leading to insufficient colostrum and milk production (Mellor and Murray, 1985). Proportion of trauma-associated mortality was lower in lambs older than 7 days of age compared to neonatal lambs in the present study, which might be due to older lambs being strong enough to escape from some types of trauma, such as being laid on by the ewe, which was reported as a common cause of trauma during the early period of life (Green and Morgan, 1993).

Congenital abnormalities in the cardiovascular system, such as an atrioventricular septal defect, patent foramen ovale and persistent ductus arteriosus, were not uncommonly found in the present study. This finding was consistent with the report of Green and Morgan (1994). Other systems affected by congenital defects included the musculoskeletal, gastrointestinal, urinary and neurological systems. The abnormalities found included arthrogryposis, body wall defect, diaphragmatic hernia, atresia ani, megaesophagus, megacolon, ectopic urethra, cerebrum herniation and spina bifida. Most lambs with these congenital defects could survive for more than one day. One lamb with the persistent ductus arteriosus died on its 68th day. Another study reported that lambs with congenital defects were more likely to die within a couple of days (Christley et al., 2003); however the most frequent congenital abnormalities found in that study were in the central nervous system, which could be more critical to life than certain cardiovascular defects. On the other hand, lambs have been reported to survive for more than three weeks in another study in which heart defects were the most common defects found (Green and Morgan, 1994). Severity of defects would dictate survival durations.

Deaths attributed to nutritionally-related diseases, such as NMD, intestinal volvulus, ruminal acidosis and urinary calculi, were rarely found in lambs younger than 48 hours in the present study, perhaps because neonatal lambs mainly depend on suckled colostrum, and milk (or colostrum) aspiration was dealt with as a separate mortality category. Dystocia-related deaths were mostly found in lambs dying pre-partum to a few hours after birth. It was not found to be a mortality cause in lambs dying on the 3rd day and later; however, this should not be interpreted to mean that the sequelae of dystocia can not contribute to deaths in older lambs. Among lambs dying within the first 24 hours, the cause of death in the 3 heaviest lambs was dystocia. A previous study reported that lambing difficulty was also a main cause of death in heavy lambs (Sawalha et al., 2007). Nutritional management in late-gestation ewes is a significant factor for lamb survival associated with birth weight (Russel et al., 1981).

The primary causes of lamb mortality are usually not consistent between flocks, and vary depending on environmental condition and flock management (Nash et al., 1996). The primary cause of mortality could not be determined in almost one third of lambs sent to the laboratory, but finding an etiology depended on the age of the lamb; the percentage of “open diagnoses” lambs was lower in 3 to 90 days of age lambs compared to younger lambs (Table 2.3). The same trend was reported in Arsenault (2002).

2.6 Conclusions

In summary, this study of laboratory submitted lambs provided valuable (albeit biased) information to guide further investigations into lamb mortality in PEI flocks. Prior to this study, there were no data available on causes of lamb mortality in the Maritimes. The value of post-mortem examination as a tool for mortality investigations was confirmed: 71% of submissions resulted in identification of a primary cause of death, with the likelihood of diagnosis being higher as lambs aged. Of 367 submitted lambs that were born alive, respiratory lesions were present in two-thirds. The most frequent primary cause of death was infection, with the two most frequent sites of infection being the gastrointestinal system and then the respiratory systems. The predominance of infection, particularly in the older lamb submissions, deserves further investigation as it may suggest PEI flocks are at higher risk of pathogen challenge and/or have lowered resistance to infection, perhaps because of poor management of transfer of passive immunity, nutrition and/or immunization programs. Stillbirths, infectious abortion and starvation-hypothermia, appeared to be underrepresented compared to data reported from other regions, and the reasons for this finding deserve further investigation.

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Table 2.1 Percentages of lambs within the systems affected by the primary causes of death, by age of lambs at death, for 343 born-alive lambs submitted for post-mortem examination to the Atlantic Veterinary College Diagnostic Services between January 2005 and May 2014.

Systems affected	< 1 day (%)	1-2 days (%)	3-7 days (%)	8-30 days (%)	31-90 days (%)	Overall (%)
Gastrointestinal[1]	2.4	4.6	14.0	33.7	37.0	25.1
Respiratory	0.0	7.0	14.0	7.9	15.0	10.2
Neurological	2.4	2.3	4.6	3.4	8.7	5.2
Musculoskeletal	7.3	9.3	2.3	5.6	1.6	4.4
Cardiovascular	0.0	0.0	4.6	10.1	4.7	5.0
Hepatic	0.0	7.0	7.0	1.1	0.0	2.0
Urinary	0.0	0.0	4.6	1.1	1.6	1.5
Multiple systems (two or more)	29.3	14.0	18.6	19.1	15.7	18.4
Open	58.5	55.8	30.2	18.0	15.7	28.3
Lambs submitted	41	43	43	89	127	343

[1]Involvement of the gastrointestinal system was less apparent in lambs < 3 days compared to older lambs ($p < 0.001$)

Table 2.2 Percentages of lambs within the primary causes of death, by submitted year, for 367 born-alive lambs submitted for post-mortem examination to the Atlantic Veterinary College Diagnostic Services between January 2005 and May 2014.

Causes of death	2005 (%)	2006 (%)	2007 (%)	2008 (%)	2009 (%)	2010 (%)	2011 (%)	2012 (%)	2013 (%)	2014 (%)	Overall (%)
Infection	44.4	18.2	44.0	50.0	32.1	58.6	44.2	57.1	36.2	23.8	41.1
Trauma	0.0	4.6	4.0	3.1	5.7	6.9	2.3	5.4	6.4	14.3	5.7
Congenital defect	0.0	18.2	8.0	3.1	3.8	3.4	4.6	5.4	2.1	0.0	3.8
Starvation	0.0	4.6	8.0	6.2	0.0	3.4	2.3	1.8	0.0	4.8	2.7
Nutritionally-related*	0.0	0.0	8.0	9.4	3.8	13.8	4.6	3.6	19.1	11.9	7.9
Dystocia	0.0	0.0	0.0	9.4	5.7	3.4	0.0	1.8	0.0	0.0	2.2
Milk aspiration	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	4.8	0.8
Other	0.0	13.6	0.0	6.2	11.3	10.4	16.3	5.4	0.0	7.1	7.9
Open	55.6	40.9	24.0	12.5	37.7	0.0	25.6	19.6	36.2	33.3	27.8
Lamb submitted	18	22	25	32	53	29	43	56	47	42	367

*Nutitionally-related diseases included nutritional muscular dytrophy, intestinal volvulus, ruminal acidosis, urinary calculi, etc.

Table 2.3 Percentages of lambs within the primary causes of death, by age of lambs at death, for 343 born-alive lambs submitted for post-mortem examination to the Atlantic Veterinary College Diagnostic Services between January 2005 and May 2014.

Causes of death	< 1 day (%)	1-2 days (%)	3-7 days (%)	8-30 days (%)	31-90 days (%)	Overall (%)
Infection[1]	4.9	16.3	27.9	42.7	62.2	40.2
Nutritionally-related*	2.4	0.0	4.6	12.4	10.2	7.9
Trauma[1]	14.6	9.3	11.6	3.4	0.8	5.5
Congenital defects	0.0	9.3	2.3	7.9	1.6	4.1
Starvation	0.0	0.0	4.6	5.6	2.4	2.9
Dystocia	17.1	2.3	0.0	0.0	0.0	2.3
Milk aspiration	0.0	2.3	4.6	0.0	0.0	0.9
Other causes	2.4	4.7	14.0	9.0	6.3	7.3
Open[2]	58.5	55.8	30.2	19.1	16.5	28.9
Lambs submitted	41	43	43	89	127	343

[1]The proportions of lambs with infection and trauma being identified as the primary causes of death were significantly different in lambs dying after 7 days compared to younger lambs ($p < 0.001$).

[2]The proportions of lambs with no identified cause of death were significantly different in lambs dying after 2 days compared to younger lambs ($p < 0.001$).

*Nutitionally-related diseases included nutritional muscular dystrophy, intestinal volvulus, ruminal acidosis, urinary calculi, etc.

Table 2.4 Descriptive statistics of age of lambs (days) within the primary causes of death for 343 born-alive lambs submitted for post-mortem examination to the Atlantic Veterinary College Diagnostic Services, between January 2005 and May 2014, stratified by primary cause of death.

Causes of death	Minimum (days)	Median (days)	Maximum (days)	Mean (days)	Standard deviation (days)	Number
Nutritionally-related	0.5	30.0	86.0	37.8	23.2	27
Infection	0.0	35.0	90.0	36.8	24.2	138
Starvation	3.0	23.5	90.0	31.9	32.3	10
Other causes	0.5	15.0	90.0	27.1	28.4	25
Congenital defect	1.0	18.0	68.0	19.0	19.2	14
Open	0.0	3.0	90.0	15.5	21.7	99
Trauma	0.0	1.0	49.0	6.5	11.7	19
Milk aspiration	1.0	5.0	5.0	3.7	2.3	3
Dystocia	0.0	0.0	2.0	0.3	0.7	8
Overall	0.0	21.0	90.0	26.3	25.5	343

*Nutitionally-related diseases included nutritional muscular dystrophy, intestinal volvulus, ruminal acidosis, urinary calculi, etc.

Table 2.5 Descriptive statistics of age of lambs (days) dying due to infection for 138 born-alive lambs submitted for post-mortem examination to the Atlantic Veterinary College Diagnostic Services between January 2005 and May 2014, by month of submission.

Months	Minimum (days)	Median (days)	Maximum (days)	Mean (days)	Standard deviation (days)	Number
January	0.0	17.0	90.0	18.6	21.3	20
February	1.0	32.0	50.0	29.0	13.6	33
March	1.0	14.0	79.0	28.0	25.5	21
April	17.0	49.0	81.0	49.8	20.3	13
May	0.5	39.5	90.0	38.9	21.8	14
June	30.0	54.5	81.0	53.9	13.9	16
July	28.0	78.0	90.0	71.6	19.9	10
August	75.0	75.0	75.0	75.0	0.0	2
September	3.0	29.5	56.0	29.5	37.5	2
October	24.0	24.5	25.0	24.5	0.7	2
November	15.0	15.0	15.0	15.0	0.0	1
December	18.0	26.5	42.0	28.2	11.0	4
Overall	0.0	35.0	90.0	36.8	24.2	138

Table 2.6 Percentages of antimicrobial sensitivity test results of *Mannheimia haemolytica* isolates, from 14 born-alive lambs dying due to infection, submitted for post-mortem examination to the Atlantic Veterinary College Diagnostic Services between January 2005 and May 2014, by sensitivity category.

Antibiotics	Sensitive (%)	Intermediate sensitive (%)	Resistant (%)	Number of isolates
Ceftiofur	100	0	0	10
Erythromycin	20	60	20	10
Oxytetracycline	100	0	0	10
Penicillin	100	0	0	10
Streptomycin	40	0	60	10
Trimethoprim-Sulfa	100	0	0	10
Florfenicol	100	0	0	10
Tulathromycin	33	44	22	9
Tilmicosin	50	0	50	2
Overall	74	12	14	81

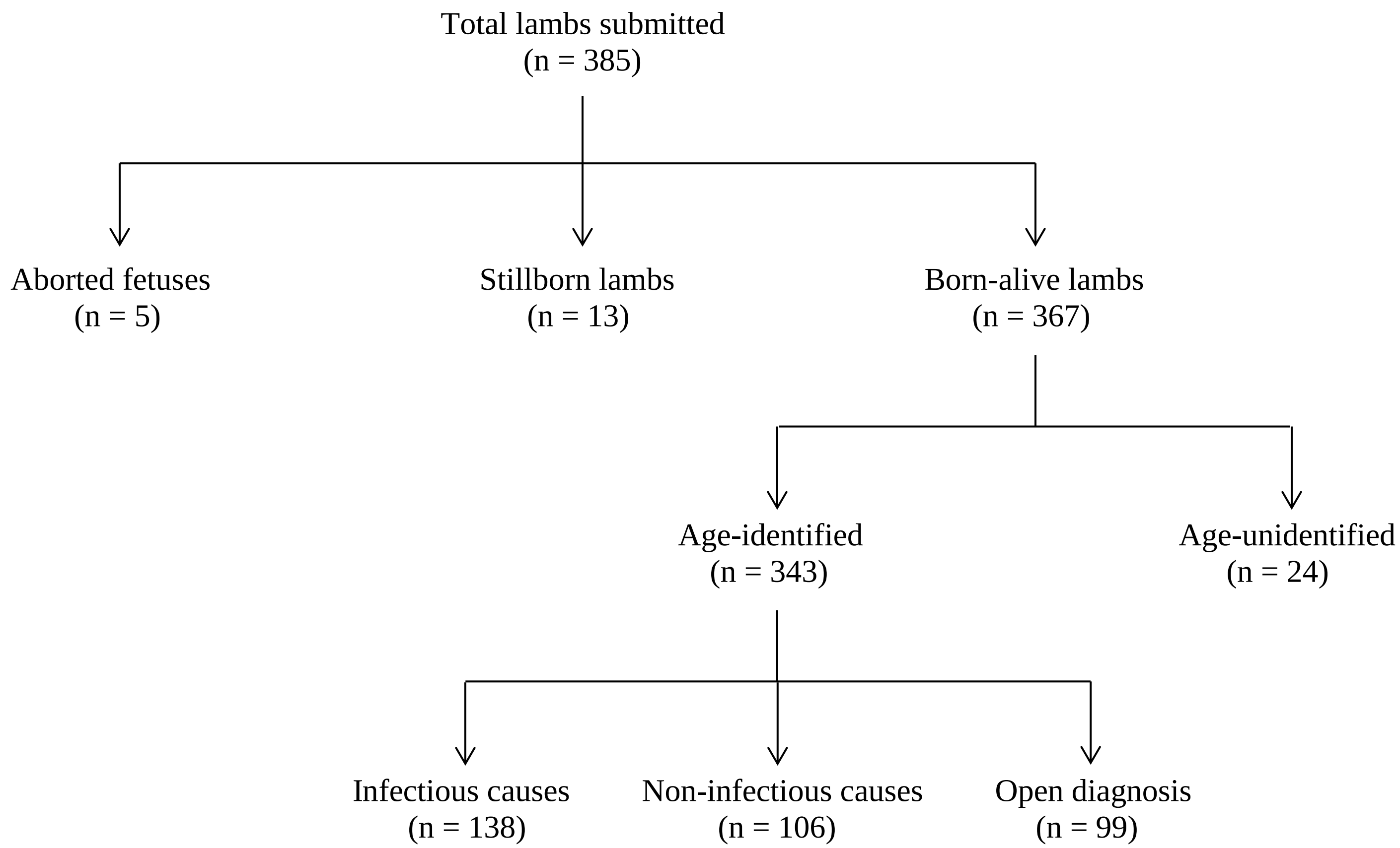

Figure 2.1 Diagram of numbers of lambs submitted for post-mortem examination to the Atlantic Veterinary College Diagnostic Services, between January 2005 and May 2014.

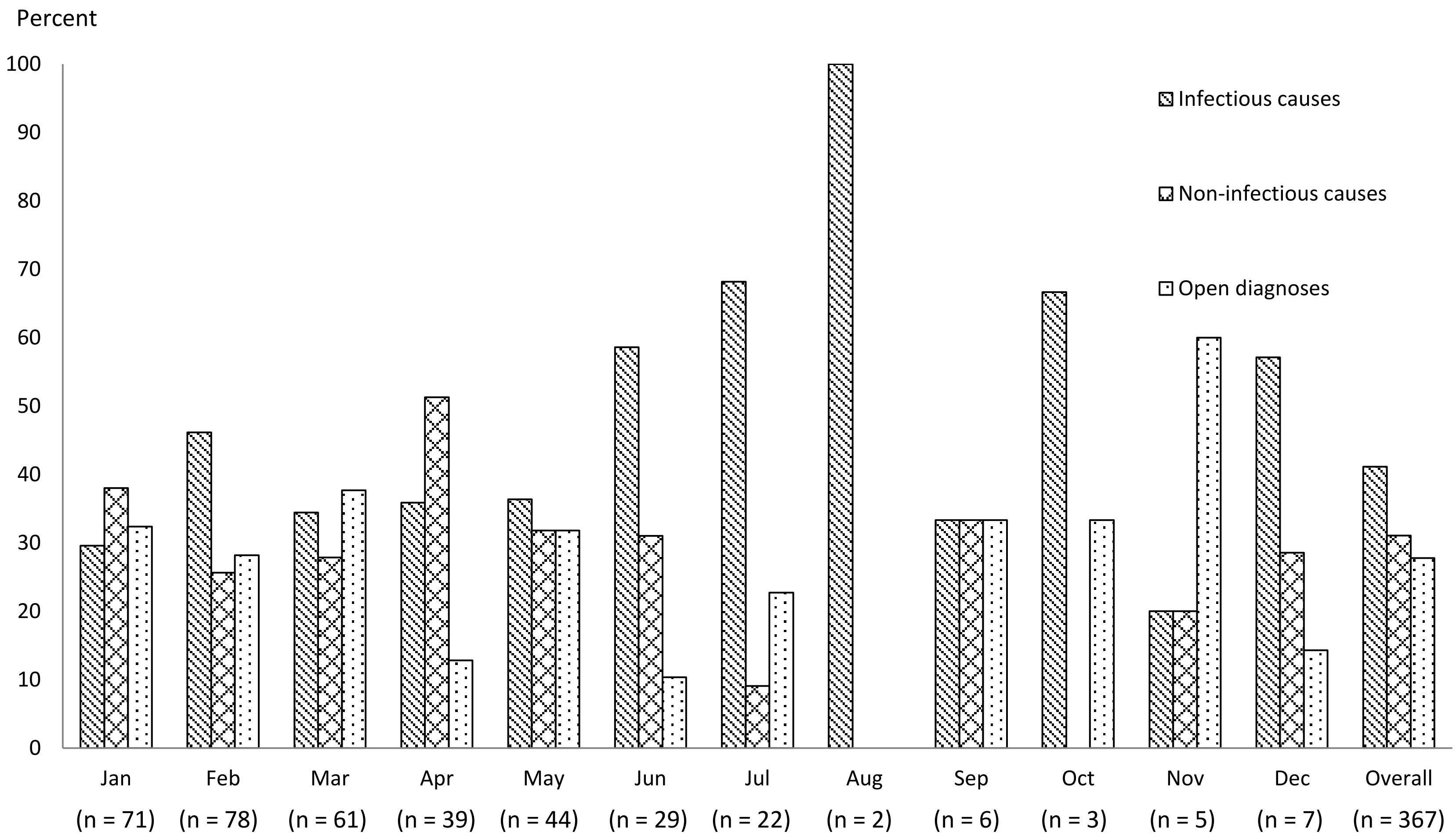

Figure 2.2 Percentages of lambs dying from infectious causes, non-infectious causes and open diagnoses, by calendar month, for 367 born-alive lambs submitted to the Atlantic Veterinary College Diagnostic Services between January and May 2014.

CHAPTER 3

A SURVEY OF LAMBING MANAGEMENT PRACTICES IN PRINCE EDWARD ISLAND FLOCKS IN 2014-2015

3.1 Abstract

Various sheep flock management practices are determinants of lamb mortality. In order to identify the most influential factors affecting lamb mortality, current management practices need to be examined. The objectives of this study were to describe flock characteristics and management practices in PEI sheep flocks, especially practices thought to be associated with lamb mortality. The study was conducted in 51 lambing groups of 37 sheep flocks during 2014 to 2015. Each lambing group was visited twice, at one to three weeks before and after lambing. Management data were collected by questionnaires and flock observations. Pre- and post-partum ewe forages were collected for feed quality analyses. Ten percent of ewes in each group were physically examined. Blood and fecal samples were collected from eight late-gestation ewes in each group. Serum biochemistry profiles, and selenium and vitamin E concentrations were analyzed. Fecal tests were performed to detect helminth eggs. Most PEI sheep flocks were small (mean number of breeding ewes/flock = 89), crossbred flocks. The typical forage used was ensiled forage. An indoor lambing system was used in all participant groups, and most lambed in the natural lambing season. Nutrients available in rations offered to ewes were often insufficient to meet their demands. In particular, supplementation of selenium and vitamin E should have been provided where the rations contained inadequate concentrations (94% of groups). Helminth and coccidia control programs were often (57% of flocks) inappropriate to maintain good health status. Navels of newborn lambs were often (38% of flocks) not disinfected, and weak lambs tended to receive inadequate colostrum supplementation (< 100 ml of colostrum at first

feeding; 65%). These inadequate health statuses and improper management practices should be modified.

3.2 Introduction

Sheep producers expect a high level of lamb survival and performance in order to improve profit margins. Flock management and environmental factors, and their associations with level of lamb survival, have been examined in various countries (Chaarani et al., 1991; Southey et al., 2001; Binns et al., 2002; Sawalha et al., 2007). For example, ewe replacement practices and nutrition of replacement ewe lambs have been shown to influence the risk of dystocia-associated lamb mortality (Haughey, 1991; Hamilton Prime Lamb Breeders, 2005). Lamb survivability is related to health status of ewes (Christley et al., 2003); optimal feeding of ewes before breeding and during pregnancy, especially during the last trimester, has positively influenced lamb survival (Binns et al., 2002; Thompson, 2011; Dwyer et al., 2016). The physiologic requirements of pregnant ewes depend on litter size; adjustment of the ration for the number of fetuses can reduce lamb losses (Dwyer et al., 2016). Environmental conditions such as ambient temperature and presence of pathogens at the time of lambing influence mortality by hypothermia and infectious diseases (Bird et al., 1984; Chniter et al., 2013). Furthermore, supervision of the lambing flock and timeliness of assistance are other factors influencing lamb survival (Binns et al., 2002). Feeding management practices for neonatal lambs, particularly colostrum and milk consumption, are closely associated with lamb morbidity and mortality (Mandal et al., 2007). Management of rams also has some relationships to lamb survival, in particular the effects of genetic selection and breeding management on lambing ease (Haughey, 1991; Hamilton Prime Lamb Breeders, 2005).

Because management practices employed in flocks are largely decided by the sheep producers themselves, the characteristics of the producers, such as their knowledge and attitudes, indirectly affect lamb survival (Willock et al., 1999). However, the risk of lamb survival, and factors related to lamb survival, differ greatly by flock; certain management strategies may be useful in some flocks but not in others. Improving lamb survival requires that the causes of lamb mortality be identified and appropriate management strategies put into place (Elliott et al., 2011; Dwyer et al., 2016). Good record keeping and a qualified flock advisor are appropriate strategies for increasing productivity of all flocks (Dwyer et al., 2016).

To date, there has been no comprehensive study of lambing management conducted in Atlantic Canadian flocks. Sheep production systems employed by producers in Prince Edward Island (PEI) can be considered typical of the Atlantic region (PEI Department of Agriculture, 2015). According to Statistics Canada (January 1, 2015), there were 3800 adult breeding sheep in PEI. The number of lambs marketed increased from 900 head in 2004 to 2400 head in 2014 (Department of Agriculture and Fisheries, PEI, 2015). Sheep producers have identified unacceptably high lamb mortality as a significant concern impeding the profitability of the industry. In order to identify the most influential factors affecting lamb mortality, current management practices need to be examined. The objectives of this study were to describe flock characteristics and management practices in sheep flocks in PEI, especially those practices previously identified in the literature as associated with lamb survival in other lamb producing regions.

3.3 Materials and methods

3.3.1 Flocks

The sheep flocks used in this study were sampled from the target population, that being flocks located in PEI. Inclusion criteria were flock size (at least 12 breeding ewes) breed (use at least 1 meat breed in their genetic program). To identify volunteer flocks for participation in this study, a brief survey was included in a mail-out to all sheep producers listed in the database maintained by the PEI Department of Agriculture and Fisheries (n = 100). A 23% response rate was obtained, yielding 23 flocks willing to cooperate. A further 19 producers, who were clients of the Atlantic Veterinary College, and who did not already respond to the survey questionnaire, were contacted via telephone and/or e-mail. Of the 42 flocks where contact was made, 33 met the selection criteria and were willing to participate. Another 4 participant flocks were obtained from snowball sampling, giving a final total of 37 participating flocks. For flocks that had > 1 distinct lambing period between September 2014 and August 2015, each lambing period was considered a unique lambing group for the purposes of this study.

3.3.2 Management practices

Each flock was visited two times per lambing group. Visits were scheduled to be one to three weeks before the expected date of the start of lambing, and again two to four weeks after the first lambing occurred for the respective lambing group. During each visit, an in-person questionnaire and a flock visit form were completed.

The questionnaire for the first visit was composed of 101 questions. It was divided into sections including characteristics of the principal manager, flock characteristics, housing, and management. Questions relating to management were grouped as follows: waste, feed and water, preventative health and biosecurity, breeding, late-gestation ewes, lambing ewes, neonatal lambs, rams, and flock performances during the last three years. Seven questions about lactating ewes and lamb management were asked during the second visit. The questionnaires were pre-tested by a focus group of three experienced sheep producers and three expert advisors.

Some management practices and environmental conditions were observed and recorded during each visit, including stocking density, trough management and visual assessment of forage. Stocking density was classified into low, medium or high by visual inspection, comparing estimated space available with space recommended for a 60-90 kg live weight ewe (1.2-1.4 m^2; Defra, 2003). Linear bunk space for a ewe was considered adequate if it was > 33 cm (Nagy and Pugh, 2012). The principal investigator and two trained research assistants completed all forms.

3.3.3 Forage composition

Forages fed to late-gestation ewes were sampled using an electric powered core sampler, taking at least three core samples from the next two to three bales to be fed to the lambing group, and thoroughly mixing the samples prior to submission. Forage fed to post-partum ewes was sampled if it was not the same as forage used pre-partum. Forage samples were submitted to the PEI Analytical Laboratories, Department of Agriculture, for routine feed quality analyses. Forage nutritional value was classified as adequate if crude protein was > 9% and total digestible nutrients was > 55%. The latter breakpoint was inferred using the following rationale: where forage total digestible nutrients is < 55%, the energy requirements of gestational and lactating ewes cannot be easily met without feeding > 50% of ration dry matter in the form of a grain (Saskatchewan Sheep Development Board, n.d.). We classified ensiled forage pH as acceptable where pH < 4.5, and acid detergent insoluble protein was ≤ 12% of crude protein (Schroeder, 2012). Copper concentration of forages was considered adequate to meet requirements if it was > 4.3 ppm dry matter (Underwood and Suttle, 1999).

3.3.4 Late-gestation ewe health

Convenience sampling was used to sample late-gestation ewes in each lambing group. Ten percent of flocks > 80 ewes, or at least 8 ewes in flocks ≤ 80 ewes, were restrained for physical examination, including body condition scoring (BCS), mucus membrane color to estimate level of anemia (FAMACHA© score), cleanliness scoring (fecal soiling on udders and legs), incisor abnormalities, and udder pathology. The five-point scaling system of BCS (Russel et al., 1969) (1= extremely thin; 5 = fat), with half scores, was used to assign a BCS score to late-gestation ewes. The five-point scaling system of FAMACHA© score (Bath et al., 2001) (1 = red; 5 = white) and four-point hygiene scale adapted from the score used in dairy cows (Schreiner and Ruegg, 2002) (1 = very little soiled; 4 = completely soiled) were used. Udders of ewes were examined by palpation. A sample of eight ewes from each group was held for blood and fecal collection – these were systematically random sampled from the ewes selected for physical examination. Blood samples were collected from the jugular vein of ewes and stored in vacuum tubes with a clot activator for serum sample separation (BD

Vacutainer SST Venous Blood Collection Tube, Serum Tube/Transport Tube, Clot Activator/Gel, 16 x 100 mm, 8.5 mL, Conventional Closure, Plastic Tube). The samples were centrifuged at 2500 revolutions per minute for 10 minutes and were then kept in 2 microcentrifuge tubes. One of the duplicate serum samples was stored at -20 °C for serum biochemistry and selenium analyses, and the other at -80 °C for vitamin E analysis, until all samples were taken and the analyses were performed. Fecal samples were taken directly from the rectum using gloved fingers and lubricant, and kept in the refrigerator until examination.

The serum samples kept at both -20 °C and -80 °C were submitted to the Animal Health Laboratory, Laboratory Services Division, University of Guelph, for ovine serum biochemistry profiles using the Cobas® 6000 c501 analyzer (Roche Diagnostics). Serum selenium and vitamin E were analyzed using inductively coupled plasma mass spectrometry analysis and high performance liquid chromatography, respectively, following the standard operating procedure of the Animal Health Laboratory, University of Guelph. The fecal samples were submitted to the Parasitology Laboratory, Atlantic Veterinary College Diagnostic Services, and examined within three days. A simple fecal flotation was performed, and each sample was classified as positive if at least one helminth egg was detected. A subsequent McMaster fecal egg count was performed on positive samples, and results were reported as total eggs per gram (epg) of feces (the lower detection level is 50 epg). Helminth eggs were classified by genus or species, as appropriate. All parasitological procedures were based on Zajac and Conboy (2011).

3.3.5 Data analysis

The study protocol was reviewed and approved by the University of PEI Animal Care Committee (14-005 (6005670), September 2014 to August 2015). Epi Data™ 7 (CDC, Atlanta, GE, US) was used for data entry. Data were then imported into Stata 13 (StataCorp LP, College Station TX, US). Descriptive statistics were calculated. Numbers of producers responding to the questions or numbers of flocks observed with a certain management practice were used as denominators for flock-level data, while numbers of lambing groups were used as denominators for group-level data. Unless otherwise indicated, the central tendency of a continuous measure is presented in the following format: mean (range) unit.

Pearson's correlation coefficients between energy-related serum biochemical substances (e.g. glucose, cholesterol) at the ewe level and lambing group level were calculated. Prevalence of intestinal helminth infection of each group was calculated. The Mann-Whitney test was performed to compare the fecal egg counts and prevalence of helminth infection in partially and totally confined lambing groups. Chi-square test was performed to evaluate the association between FAMACHA© score ≥ 3 and serum total protein < 60 g/L. The associations between presence of Trichostrongyle eggs and FAMACHA© score ≥ 3 was also evaluated using the Chi-square test. The Student's t-test was used to evaluate the association between the presence of Trichostrongyle eggs and serum total protein concentration.

3.4 Results

There were 51 lambing groups in 37 cooperating sheep flocks. These lambing groups lambed in different flocks or in the same flock but during different periods during the period of September 2014 to August 2015. Most flocks (67.6%, 25/37) had only 1 lambing group during the year-long study. Ten (27.0%) and 2 (5.4%) flocks had 2 and 3 lambing groups in the study period, respectively. Out of 36 flocks having at least 1 lambing group in the previous year, proportions of flocks having 1, 2, 3 and 4 distinct lambing groups in the previous year were 44.4% (16/36), 44.4% (16/36), 8.3% (3/36) and 2.8% (1/36), respectively, showing that fewer flocks had multiple lambing groups the study year compared to the previous year. One flock was lost to follow-up; this flock reported having two lambing groups in the study period but the producer could not be contacted for the second and subsequent visits. Thus, only data from the first visit of the first lambing group were included in this study.

3.4.1 Characteristics of the principal flock manager

There were more male principal managers (59.4%, 22/37) than female, and 54.0% (20/37) were older than 50 years of age. Proportions of managers in the age range of 21-30, 31-40 and 41-50 years old were 13.5% (5/37), 13.5% (5/37) and 18.9% (7/37), respectively. Around one-fourth of managers (24.3%, 9/37) had a post secondary-school degree or certificate in agriculture. Seventy percent owned other agricultural enterprises. Almost half of the managers (48.6%, 18/37) had more than 10 years of experience in sheep farming, with 16.2% (6/37), 27.0% (10/37) and 8.1% (3/37) having 6-10 years, 3-5 years and 0-2 years of experience, respectively. Only 10.8% (4/37) of study producers hired temporary workers.

3.4.2 Flock characteristics

Mean flock size was 89 (13-250) and 4 (1-10) for breeding ewes and rams, respectively. In total, there were 3294 ewes and 135 rams in the study flocks. Three flocks (8.1%) had only 1 breed (Hampshire, Suffolk and Black Welsh Mountain). The other 34 flocks incorporated 2 or more of the following breeds: Suffolk, Dorset, Canadian Arcott, Rideau, Texel, Cheviot, Hampshire, Ile de France, Border Leicester, Corriedale, Finnsheep, Romanov, Shetland and Southdown. All of the study producers sold market lambs. About one-third (35.1%, 13/37) and one-fifth (21.6%, 8/37) cited fleece and breeding stock, respectively, as meaningful sources of income. Other sources of income cited were milk (8.1%, 3/37) and hide (2.7%, 1/37). The proportions of groups lambing in autumn, winter and spring were 23.5% (12/51), 41.2% (21/51) and 35.3% (18/51), respectively. The number of lambing groups in each month during the study period, September 2014 to May 2015, is shown in Fig. 3.1. Most flocks had a lambing period during January to May.

3.4.3 Housing and waste management

Sixty-five percent (24/37) of study producers allowed their ewes to graze pasture at some point each year. The proportions of flocks having concrete flooring in the lambing area, claiming pen area, and mixing pen area were 48.6% (18/37), 51.4% (19/37) and 51.4% (19/37), respectively. Dirt floors were the most common alternative to concrete. Most producers (86.5%, 32/37) cleaned lambing areas between lambing groups. Bedding addition only, manure removal only, manure removal with bedding addition, and manure removal with addition of disinfectant were implemented in 31.2% (10/32), 12.5% (4/32), 34.4% (11/32) and 21.9% (7/32) of study flocks where the lambing areas were cleaned between lambing groups. Calcium oxide (lime) was the disinfectant of choice used in every flock where a disinfectant was used in the lambing area. Manure was removed from barns from once a year to once a week, with the mean frequency of removal being six times per year. Out of the 37 study flocks, 8 had mechanical ventilation systems in their barns. Ewes of 21.6% (8/37) of flocks were allowed access to outdoor loafing areas during the indoor period. Out of 50 lambing groups observed, 38 were kept at medium stocking density, whereas 7 and 5 groups were kept at low and high stocking densities, respectively.

3.4.4 Feeding management

More than 80% (30/37) of producers fed round bale ensiled forage to their late-gestation ewes. None were using bunker ensiled forage. Nineteen percent (7/37) offered only hay, while 13.5% (5/37) offered both ensiled forage and hay. Proportions of producers offering forage once a day, twice a day, more than twice a day, and free-choice were 5.4% (2/37), 40.5% (15/37), 10.8% (4/37) and 43.2% (16/37), respectively. Free-choice means that forage was immediately set out when the ewes had finished what was provided. None of the late-gestation ewes in any study flock were given access to pasture. The mean linear bunk space per ewe was 37 cm, ranging from 10 to 64 cm. Two-thirds (25/37) of producers fed farm-grown grain(s) to late-gestation ewes, whereas 2.7% (1/37) and 29.7% (11/37) used a commercial feed, and a farm-grown grain(s) with a commercial feed, respectively. Amounts of grain fed to late-gestation ewes from 6 weeks before lambing to the time of lambing are shown in Table 3.1. The amount at lambing was approximately two times the amount fed at six weeks before lambing. There was one group of late-gestation ewes that did not receive grain during this period.

Almost half (45.9%, 17/37) of study producers offered supplementary protein to their late-gestation ewes, such as 36% protein pellet and soybean meal. The mean amount of protein supplement fed was 0.25 (0.02-0.68) kg per ewe per day. One producer (2.7%) adjusted the late-gestation ewe ration according to the number of fetuses, while 21.6% (8/37) grouped their late-gestation ewes, and fed them according to BCS. Late-gestation ewes of all flocks were offered a mineral supplement. The proportions of producers using a loose mineral, a salt block with cobalt-iodine, and a salt block with multiple trace elements to late-gestation ewes was 83.8% (31/37), 67.6% (25/37), and 5.4% (2/37), respectively. Other supplements, such as plain white salt, were used in 10.8% (4/37) of flocks. Most producers (89.2%, 33/37) offered a mineral supplement free-choice to late-gestation ewes, whereas 8.1% (3/37) and 2.7% (1/37) offered a mineral supplement daily and weekly, respectively.

Water was provided to late-gestation ewes by trough, bucket, and nipple or automatic watering system in 51.4% (19/37), 27.0% (10/37) and 21.6% (8/37) of study flocks, respectively. Water containers were cleaned once a day, more than once a week, once a week, and less than once a month in 35.1% (13/37), 13.5% (5/37), 24.3% (9/37) and 10.8% (4/37) of flocks, respectively. Some producers (16.2%, 6/37) cleaned water containers based on degree of soilage.

There was variability in feeds provided to post-partum ewes. Regarding forages, 75% (27/36) of observed flocks received only ensiled forage, whereas 16.7% (6/36), 5.6% (2/36) and 2.8% (1/36) were offered only hay, hay with ensiled forage, and hay together with access to grazing, respectively. Forage was set out in the feeders once, twice, more than twice a day, and free-choice in 5.6% (2/36), 50.0% (18/36), 16.7% (6/36) and 27.8% (10/36) of study flocks, respectively. Almost all flocks (97.2%, 35/36) were fed grain daily. The mean amount of grain fed was 0.77 (0.00-1.80) kg per head per day. Farm-grown grain(s), commercial feed, and farm-grown grain(s) combined with a commercial feed were used in 61.1% (22/36), 8.3% (3/36) and 30.6% (11/36) of flocks, respectively. Mineral was supplemented in 94.4% (34/36) of flocks. Most minerals supplemented were offered free-choice (83.3%, 30/36), while others offered the mineral daily. Loose mineral and a cobalt-iodine salt block were used in 77.8% (28/36) and 33.3% (12/36) of flocks, respectively.

3.4.5 Forage composition

Forty-four crops of round bale ensiled forage and seven crops of hay were sampled during the first visits. During the second visits, a further 8 crops of round bale ensiled forage and 3 crops of hay were sampled from 11 lambing groups where forage had been changed. None of the forages were chopped. None of the samples were excessively contaminated with weeds, mold, soil or other pests, and all had a normal odour. The nutritional values of the forages offered to the late-gestation ewes are shown in Table 3.2. The nutritive values of lactating ewe forages (43 ensiled forages and 8 hay samples) were similar to late-gestation ewe forages. Two percent (3/102) of forage samples contained < 55% total digestible nutrients, and 13.7% (14/102) of forage samples had < 9% crude protein - 64.3% (9/14) of the low protein samples were hay samples. Forty-two percent (36/86) of ensiled forage samples had an acid detergent insoluble (or heat-damaged) protein > 12% of crude protein. Almost all (96.5%, 81/86) ensiled forage samples had a pH $\geq$ 4.5.

The copper concentration in forages showed great variation, with 24.5% (25/102) of forage samples having a copper concentration below that considered adequate to meet requirements (4.3-28.4 ppm dry matter; Underwood and Suttle, 1999).

3.4.6 Biosecurity

Most producers introduced 1 or more new sheep to the flock over the previous 3 years (86.5%, 32/37) while 24.3% (9/37) of producers introduced only rams. Twenty-three out of 32 producers introducing new animals reported enquiring about the health status of the flock of origin prior to purchase. Diseases of concern to producers when introducing new animals included the following: footrot, Maedi-visna, scrapie, caseous lymphadenitis, genetic defects, gastrointestinal parasites, external parasites and abortion diseases. Seventy-eight percent (25/32) of producers reported isolating purchased animals for some period after arrival to the flock. The duration of isolation ranged from 14 to 60 days, the mean being 28 days. Most producers (64.9%, 24/37) isolated only some sick sheep, while 29.7% (11/37) isolated every case; 5.4% (2/37) did not isolate any sick sheep. Five flocks (13.5%) had a visitor restriction policy. Most producers (64.9%, 24/37) removed the placenta(s) from the lambing area soon after birth.

Many producers farmed other livestock that had opportunity to contact the sheep flock, specifically cattle (24.3%, 9/37), poultry (16.2%, 6/37), goats (13.5%, 5/37), horses (5.4%, 2/37) and llamas (2.7%, 1/37). A small proportion of producers (10.8%, 4/37) reported that they co-grazed their sheep with farm animals from other flocks. Sheep were co-grazed with cattle, goats and both cattle and goats in two, one and one flock, respectively. Dogs, rodents, and cats were animals commonly in-contact with sheep in 78.4% (29/37), 64.9% (24/37) and 54.0% (20/37) of study flocks, respectively.

3.4.7 Ram management

Six producers (16.2%) shared their rams with other flocks. Breeding soundness examination of rams was not performed in any flock in the study. However, 59.4% (22/37) of producers increased energy and protein density in the ram ration before breeding season. Ram rotation within the breeding group was performed in 7 flocks, with rotation period ranging from 4 to 60 days. Rams in 13 out of 51 breeding groups were continuously left with the ewes after the first exposure.

3.4.8 Breeding and late-gestation management

The first quartile, median and third quartile of reported ages of ewes at first breeding were 8, 8 and 12 months (range: 6-24 months). The first quartile, median and third quartile of reported weights of ewes at first breeding were 45, 50 and 54 kg (range: 32-68). Flushing (offering a ration high in protein and energy for a few weeks prior to breeding) was implemented in 83.8% (31/37) of study flocks. Almost half (45.9%, 17/37) of producers bred their first-time breeders separately from older ewes. There were 25 and 2 groups containing only ewes and ewe lambs, respectively; whereas, 24 groups contained both ewes and ewe lambs. The mean numbers of ewes and ewe lambs bred in each breeding group were 56 (7-170) and 15 (1-66), respectively. The mean total number of ewes bred in each group was 62 (10-180).

Eleven of 51 breeding groups were subjected to some form of estrous induction prior to breeding. Most (54.5%, 6/11) were induced into estrous during the anovulatory season; 36.4% (4/11), and 9.1% (1/11) of these breeding groups were induced during the transition period and during the ovulatory season, respectively. Seven of 11 estrous-induced groups were induced with intra-vaginal progesterone (CIDR 330, Zoetis), whereas photoperiod control, oral progestagen (melengesterol acetate), light control with oral progestagen, and intra-vaginal progesterone with injectable prostaglandin were each used in one breeding group.

The mean ewe-to-ram ratio of the most recent breeding season of 51 groups was 23:1 (5:1 to 100:1). Seventy percent of breeding groups (36/51) used a single-sire mating system, and 29.4% (15/51) were bred using a group mating system (> 1 ram placed with a group of ewes). Marking harnesses were used on rams in 70.6% (36/51) of breeding groups.

The first quartile, median and third quartile of length of the breeding period were 42, 60 and 150 days (range: 6-150 days). There were 13 groups in which rams were left with ewes until lambing. The first quartile, median and third quartile of length of the breeding period were 40, 60 and 60 days (range: 6-120), respectively, if those groups always containing rams were excluded.

Forty-eight percent (24/50) of groups of late-gestation ewes were not subdivided. Other groups of late-gestation ewes were subdivided based on breeding subgroup (20.0%, 10/50), age (14.0%, 7/50), BCS (6.0%, 3/50) and expected date of lambing (2.0%, 1/50). Pregnancy diagnosis was performed in every ewe in 13.5% (5/37), and in some ewes in 5.4% (2/37) of study flocks. In one flock scanning to determine fetal numbers was also performed. The mean average 3-year pregnancy rate reported by flocks performing pregnancy diagnosis was 86.2% (73%-95%).

The mean proportion of ewes reported to have aborted annually over the past 3 years was 1.9% (0%-7.5%). The proportion of producers that sheared and crutched their late-gestation ewes in preparation for lambing was 45.9% (17/37) and 8.1% (3/37), respectively. The proportions of producers that checked for mastitis using visual, manual and milk inspection before lambing, at lambing, and at weaning are displayed in Table 3.3. Udder health was checked at lambing in almost all flocks. Thirty-two percent (12/37) of producers in the study maintained a separate group of close-up ewes (i.e. those ewes considered very close to lambing).

3.4.9 Health management

Proportions of flocks using a killed multi-valent clostridial vaccine in ewes, rams and lambs were 64.9% (24/37), 48.6% (18/37) and 40.5% (15/37), respectively. Proportions of producers using a killed single-valent *Corynebacterium pseudotuberculosis* vaccine in ewes, rams and lambs were 16.2% (6/37), 10.8% (4/37) and 13.5% (5/37), respectively. About one-third (35.1%, 13/37) of producers did not vaccinate their ewes at all, while 48.6% (18/37) and 59.4% (22/37) did not vaccinate rams and lambs, respectively. Almost all (95.8%, 23/24) of the producers vaccinating ewes for clostridia injected the vaccine between 2 weeks and 1 month before the expected start of the lambing period. No producers used a vaccine against abortion pathogens such as *Chlamydia abortus* and *Campylobacter* spp., the only other vaccine category currently licensed for use in sheep in Canada.

Selenium and vitamin E injectable supplements were administered to ewes, rams and lambs in 18.9% (7/37), 8.1% (3/37) and 78.4% (29/37) of participant flocks, respectively. For the 27 producers who answered the question about age of lambs when the selenium and vitamin E supplemetation was delivered, 25.9% (7/27) gave the injection soon after birth, while 18.5% (5/27), 22.2% (6/27), 18.5% (5/27), 11.1% (3/27) and 3.7% (1/27) of the producers administered the injection at 1, 2, 3, 4-7 and 30 days of age, respectively. Coccidiostats, including monensin, lasalocid and decoquinate, were provided to ewes, rams and lambs in 18.9% (7/37), 5.4% (2/37) and 43.2% (16/37) of study flocks, respectively. Vitamin A and D injectable products were administered to ewes and lambs in 2.7% (1/37) and 8.1% (3/37) of flocks, respectively. In ewes, vitamin B complex injections were given in 2.7% (1/37) of flocks. In lambs, chlortetracycline (2.7% of flocks, 1/37) and a probiotic (2.7% of flocks, 1/37) were incorporated into creep feed (supplemental feed).

Macrocyclic lactones (ivermectin, doramectin and moxidectin) were used for deworming ewes and rams in more than half of study flocks (59.4%, 22/37 and 54.0%, 20/37, respectively). This anthelmintic family was used in lambs in 29.7% (11/37) of study flocks. Benzimidazoles, including fenbendazole and albendazole, were used in 35.1% (13/37), 27.0% (10/37) and 29.7% (11/37) of flocks for ewes, rams and lambs, respectively. Levamisole was used for ewes and lambs in 5.4% (2/37) and 2.7% (1/37) of flocks, respectively, whereas levamisole was not used in rams from any flock. One producer used organic products (diatomaceous earth and minced garlic) for deworming. Some producers had not dewormed their ewes (24.3%, 9/37), rams (32.4%, 12/37) and lambs (43.2%, 16/37) anytime in the past 3 years. Thirty-nine percent (11/28) of the producers that dewormed ewes did so once a year. Eleven percent (3/28) and 7.1% (2/28) of producers treated their ewes 2 times a year, and 3 times a year, respectively, while 42.9% (12/28) of treating producers dewormed their flocks when they perceived a need to do so, based on observation of the ewes, or veterinary advice.

Thirteen producers who routinely dewormed their ewes did so based on pasture availability: eight flocks were dewormed prior to coming off pasture. Four flocks were dewormed while on pasture. One flock was dewormed before being turned out onto pasture. Three, two, one and one producer dewormed ewes before breeding, before lambing, after lambing and after lambs were weaned, respectively. In 25 flocks in which rams were dewormed, 28.0% (7/25), 16.0% (4/25) and 8.0% (2/25) were treated with an anthelmintic 1, 2, and 3 times a year, respectively. Forty-four percent (11/25) of producers dewormed their rams when they perceived a need to do so.

Ten of 21 producers (47.6%) deworming their lambs treated them once a year, while 9.5% (2/21) and 42.9% (9/21) of these producers treated their lambs 3 times a year, and when they perceived a need to do so based on observation or veterinary advice, respectively. More than one-third (35.1%, 13/37) of study producers suspected their sheep carried anthelmintic resistant helminths. Macrocyclic lactone, benzimidazoles and levamisole resistance were suspected in 69.2% (9/13), 46.2% (6/13) and 7.7% (1/13) of the suspected flocks, respectively.

External parasite control products were used in 64.9% (24/37) of study flocks, however, only two producers used products other than macrocyclic lactones (e.g. permethrin).

More than 94% (34/36) of producers trimmed hooves of their sheep; 61.8% (21/34), 17.6% (6/34) and 20.6% (7/34) reported trimming once a year, twice a year, and when they perceived a need to do so. Approaches used for foot problem prophylaxis and/or treatment in study flocks were antimicrobial drugs (10.8%, 4/37), zinc sulphate footbath (10.8%, 4/37), provision of dry housing surfaces (8.1%, 3/37), copper sulphate footbath (5.4%, 2/37) and genetic selection (5.4%, 2/37).

Common problems of ewes reported by study flock managers with at least 1 year of sheep farming were mastitis, pneumonia, gastrointestinal parasitism, neurological diseases and wasting diseases, which were reported in 41.7% (15/36), 27.8% (10/36), 19.4% (7/36), 16.7% (6/36) and 8.3% (3/36) of study flocks, respectively. Other problems were caseous lymphadenitis (2.8%, 1/36) and footrot (2.8%, 1/36). Coccidiosis was the most common problem of lambs identified by producers in 52.8% (19/36) of flocks in this study. Other cited problems of lambs were neurological diseases (13.9%, 5/36), pneumonia (11.1%, 4/36), diarrhea (11.1%, 4/36), gastrointestinal parasitism (2.8%, 1/36), urinary calculi (2.8%, 1/36) and nutritional muscular dystrophy (2.8%, 1/36). One-fourth (9/36) of producers reported no common health problem in both their ewes and lambs.

Most sheep producers (86.5%, 32/37) treated individual sick sheep based on their own decision without veterinary advice; 13.5% (5/37) of producers based treatments on veterinary advice. Eighty percent (28/36) of producers kept some form of flock records. Three-quarters (27/36) reported that they regularly analysed performance of their flocks (e.g. average number of lambs born per ewe, average birth weight), and 58.3% (21/36) had routinely set performance goals.

Seven of 37 (18.9%) producers lost sheep during the last 3 years to predators. Coyotes and eagles were responsible for losses in 6 (85.7%, 6/7) and 3 (42.8%, 3/7) flocks, respectively. Measures used to mitigate predator attack in these flocks included hunting of predators (57.1%, 4/7), improved fencing (42.8%, 3/7), and running guardian animals with the flock (28.6%, 2/7).

Proportions of ewes culled each year in study flocks ranged between 0% to 32.5%, with a mean of 9.8% and a median of 8.0%. More than half of 36 producers having at least 1 year of sheep farming experience used old age (69.4%, 25/36), teat and udder problems (66.7%, 24/36) and infertility (52.8%, 19/36) as reasons for culling. Reproductive problems, poor lamb performance, unacceptable habits, physical problems, and genetic defects were reported as reasons for culling in 47.2% (17/36), 33.3% (12/36), 30.6% (11/36), 22.2% (8/36) and 11.1% (4/36) of study flocks, respectively. Diseases such as caseous lymphadenitis, footrot, pneumonia and nerve damage, were reported as reasons for culling in 11.1% (4/36) of study flocks. The mean proportion of ewes per year reported to have died in flocks in the past 3 years was 4.4%, ranging from 0 to 25.0%.

3.4.10 Late-gestation ewe health

Physical examination and sample collection were not possible in 3 groups of ewes because some ewes had already lambed when they were visited; data from 438 late-gestation ewes from 48 groups in 34 flocks were used in the analysis of the physical examination findings. The proportion of ewes having an ideal BCS for late-gestation ewes (3.0 or 3.5) was 54.1% (237/438) (Fig. 3.2). The proportion of ewes having a FAMACHA© normal score (1 or 2) was only 35.4% (155/438) (Fig. 3.3). Legs and udders of 54.8% (240/438) of ewes were not soiled, or were very slightly soiled (Fig. 3.4). Fifteen ewes (3.4%) had 1 or more adult incisors lost, and only 1 ewe (0.2%) had an abnormal udder on palpation.

Blood and fecal samples were collected from 384 and 383 ewes, respectively. Ewe-level summary statistics for serum biochemistry profiles of late-gestation ewes, with reference ranges, are shown in Table 3.4. More than half of samples had lower serum selenium, vitamin E, calcium or total protein concentrations, and almost one-quarter had higher β-hydroxybutyrate (BHBA) than reference ranges. There were some significant correlations between ewe level energy-related substances, including glucose, cholesterol, BHBA and non-esterified fatty acids (NEFA) (Table 3.5). The strongest correlation was the correlation at the ewe level between BHBA and NEFA ($r = 0.60$, $p < 0.001$). The correlation between these 2 substances at the group level was also significant ($r = 0.60$, $p < 0.001$), whereas other correlations between energy-related substances at the group level were not significant.

Lambing-group level summary statistics for serum biochemistry profiles of late-gestation ewes are presented in Table 3.6. Almost all groups had at least one ewe with low selenium, vitamin E, calcium or total protein concentration when compared to reference ranges. The group medians of these substances were below the reference ranges in more than half of study groups.

Eggs of gastrointestinal helminths were found in 52.5% (201/383) of samples submitted, but the prevalence differed depending on the housing system ($p < 0.001$); eggs were found in 65.6% (168/256) and 26.0% (33/127) of samples from partially confined ewes and totally confined ewes, respectively. When restricting the analysis to the Trichostrongyle eggs, the prevalence were 61.7% (159/256) and 15.0% (19/127) in partially confined ewes and totally confined ewes ($p < 0.001$). The prevalence of gastrointestinal helminths was highest in samples collected in October 2014 and lowest in samples collected in May 2015. Helminth eggs were not detected in 5 groups (10.4%) representing 3 flocks (8.8%). From 160 positive samples where eggs were enumerated by the McMaster fecal egg count technique, the mean number of eggs per gram of feces was 364, ranging from 50 to 5450 epg. Only 8.1% (13/160) of positive samples had an egg count ≥ 1000 epg. A graph of median eggs per gram of feces in positive samples within each calendar month is shown in Fig. 3.5. The level varied considerably by month, with the highest counts being in November, April and May. The fecal eggs counts were not different between samples from partially confined ewes and totally confined ewes ($p = 0.64$). Types of helminth eggs identified, other than Trichostrongyles, included *Moniezia* spp., *Strongyloides* spp. and *Trichuris* spp.

There was no association between presence of Trichostrongyle eggs and FAMACHA© score ≥ 3 ($p = 0.14$). Additionally, the association between presence of Trichostrongyle eggs and serum total protein concentration was not significant ($p = 0.87$). However, ewes with a FAMACHA© score ≥ 3 were more likely to have a serum total protein concentration < 60 g/L ($p < 0.001$).

3.4.11 Lambing and lamb management

Ewes in all 37 study flocks routinely lambed indoors. The mean frequency of checking ewes close to lambing was 8 (3-24) times per day. The common reasons producers decided to intervene at lambing were as follows: prolonged lambing, malpresentation or malposture (backward, leg out), fetopelvic disproportion (e.g. a large single lamb or a ewe with a small pelvic size), and multiple lambs presented at the same time. The mean proportion of lambing ewes that required assistance over the last 3-year period was 15.6% (0%-40%).

Rubbing was used to stimulate breathing in newborn lambs in 67.6% (25/37) of study flocks. Other techniques used to stimulate breathing were: clearing mucus from the nasal passage, swinging, percussing, putting straw up the nostrils, putting water in the ears and dipping in cold water. One-fourth (24.3%, 9/37) of producers weighed the lambs during the first 24 hours of life. About half (51.4%, 19/37) of the producers disinfected the navel of every newborn lamb. A portion of lambs was disinfected in 10.8% (4/37) of study flocks, while 37.8% (14/37) of producers did not practice navel dipping at all. Most producers (78.3%, 18/23) that employed dipping provided new dip for each lamb. Almost all of these producers used an iodine solution for disinfection, except one that used scarlet oil (parachlorometaxylenol).

All producers provided assistance for any lamb that did not readily nurse from its dam. The mean waiting time until intervention was 63 (0-300) minutes. Supplementary colostrum was provided in 86.5% (32/37) of flocks. Most often producers providing colostrum supplementation used colostrum from the dam (62.5%, 20/32), and colostrum from another recently lambed ewe (59.4%, 19/32). Other sources of supplementary colostrum used were cow colostrum (40.6%, 13/32) and a commercial colostrum replacement product (bovine sourced) (40.6%, 13/32). When supplementing colostrum, 21 producers (65.6%) used > 1 source of colostrum, whereas 4 producers (12.5%) used only a commercial colostrum replacement product. Twelve percent (4/32) of producers supplementing colostrum used fresh colostrum stored in the refrigerator and 75.0% (24/32) used frozen colostrum. Refrigerated fresh colostrum was usually stored for < 2 days, with 30 days as the longest period. Frozen colostrum was stored for less than one year. Twenty of the 24 producers who stored colostrum in freezers thawed the colostrum in a warm water bath. Other methods included counter top (8.3%, 2/24), and microwave oven (8.3%, 2/24).

The reported mean proportion of lambs requiring supplementary colostrum was 10.7% (1%-50%). Producers reported that lambs were fed on average 84 (20-200) ml of supplementary colostrum the first time they received colostrum. More than half (65.6%, 21/32) of producers offering a colostrum supplement used a graduated bottle to measure the amount of colostrum, while 31.2% (10/32) used a syringe. Equipment used for colostrum and milk feeding were bottle and teat (94.6%, 35/37), tube (43.2%, 16/37), automatic feeder (5.4%, 2/37), and bucket (2.7%, 1/37). Ten producers (27.0%) routinely fostered lambs onto other lactating ewes when unable to be raised by their dams. Techniques used for fostering in these flocks were odour-masking of the lamb to the ewe (60.0%, 6/10) and ewe restraint (60.0%, 6/10).

Techniques used to warm hypothermic lambs were: a warm place (51.4%, 19/37), heat lamp (37.8%, 14/37), warming bag (16.2%, 6/37), blanket (16.2%, 6/37), warm milk (13.5%, 5/37), warm water (13.5%, 5/37), warming box (13.5%, 5/37), hair dryer (10.8%, 4/37), rubbing (5.4%, 2/37), and heat pad (2.7%, 1/37). One-fourth (9/37) of producers routinely measured the rectal temperature of lambs appearing weak. Producers treated weak lambs by bottle feeding milk or colostrum, tube feeding milk or colostrum, tube feeding a dextrose solution, and by injection of dextrose, intraperitoneally, in 89.2% (33/37), 64.9% (24/37), 8.1% (3/37) and 13.5% (5/37) of study flocks, respectively. The mean age of lambs when creep feed was first offered was 11 (0-28) days. The proportions of producers which used a commercial creep feed, a farm-grown grain(s), and a farm-grown grain(s) mixed with a commercial feed were 47.2% (17/36), 19.4% (7/36) and 33.3% (12/36), respectively. A free-choice feeding system was used for creep feed in 80.6% (29/36) of participating flocks The proportion of producers who fed the creep ration once a day and twice a day were 2.8% (1/36) and 16.7% (6/36), respectively. Twenty-nine flocks (80.6%) were provided with a mineral supplement. Ninety percent (26/29) of these flocks were offered a mineral supplement to lambs free-choice, while 6.9% (2/29) and 3.4% (1/29) offered it daily and weekly, respectively. Loose mineral and salt blocks (for sheep) with cobalt and iodine were used in 75.9% (22/29) and 27.6% (8/29) of flocks offered mineral supplement to lambs, respectively.

Twenty-nine producers (78.4%) applied visual identification to their lambs. For the 26 producers answering the question about age of the lamb when the identification was applied, half applied the identification within the first 24 hours. Others identified their lambs on the second day and up to two weeks after birth. An ear tag was used in 72.4% (21/29) of flocks providing lamb identification. Twenty-four percent (7/29) and 3.4% (1/29) of producers used paint (livestock paint or paint branding) and yarn to identify their lambs, respectively. Eight percent of producers (3/37) left all lambs undocked. Proportions of producers that docked the tail of all lambs, and only female lambs, were 91.2% (31/34) and 8.8% (3/34), respectively. More than 90% (29/32) of producers docked using an elastic band, while a few flocks (9.4%, 3/32) used an electric hot docker. Tail-docking was performed on the 1st, 2nd, 3rd to 5th, 7th and 14th day in 18.2% (6/33), 24.2% (8/33), 12.1% (4/33), 33.3% (11/33) and 12.1% (4/33) of flocks that tail docked, respectively. Only 21.6% (8/37) of producers castrated their ram lambs. Almost all (87.5%) of these 8 producers used elastic bands for castration, whereas a burdizzo was used in 1 flock. Lambs were castrated at 1 day, 2 days, 1 week, 2 weeks and 8 weeks of age in 12.5% (1/8), 37.5% (3/8), 25.0% (2/8), 12.5% (1/8) and 12.5% (1/8) of these flocks, respectively.

The mean frequency of checking ewes and lambs after parturition was 5 (1-24) times per day. Out of 36 producers having at least 1 year of sheep farming experience, 1 producer did not use claiming pens to facilitate ewe-lamb bonding. Another 2 producers used claiming pens but animals stayed in them < 24 hours. Sixteen producers (44.4%) allowed their lambs to stay in claiming pens for at least 1 day. Staying in claiming pens for at least 2 days, at least 3 days, and longer than 3 days was reported in 16.7% (6/36), 16.7% (6/36) and 13.9% (5/36) of flocks, respectively. The most common reason to wean lambs was age (54.0%, 20/37), while approximate weight was used in 48.6% (18/37) of flocks. On average, lambs were weaned at 10 (4-22) weeks of age or 20 (11-36) kg. Nineteen percent (7/37) decided to wean based on pasture availability. Other minor reasons for making a weaning decision were time of year, ewe performance (poor performance), and milk demand.

3.5 Discussion

This is the first comprehensive description of flock characteristics and management practices in sheep flocks in Atlantic Canada, focused on practices thought to be associated with lamb mortality. According to Statistics Canada (2015), the numbers of ewes and rams in PEI in January 2015 were about 3700 and 100, respectively. Thus, we estimate that 89% of ewes and almost all rams in PEI were included in the present study, suggesting that the study population of more than one-third of the 100 PEI sheep flocks was highly representative of the target population (PEI sheep flocks engaged in lamb production, having ≥ 12 ewes).

3.5.1 Characteristics of the principal flock manager

Although the principal managers of study flocks were split evenly between males and females, almost all managers reported receiving assistance from other family members. Very few flocks hired extra workers, perhaps reflective of the economics of small flocks.

Around half of principal managers were older than 50 years of age, and had been involved in sheep raising for more than a decade, suggesting a resource of experienced producers for the industry in PEI. However, most did not have a formal agricultural education. The published association of experience and education with lamb mortality is interesting. Holmøy et al. (2012) reported that the risk of lamb mortality was lower in flocks managed by producers having experience in sheep farming for > 15 years. On the other hand, the risk was higher in flocks managed by producers having formal agricultural education, which was perhaps a result of under-reported mortality from producers without formal agricultural education (Holmøy et al., 2012). The same study also reported that producers having agricultural education were more likely to have less experience and practical skills in sheep farming because they were younger than producers who did not have a degree in agriculture. A study on attitudes of sheep producers conducted in Australia (Elliott et al., 2011) found that the producers recognized negative impacts of lamb mortality and desired to improve survival in their lamb crops. They had a tendency to accept new strategies that could help them achieve better lamb survival. Positive attitudes of producers were important in increasing lamb survival because producer characteristics, attitudes and perceptions of control tend to influence strategic management decisions. Although information on management is widely available to sheep producers, face-to-face and practical learning approaches were preferred. The most valued sources of knowledge were veterinarians or flock advisors working directly with them (Ingram, 2008; Dodunski, 2014).

3.5.2 Flock characteristics

British flocks with > 900 ewes had higher lamb losses after the first 24 hours compared to flocks with ≤ 900 ewes, and this was believed to be due to a lack of appropriate supervision (Binns et al., 2002). In the present study, the largest flock was 250 head of breeding ewes, which is much lower than the size required for classification as a large flock in the British study.

Almost all of our study flocks were engaged in crossbred commercial lamb production. Lower mortality risks for crossbred lambs, compared to purebred, have been reported, perhaps due to a lower risk of dystocia, starvation, hypothermia and gastrointestinal disorders in crossbred lambs when compared to purebred lambs (Smith, 1977; Wiener and Woolliams, 1983; Yapi et al., 1990; Nash et al., 1996; McHugh et al., 2016). The breeds contributing to the genetic make-up of our crossbred study population were most commonly Suffolk, Dorset, Canadian Arcott, Rideau and Texel. Stillbirth, lambing difficulty, litter size and behavior of lambs, which are related to lamb mortality, tend to be influenced by sire and dam breed; lamb survival varies among breeds of ewes and lambs (Smith, 1977; Johnston et al., 1999; Carson et al., 2001). For instance, lamb survival in Canadian Arcotts was greater when compared to Suffolks (Demirören et al., 1995). Thus, genetic selection can be employed to improve lamb survival (Hatcher et al., 2010). However, Southey et al. (2001) reported that heritability of lamb survival is quite low (0.03-0.21).

In many pastoral management systems, such as that used in Ireland, spring is the most common lambing season (McHugh et al., 2016). In PEI, where partial or total confinement systems are more common, the proportion of lambing groups giving birth in winter (December 2014 to March 2015) was highest but quite close to the proportion of groups lambing in spring (Fig. 3.1). Season of breeding and lambing are factors that can influence lamb mortality (Petersson and Danell, 1985; Fisher, 2004). Various factors are believed to be responsible for the differences in lamb mortalities among seasons. Lambs born in spring had greater birth weights than lambs born in autumn or winter, perhaps due to seasonal differences in maternal nutrition and development of the placenta (Morris et al., 1993; Jenkinson et al., 1995). Amount of milk produced from ewes lambing in autumn was lower than ewes lambing in spring, which might be a result of seasonal differences in endocrine function (Jenkinson et al., 1995). Logically, lambs born in autumn and winter had a greater tendency to suffer from hypothermia (Fisher, 2004). Lambs born in December, which is a winter month in Ireland, were more likely to die within the first 24 hours than lambs born in other months (McHugh et al., 2016). Therefore, spring lambing seems to be preferable. However, Sormunen-Cristian and Suvela (1999) found highest birth mortality in lambs born during spring months, which was associated with the detrimental effects of larger litter sizes observed in the spring. In addition, the post-weaning mortality of autumn born lambs was lower than lambs born in other seasons. Breeding out-of-season for autumn lambs might be an effective strategy for reducing mortality typically associated with the larger litter sizes of ewes lambing in winter and spring (Fisher, 2004). The proportion of groups of ewes lambing in autumn, winter and spring were 22.7%, 44.7% and 32.6%, respectively, in the present study. In west Texas, litter sizes of ewes bred in summer were reported to be low, which might be a result of high early embryonic death, which was thought to be partly due to the high ambient temperature (Huston, 1983). Nevertheless, heat stress is not a considerable problem in PEI because temperatures exceed 30 °C only a few days of each year (Fuquay, 1981). Male fertility has been another factor related to litter size, which is normally highest in autumn, and can be affected by summer environmental temperatures (Derycke et al., 1990).

Splitting breeding groups, and spreading breeding and lambing activities, has been recommended in order to spread labour requirements over more than one period (Andrewes and Taylor, 1986; Hawkins et al., 1989). Producers in the present study often employed multiple distinct lambing periods; one-third of study flocks had more than one lambing period for one or more of the following reasons: management of labour, management of lambing facilities, access to rams, or as a lamb marketing strategy.

3.5.3 Housing and waste management

Almost one-third of our study producers employed a total confinement system, which is greater than the 11% found in a previous study of Canadian flocks (Dohoo et al., 1985). Effective shelter from inclement weather was available in every study flock. Previous research suggests that there can be a considerable variation in lamb survival; differences of ambient environmental conditions, pathogen contamination and management were reasons for the variation (Nash et al., 1996; Dwyer et al., 2016). Many studies have found a positive influence of shelter on lamb survival by reducing mortality caused by starvation-exposure and predators (Upreti, 1989; Dwyer, 2008). However, risk of infection was higher for animals maintained indoors versus outdoors (Binns et al., 2002). Time spent under shelter was longer for lactating ewes when compared to ewes at other stages, presumably reflective of mothering behavior (Pollard et al., 1999).

Binns et al. (2002) found lower first-day mortality in flocks where bedding material was added to mothering pens more than once a day compared to daily. In the present study, almost all producers cleaned the lambing area between lambing groups, and bedding was commonly added as part of the cleaning process. Unfortunately, frequency of adding bedding was not included in our questionnaire, which was an oversight.

Lamb injury occurred more frequently in high stocking density flocks (Dwyer, 2008). The high density ($\geq$ 10000 ewes/km^2) also increased the incidence of mis-mothering (Alexander et al., 1983). Only 10% of our study groups were considered high stocking density and, therefore, it is unlikely to contribute much to lamb mortality in the study population.

3.5.4 Feeding management

There is considerable evidence that feeding management of ewes before breeding, in early gestation and in late gestation is crucial for minimizing lamb mortality. Birth weight and vigor of lambs are impacted by ewe nutrition (Kleeman et al., 1990; Chaarani et al., 1991; Berger, 1997; Christley et al., 2003; Mandal et al., 2007). Both the length and severity of suboptimal nutrient supply to pregnant ewes are known factors contributing to lowered lamb birth weight (Rooke et al., 2015). Thermoregulation of lambs was improved by better maternal nutritional status (Ojha et al., 2013). Nutritional status of pregnant ewes affects lambing ease, maternal behavior, ewe-lamb relationship, and quantity and quality of colostrum produced (Dwyer, 2003; Terrazas et al., 2012; Dwyer et al., 2016). The drawback to overfeeding late-gestation ewes is the increased incidence of oversized single fetuses and subsequent increased risk of dystocia (Dwyer et al., 2016).

Offering a combination of ensiled forage and hay decreased mortality within the first five days of life elsewhere (Holmøy et al. 2012). The advantage of offering freshly opened ensiled forages two or more times per day was also revealed in the same study. Presenting fresh forage frequently encourages dry matter intake because of higher palatability and lower competition. In the present study, combination feeding of ensiled forage and hay was not commonly practiced (five flocks). Only two producers offered forage to late-gestation ewes less than twice per day.

None of the producers allowed their gestating ewes to graze pastures. A review article mentioned that grazing ewes may be at greater risk for undernutrition compared to non-grazing ewes, due to pasture shortages, variable pasture quality, and harsh environmental conditions (Dwyer et al., 2016).

The mean measure of linear bunk space in the present study approximated 37 cm per ewe, which was greater than the 33 cm recommended by Nagy and Pugh (2012). Sixteen lambing groups had less bunk space than recommended, possibly resulting in problems associated with competition, especially where feed is not offered ad lib.

Almost half of producers in the present study provided a protein supplement to late-gestation ewes. Protein supplements fed in amounts proportional to the number of fetuses were beneficial to birth weight and lamb survival in one study (Lynch et al., 1990). Haughey (1991) reported that some management practices interrupted ewe-lamb relationship, such as providing supplements to lambing or early post-partum ewes, which consequently increase lamb mortality.

Nutritional requirements of ewes six weeks before lambing through to lambing are high when compared to the early and mid-gestation periods due to the higher demands of fetal development (NRC, 2007). In the present study, ewes of almost all lambing groups received an allowance of grain when they were close to lambing. The amount of grain offered was generally increased in the final six weeks of gestation (Table 3.1). The proportion of lactating ewe groups obtaining grain in the present study (96%) was similar to the proportion previously reported for Canadian flocks (92%) (Dohoo et al., 1985). However, the amount of grain fed in many flocks was low compared to the 0.5 to 1.0 kg per ewe per day that has been recommended (Rankins and Pugh, 2012). At 6 weeks before lambing, only 35% of our study flocks were fed ≥ 0.5 kg of grain per ewe per day. The proportion of flocks receiving at least 0.5 kg of grain per ewe per day gradually increased until it was 86% at the time lambing began in the group. The considerable proportion of ewes with BHBA above the reference range (Table 3.4) can likely be attributed to this poor grain feeding practice.

Fetal number influences nutritional requirements of ewes in late gestation. Similarly, the need for weight gain in low body condition ewes will also increase requirements. On the other hand, the ration provided to ewes should not lead to over-conditioning during late gestation (Elliott et al., 2011; Dwyer et al., 2016). Very few of our study producers offered feed based on the BCS of ewes, and only one flock included the number of fetuses as a factor for ration calculation.

The average amount of grain fed to lactating ewes was 0.2 kg higher than that fed at the week of expected lambing. This feeding was consistent with the higher energy and protein requirements of lactating ewes compared to late-gestation ewes (NRC, 2007).

A loose mineral designed for sheep was the most common type of mineral used in our study flocks. Rocks et al. (1982) reported that sheep preferred loose mineral when compared to a mineral block. In addition, Rankins and Pugh (2012) proposed that provision of mineral blocks should be avoided because it can damage teeth. However, teeth damage was uncommon in ewes examined in the present study, although mineral blocks were widely used.

3.5.5 Forage composition

From visual assessments, forages used in our study flocks had no significant contamination with weeds, dirt, manure and mold. Ensiled forage samples, on average, had enough energy and protein for late-gestation ewes, but there was great variation in forage quality (Table 3.2). Because the capacity of the rumen becomes restricted as pregnancy progresses, and there is a higher nutrient demand for fetal growth, forage intake of ewes in the last trimester of pregnancy is often insufficient to meet their needs. Supplementation with grain is required at this stage (Rankins and Pugh, 2012). The amount of grain should be based on the number of fetuses, BCS, or blood ketone concentration (Chniter et al., 2013; Dwyer et al., 2016). Care must be taken, however, as overfeeding during late gestation can lead to dystocia (Hamilton Prime Lamb Breeders, 2005).

Nutrients required for lactating ewes are higher than for late-gestation ewes (NRC, 2007). In the present study, on average, nutrient components of hay samples offered to lactating ewes were better than those offered to late-gestation ewes. Conversely, nutrient components of ensiled forage samples for lactating ewes were very similar to ensiled forage samples for late-gestation ewes.

The actual intake of forage dry matter and grain could not be evaluated in the present study. However, it can be inferred from the forage quality that most hay-fed flocks and some flocks fed ensiled feeds were unlikely to have had their late-gestation protein requirements met. Additionally, many of the ensiled forage samples contained > 12% acid detergent insoluble protein (Table 3.2) reflecting a suboptimal ensiling process, resulting in a proportion of the total protein being unavailable to the animal (Schroeder, 2012). Poorly ensiled forage can predispose to the ingestion of pathogenic bacteria, such as *Listeria monocytogenes*. Vilar et al. (2007) reported that dairy cattle fed ensiled forage having pH ≥ 4.5 were at a higher risk of listeriosis compared to those fed ensiled forage having pH < 4.5. In the present study, most ensiled forage samples from late pregnant and lactating ewes had pH ≥ 4.5 (Table 3.2). This may also have contributed to the reported presence of listeriosis in a few lambs submitted for post-mortem examination during 2005 to 2014 (Chapter 2).

About one-quarter of the forage samples had insufficient copper (< 4.3 ppm dry matter) to meet sheep requirements without receiving additional copper from other sources (Underwood and Suttle, 1999). However, grains and mineral supplements were not included in feed analyses of the present study. These results suggest that judicious copper supplementation may be required in some flocks in PEI. Animal copper concentrations should be measured before instituting copper supplementation. Also, because many interacting factors can alter dietary copper availability and retention, the copper status of sheep flocks is best determined using a hepatic tissue concentration.

3.5.6 Biosecurity

A high proportion (72%) of producers inquired about the health status of flocks of origin when purchasing animals. Furthermore, a high proportion of producers isolated purchased animals (78%); these observations suggest that producers are aware of the risks of introducing disease into their flocks. Forty percent of US sheep producers introducing new animals implemented an isolation strategy; the average time for isolation was 26 days (USDA, 2012). The length of isolation in the present study (14-60 days) was shorter than the US study but not < the 14-day recommendation (Scharko et al., 2012). Sick sheep were also isolated in almost all of our study flocks. Eales et al. (1983) recommended isolating animals suffering from infectious disease. Many producers in the present study isolated only animals believed to have a contagious disease. Control of human traffic is a one of the important biosecurity practices (Scharko et al., 2012). This practice was implemented in 14% of flocks in the present study.

Sheep in most participant flocks had contact with dogs, rodents and cats (78%, 65% and 54%, respectively). Dogs and cats were reported as the most frequent animals that could access sheep barns in the US (USDA, 2012). Dogs can cause trauma-associated lamb mortality (Dwyer, 2008). Toxoplasmosis is one of the main infectious causes of abortion and stillbirth in sheep, which is mostly transmitted by cats. Control measures that limit cat number in barns, and in particular kittens, may help reduce this risk (Berger, 1997).

Placenta is a source of transmission of some abortive diseases, including chlamydiosis, campylobacteriosis, and coxiellosis (Edmondson et al., 2012). Most producers in the present study removed placenta promptly from the lambing areas, which may have been helpful in reducing transmission of abortive diseases within the herds.

Some study producers allowed their sheep to graze in the same area with cattle and goats. Benefits of mixed species grazing include better pasture management (Scharko et al., 2012). Co-grazing with cattle can also reduce parasite contamination; however, some diseases (e.g. salmonellosis) can be transmitted between the two species (Miller et al., 2012).

3.5.7 Ram management

Breeding soundness examination should be performed before the breeding season to ensure reproductive ability of rams (Edmondson et al., 2012). Nevertheless, none of our study producers performed such an examination. Some producers shared rams with other flocks. A main advantage of sharing rams is increasing the genetic diversity of flocks (Sorensen and Norberg, 2008). However, the health status of shared rams should be checked, and biosecurity measures put into place, in order to avoid disease transmission. A wide range of diseases needs to be considered in shared rams, such as internal parasites, caseous lymphadenitis, footrot and venereal diseases causing abortion (toxoplasmosis, chlamydiosis and campylobacteriosis). Rams used for breeding should be selected for positive Expected Progeny Differences for prolificacy, unassisted lambing and growth (Carson et al., 2001; Hamilton Prime Lamb Breeders, 2005; Elliott et al., 2011; Dwyer et al., 2016). A 12% to 14% protein diet with high energy should be introduced to rams approximately 1 month before breeding (Rankins and Pugh, 2012). This practice was followed by most producers in the present study.

3.5.8 Breeding and late-gestation management

Breeding management can have profound effects on lamb survival (Piwczynski et al., 2012). In Irish sheep, multi-breed ewes first giving birth at < 18 months of age were more likely to experience lambing difficulties than sheep giving birth at 18 months of age or older, and their lambs had a higher risk of mortality (McHugh et al., 2016). These findings suggest that ewe lambs should not be bred before 13 months of age in order to optimize lamb survival. Demirören et al. (1995) reported that ewe lambs in North America were mostly bred after 8 months of age. However, breed is a major factor for determining appropriate age for first lambing, because different breeds have different growth rates. Additionally, time needed for mammary gland development varies by breed. In the present study, the mean age of ewes at first breeding was 11 months, and 86% of flocks started breeding their ewe lambs before 13 months of age. In addition, 44% of flocks first exposed ewe lambs to rams at 8 months of age or younger. It is possible that the economics of lamb production might have prompted an earlier breeding date in some flocks and/or had predominantly faster growing breeds compared to the studies mentioned above.

Dystocia is a major cause of perinatal lamb mortality (Cloete et al., 1998); small pelvic size is a common predisposing factor (Hamilton Prime Lamb Breeders, 2005). Replacement ewe lambs should be properly selected from ewes in which lambing assistance is not required, and they should be fed to maximize pelvic size to avoid lambing difficulties (Haughey, 1991). Ewe lambs should also not be bred to rams of large breeds in order to avoid lambing difficulties. (Berger, 1997). Carson et al. (2001) reported higher pre-weaning mortality in ewes having a lower BCS at breeding. Moreover, a positive association between ewe weight at the time of breeding, the birth weight of their lambs, and lamb survival, has been reported in several studies (Thomson et al., 2004; Piwczynski et al., 2012; Aktas and Dogan, 2014). Feeding thin ewes an increased ration prior to breeding was found to be useful for improving lamb survival (Binns et al., 2002). Flushing pre-breeding ewes, which might decrease lamb losses, was implemented in most participant flocks (84%). Flushing before and during breeding improves litter size by increasing ovulation rate. However high lamb losses might be encountered due to a higher proportion of larger litters (Gama et al., 1991; Christley et al., 2003). Mortality risks of lambs born to primiparous ewes was higher than lambs born to multiparous ewes because of underdeveloped maternal behavior and body structure of young ewes (Berger, 1997; McHugh, 2016). Many flocks in the present study bred nulliparous ewes separately (46%), which might allow for closer supervision of this group of ewes at lambing.

A study conducted in Australia found that flocks having < 200 ewes at lambing had lower lamb mortality than flocks having > 400 ewes (Thompson, 2011). In the present study, there was no flock with > 180 ewes bred at the same time, and the largest of our flocks had only 250 ewes. The ratio of rams to ewes can be important for flock reproductive performance. Recommended ram-to-ewe ratios range from 1:5 to 1:50, depending on the reproductive management system used (Edmondson et al., 2012). Rams from ewes having high maternal ability are preferred (Haughey, 1991). In the present study, the highest ram-to-ewe ratio was 1:100 which is considered suboptimal. Single-sire mating was employed in 70% of breeding groups. Some producers left their rams with ewes continuously resulting in long lambing periods, which can lead to inappropriate supervision at lambing (Elliott et al., 2011). A marking harness is helpful to monitor mating in flocks and was used in 71% of our study groups.

Many participants separated pregnant ewes into smaller groups based on various factors, and some separated ewes close to lambing from their flock mates. Grouping ewes close to lambing, for example separating younger ewes from older ewes, has been found to be advantageous. Lambs born to ewes between 2 and 6 years of age required less attention than lambs from younger and older ewes (Sawalha et al., 2007).

A small proportion of our flocks utilized pregnancy diagnosis, and fetal counts were determined in only one flock in the present study. Determination of fetal count is a valuable technique for feeding management of late-gestation ewes to improve lamb survival (Dwyer et al., 2016).

Abortion risks reported by the producers in the present study were higher than previous reports from Peruvian and Canadian flocks (Ameghino et al., 1984; Dohoo et al., 1985), but were within the 5% target (Edmondson et al., 2012). There were 2 producers reporting an abortion risk higher than 5%, suggesting endemic disease in these flocks. Vaccines against common abortive diseases of sheep, such as chlamydiosis, campylobacteriosis and toxoplasmosis are available in some countries (Edmondson et al., 2012). Vaccination against abortion-causing diseases was not routinely performed, likely because epizootic abortion was not identified as an important cause of mortality in our study flocks.

Approximately half of our study producers sheared their ewes before lambing. Producers are encouraged to shear before lambing to improve ewe energy intake, shelter-seeking behavior, and mothering success (Nowak and Poindron, 2006; Elliott et al., 2011). Shearing mid-pregnancy also has been found to increase birth weights and weaning weights of twin lambs (Kenyon et al., 2004).

3.5.9 Health management

Vaccination against clostridial pathogens has been recommended for late-gestation ewes in the last month of pregnancy, principally to protect their lambs (Eales et al., 1983). A multi-valent clostridial vaccine was administered to pregnant ewes in approximately two-thirds of our study flocks, whereas less than 50% of flocks used the vaccine for rams and lambs. A previous survey reported that 87% of flocks used clostridial vaccine (Dohoo et al., 1985), which is higher than the 70% of producers administering the vaccine in the present study, suggesting some complacency among producers with respect to the risk of clostridial disease.

Caseous lymphadenitis vaccine was another vaccine used in study flocks. A study of the efficacy of caseous lymphadenitis vaccination in sheep found that both experimental and commercial vaccines reduced occurrence of disease (Fontaine et al., 2006). The first dose of vaccine should be administered at 6 to 8 weeks of age and the booster 4 to 5 weeks later. Pregnant ewes should be vaccinated annually before lambing on a similar schedule to the clostridial vaccine (Windsor, 2011). In a previous survey in

Canada, a footrot vaccine and a contagious ecthyma vaccine were used in 8% and 3% of flocks, respectively (Dohoo et al., 1985). These vaccines are no longer available in Canada, thus there were no reports of their use by producers in the present study.

Insufficient selenium in soil has been reported in Canada (Hidiroglou et al., 1968; Beauchamp et al., 1969). The vitamin E requirement is higher in growing sheep, so deficiency can be expected in fast-growing lambs with restricted access to fresh green forage (Gabbedy et al., 1977; Steele et al., 1980). Almost 80% of study flocks administered an injectable selenium and vitamin E product to their lambs, mostly within the first week of life. Serum immunoglobulin (Ig) G of lambs treated with vitamin E immediately after birth was higher than in untreated lambs (Gentry et al., 1992). The immune response of older lambs was also improved following selenium administration (Kumar et al., 2009). Although benefits of supplementation of vitamin E in ewes on lamb survival have been reported (Kott et al., 1983; Capper et al. 2006), only 19% of our study flocks administered injectable selenium and vitamin E to pregnant ewes. However, almost all producers provided mineral supplements to late-gestation ewes, and some of these supplements contained selenium. Transplacental transfer of selenium readily occurs (Koller et al., 1984), but only a small amount of vitamin E is transferred via this route (Njeru et al., 1994). In one study, the concentrations of selenium and vitamin E in the colostrum of ewes injected with these micronutrients before lambing were higher than in non-injected ewes; however, serum selenium of lambs was not affected by selenium supplementation of ewes (Cuesta et al., 1995).

Several coccidiostats were added to the feed of study flocks; coccidiostats were most often added to lamb rations, suggesting that coccidiosis was perceived to be an important risk factor in lambs in PEI study flocks. Coccidiosis is a common disease in Canadian sheep flocks; it accounted for 5% of total sheep losses, almost exclusively in lambs (Dohoo et al., 1985). According to the survey from the present study, more than half of participants reported that coccidiosis was the major cause of lamb morbidity in their flocks. The amount of oocysts shed by ewes tends to be increased around parturition, presumably because of a suppressed immune status of the ewe at this time. This shedding by ewes is an important source of oocysts for lamb infection (Coop and Wright, 2000). In addition to maintaining a clean and dry environment, addition of coccidiostats to the feed of pregnant ewes has been suggested as a strategy to reduce transmission to lambs (Rankins and Pugh, 2012). Interestingly, Binns et al. (2002) found that high stillbirth rate (> 5%) was associated with routine use of coccidiostats in a cross-sectional study. Rather than a direct causal effect, flocks with poor management might have increased use of coccidiostats and a higher risk of stillbirth.

Intestinal parasitism has been found to be a common cause of death in older lambs (Green and Morgan, 1994). It was responsible for almost 9% of total deaths in Canadian sheep flocks, with around half of these deaths occurring in lambs (Dohoo et al., 1985). In the United States (US), 13% of producers lost ewes and lambs to internal parasites during 2010 (USDA, 2012). Anthelminthic products used in those flocks were levamisole and dewormers of the benzimidazole family. Macrocyclic lactones were the anthelminthic group most frequently used in our study flocks. Some producers treated their animals with > 1 of these families of drug (35.1%), while some had not used an anthelminthic drug in the last 3 years (13.5%). The proportion of flocks where an anthelminthic drug was not used was much higher than the 1% reported in a survey conducted > 30 years ago (Dohoo et al., 1985). Almost all of these flocks were total confinement flocks. The general trend towards organic rearing systems, and less use of pharmaceuticals, might partly explain the lower proportion of anthelminthic use in our flocks (only one of the flocks in the present study was a certified organic flock). Targeted selective anthelmintic treatment, based on the color of the palpebral conjunctiva, BCS, fecal consistency, and fecal egg count or flotation, was implemented in some study flocks, leading to lower anthelminthic use. The aim of this approach is to manage (rather than eliminate) the flock helminth load while slowing down development of anthelminthic resistance (Besier and Love, 2003; Kenyon et al., 2009). Anthelmintic resistance of gastrointestinal nematodes has been a common problem in small ruminant populations around the world (Jackson and Coop, 2000; Kaplan, 2004; Falzon et al., 2013b). This problem was suspected by 35% of producers participating in the present study, based either on producer observation of lack of improvement post treatment or by monitoring post-treatment fecal egg counts conducted by a diagnostic laboratory.

According to a previous Canadian survey, the proportion of producers treating for external parasites was relatively high at 70% (Dohoo et al., 1985). Conversely, external parasite control products were used in only one-third of flocks in a US study (USDA, 2012). The proportion of producers using external parasite control products in the present study was similar to the Dohoo et al. (1985) study (65%), and no producers considered external parasites a major health threat.

Footrot was the main cause of morbidity in adult sheep (Dohoo et al., 1985). In the present study, the high proportion of flocks providing preventive and treatment measures for foot problems, including hoof trimming, suggested that foot problems were also common.

Mastitis was the most common health problem self-reported by our sheep producers, and it was also one of the main causes of morbidity in the study by Dohoo et al. (1985). Surprisingly in the present study, only one udder abnormality was observed in late-gestation ewes, despite this disorder being cited as important by most producers, suggesting that producers in the present study tended to cull ewes with udder problems from the flock prior to the subsequent breeding period. Chronic mastitis diminishes colostrum and milk production which impacts passive transfer of immunity (Christley et al., 2003). Udder problems also increase risk of lamb starvation and hypothermia (Berger, 1997). Lambs born to ewes with low milk yield are more likely to have poor weight gain (Atashi et al., 2013).

A considerable proportion of our study producers reported other common diseases in their flocks, including pneumonia, intestinal helminths and neurological diseases. According to Dohoo et al. (1985) and Yapi et al. (1990), pneumonia was the second most common cause of lamb mortality. However, it tends to be more common in older lambs compared to neonatal lambs (Green and Morgan, 1994). The pathogens most frequently implicated in pneumonia are normal flora (eg. *Mannheimia haemolytica*), thus the occurrence and seriousness of the disease in flocks will depend on environmental conditions and management practices (Nash et al., 1997). In the study by Dohoo et al. (1985), pneumonia was the most common cause of death in ewes (0.8% of flocks), while 23% of US producers experienced ewe losses from respiratory problems (USDA, 2012).

Neurological disorders reported in our survey were based only on clinical signs, and the exact causes were not determined. One neurologic disorder which was listed as a common cause of death in a previous survey is listeriosis (Dohoo et al., 1985); the widespread feeding of round bale ensiled forage observed in the present study might have predisposed to this disease in some of the neurological cases reported.

In the present study, sick sheep were mostly treated by producers with no input from the veterinarian (86%). According to Moon et al. (2010), antimicrobials were used in more than two-thirds of sick animals. Producers were unable to report the types of treatments used in the present study. Treating as soon as possible with guidance from veterinarians was suggested by Eales et al. (1983). Sick lambs receiving prompt attention had a lower probability of death than if there was a delay in treatment (Binns et al., 2002). Most producers (80%) in the present study reported that they maintained flock records, but not all of them analysed flock performance from the records. Record-keeping is important to improve herd performance (Dwyer et al., 2016). Eales et al. (1983) recommended that producers maintain and interpret two types of records, the first being records which relate to the general history of the flock, and the second being records which provide detailed information on each lamb that dies. Factors associated with lamb mortality could be identified from these records. Setting a target for maximum flock productivity was also recommended, but < 60% of our study producers had goals for their flocks.

Nineteen percent of participating producers experienced losing their sheep to predators, including coyotes and eagles, over the last few years. Coyotes were the most common predator, but eagles were uncommon in American sheep flocks (USDA, 2012).

The proportion of flocks affected by predators was higher than the 7% reported in a previous survey in Canada (Dohoo et al., 1985), but it was lower than the 23% reported in the US (USDA, 2012). Young lambs were more likely to be attacked (Bleich, 1999). In the present study, the details of sheep attacks were not included in the questionnaire. Lamb strength and maternal protection are necessary to avoid predation (Hewson and Verkaik, 1981). Guardian animals, such as alpacas, have been used to protect sheep from predators (Elliott et al., 2011); however, only dogs were used in our participant flocks.

The average reported culling rate per year in our study flocks was 10%, which was lower than the 14%-18% previously reported in Canada and US (Dohoo et al., 1985; USDA, 2012). These low reported culling rates might be due to healthier flocks, flocks in the process of expansion, or under-reporting. Four producers in the present study had not culled animals in the last 3 years, while more than 20% of animals were culled in 1 flock over the last 3 years. Binns et al. (2002) found an association between lamb mortality after 24 hours of age and the annual ewe turnover. Producers replacing > 20% of their ewes per year tended to have higher lamb mortality (> 3%) compared to other flocks with lower ewe turnover (OR = 1.5). High lamb mortality in these flocks was associated with early culling and death of ewes.

The most common causes of culling in the present study flocks were old age, mastitis and failure to conceive, similar to previous surveys (Dohoo et al., 1985; USDA, 2012). Proportions of culls based on reproductive system disorders were considerable in the present study and the previous surveys (Dohoo et al., 1985; USDA, 2012). Poor maternal behavior and the requirement for perinatal intervention should also be considered as reasons for culling (Haughey, 1991; Elliott et al., 2011). The mean proportion of ewes that were reported to have died each year in flocks in the present study was 4%, which did not exceed the < 5% goal for adult sheep losses (Turner et al., 1959), and was lower than the 5% ewe mortality reported by the USDA (2012). The main reasons for ewe deaths reported in our study flocks were lambing-related problems, internal helminths, and age-related illnesses. Pneumonia and predator attacks were the two most common causes of death in ewes. In Dohoo et al. (1985), approximately 20% and 13% of flocks lost their ewes due to these reasons, respectively. In the US, 13% of producers lost ewes to predators, but the proportion varied by flock type, size and region (USDA, 2012). Age-related illnesses and lambing-related problems were the major non-predator causes of death in the US (USDA, 2012).

3.5.10 Late-gestation ewe health

The incidence of dystocia was lower in ewes having an optimal BCS versus over- and under-conditioned ewes (Carson et al., 2001; Thompson, 2011). Birth weight of lambs, in part, depends on the BCS of the ewe (Christley et al., 2003). A study in Australia found an association between BCS of ewes at lambing and lamb survival (Thompson, 2011). Just over half of late-gestation ewes examined in the present study were in an appropriate BCS for their physiological status (Fig. 3.2). Under-conditioning (38%) was consistent with a low amount of grain fed to late-gestation ewes in many flocks. Thin ewes are more susceptible to pregnancy toxemia, and they produce poorer quality colostrum compared to ewes in good body condition (Fragkou et al., 2010). Moreover, ewes in poor body condition are more likely to abandon their lambs (Thompson, 2011). On the other hand, over-conditioned ewes are also predisposed to pregnancy toxemia (Robinson, 1973). Thus, producers should pay more attention to maintaining ewe BCS at 3.0 to 3.5 during late gestation, by providing appropriate quantity and/or quality of ration to subgroups of ewes needing better or poorer nutrition, in order to avoid considerable lamb mortality.

Although mastitis was reported as the most common health problem of ewes in participant flocks, almost all ewes sampled had normal udders on palpation. This may be due to culling practices; ewes with chronic mastitis may have been culled prior to breeding. Studies have shown that colostrum production is decreased with mastitis, and ewes with teat and udder problems are less willing to allow their lambs to nurse. Lambs born to ewes with udder abnormalities had a higher risk of pre-weaning mortality than lambs born to normal ewes (Green and Morgan, 1993; Christley et al., 2003).

The serum selenium concentration of ewes was low in 74% of ewes sampled (Table 3.4). It is well established that there is insufficient selenium in the soil in PEI, and supplementation is therefore required. Lambs born to ewes supplemented with oral selenium during mid and late gestation had a higher pre-weaning growth rate and a greater chance of surviving the perinatal period (Munoz et al., 2009). Subclinical selenium deficiency in pregnant ewes can cause muscular dystrophy in lambs (Langlands et al., 1991). Clearly, selenium supplementation practices were inadequate in many of our study flocks, and there was perhaps an overreliance on injection of selenium and vitamin E products, which provides only transient supplementation rather than consistent supplementary selenium in the ration.

Most of the summary statistics for all other parameters reported from the ewe blood profiles were within normal range (Table 3.4). The median vitamin E concentration was slightly lower than the reference values (2.20 vs 2.32-6.96 µmol/l), and 55% of sampled ewes were vitamin E deficient. This is not surprising as all of the flocks had been off pasture during pregnancy, and only 19% of producers provided vitamin E supplements to their ewes. Fresh green grass is an excellent source of vitamin E for sheep, but vitamin E degrades rapidly in stored forage (McDowell et al., 1996; Kostadinovic et al., 2013). In the present study, ewes were fed primarily stored forages with a small proportion of grain. The requirement for vitamin E is increased during the peri-parturient period due to an accumulation of reactive oxygen species associated with pregnancy (Goff and Stabel, 1990). Vitamin E supplementation in the feed of gestation ewes a few weeks prior to lambing was shown to improve pre-weaning survival of lambs born early in the lambing season (Kott et al., 1998). Menzies et al. (2003) reported 10% and 90% of liver tissues of culled ewes and lambs were below the reference values, respectively. These results suggest that strategies should be developed to assess and enhance vitamin E intake in PEI flocks.

Almost two-thirds (66%) of sampled ewes had serum calcium concentrations below the reference value (Table 3.4). Additionally, the level measured is the total calcium, which does not reflect the amount of active free calcium in the circulation. Because proportions of calcium in our forage samples were adequate, a possible cause of insufficient calcium concentration is a low availability due to interactions with other minerals (NRC, 2007). A high calcium demand in late-gestation ewes is a result of considerable growth of lambs during the last trimester (NRC, 2007). Lima et al. (1993) found a decline of lamb skeletal mineralization in ewes offered low calcium diet. Signs of hypocalcaemia are associated with impaired muscular function, such as stiff gait, tremor and bloat (Rankins and Pugh, 2012).

BHBA and NEFA, which are products of using fat as a source of energy, were significantly positively correlated (Table 3.5) as reported by Karagiannis et al. (2014). The association suggests that both BHBA and NEFA can be used to monitor energy balance. However, the correlation coefficient found in the present study was higher in Karagiannis et al. (2014).

About half of the fecal samples taken from the ewes were positive for helmith eggs on the simple fecal floatation test, suggesting some degree of gastrointestinal parasitism. This was consistent with gastrointestinal parasitism being reported as one of the common problems in ewes in our study flocks. The mean numbers of eggs per gram of feces found in most samples was below the threshold (1000 epg) for nematode treatment (Gonzalez-Garduno et al., 2014). The mean number of eggs per gram of feces in each month of the present study varied considerably (Fig. 3.5). The numbers, however, cannot be directly compared because the samples originated from different flocks, each of which utilized different management systems. Two studies in Canadian sheep flocks reported greater fecal eggs count in ewes during May to June (Ayalew and Gibbs, 1973; Mederos et al., 2010). Unfortunately, in the present study there was no sample collected during that period. Another study conducted in Ontario, Canada, reported that both environmental and ewe physiological factors affect the rise of gastrointestinal nematode eggs during periparturient period (Falzon et al., 2013a).

The fecal egg count results presented above are proportions of ewes with eggs present. Helminths eggs were more commonly found in fecal samples from partially confined flocks compared to totally confined flocks, and the numbers of eggs per gram of feces were lower in flocks using the total confinement system (Fig. 3.5). In addition, all three flocks in which helminths eggs were not found were totally confined flocks. This finding is in agreement with results found by Bush and Lind (1973), whereby Trichostrongyle eggs were found in 15% of fecal samples from flocks reported to utilize a total confinement system compared to 62% in partially confined sheep.

Nematodes are the most common gastrointestinal helminths affecting Canadian sheep flocks (Ayalew and Gibbs, 1973; Falzon et al., 2014), which is consistent with the findings of this report. *Haemonchus contortus,* a member of the Trichostrongyle family, is a common sheep pathogen. The FAMACHA© system was used to assess degree of anemia, which is a common consequence of haemonchosis (van Wyk and Bath, 2002). However, a significant association between presence of Trichostrongyle eggs and FAMACHA© score ≥ 3 was not found in the present study. Additionally, total serum protein concentration was not significantly associated with presence of Trichostrongyle eggs. These findings might result from the low level of infection of ewes at the time they were sampled; only 12 ewes had > 1000 epg. Conversely, the value of the epg of feces in the present study, and the correlation with true gastrointestinal helminth burden, can be questioned, since most fecal samples were taken during the months characterised by variable hypobiosis, superimposed on a physiologic period characterised by a variable pre-partum rise in egg shedding. Relationships between egg counts, serum total protein, and anemia may also be weak because it was not possible to speciate the

Trichostrongyle eggs to determine the actual burden of *Haemonchus contortus*. However, our finding of a significant negative relationship between the FAMACHA© score and serum total protein in late-gestation ewes provides some evidence that the FAMACHA© score, as applied in the present study, may have reflected helminth burdens in the weeks prior to sampling.

3.5.11 Lambing and lamb management

Management of ewes and lambs at lambing is critical to prevent infectious diseases in the newborn. Sanitation is particularly important (Eales et al., 1983). All producers in the present study used an indoor lambing system. Because exposure to cold weather is a common cause of mortality, lambing indoors minimizes the incidence of early neonatal death (Binns et al., 2002; Thompson, 2011). Death attributed to injury, starvation, hypothermia and predation were also lower in the indoor lambing system. On the other hand, lambing indoors may lead to a higher incidence of infectious diseases, specifically in flocks with poor hygienic practices (Rowland et al., 1992; Dwyer et al., 2016). In the present study, ewes close to lambing were monitored at least three times per day, with a mean frequency of eight times per day. The risk of stillbirths and neonatal mortality, particularly parturition-associated deaths, was lower in flocks regularly checked during both the day and night hours (Eales et al., 1983; Holmøy et al., 2012). Substantial labour is required for good supervision (Fisher, 2004). In the present study, some producers hired workers and some received help from students during the lambing period. Low lamb mortality in the study of Sormunen-Cristian and Suvela (1999) was also attributed to student labour. Close supervision has been beneficial on lamb survival; however, it should not interfere with the development of bonding behavior between the ewes and their lambs (Haughey, 1991).

All producers in the present study provided lambing assistance to ewes as required. Sixteen percent of lambings were reported to require an intervention. The proportion of parturitions in which assistance was provided was up to 40% in some flocks in the present study, while 32% was reported in a previous study (McHugh et al., 2016). The breed of lamb and the pelvic size of the ewe are common factors related to the need for assistance (McSporran and Fielden, 1979; Grommers et al., 1985). McHugh et al. (2016) found that the risk of lamb mortality increased with the degree of lambing difficulty.

Quality, quantity and timing of colostrum intake are critical determinants of passive transfer of antibodies, and thus, lamb survival. Ten to twenty percent of lamb body weight of high quality colostrum should be consumed within 3-12 hours after birth (Rankins and Pugh, 2012). Nursing directly from the dam’s udders is considered optimal, but supplementation is critical if there is inadequate intake within two hours of birth (Dwyer et al., 2016). Weak lambs should receive prompt intervention to ensure survival (Nash et al., 1996).

According to Eales et al. (1983), navel disinfection is recommended, especially for outdoor lambing. However, in a study in Norway, navel disinfection had no impact on lamb survival. This finding might be due to the fact that infection was uncommon in the Norwegian sheep flocks (Holmøy et al., 2012), or consequences of navel infection were not severe enough to be a cause of lamb mortality. Approximately 60% of flocks in the present study used navel disinfection.

Failure of passive transfer is a major factor associated with lamb mortality (Christley et al., 2003; Dwyer et al., 2016). Several sources of colostrum were used for supplementation in our study flocks. An on-farm source of colostrum (from their own ewes) was the producers' first choice. Colostrum from goats can also be used for lambs. Body weight gain, IgG and IgM concentrations of lambs receiving goat colostrum were not different from lambs receiving sheep colostrum; however the complement system was less active in lambs receiving goat colostrum (Hernandez-Castellano et al., 2015a). Goat colostrum was not used in our study flocks, while ovine colostrum was used in 41% of study flocks. Tsiligianni et al. (2012) reported that, although concentrations of total proteins, albumin and total globulins in lambs receiving cow colostrum were lower than lambs receiving sheep colostrum at some sampling times, none of lambs receiving cow colostrum had disrupted growth or died during the first three weeks. These indicated that transfer of passive immunity and nutrients provided by cow colostrum can be sufficient for lambs. However, hemolytic anemia has been identified as a possible cause of mortality in lambs offered bovine colostrum (Winter, 2011).

Commercial colostrum products are bovine or ovine in origin. According to Quigley et al. (2002), bovine IgG in commercial colostrum could be absorbed in circulation of newborn lambs. Blood IgG concentrations at 24 hours were lower in lambs that received commercial colostrum compared to lambs that received dam's colostrum, but higher than in lambs that received a commercial lamb milk replacer. Additionally, the average IgG concentration in lambs that received commercial colostrum was sufficiently maintained over the two-week study period. Active immunity of lambs is fully developed at 2 weeks of age (Tizard, 1992), so the effect of bovine-derived commercial colostrum may be adequate to cover the period that passive immunity is required. Provision of commercial colostrum is better than ignoring colostrum supplementation. However, for the nutritional aspect of commercial colostrum replacers, a study on cow commercial colostrum products found that the compositions of the products varied (Li et al., 2004). Based on the studies mentioned above, sheep colostrum is the best source of passive immunity for newborn lambs.

Freezing was the most common method used for colostrum storage in the present study. The concentration of Ig in goat colostrum was not affected by freezing at -20 $^{\circ}$C (Arguello et al., 2003). In human colostrum, concentrations of IgA, Interleukine-8 and transforming growth factor-β1 were reduced when colostrum was stored at -80 $^{\circ}$C for 12 months (Ramirez-Santana et al., 2012). A microwave oven was used for thawing in two flocks in the present study. A study on bovine colostrum found that IgG and IgM concentrations in colostrum were not affected by thawing in a microwave oven (750 watt, 17 minutes) compared to thawing in water bath, but total protein and IgA were reduced by microwave thawing (Jones et al., 1987). Caprine colostral IgG concentration was not different in samples thawed by water bath, refrigeration, room temperature and microwave oven at 55 $^{\circ}$C final temperature (Arguello et al., 2003).

It was reported in the present study that approximately 10% of lambs required colostrum supplementation based on producer’s perceptions. In the first 2 days of life in goat kids, 4 g of IgG/kg of body weight was required to obtain adequate passive immunity for disease protection from colostrum (Castro et al., 2005; Morales-delaNuez et al., 2009; Moreno-Indias et al., 2012). A study conducted in lambs found that lambs that obtained colostrum providing 4 g of IgG/kg of body weight had lower blood IgG, compared to those that nursed directly from the dams and those obtaining 8 g of IgG/kg of body weight. Conversely, blood IgG concentrations of lambs receiving 8 g of IgG/kg body weight were not different from the IgG concentrations of lambs that nursed from the dams (Hernandez-Castellano et al., 2015b). Within the first 18 hours of a lamb's life, at least 50 ml of colostrum per kilogram of body weight is required for effective passive transfer, and the colostrum should contain ≥ 50 g/l IgG (Dwyer et al., 2016). Plasma Ig < 800 mg/dl or serum IgG < 7 mg/ml indicate failure of passive transfer (Cebra and Cebra, 2012; Boucher, 2014). Sawyer et al. (1977) used an IgG concentration of 2 standard deviations below the mean (8.63 mg/ml) as a cut-off for lamb failure of passive transfer. Four percent of their study population were classified as failure of passive transfer. Lambs with failure of passive transfer are more susceptible to infectious diseases. Three to four times higher lamb mortality was reported in lambs having < 10 mg/ml IgG compared to lambs having ≥ 10 mg/ml IgG (Gilbert et al., 1988). Routine measurement of lamb IgG would help to alleviate losses due to failure of passive transfer; however, it is impractical and not economical. Based on the estimated amount of colostrum fed at first feeding reported by producers in the present study, lambs were fed an average of just 84 ml, and some received as little as 20 ml in total, which does not meet requirements for optimal passive transfer. Producers may not be aware of the requirements for colostrum intake in the first 24 hours. However, most producers did not measure the exact amounts of colostrum, so there may have been errors in reported estimates.

When birth weights of lambs are taken into consideration, lamb mortality is lowest in lambs having intermediate birth weight (3.5-4.0 kg in Scottish Blackface sheep), compared to heavier and lighter lambs. Small lambs are susceptible to hypothermia, whereas large lambs are susceptible to dystocia-related mortality (Sawalha et al., 2007). Despite the fact that birth weight of the lamb is an important predictor of neonatal lamb mortality (Huffman et al., 1985; Gama et al., 1991), few producers in the present study routinely weighed their lambs.

All non-nursing lambs received assistance within five hours of birth; however most received assistance within an hour. Owens et al. (1985) found lamb mortality risk increased with every minute a lamb took to stand and find the udder. The time spent to stand and find the udder can be longer if ewes and lambs were disturbed by human intervention from inappropriate management (Dwyer et al., 2016).

All producers provided some degree of support to weak lambs. Bottle feeding was done by almost all producers (95%), while a large proportion of producers delivered milk via oesophageal feeding tube (43%). Holmøy et al. (2012) found lower neonatal mortality in flocks provided with suckling assistance only, or both suckling assistance and tube feeding, compared to lambs in flocks in which there was no intervention. However, lambs in flocks in which only tube feeding was provided had higher mortality than flocks provided with suckling assistance only. Possible explanations for this observation in this cross-sectional study may be that routine tube feeding was mainly used in flocks with a high incidence of lamb mortality (i.e. tube feeding was being used to combat a mortality problem), or that this practice may have interfered with the suckling instinct of lambs (Holmøy et al., 2012). These findings suggest that tube-fed lambs may still be weak when returned to the ewes and require further assistance. In general, weak lambs are at risk of infectious diseases because of failure of passive transfer and/or a suppressed immune system. The risk of infectious diseases may be increased in tube-fed lambs, especially if equipment used is contaminated with pathogens (Dwyer et al., 2016). Thus, cleanliness of equipment is important in risk reduction (Eales et al., 1983). Additionally, incorrect feeding tube placement, which usually occurs in very weak lambs with a poor protective reflex, can also cause a serious complication to tube feeding (Eales et al., 1987).

Fostering lambs to another ewe was not a widespread practice in our study flocks. Because ewes have a selective natural bond to their lambs, this method is sometimes not successful. Success of this practice is determined by lamb health and vigor, foster ewe maternal and acceptance behavior, and milk production (Snowder and Knight, 1995). Lambs that receive insufficient care by ewes require human intervention (Poindron et al., 1984; Binns et al., 2002; Dwyer et al., 2016). A higher incidence of lamb mortality was observed in flocks where fostering was implemented (Binns et al. 2002), however this observation may be a reverse causality because the study was cross-sectional. Additionally, it may be confounded by the finding that this method was more often employed in flocks having a high proportion of multiple births (Dwyer et al., 2016).

Hypothermia and hypoglycemia are common causes of neonatal mortality. Early detection and treatment ameliorates these losses (Eales et al., 1983). Measurement of rectal temperature was not routinely used in our study flocks although it is a practical technique to detect at-risk lambs. Hypothermic lambs should be protected from cold and wind exposure. For instance, a heat lamp, hot water container and/or blanket can be used to alleviate the hypothermic condition (Cebra and Cebra, 2012). A wide range of approaches for dealing with hypothermic lambs was used in our study flocks. Other than colostrum and milk, administration of dextrose solution for hypoglycemic lambs was used in a small proportion of our study flocks. Some producers administered the solution by tubing, while some injected it into the peritoneal space.

Dohoo et al. (1985) reported that between 1982 and 1983, the proportions of lambs first offered creep feed (supplemental feed) at < 2 weeks, 2-3 weeks, and over 3 weeks of age, were 32%, 49% and 18%, respectively. In the present study, producers tended to offer creep feed earlier, with 56% first offering creep feed before 2 weeks of age, 36% at 2-3 weeks of age, and only 8% waiting until lambs were at least 3 weeks of age. Creep feed is beneficial in rumen development, thus it should be offered to lambs as soon as possible (Rankins and Pugh, 2012). Commercial creep feeds were used more commonly than farm-grown grains in our study flocks, which was in contrast to the high proportion of farm-grown grains used in late-gestation ewes.

Over two-thirds of our producers applied some form of identification to their lambs during the first two weeks. Identifying the lambs allows producers to keep accurate records of the ewes and their offspring, which is useful for culling purposes and for selection of replacement animals. In Canada, permanent identification with an approved unique identification number ear tag is legally required for sheep leaving the flock.

Eighty-two percent of producers performed tail-docking, and the procedure was done when lambs were 2-14 days of age, which is the age recommended by Reilly et al., (2012). However, tail docking before 7 days of age is recommended in the Code of Practice for Sheep (NAFCC, 2013). Docking by electronic hot docker causes less adverse effects than docking by elastrator band (Reilly et al., 2012). Most producers in the present study used the latter method, likely due to convenience. Castration was not commonly performed in our study flocks. In flocks in which castration was performed, the procedure was done within the first eight weeks of life using an elastrator band in most cases - only one producer used a burdizzo. NFACC (2013) suggests performing castration by 7 days of age. A common drawback of castration in young ram lambs is an underdevelopment of the urethra, which predisposes to urolithiasis (Bani Ismail et al., 2007). However, Nash et al. (1996) reported lower mortality after the first day of life until weaning in ram lambs castrated at 30 days of age compared to ewe lambs.

Tetanus is a common consequence of both tail-docking and castration; therefore tetanus prophylaxis is required in flocks in which these procedures are performed. If lambs are born to ewes that are not vaccinated during late gestation, they should receive both antitoxin and vaccine when the procedures are performed, whereas only antitoxin is required in lambs born to vaccinated ewes (Edmondson et al., 2012; Reilly et al., 2012). In the present study, clostridial a vaccine was administered to lambs in 33% of flocks in which tail docking was performed, and in 62% of flocks in which castration was performed.

Claiming pens were used in 97% of our study flocks, which was similar to 94% in a previous Canadian report (Dohoo et al., 1985). Most lambs in the present study stayed in the pen for one to three days. In many flocks, duration of stay of lambs in claiming pens depended on the size of the litter; singleton lambs stayed in claiming pens for fewer days than twins or triplets. Staying in the pen allows normal maternal bonding to occur, which is beneficial for lamb survival (Nowak and Poindron, 2006; Dwyer et al., 2016). Adequate-sized lambing areas and claiming pens were important to prevent lamb losses caused by mis-mothering and trauma (Berger, 1997; Dwyer et al., 2016). In the present study, the proportions of ewes weaned at less than 7, 7-8, 9-12 and > 12 weeks of age were 14%, 31%, 40% and 14%, respectively, which were similar to the 10%, 28%, 39% and 22% found in the study by Dohoo et al. (1985). However, more producers in the present study (49%) based their decision to wean on weight of lambs rather than age.

3.6 Conclusions

The typical flock in PEI is a small, crossbred, partial or complete confinement operation, employing lambing both in- and out-of-season, with heavy reliance on ensiled forage, with the objective of maximizing lambs marketed per ewe per year. The population of sheep producers in PEI is diverse, with both young inexperienced producers and producers with many years of experience.

In general, PEI ewe flocks were found to be in good health, and there was overall a strong understanding of health management and biosecurity practices. Producers utilized many management strategies to maximize lamb production, including cross-breeding and enhanced pre-breeding nutrition. Nonetheless, our findings point to a number of areas of flock management that should be further examined, with the objective of enhancing lamb survival.

The amount of grain offered to ewes close to lambing was often inadequate to meet the energy and protein demands of late-gestation and lactating ewes. This inadequacy, combined with highly variable forage quality, may, in part, explain the large proportion of ewes that were in poor body condition prior to and at the time of lambing, and the large proportion of lambing groups with at least one high BHBA value.

Forage analysis was not routinely performed by the producers enrolled in this study - forage testing is essential for providing a balanced ration to optimize the health and productivity of the sheep flock, and regular testing should be encouraged.

Mineral and vitamin nutrition is suboptimal in many PEI flocks. Selenium and vitamin E supplementation practices warrant careful reassessment, as a large proportion of the study flocks had insufficient intake of these trace elements to meet requirements.

In addition to poor body condition, helminth burdens were present in most ewes sampled, and high FAMACHA© scores with low serum total protein were common findings in the present study flocks. These findings, together with the perceived lack of efficacy of the anthelmintic products presently on the market, suggest that different helminth management strategies may need to be examined and put into practice in many of these flocks. Coccidiosis was reported as a common health problem in lambs in many flocks, yet coccidiosis prevention products were not routinely used. Developing practical control measures for coccidiosis in PEI flocks should be a top priority.

Several routine practices relating to neonatal lamb management warrant further examination in PEI flocks: navel disinfection was not routinely applied; and in most flocks, the volume of colostrum fed to newborn lambs requiring assistance did not meet the needs of a newborn lamb to provide adequate nutrients and immune-related factors.

Multivariate analysis of prospective data from these flocks, relating management practices and other flock-level factors to actual lamb survival, will shed further light on the management practices most associated with high lamb survivability.

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Table 3.1 Descriptive statistics summarizing the amount of grain fed in the late-gestation period (kg per head per day) in 51 lambing groups of 37 Prince Edward Island sheep flocks during 2014 to 2015.

Weeks before lambing	Minimum	First quartile	Median	Third quartile	Maximum	Mean	Standard deviation
6	0.0	0.0	0.2	0.4	1.0	0.3	0.3
5	0.0	0.0	0.2	0.4	1.0	0.3	0.3
4	0.0	0.2	0.4	0.4	1.1	0.4	0.3
3	0.0	0.2	0.4	0.4	1.1	0.4	0.3
2	0.0	0.4	0.4	0.7	1.1	0.5	0.3
1	0.0	0.4	0.4	0.9	1.4	0.6	0.3
0	0.0	0.4	0.5	0.9	1.4	0.6	0.3

Table 3.2 Descriptive statistics summarizing feed compositions of late-gestation forage samples of 51 lambing groups in 37 Prince Edward Island sheep flocks during 2014 to 2015.

Parameter	Ensiled forage (n = 44)						Hay (n = 7)				
	Unit	Min	Med	Max	Mean	Standard deviation	Min	Med	Max	Mean	Standard deviation
Dry matter	%	26.10	62.80	89.00	61.77	14.97	84.76	87.50	90.30	87.50	1.73
Crude protein	%	7.99	12.36	21.70	13.56	3.01	6.52	8.47	10.45	8.64	1.30
Acid detergent insoluble protein	%	6.02	11.45	20.52	11.77	3.19	-	-	-	-	-
Acid detergent fiber	%	25.32	33.94	40.88	33.54	3.46	34.72	37.75	38.26	37.03	1.42
Total digestible nutrient	%	53.43	60.94	76.64	61.82	4.51	56.60	57.35	60.13	58.00	1.26
Net energy intake	Mcal/kg	1.14	1.35	1.60	1.36	0.10	1.23	1.24	1.33	1.26	0.04
Digestible energy	Mcal/kg	2.28	2.67	3.17	2.70	0.20	2.43	2.45	2.63	2.50	0.08
Calcium	%	0.22	0.73	1.41	0.79	0.30	0.22	0.41	0.55	0.39	0.11
Phosphorus	%	0.18	0.27	0.41	0.27	0.05	0.18	0.22	0.26	0.22	0.03
Magnesium	%	0.12	0.24	0.41	0.24	0.06	0.13	0.18	0.23	0.18	0.03
Potassium	%	0.91	1.90	2.93	1.93	0.42	1.27	1.36	1.69	1.44	0.17
Copper	ppm	1.52	5.20	10.12	5.64	1.82	3.26	3.98	5.16	4.00	0.62
Zinc	ppm	10.45	19.84	40.29	20.68	5.67	9.57	13.88	22.68	14.72	4.26
pH	-	4.30	5.40	6.50	5.39	0.47	-	-	-	-	-
Net energy for gain	Mcal/kg	0.55	0.68	1.10	0.71	0.12	0.58	0.63	0.66	0.63	0.03
Net energy for maintenance	Mcal/kg	1.25	1.38	1.80	1.41	0.12	1.28	1.33	1.37	1.33	0.03

Table 3.3 Percentage distribution of udder inspection by visual and manual methods, and inspection of the milk and/or colostrum when checking for mastitis at three periods for 37 sheep flocks in Prince Edward Island during 2014 to 2015.

Period	Visual	Manual	Milk/colostrum
Before lambing	59.5%	27.0%	N/A
At lambing	94.6%	94.6%	91.9%
At weaning	64.9%	43.2%	16.2%

Table 3.4 Descriptive statistics of late-gestation serum biochemistry profiles from 384 individual ewes in 37 Prince Edward Island sheep flocks during 2014 to 2015.

Parameter	Unit	Median	Mean	Standard deviation	Reference from the laboratory*	Reference from publications**	Number of samples tested	Percent of samples below the reference range	Percent of samples above the reference range
Selenium	µg/ml	0.072	0.079	0.043	**0.11-0.16**	**0.11-0.16**	382	74.08*	2.88*
Vitamin E	µmol/l	2.20	2.35	1.09	**-**	**2.32-6.96**	379	55.15**	0.26**
Calcium	mmol/l	2.37	2.34	0.20	**2.43-2.91**	**2.88-3.20**	383	65.80*	0*
Phosphorus	mmol/l	1.45	1.50	0.35	**1.1-2.54**	**1.62-2.36**	383	10.18*	0.78*
Magnesium	mmol/l	1.00	1.01	0.11	**0.89-1.28**	**0.90-1.15**	383	5.74*	2.35*
Sodium	mmol/l	147.00	147.03	286.00	**143-153**	**139-152**	383	3.66*	1.31*
Potassium	mmol/l	4.90	4.91	0.46	**4-5.5**	**3.9-5.4**	383	1.31*	6.53*
Chloride	mmol/l	106.00	105.56	3.25	**102-113**	**95-103**	383	10.70*	0.52*
Total protein	g/l	62.00	62.34	5.91	**65-90**	**60-75**	383	66.06*	0.52*
Albumin	g/l	32.00	32.12	3.35	**29-47**	**24-30**	383	14.36*	0*
Globulin	g/l	29.00	30.22	5.87	**26-52**	**35-57**	383	19.58*	0.52*
Urea	mmol/l	4.60	4.58	1.82	**3.4-12.0**	**2.86-7.14**	383	26.63*	0*
Creatinine	µmol/l	73.00	74.95	18.05	**28-100**	**106.08-167.96**	382	0*	7.07*

Parameter	Unit	Median	Mean	Standard deviation	Reference from the laboratory**	Reference from publications*	Number of samples tested	Percent of samples below the reference range	Percent of samples above the reference range
Glucose	mmol/l	3.20	3.30	0.61	**2.6-4.4**	**2.78-4.44**	383	5.48*	4.96*
Cholesterol	mmol/l	1.78	1.82	0.36	**1.13-2.57**	**1.35-1.97**	383	2.35*	3.66*
Total bilirubin	µmol/l	1.00	1.30	0.84	**0-2**	**1.71-8.55**	383	0*	7.05*
Conjugated bilirubin	µmol/l	0	0.44	0.53	**-**	**0-4.62**	383	0**	0**
Free bilirubin	µmol/l	1.00	0.86	0.70	**0-1**	**0-2.05**	383	0*	9.14*
Alkaline phosphatase	Unit/l	85.00	97.80	53.81	**0-247**	**68-387**	383	0*	2.35*
Gamma-glutamyl transferase	Unit/l	53.00	55.27	14.82	**17-77**	**20-52**	383	0.26*	6.79*
Aspartate aminotransferase	Unit/l	79.00	84.59	25.68	**64-158**	**60-280**	383	9.66*	1.57*
Creatine kinase	Unit/l	122.00	295.73	651.62	**23-313**	**8-13**	383	0*	19.84*
Glutamate dehydrogenase	Unit/l	6.00	13.05	29.56	**0-25**	**20**	383	0*	7.57*
β-hydroxybutyrate	µmol/l	466.50	545.83	453.32	**185-605**	**0-700**	382	0.52*	23.30*
Non-esterified fatty acids	mmol/l	0.26	0.35	0.32	**0.0-0.3**	**0.06-0.61**	382	0*	42.15*
Haptoglobin	g/l	0.36	1.02	1.81	**0.0-0.8**	**0.49-0.67**	382	0*	20.94*

Reference publications used: Jackson and Cockcroft, 2002; Menzies et al., 2004; Christian and Pugh, 2012; Wells et al., 2013; Anoushepour et al., 2014

Table 3.5 Pearson's correlation coefficients and p-values of the correlations between energy-related serum biochemical parameters at the ewe level from late-gestation ewes in Prince Edward Island during 2014 to 2015 (n = 384).

Parameter	Glucose	Cholesterol	β-hydroxybutyrate	Non-esterified fatty acids
Glucose	1.000	-	-	-
Cholesterol	-0.088 (0.085)	1.000	-	-
β-hydroxybutyrate	-0.225 (< 0.001)	0.167 (0.001)	1.000	-
Non-esterified fatty acids	-0.133 (0.010)	0.163 (0.001)	0.598 (< 0.001)	1.000

Table 3.6 Descriptive statistics of medians of group-level serum biochemistry profiles from late-gestation ewes in 34 Prince Edward Island sheep flocks during 2014 to 2015 (48 lambing groups, n=8 ewes per group).

Parameter	Unit	Minimum	Median	Maximum	Mean	Standard deviation	Percent of groups having ≥ 1 low value	Percent of groups having ≥ 1 high value	Percent of groups with median below the reference range	Percent of groups with median above the reference range
Selenium	µg/ml	0.005	0.075	0.170	0.077	0.038	93.75	4.17	83.33	2.08
Vitamin E	µmol/l	1.10	2.22	5.05	2.29	0.74	95.83	0	54.17	0
Calcium	mmol/l	2.02	2.36	2.54	2.34	0.12	97.92	0	77.08	0
Phosphorus	mmol/l	1.10	1.45	2.01	1.49	0.22	56.25	6.25	2.08	0
Magnesium	mmol/l	0.90	1.00	1.15	1.00	0.07	35.42	14.58	0	0
Sodium	mmol/l	144.50	147.00	153.00	147.01	1.70	22.92	6.25	0	0
Potassium	mmol/l	4.00	4.90	5.30	4.89	0.23	4.17	39.58	0	0
Chloride	mmol/l	101.00	106.00	111.50	105.76	2.09	52.08	2.08	2.08	0
Total protein	g/l	56.00	61.25	69.50	62.08	3.44	95.83	4.17	75.00	0
Albumin	g/l	27.00	32.00	36.50	32.22	2.23	56.25	0	6.25	0
Globulin	g/l	25.00	29.50	36.50	29.77	3.09	68.75	4.17	4.17	0
Urea	mmol/l	0.85	4.35	7.65	4.49	1.53	52.08	0	27.08	0
Creatinine	µmol/l	48.00	76.25	134.50	74.74	15.13	0	33.33	0	2.08

Parameter	Unit	Minimum	Median	Maximum	Mean	Standard deviation	Percent of groups having ≥ 1 low value	Percent of groups having ≥ 1 high value	Percent of groups with median below the reference range (n=48)	Percent of groups with median above the reference range (n=48)
Glucose	mmol/l	2.45	3.22	4.30	3.25	0.38	27.08	27.08	2.08	0
Cholesterol	mmol/l	0.94	1.80	2.55	1.81	0.25	8.33	18.75	2.08	0
Total bilirubin	µmol/l	1.00	1.00	5.00	1.21	0.63	0	31.25	0	2.08
Conjugated bilirubin	µmol/l	0.00	0.00	1.00	0.41	0.47	0	0	0	0
Free bilirubin	µmol/l	0.00	1.00	3.00	0.93	0.42	0	43.75	0	2.08
Alkaline phosphatase	Unit/l	44.50	81.50	187.50	90.02	31.80	0	14.58	0	0
Gamma-glutamyl transferase	Unit/l	40.50	53.75	78.00	54.78	8.81	2.08	31.25	0	2.08
Aspartate aminotransferase	Unit/l	65.50	79.00	108.00	81.43	9.08	54.17	10.42	0	0
Creatine kinase	Unit/l	73.00	124.25	391.00	144.43	69.58	0	77.08	0	6.25
Glutamate dehydrogenase	Unit/l	2.50	6.00	94.00	9.03	13.02	0	37.50	0	2.08
β-hydroxybutyrate	µmol/l	283.00	462.25	995.00	477.46	128.45	4.17	68.75	0	8.33
Non-esterified fatty acids	mmol/l	0.06	0.24	0.98	0.30	0.20	0	85.42	0	35.42
Haptoglobin	g/l	0.19	0.36	5.50	0.77	1.19	0	52.08	0	16.67

Reference publications used: Jackson and Cockcroft, 2002; Menzies et al., 2004; Christian and Pugh, 2012; Wells et al., 2013; Anoushepour et al., 2014

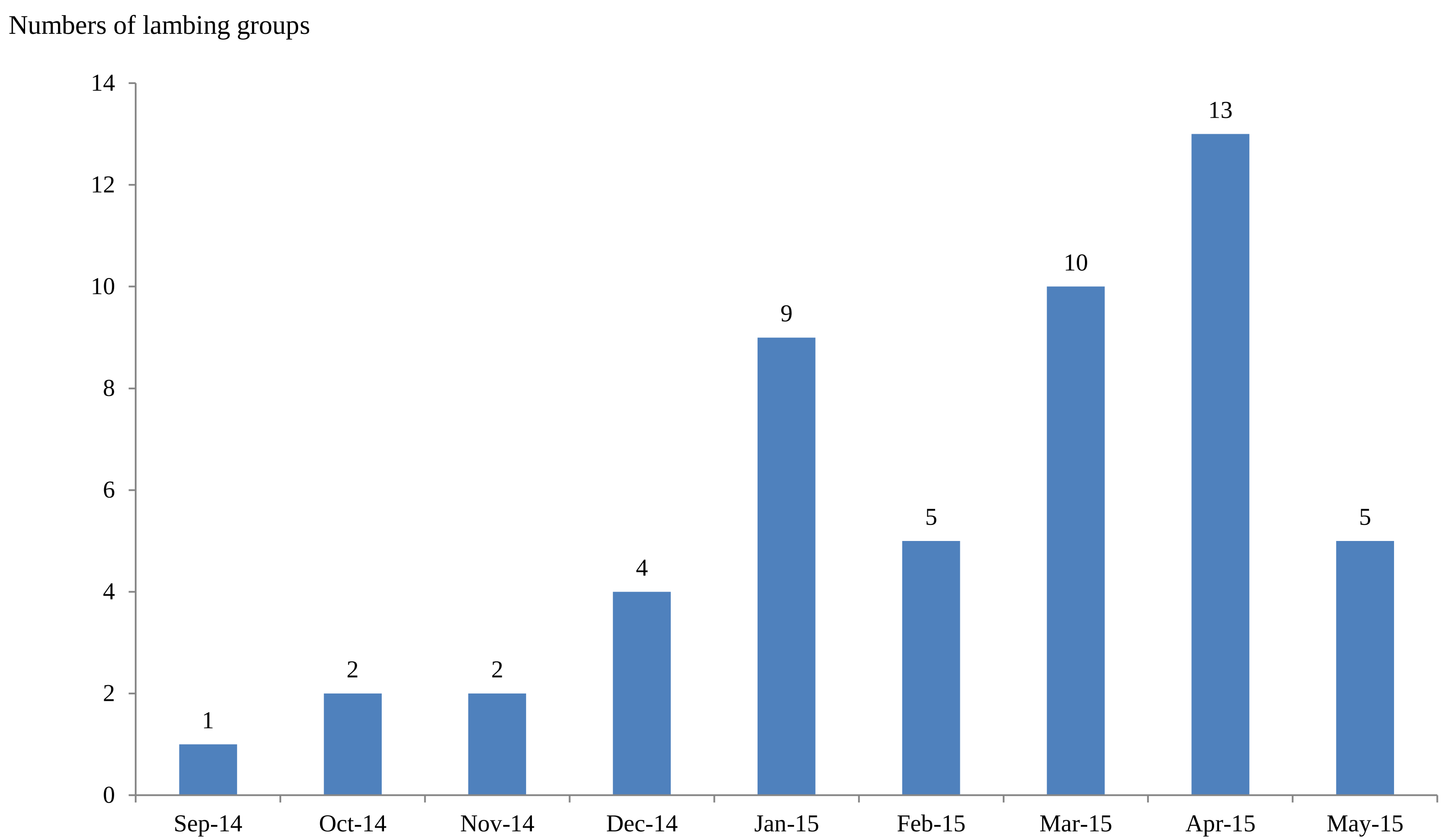

Figure 3.1 Number of study groups lambing each month in Prince Edward Island during the study period from September 2014 to May 2015.

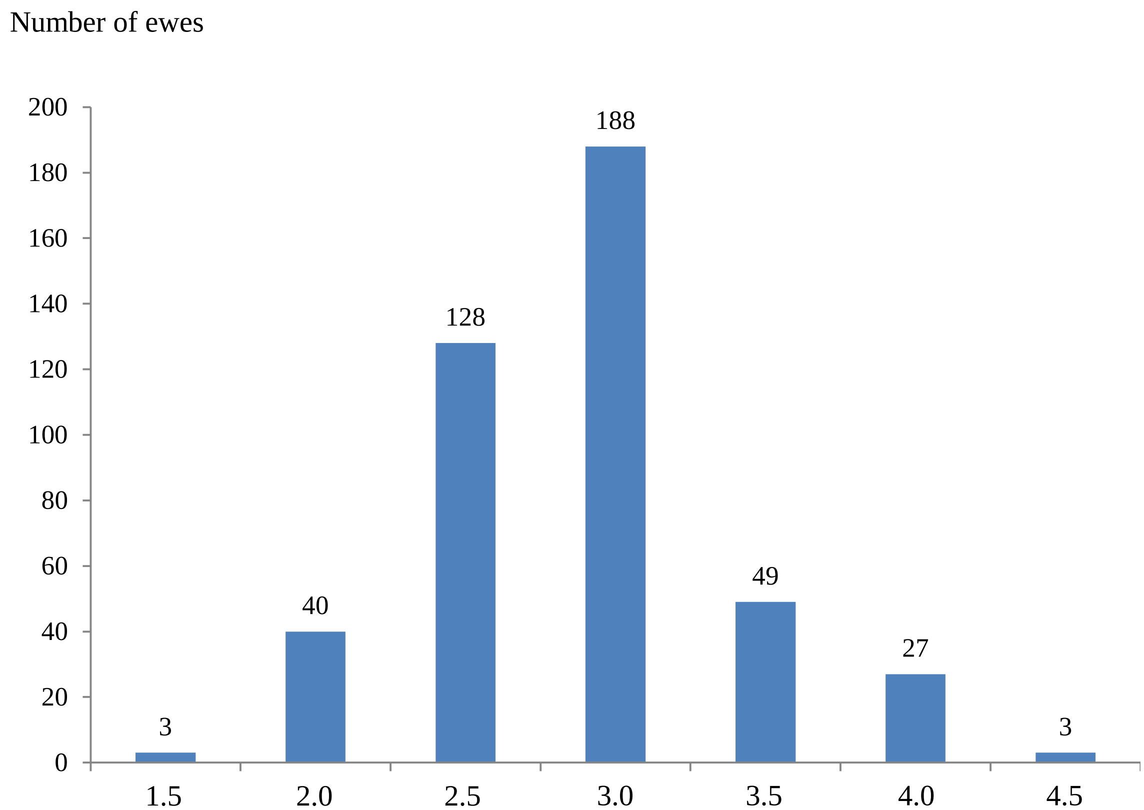

Figure 3.2 Distribution of body condition score (5-point scaling system; Russel et al., 1969) of 438 late-gestation ewes from 48 lambing groups in Prince Edward Island during 2014 to 2015.

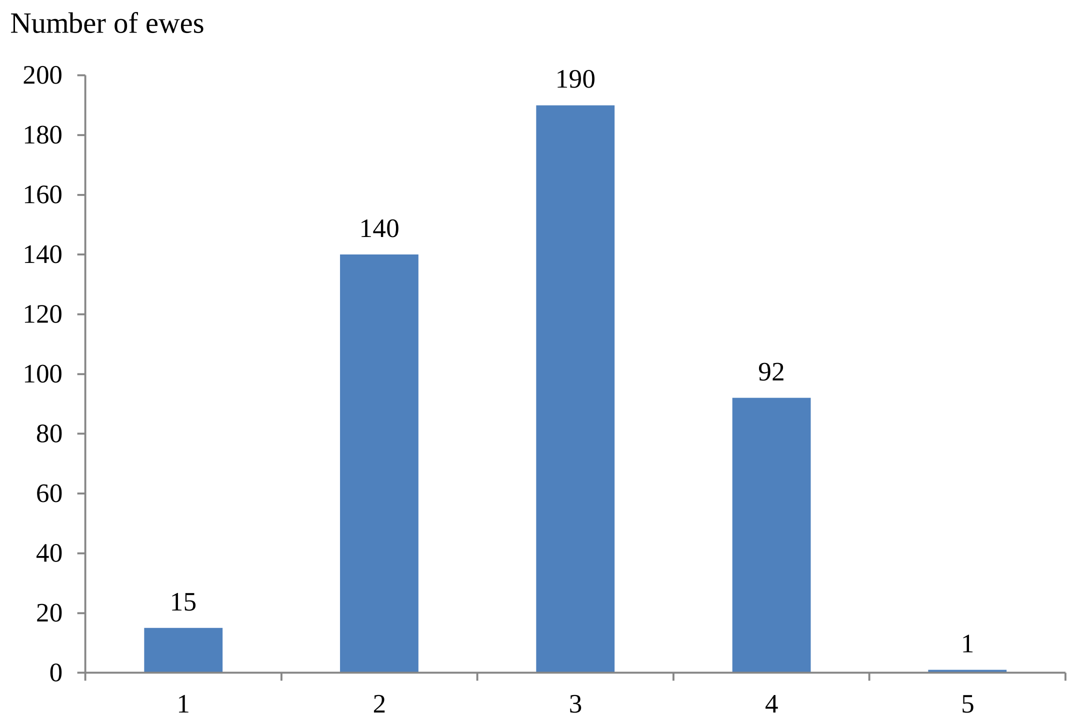

Figure 3.3 Distribution of FAMACHA© score (5-point scaling system where 1 = red and 5 = white; Bath et al., 2001) of 438 late-gestation ewes from 48 lambing groups in Prince Edward Island during 2014 to 2015.

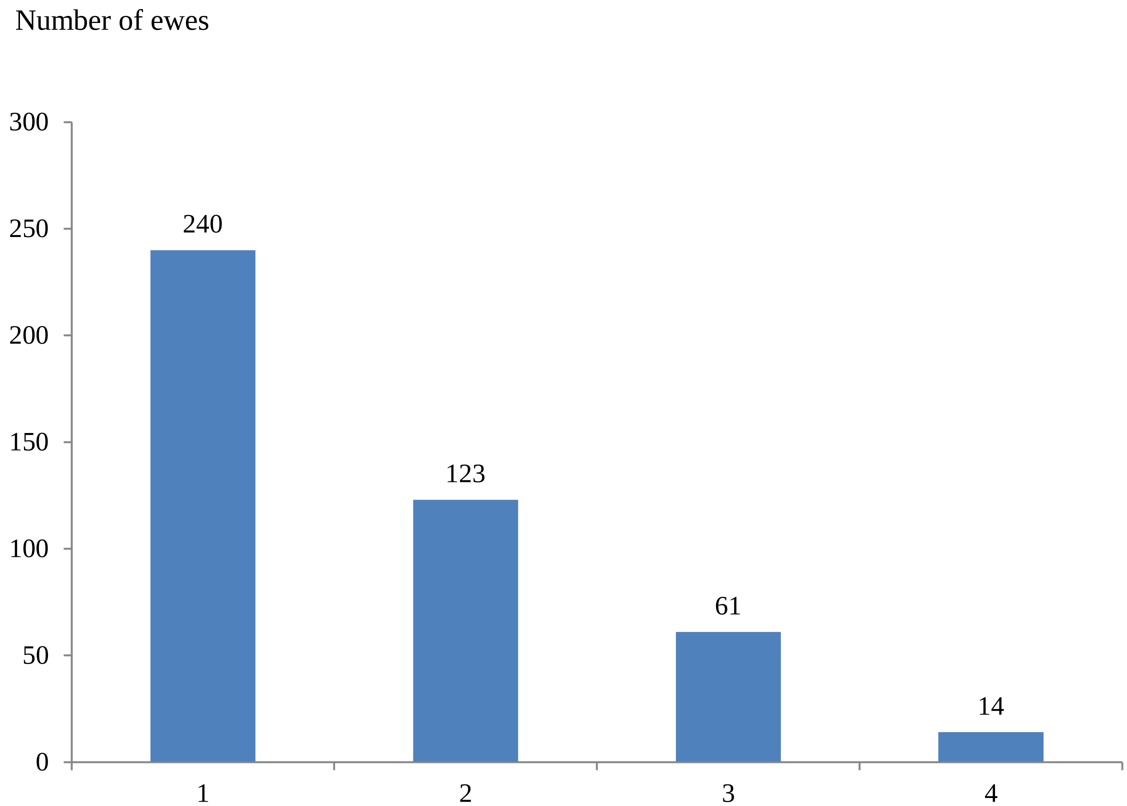

Figure 3.4 Distribution of hygiene score (4-point scaling system where 1 = very little soiled and 4 = completely soiled; Schreiner and Ruegg, 2002) of 438 late-gestation ewes from 48 lambing groups in Prince Edward Island during 2014 to 2015.

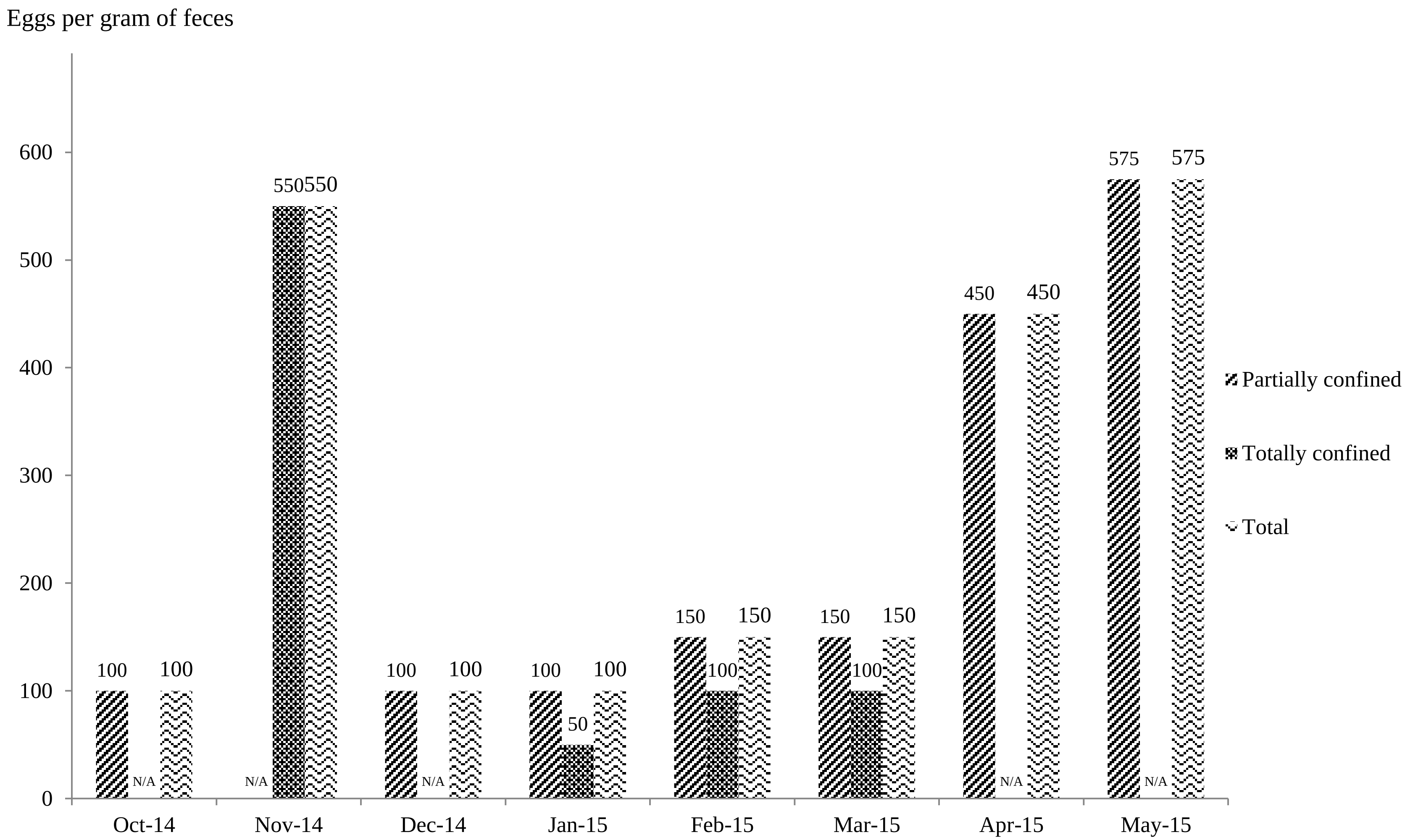

Figure 3.5 Median numbers of gastrointestinal helminth eggs per gram of feces from 160 late-gestation ewes in Prince Edward Island, classified by month at sampling and management system during October 2014 to May 2015.

CHAPTER 4

SELENIUM AND VITAMIN E STATUS OF LATE-GESTATION EWES AND THEIR LAMBS IN PRINCE EDWARD ISLAND

4.1 Abstract

Maintaining sufficient selenium and vitamin E in late-gestation ewes and lambs is important for maximizing flock productivity. However, based on environmental conditions and management practices in Prince Edward Island, Canada, deficiency of these micronutrients is likely to be common. The objectives of this cross-sectional study were to assess the selenium and vitamin E status of late-gestation ewes and lambs, and to determine factors associated with their status in lambs. Data on mineral/vitamin-related management practices were collected from 50 lambing groups in 36 flocks. Selenium and vitamin E concentrations were determined in 384 serum samples of late-gestation ewes (48 lambing groups), and 320 liver samples of lambs submitted for necropsy (44 lambing groups). Selenium and vitamin E deficiencies were common in the study flocks, especially in late-gestation ewes. Injection of a selenium and vitamin E supplement to lambs improved the status of both micronutrients ($p < 0.001$); however, the effect decreased with time ($p \leq 0.001$). Moreover, lamb selenium and vitamin E concentrations were positively associated with the group mean time between supplementation and death. In untreated lambs, the concentration of both nutrients varied by age ($p < 0.05$). The concentrations increased after birth to peak at around 3 weeks of age, and then declined. The selenium concentration in untreated lambs was positively associated with the selenium concentration in late-gestation ewes, but this association in aborted fetuses and stillborn lambs was stronger than in born-alive lambs. The liver vitamin E concentration was lower in aborted fetuses and stillborn lambs compared to born-alive untreated lambs ($p = 0.012$). Protein and mineral supplement practices of late-gestation ewes were also associated with lamb vitamin E concentration

(p = 0.015 and 0.031, respectively). These findings indicate that proper nutritional management in late-gestation ewes and lambs, and timely injectable and dietary supplementation of selenium and vitamin E in lambs are required to ensure satisfactory selenium and vitamin E status of lambs.

4.2 Introduction

Selenium and vitamin E are important micronutrients that are essential for ensuring optimal health and performance of sheep. There are complex links between the metabolic function of these two nutrients (Finch and Turner, 1996; NRC, 2007), with both having well-established and distinct roles in the antioxidant protection of cells (Chow, 2001; Chiaradia et al., 2002), and in humoral and cell-mediated immune function (Ramos et al., 1998).

Placental transfer of selenium is important in determining the selenium status of neonatal lambs (Koller et al., 1984). On the other hand, only small amounts of vitamin E are transferred from the dam to the fetus via the placenta; colostral transfer is the primary source of vitamin E for neonatal lambs (Njeru et al., 1994). The selenium status of newborn lambs, and colostral selenium concentration, are closely related to the dietary selenium intake of ewes (Ammerman and Miller, 1975; Davis et al., 2006). Concentrations of vitamin E in colostrum and in the serum of neonatal lambs also depend on the vitamin E intake of the ewe (Pehrson et al., 1990). Daily requirements of selenium and vitamin E for late-gestation ewes are approximately 0.03 mg and 5.6 IU/kg of body weight, respectively (NRC, 2007).

The effects of selenium and vitamin E deficiencies can be both clinical and subclinical in nature, and have been studied most commonly through supplementation trials, particularly in late-gestation ewes and lambs (Hatfield et al., 2000). Documented benefits of supplementation in deficient flocks include improved selenium and vitamin E blood and tissue concentrations, enhanced antioxidant function, and improved passive transfer of immunoglobulin and immune responses of ewes and their lambs. Decreased stillbirth and mortality, increased vigor and growth rate, and decreased incidence of nutritional muscular dystrophy (NMD) have also been noted in lambs as a result of maternal supplementation (Jenkins et al., 1974; Finch and Turner, 1989; Pehrson et al., 1990; Gentry et al., 1992; Njeru et al., 1994; Kott et al., 1998; Ramos et al., 1998; Rooke et al., 2004; Capper et al., 2005; Hamam and Abou-Zeina, 2007; Munoz et al., 2009; Chalabis-Mazurek and Walkuska, 2014; Moeini and Jalilian, 2014; Donnem et al., 2015). However, results for trials have varied, perhaps in part due to a variety of study designs, including variation in the timing, duration, concentration, and chemical form of the experimental supplement, but also in the amount of selenium and vitamin E in the basal diet. Stress exposure, concurrent disease, age and breed of the experimental animals, and the number of animals in the study are also sources of between-study variation in response to supplementation (Phillippo et al., 1987; Daniels et al., 2000; Palmieri and Szarek, 2011; Liu et al., 2014).

NMD, also known as white muscle disease, is a disorder of skeletal muscle in sheep, often associated with insufficient dietary selenium and/or vitamin E, and is the most common and easily identifiable clinical manifestation of these deficiencies. NMD generally occurs in lambs during the first six months of life but can occur prenatally.

Oxidative damage to metabolically active skeletal muscle occurs. Most often affected are cardiac, diaphragmatic and glossal muscles as well as less active locomotory muscles, resulting in impaired mobility and suckling of affected lambs. In cases where muscles of respiration are affected, dyspnea can be observed (Bostedt and Schramel, 1990; Radwinska and Zarczynska, 2014). Risk of mortality is increased if cardiac muscle is affected (Ramirez-Bribiesca et al., 2005).

Herbage concentration of selenium is closely related to the concentrations of selenium in the soil in which the plant grows. Plants grown in soils containing < 0.6 mg/kg selenium are likely to have herbage concentrations < 0.1 mg/kg selenium, which is considered insufficient for optimal livestock production (Gupta and Winter, 1975). According to Gupta and Gupta (2000), soils in Atlantic Canada tend to contain selenium at insufficient concentrations to meet livestock requirements, containing only 0.1-0.6 mg/kg. In support of this regional soil deficiency, the selenium status of lambs in eastern Canada has been shown to be lower than in lambs in other parts of the country (Hoffman et al., 1973).

Fresh green forage is the main source of vitamin E for sheep (McDowell, 2000). Because of the poor stability of vitamin E, any processing and/or storage of forage leads to significant breakdown of this vitamin; thus, stored forages, such as hay and silage, do not provide sufficient vitamin E to meet dietary requirements of animals even though the standing crop would meet requirements (Menzies et al., 2004; Liu et al., 2014). Canadian sheep are highly dependent on stored forages at least during the winter months and exclusively in total confinement flocks. Vitamin E deficiency can occur in lambs born to ewes fed only stored feeds (Menzies et al., 2004). A survey of liver vitamin E concentrations in market lambs conducted in Ontario, Canada, found that most samples were in a range considered deficient (Menzies et al., 2003).

Based on our knowledge of soil selenium content and forage management practices in Prince Edward Island (PEI), sheep in this province are likely to be at risk of selenium and/or vitamin E deficiencies; however, this hypothesis has not been previously tested. Therefore this study was conducted: 1) to assess selenium and vitamin E status of late-gestation ewes in commercial market lamb flocks in PEI; 2) to determine the selenium and vitamin E status of livers in lambs submitted for necropsy; and 3) to determine factors influencing lamb liver selenium and vitamin E concentrations, including supplementation practices.

4.3 Materials and methods

4.3.1 Animals and samples

During the predicted lambing periods between September 2014 and August 2015, 36 PEI sheep flocks with a flock size range of 13 to 250 breeding ewes were visited, and management data and blood samples of late-gestation ewes were collected as described in Chapter 3. Data relating to the treatment of ewes any time of the year and post-partum lambs with injectable selenium and vitamin E products, including the approximate ages of the lambs treated, feeding management practices, pastured or confined, type of forage, type of concentrate, protein supplementation, and type and method of oral mineral supplement in late-gestation ewes were selected to determine their effects on lamb liver selenium and vitamin E concentrations.

As part of a larger study on lamb mortality using the same lambing groups, 320 lambs that were aborted, stillborn or died up to 8 weeks of age were submitted during the year for post-mortem examination at the Atlantic Veterinary College Diagnostic Services, University of PEI. The following information was recorded for each submitted lamb: flock of origin, lambing group, gender, and the date and age at the time of death. Routine diagnostic processes were performed, and each mortality was categorized as one of the following: abortion, stillbirth, or born alive and died. At least five grams of liver tissue were collected from these lambs after the examination was completed.

Ewe serum samples were processed and analyzed for selenium and vitamin E concentrations as mentioned in Chapter 3. Liver samples were split in half; samples for selenium analysis were stored at -20 °C, and samples for vitamin E analysis were stored at -80 °C. The selenium concentration in liver tissue of dead lambs was analyzed using the spectrofluorophotometry technique. The standard operating procedures of the Animal Health Laboratory, University of Guelph, were followed for the liver selenium analysis. Concentration of vitamin E in liver tissue of lambs was determined by high performance liquid chromatography at the Diagnostic Center for Population and Animal Health, Michigan State University.

All study protocols were reviewed and approved by the University of PEI animal care committee. The consent form was reviewed and signed by cooperating flock owners.

4.3.2 Statistical analysis

Descriptive statistics were calculated for selenium and vitamin E concentrations in serum of late-gestation ewes and in liver of dead lambs, stratified by history of treatment with an injectable selenium and vitamin E product. Lambs were categorized as "treated" if the time of lamb death was after the time of lamb treatment with an injectable selenium and vitamin E product. Lambs were categorized as "untreated" if the lamb death was before the time of lamb treatment with an injectable selenium and vitamin E product, or if the lamb was born in groups in which the treatment was not provided to lambs. The selenium and vitamin E status of ewes and lambs were classified as deficient using the concentrations recommended by Puls (1994a, 1994b) and Menzies et al. (2003), respectively.

The Mann-Whitney test was used to compare selenium and vitamin E concentrations in untreated and treated ewes (lambing group-level) and lambs (individual-level) because the concentrations were not normally distributed, and no transformation could make them normal. Correlations between tissue concentrations in ewes and lambs were evaluated based on Pearson's correlation coefficients.

For the purpose of modeling ewe factors associated with selenium and vitamin E concentrations in lamb livers, it was not possible to take serum samples from all ewes in the study, nor was it possible to take serum samples only from ewes of dead lambs because of the unpredictability of the dead lambs. As a result, paired dam and dead offspring samples could not be obtained in this study. Thus, the median value for ewe serum selenium and vitamin E concentrations for each lambing group was used as representative of the nutrient status of the respective ewe group for each dead lamb.

Lamb liver selenium and vitamin E concentrations were the principle outcomes of interest in this study. Factors affecting liver selenium and vitamin E concentrations in lambs were initially identified using four separate linear mixed models for selenium and vitamin E concentrations in the untreated and treated lambs (regardless of ewe treatment), because this permitted testing of the effect of length of time between the lamb treatment and death in the treated lambs. In addition, two separate linear mixed models (one for selenium concentration and one for vitamin E concentration) which included all lambs were fitted; lamb treatment was considered as a fixed effect. A natural logarithmic transformation was used for lamb liver selenium and vitamin E concentrations to normalize the continuous outcome variables in order to meet model assumptions.

While there was clustering of lambing groups within flock, this affected only the 12 flocks that had > 1 lambing group. Lambing group was entered into models as a random effect, while the other variables, including treatment status of ewes, feeding management of ewes, month of flock visit, lambing group's median ewe serum selenium and vitamin E concentration, lamb gender, age of lamb at death, and type of death (abortion/stillbirth or post-partum death) were considered fixed effects.

For building the models, independent variables were first analyzed using univariate linear mixed regression analysis. The final models were built using a backward elimination procedure, starting with all variables regardless of p-values from the univariate analysis, and ending with statistically significant variables determined by p-value < 0.05. All possible 2-way interactions between main effects were explored, and confounding factors were evaluated if they met conceptual and statistical criteria (changed model coefficients by 20% or more). Random slope and contextual effects were also evaluated for lambing group mean of continuous variables at the individual lamb level. Intra-class correlation coefficient at lambing group level of each model was computed. The final models were checked for model assumptions. Stata 14 (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, Tx: StataCorp LP) was used for all statistical analyses.

4.4 Results

4.4.1 Descriptive statistics

Descriptive statistics of flock management practices can be found in Chapter 3. Of 320 dead lambs (from 44 lambing groups; 6 lambing groups did not submit dead lambs) submitted for post-mortem examination, the proportions of abortions and stillbirths were 6.2% and 26.2%, respectively. Based on the 315 lambs where age at death was provided, the mean and median ages of lambs at death were 6 (±12) and 0.5 days, respectively. Based on the 297 lambs for which gender was recorded, the proportions of male and female lambs were 52.2% and 47.5%, respectively, with one intersex. Based on reported age at death and the reported age of selenium and vitamin E administration, 26.6% (77 of 289) of the submissions had received a selenium and vitamin E injection prior to death. Sixty-four percent of submitted lambs were untreated and born to untreated ewes, while 7.3% were treated lambs born to treated ewes. The proportions of untreated lambs born to treated ewes and treated lambs born to untreated ewes were 9.3% and 19.4%, respectively. For the 77 lambs that were treated with selenium and vitamin E prior to death, the mean and median times between injection and death were 11 (±16) and 2 days, respectively.

There were 384 ewe serum samples from 48 lambing groups and 307 lamb liver samples from 44 lambing groups. However, 27 liver samples provided insufficient material for both selenium and vitamin E analysis, leaving only 280 samples for vitamin E analysis. Sixteen serum samples and one liver sample had vitamin E concentrations less than the lowest detectable level; 0.45 µg/g, half the limit of detection, was assigned as the concentration for these samples for the purposes of statistical analyses.

Descriptive statistics for selenium and vitamin E concentrations of lamb liver, along with lambing group's median ewe and individual ewe serum concentrations, are displayed in Table 4.1 and 4.2. When cut-offs recommended by Puls (1994a, 1994b) were used, more than half of individual ewes and ewe lambing groups (74.1% and 83.3%, respectively) had selenium concentrations below the reference range; however most were still above the deficient range. Serum vitamin E concentration in most individual ewes and ewe lambing groups (55.1% and 54.2%, respectively) were in the deficient range. Forty percent of lambs had liver selenium in the deficient range, and 13.9% of lambs had liver vitamin E in the deficient range (Menzies et al., 2003). There were only 3 groups of late-gestation ewes having both selenium and vitamin E concentrations in the deficient ranges, whereas there were 17 lambs that had concentrations in the deficient range of both nutrients concurrently.

4.4.2 Inferential statistics

Ewe lambing group median serum selenium concentration in those groups that had received a selenium and vitamin E injection were significantly higher than groups where no such treatment had been administered ($p = 0.048$). However, the vitamin E concentrations were not different when these groups were compared ($p = 0.66$). Both liver selenium and vitamin E concentrations in treated lambs were significantly higher than untreated lambs regardless of the treatment status of ewes ($p < 0.001$ and $p < 0.001$).

The Pearson's correlation coefficient for the relationship between individual serum selenium and vitamin E concentrations of all 379 late-gestation ewes was low (0.01, $p = 0.86$), whereas the correlation between selenium and vitamin E concentrations in liver samples of lambs was high (0.62, $p < 0.001$). When the correlation was analyzed separately for individual ewes either receiving ($n = 64$) or not receiving ($n = 315$) an injectable selenium and vitamin E product, the correlation coefficients for treated and untreated ewes were 0.27 ($p = 0.03$) and -0.01 ($p = 0.80$), respectively.

4.4.3 Model factors

Independent variables retained in the final models for untreated lamb liver selenium and vitamin E concentrations are displayed in Table 4.3 and 4.4, respectively. The selenium concentration was associated with age of lamb at death, and the interaction between the group median ewe serum selenium concentration and type of death. Age of lamb at death and type of death were also in the final model for lamb liver vitamin E concentration. The fixed effect of lamb age at death on the liver concentration of both nutrients was quadratic, with a negative coefficient of the quadratic term. The concentrations increased with age at death until around day 20 after birth, and then decreased (Fig. 4.1b and 4.2).

Aborted fetuses and stillborn lambs had lower liver selenium and vitamin E than born-alive lambs. In the selenium model (Table 4.3), the significant term for the interaction between group median ewe serum selenium concentration and type of death indicates that ewe selenium status was associated with lamb liver selenium concentration to a greater extent in aborted fetuses and stillborn lambs when compared to born-alive lambs dying later (steeper slope of the line in Fig. 4.1a). For lamb liver vitamin E, the concentration in aborted fetuses and stillborn lambs was 35% lower than born-alive lambs dying later. Intra-class correlation coefficient at the lambing group level was 0.64 and 0.08 for the selenium and vitamin E models, respectively. There was no significant contextual effect in either model.

For lambs that received supplementation before death, both models for liver selenium and vitamin E concentrations in lamb livers depended only on the time from supplementation to death, with a negative association (Table 4.5). However, there was a significant contextual effect in both models whereby, between groups, liver selenium and vitamin E concentrations was associated with group mean time between lamb treatment and lamb death, with a positive association. Therefore, the liver selenium and vitamin E concentrations were higher with shorter time between individual lamb treatment and lamb death, but higher with higher group mean time between lamb treatment and lamb death. The intra-class correlation coefficient at lambing group level was 0.06 in the selenium model, and was negligible in the vitamin E model.

The model for lamb liver selenium concentration, including both untreated and treated lambs, is displayed in Table 4.6. The effect of lamb age at death on liver selenium was quadratic. The concentration reached its peak at around 3 weeks of age at death, and then declined (Fig. 4.3). Lamb liver selenium was positively associated with the group median late-gestation ewe serum selenium concentration. A 0.01 μmol/l increase in late-gestation ewe serum selenium concentration was associated with an

8.3% greater liver selenium concentration. When a lamb had received an injectable supplement, the liver Se concentration was increased by 110.7%. Month of visit was another factor associated with lamb selenium concentration in the final model. The concentration in February, April or May was greater than March when multiple monthly comparisons were taken into account. The intra-class correlation coefficient at the lambing group level in this model was 0.06.

Table 4.7 displays factors associated with lamb liver vitamin E concentration in the final model, which included all lambs regardless of treatment status. Providing a commercial protein supplement, and offering oral mineral supplement free-choice to late-gestation ewes, increased the concentration in lamb liver by 48.70% and 68.57%, respectively. Lambs that died pre-partum (abortions) and during the birth process (stillbirths) had a lower concentration by 47.11%. In addition, lambs treated with an injectable selenium and vitamin E product before death had 117.10% greater vitamin E concentration in their liver compared to untreated lambs. The intra-class correlation coefficient at the lambing group level in this model was negligible.

4.5 Discussion

This study was able to determine: 1) the selenium and vitamin E status of late-gestation ewes in commercial market lamb flocks in PEI; 2) the selenium and vitamin E status of livers in lambs submitted for necropsy in PEI; and 3) factors influencing lamb liver selenium and vitamin E concentrations, including supplementation practices in PEI, where selenium and vitamin E would be expected to be deficient.

4.5.1 Descriptive statistics

A wide range of positive effects of selenium and/or vitamin E supplementation of ewes on their lambs has been reported. These include decreasing stillbirth risk (Donnem et al., 2015), increasing lamb health status (Pehrson et al., 1990; Cuesta et al., 1995; Liu et al., 2014; Moeini and Jalilian, 2014), birth weight (Lekatz et al., 2010), daily weight gain (Gentry et al., 1992; Munoz et al., 2009; Moeini and Jalilian, 2014), immunological status (Gentry et al., 1992; Rooke et al., 2004; Munoz et al., 2009; Moeini and Jalilian, 2014), vigor (Munoz et al., 2009) and survival risk (Gentry et al., 1992; Kott et al., 1998; Munoz et al., 2009). Gentry et al. (1992) found that effects of vitamin E supplementation to late-gestation ewes on pre-weaning lamb performance were more apparent than effects of direct supplementation to lambs. However, only one-sixth of the cooperating lambing groups in the present study reported using injectable selenium and vitamin E in ewes. In the present study, lambs in 74% of the groups received selenium and vitamin E injections, which is similar to the 77% reported by Dohoo et al. (1985).

According to previous studies, immunological effects of subcutaneous selenium and vitamin E injection to early post-partum lambs were less evident after 6 weeks of age. Alterations of selenium metabolism by microorganisms in the fully developed rumen might be responsible for this difference (Hudman and Glenn, 1985; Finch and Turner, 1989). According to Menzies et al. (2003), injectable selenium and vitamin E was the most frequent method of selenium and vitamin E supplementation, and birth was the most common time for administration. Of producers that injected lambs in the present study, 97% reported administering the injection within the first week of life, which is the proper timing for selenium and vitamin E supplementation.

Menzies et al. (2003) reported that 3.3% and 42.6% of lamb and ewe liver samples from slaughter houses in Ontario, Canada, had selenium concentrations in the marginal ranges, but no sample was in the deficient ranges. In contrast, 40.4% and 37.5% of lamb samples in the present study were in the deficient and marginal ranges, respectively, using the same cut-off values utilized by Menzies et al. (2003). For ewes, 10.4% and 47.9% of ewe lambing groups had median serum selenium concentrations in the deficient and marginal ranges, respectively, based on the cut-off values proposed by Puls (1994a).

For vitamin E, Menzies et al. (2003) reported that 1.7% and 10.0% of lamb and ewe samples had concentrations in the deficient ranges. In addition, a large proportion of lambs had vitamin E concentrations in the marginal range (88.3%). In the present study, 13.9% and 64.6% of lamb liver samples had vitamin E concentrations in the deficient and marginal ranges, respectively. Applying the cut-off values recommended by Puls (1994b), more than a half of the ewe lambing groups (54.2%) had median serum vitamin E concentrations in the deficient range, and 41.7% were in the marginal range.

These findings suggest more widespread and serious selenium and vitamin E deficiencies in PEI flocks than in Ontario flocks. However, the proportions of ewes and lambs having sufficient selenium concentration in the present study were higher than the proportions reported by a study in Poland (Chalabis-Mazurek and Walkuska, 2014). Because serum selenium and vitamin E concentrations increase with age (Stowe and Herdt, 1992, Puls, 1994b), and most lamb samples in the present study were collected from lambs younger than 3 days of age, selenium and vitamin E concentrations might be lower than expected. Decreasing blood vitamin E concentrations during the peri-parturient period has been reported in cows (Weiss et al., 1990; Lindqvist et al., 2011).

Parturition-associated stresses might be responsible for this reduction (Goff and Stabel, 1990); thus it is possible that selenium and vitamin E concentrations in ewes are decreased during the peri-parturient period as well. These stresses could partly explain the high proportion of ewe groups having median serum selenium and vitamin E concentrations below the normal ranges, but it is unlikely to be the sole reason, as most ewes were two to four weeks from lambing when sampled.

Based on the cut-off values used by Menzies et al. (2003), all lambs having selenium concentrations in the deficient range had vitamin E concentrations in the deficient range, whereas 44.7% of lambs having vitamin E concentrations in the deficient range had selenium concentrations in the deficient range. These findings indicate that some lambs in the present study might have received a dietary mineral supplement containing selenium, with little to no vitamin E, which is typical of most commercial mineral premixes and blocks.

4.5.2 Inferential statistics

In late gestation-ewes, individual serum selenium concentrations were not correlated with the vitamin E concentrations. In contrast, there was a moderate positive correlation between the concentrations of these nutrients in liver tissues of lambs. These are opposite to the finding reported by Menzies et al. (2003), where the association between liver selenium and vitamin E concentrations of ewes was significant, but the association in lambs was not significant. However, when selenium and vitamin E injectable supplementation was taken into account, the selenium and vitamin E concentrations in individual late-gestation ewes was positively correlated in those ewes receiving injectable selenium and vitamin E, but they were not correlated in untreated ewes. One reason for this finding might be that the concentrations of selenium and vitamin E in other nutrient sources (e.g., forages, grains, mineral mixes and blocks, injectable AD and E) do not consistently enhance the status of both of these micronutrients concurrently. In contrast, a significant correlation between selenium and vitamin E concentration was found in both untreated and treated lambs in the present study. This might be evidence of a correlation between the two micronutrients in colostrum and milk, which are the major source of nutrients during the early stage of life.

4.5.3 Model factors

Methodologically, there were three hierarchical levels in the data for the models, including flock, lambing group and lamb. Three-hundred and twenty dead lambs included in the present study belonged to 44 lambing groups in 34 flocks. Because the proportion of flocks that submitted lambs from more than one lambing group was low, we considered omitting one of these levels from the data analysis. Based on log-restricted likelihoods of the models, the models with lambing group as a random effect were better than the models with flock as a random effect. Moreover, variances at the flock level were low in all models. Thus, the models where lambing group was retained as a random effect are presented.

In the livers of untreated lambs, selenium concentrations increased in concert with increasing ewe serum selenium concentration (Table 4.3); however there was a significant interaction between ewe serum selenium concentration and type of death (Fig. 4.1a). The positive association between lamb liver selenium concentration and late-gestation ewe serum selenium concentration was more pronounced in aborted and stillborn lambs when compared to that of lambs dying in the post-partum period. Since the amount of selenium in colostrum is related to the serum concentration in late-gestation ewes (Chalabis-Mazurek and Walkuska. 2014; Moeini and Jalilian, 2014), the above findings are likely explained, in part, by changes in the micronutrient intake of lambs during the post-partum period, leading to a gradually weakening relationship between lamb post-partum selenium status and late-gestation ewe selenium status. The blood selenium concentration of late-gestation ewe influences the amount of selenium transferred to the fetuses (Bostedt and Schramel, 1990) which may also explain, in part, this interaction. Adequate selenium supply for late-gestation ewes promotes satisfactory fetal growth (Lekatz et al., 2010; Meyer et al., 2010).

Another factor associated with untreated lamb liver selenium concentration in the present study was age. The peak selenium concentration was detected in lambs dying on day 20-24 of age (Fig. 4.1b). The sharp increase during the first 4 days could be a result of the efficiency of selenium transfer via colostrum. Colostral selenium concentration is 3.5 times the concentration in milk (Pehrson et al., 1990). This association was opposite to findings in calves - the concentration in calves decreased from birth to 4 days of age, then slightly increased over the next 6 weeks, and then increased again after animals consumed an adequate amount of solid feed, but it was never as high as the concentration at birth (Bostedt and Schramel, 1990).

There was one lambing group with higher random effects when compared to other groups in the final model for liver selenium in untreated lambs. Most of the model coefficient estimates were not affected when this group was included in the model, except the effect of group median ewe selenium concentration; when this group was included, the estimate was two times higher. Additionally, the proportion of variance at the group level was 0.27 vs. 0.64 with the group excluded or included, respectively. Therefore, the final model (Table 4.3) includes this group.

There were two factors associated with liver vitamin E concentration of untreated lambs in the present study: age of lamb at death and type of death (Table 4.4). Gentry et al. (1992) reported that the greatest concentration of lamb serum vitamin E was detected on the first day of life and then decreased over time; however, the association between age and liver vitamin E concentration found in the present study was quadratic (Fig. 4.2). The greatest concentration of lamb liver vitamin E was found in lambs dying at 16 to 22 days of age. The marked increase observed during the early stages of life was possibly attributed to the greater concentration of vitamin E in colostrum compared to that of milk one week following parturition (Pehrson et al., 1990; Liu et al., 2014). Lamb vigor and suckling ability affect colostrum consumption, and consequently affect vitamin E concentrations in neonatal lambs (Liu et al., 2014). Although colostrum concentrations of vitamin E have been reported to be associated with the serum vitamin E status of ewes (Pehrson et al., 1990), group median ewe serum concentration did not remain in the final model for liver vitamin E concentration in untreated lambs, suggesting a weak effect, if any, in our flocks. Transplacental transfer is a minor source of vitamin E in neonatal lambs (Rooke et al., 2004). A lower liver vitamin E concentration in aborted fetuses and stillborn lambs compared to born-alive lambs dying later in the present study is consistent with poor placental transfer.

A quadratic relationship was observed between age and liver concentrations of both selenium and vitamin E in untreated lambs. The peak concentrations at 3 weeks of age may be explained by a gradually increasing intake of creep feed and dietary mineral supplements after birth, which tend to contain ample selenium and vitamin E, followed by increasing intake of stored forages and grains in older lambs, which tend to have lower concentrations of these micronutrients. Creep feed was offered to lambs by 3 weeks of age in 92% of the study groups. However, the timing when creep feed was replaced with stored forage and grain was unknown.

Effects of the time between selenium and vitamin E injection and the age at death were investigated by testing separate models for treated lambs in the present study. Both selenium and vitamin E concentrations in liver tissues of treated lambs depended only on the length of time after supplementation to death. However, the within-group and between-group coefficients were different. The length of time to death after supplementation within a given lamb was negatively correlated with lamb liver selenium and vitamin E concentrations. By contrast, there was a positive relationship between lamb liver selenium and vitamin E concentrations and group mean time between supplementation and death. This contextual effect diminished proportions of group level variance from both models. These results indicate that the liver selenium and vitamin E concentrations were higher with shorter time between death and individual supplementation, but higher with higher group mean time between treatment and death. This might be partly due to different dosages and time of selenium and vitamin E supplementation used in each lambing group. Further studies are required to determine an appropriate dosage and timing of selenium and vitamin E supplementation in newborn lambs, and if higher liver selenium and vitamin E concentrations in lambs might protect against early neonatal death.

It has been shown that after an initial spike in whole blood selenium concentrations in sheep, a decline in the selenium levels was observed two days after intramuscular injection (Puls, 1994a). Hidiroglou et al. (1990) found that serum vitamin E concentrations peaked by day three following injection, and continuously diminished throughout the four-week study period. Another study reported that serum vitamin E concentrations of treated ewes was higher than untreated ewes at day three post-injection, but that serum vitamin E concentrations in untreated and treated ewes were not different at day six post-injection (Gentry et al., 1992).

The directions of the effects of age and group median serum selenium concentration of late-gestation ewes on lamb liver selenium concentrations in the final model using data from all lambs, regardless of treatment, were the same as those found in the model based only on data from untreated lambs. These similarities were reasonable because about three-fourths of the study population were untreated lambs. However, the effect of serum selenium concentration in the late-gestation ewes was more pronounced in this combined model than the model for just untreated lambs.

The effect of selenium and vitamin E injection before death was also prominent in the final model of all lambs, driven primarily by the strength of the injection association within the treated lambs. An additional factor retained in this final model was month of flock visit for late-gestation ewe sampling, but there was no obvious pattern to this association and it is difficult to explain why, on average, liver selenium concentrations in lambs would be low in March and high in February, April and May.

Several nutritional management practices of late-gestation ewes were associated with lamb liver vitamin E concentrations in the final models including all lambs. Offering a commercial protein supplement, and providing oral mineral supplements free-choice were associated with increased liver concentrations of vitamin E in the lambs. Stored forages, which usually contain limited amounts of vitamin E (NRC, 2007), were the primary feedstuffs fed to the late-gestation ewes of our study flocks. Commercial protein and oral mineral supplements clearly augmented vitamin E status, as generally they contain significant amounts of vitamin E (Tarr, n.d.). Treatment with an injectable selenium and vitamin E product was highly associated with liver vitamin E concentrations in the lambs, as expected.

The differences between age-related patterns of lamb selenium and vitamin E concentrations in the present study and those of other studies might partly be attributed to different types of samples used to evaluate the concentrations (liver vs serum). Furthermore, timing and dosage of selenium and vitamin E injections to ewes and lambs are important factors associated with subsequent effects of the supplementation, such as reproductive and immunological effects (Liu et al., 2014). Our study results may have been affected by producers using injectable products in an off-label manner (e.g. under- and over-dosing), or inaccurate timing of injections used for data analyses.

According to the intra-class correlation coefficients of the models, the correlations of liver vitamin E concentrations within lambing groups were low in both untreated and treated lamb. Thus, factors at the individual level must be considered when the goal is to enhance lamb vitamin E status. For instance, in the present study, chronic disease processes leading to a reduction in colostral or milk intake might have affected liver concentrations of vitamin E. Factors at the lambing group level should also be considered with factors at the individual level in order to improve lamb selenium status in untreated lambs. Liver selenium concentrations in treated lambs were associated with one group level factor, the mean length of time after lamb supplementation.

Reported selenium and vitamin E injection use in ewes any time of the year was not associated with lamb liver selenium and vitamin E concentrations. However, the injections to the ewes at a time other than pre-parturition might have weakened an association with ewe injections during late gestation. The lamb liver selenium and vitamin E concentrations in the present study were obtained only from dead lambs. Because factors associated with the concentrations in dead and live lambs may not be the same, further studies in live lambs are required to confirm our findings.

4.6 Conclusions

In PEI sheep flocks, injectable selenium and vitamin E products were commonly used in lambs, while their use in ewes was limited. Based on the proportion of serum and liver samples that fell below the reference ranges, selenium and vitamin E deficiencies must be considered widespread in PEI flocks, especially in late-gestation ewes. Liver selenium and vitamin E concentrations were positively correlated with each other, in both untreated and treated lambs, reflecting the fact that colostrum and milk generally contain both of these micronutrients. Injection of selenium and vitamin E in neonatal lambs improved the status of both micronutrients in the livers of dead lambs; however, the effect of injection decreased with time. The positive relationship between lamb liver selenium and vitamin E concentrations and the group mean time between supplementation and death may suggest opportunities for further research to determine appropriate dosage and timing of selenium and vitamin E injection in newborn lambs.

The liver concentrations of both nutrients in lambs varied substantially with age at death, especially in untreated lambs. The concentrations peaked at approximately 3 weeks and thereafter declined, likely reflecting the transition from an all milk-based diet to one of solid feed. This observation emphasizes the importance of providing on-going dietary supplementation of selenium and vitamin E in the growing lambs.

The liver selenium concentration in lambs was positively associated with the concentration in late-gestation ewe serum, but in untreated lambs, the strength of the association varied by type of death. The relationship between selenium concentration in late-gestation ewe serum and selenium concentration in lamb livers was stronger in stillborn lambs and abortions than in live-born lambs dying later. The lamb liver vitamin E concentration was lower in stillborn lambs and aborted fetuses compared to born-alive lambs dying later. These findings point to the importance of colostrum and milk as sources of selenium and vitamin E for neonatal lambs. Moreover, protein and mineral supplement practices of late-gestation ewes were associated with lamb vitamin E concentration.

Taken together, these results point to the importance of nutritional management of late-gestation ewes, augmented by timely injectable and dietary supplementation of selenium and vitamin E, for ensuring an adequate selenium and vitamin E status in fetal, newborn and growing lambs.

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Table 4.1 Descriptive statistics for serum and liver selenium concentrations in late-gestation ewes and dead lambs, respectively, by selenium and vitamin E treatment status and overall, from 50 lambing groups on 36 flocks in Prince Edward Island in 2014 to 2015.

Parameter	Minimum	Median	Maximum	Mean	Standard deviation	Number of samples	Number of samples within deficient range*
Late-gestation ewe serum, median values representing lambing groups (µg/ml)							
Overall	0.005	0.075	0.170	0.077	0.038	48	5
Untreated ewes	0.005	0.071	0.170	0.073	0.038	40	5
Treated ewes	0.051	0.102	0.140	0.097	0.030	8	0
Late-gestation ewe serum, individual values (µg/ml)							
Overall	0.004	0.072	0.230	0.079	0.043	382	47
Untreated ewes	0.004	0.066	0.230	0.075	0.044	318	47
Treated ewes	0.032	0.100	0.160	0.097	0.033	64	0
Dead lamb liver, individual values (µg/g)**							
Overall	0.033	0.290	8.000	0.605	0.873	307**	124**
Untreated lambs	0.033	0.270	8.000	0.450	0.712	199	90
Treated lambs	0.140	0.460	5.300	0.985	1.140	77	25

*The range considered likely to be associated with clinical disease: for ewe serum selenium concentration, ≤ 0.030 µg/ml (Puls, 1994a); for lamb liver selenium concentration, ≤ 0.257 µg/g (Menzies et al., 2003)
**Because age at death and/or age of supplementation are not available in 31 lambs, their treatment status cannot be classified. These lambs are not used for computing descriptive statistics of the subgroups.

Table 4.2 Descriptive statistics for vitamin E concentrations of late-gestation ewe serum samples and dead lamb liver samples, by selenium and vitamin E treatment status and overall, from 50 lambing groups on 36 flocks in Prince Edward Island in 2014 to 2015.

Parameter	Minimum	Median	Maximum	Mean	Standard deviation	Number of samples tested	Number of samples within deficient level*
Late-gestation ewe serum, median values representing lambing groups (µmol/l)							
Overall	1.10	2.22	5.05	2.29	0.74	48	26
Untreated ewes	1.10	2.18	5.05	2.28	0.77	40	22
Treated ewes	1.60	2.42	3.35	2.36	0.58	8	4
Late-gestation ewe serum, individual values (µmol/l)							
Overall	0.90	2.20	7.80	2.36	1.06	379	209
Untreated ewes	0.90	2.10	7.80	2.36	1.09	315	179
Treated ewes	0.90	2.45	4.70	2.41	0.90	64	30
Dead lamb liver, individual values (µg/g)**							
Overall	0.89	7.96	2316.71	58.15	201.52	280**	39
Untreated lambs	0.89	7.22	703.57	24.88	81.60	183	36
Treated lambs	3.66	18.76	2316.71	141.46	358.88	72	3

*The range considered likely to be associated with clinical disease: for ewe serum vitamin E concentration, ≤ 2.32 µmol/l (Puls, 1994b); for lamb liver vitamin E concentration, ≤ 5 µg/g (Menzies et al., 2003)
**Because age at death and/or age of supplementation are not available in 25 lambs, their treatment status cannot be classified. These lambs are not used for computing descriptive statistics of the subgroups.

Table 4.3 Final model* of factors associated with natural logarithm selenium concentration in 194 lamb livers among untreated (for selenium and vitamin E) lambs from 32 lambing groups in Prince Edward Island in 2014 to 2015.

Variable	Coefficient	Standard error	p-value	95% confidence interval	
Lamb age at death (linear term)	0.0426	0.0156	0.007	0.0119	0.0733
Lamb age at death (quadratic term)	-0.0010	0.0004	0.011	-0.0018	-0.0002
Ewe serum selenium, group median	3.1031	3.5142	0.377	-3.7845	9.9908
Type of lamb death					
-Abortion/stillbirth (baseline = post-partum)	-0.2530	0.0947	0.008	-0.4387	-0.0674
Interaction between group median ewe serum selenium concentration and type of lamb death	4.4903	2.1383	0.036	0.2993	8.6814
Constant	-1.0016	0.1386	< 0.001	-1.2733	-0.7298

*Log restricted-likelihood = -177.07

Table 4.4 Final model* of factors associated with natural logarithm vitamin E concentration in 180 lamb livers among untreated (for selenium and vitamin E) lambs from 33 lambing groups in Prince Edward Island in 2014 to 2015.

Variable	Coefficient	Standard error	p-value	95% confidence interval	
Lamb age at death (linear term)	0.0732	0.0281	0.009	0.0181	0.1283
Lamb age at death (quadratic term)	-0.0020	0.0007	0.007	-0.0034	-0.0005
Type of lamb death					
-Abortion/stillbirth (baseline = post-partum)	-0.4320	0.1710	0.012	-0.7671	-0.0968
Constant	2.3800	0.1379	< 0.001	2.1097	2.6503

*Log restricted-likelihood = -260.81

Table 4.5 Final models of factors associated with selenium and vitamin E concentrations (both natural logarithm transformed) in lamb livers among treated (for selenium and vitamin E) lambs from 22 lambing groups in Prince Edward Island in 2014 to 2015.

Variable	Coefficient	Standard error	p-value	95% confidence interval	
Model for liver selenium concentration (n = 73 from 22 lambing groups; Log restricted-likelihood = -102.88)					
Time from lamb supplementation until lamb death (individual-level, days)	-0.0257	0.0079	0.001	-0.0412	-0.0103
Mean time from lamb supplementation until lamb death (group-level, days)	0.0634	0.0157	< 0.001	0.0326	0.0941
Constant	-0.9497	0.1912	< 0.001	-1.3245	-0.5750
Model for liver vitamin E concentration (n = 69 from 22 lambing groups; Log restricted-likelihood = -126.84)					
Time from lamb supplementation until lamb death (individual-level, days)	-0.0500	0 .0127	< 0.001	-0. 0749	-0. 0251
Mean time from lamb supplementation until lamb death (group-level, days)	0 .0876	0 .0235	< 0.001	0.0416	0.1337
Constant	2.9315	0 .2826	< 0.001	2.3776	3.4854

Table 4.6 Final model* of factors associated with natural logarithm selenium concentration in 271 lambs (regardless of lamb treatment status) from 38 lambing groups in Prince Edward Island in 2014 to 2015.

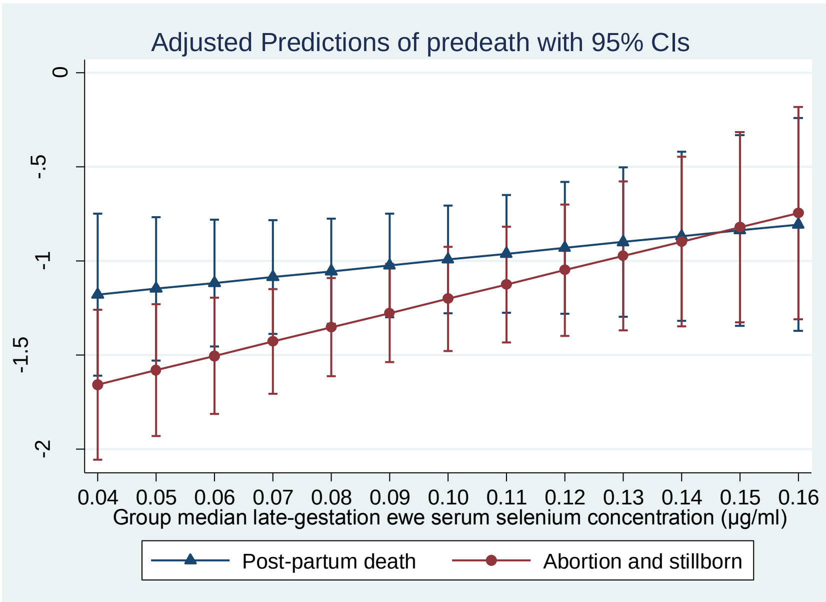
Variable	Coefficient	Standard error	p-value	95% confidence interval	
Lamb age at death (days) (linear term)	0.0414	0.0113	< 0.001	0.0193	0.0635
Lamb age at death (days) (quadratic term)	-0.0010	0.0003	< 0.001	-0.0015	-0.0005
Ewe serum selenium, group median (µmol/l)	7.9311	1.8243	< 0.001	4.3555	11.5067
Treatment status of lamb -Treated (baseline = not treated)	0.7451	0.1175	< 0.001	0.5148	0.9753
Month (baseline = March)					
-April	0.3764	0.1607	0.019	0.0614	0.6914
-May	0.7140	0.3094	0.021	0.1076	1.3203
-September	-0.0614	0.5415	0.910	-1.1226	0.9998
-October	0.2666	0.2462	0.279	-0.2159	0.7491
-November	0.2230	0.4079	0.585	-0.5764	1.0224
-December	0.3690	0.2710	0.173	-0.1622	0.9002
-January	0.3056	0.1895	0.107	-0.0659	0.6771
-February	0.6349	0.1743	< 0.001	0.2934	0.9765
Constant	-2.2417	0.1936	< 0.001	-2.6211	-1.8623

*Log restricted-likelihood = -305.20

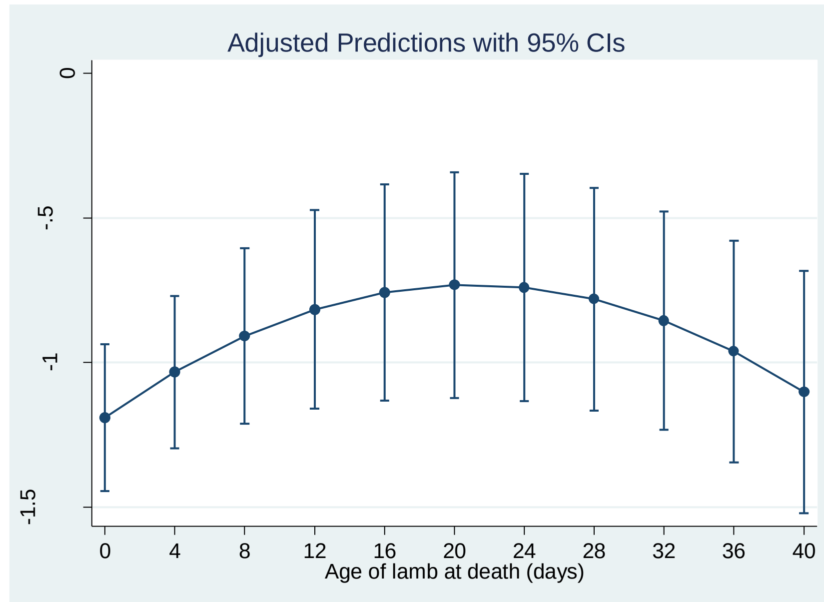
Table 4.7 Final model* of factors associated with natural logarithm vitamin E concentration in 255 lambs (regardless of lamb treatment status) from 39 lambing groups in Prince Edward Island in 2014 to 2015.

Variable	Coefficient	Standard error	p-value	95% confidence interval	
Late-gestation ewe protein supplement					
-Available (baseline = not available)	0.3968	0.1634	0.015	0.0765	0.7171
Late-gestation ewe mineral supplement					
-Free-choice (baseline = daily/weekly)	0.5222	0.2416	0.031	0.0486	0.9958
Type of death					
-Abortion/stillbirth (baseline = post-partum)	-0.6370	0.1748	< 0.001	-0.9797	-0.2944
Treatment status of lamb					
-Treated (baseline = not treated)	0.7752	0.1862	< 0.001	0.4102	1.1402
Constant	1.8644	0.2658	< 0.001	1.3435	2.3853

*Log restricted-likelihood = -403.40



(a)



(b)

Figure 4.1 Predicted effects of (a) median late-gestation ewe serum selenium concentration (age of lamb at death set at 0 days), and (b) age of lamb at death (median late-gestation ewe serum selenium concentration set at 0.08 µg/ml) on lamb liver selenium concentration (natural logarithm transformed) in untreated lambs dying pre- and post-partum, in Prince Edward Island in 2014 to 2015.

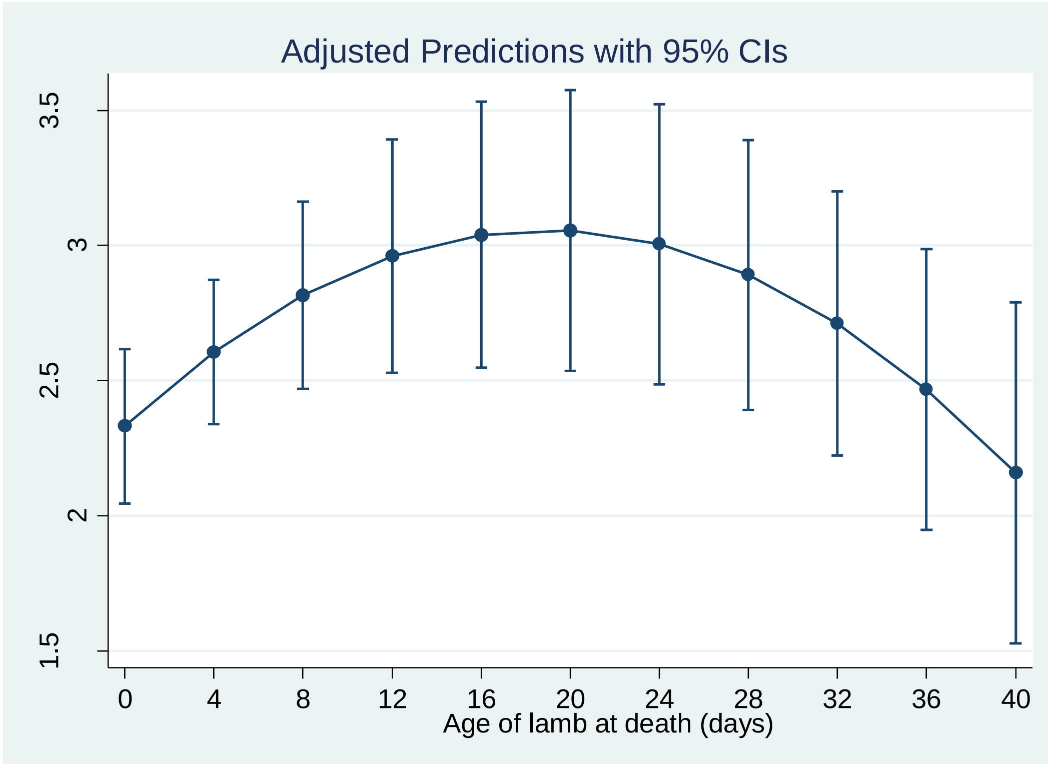


Figure 4.2 Predicted effects of age at death on lamb liver vitamin E concentration (natural logarithm transformed) in untreated lambs dying post-partum, in Prince Edward Island in 2014 to 2015.

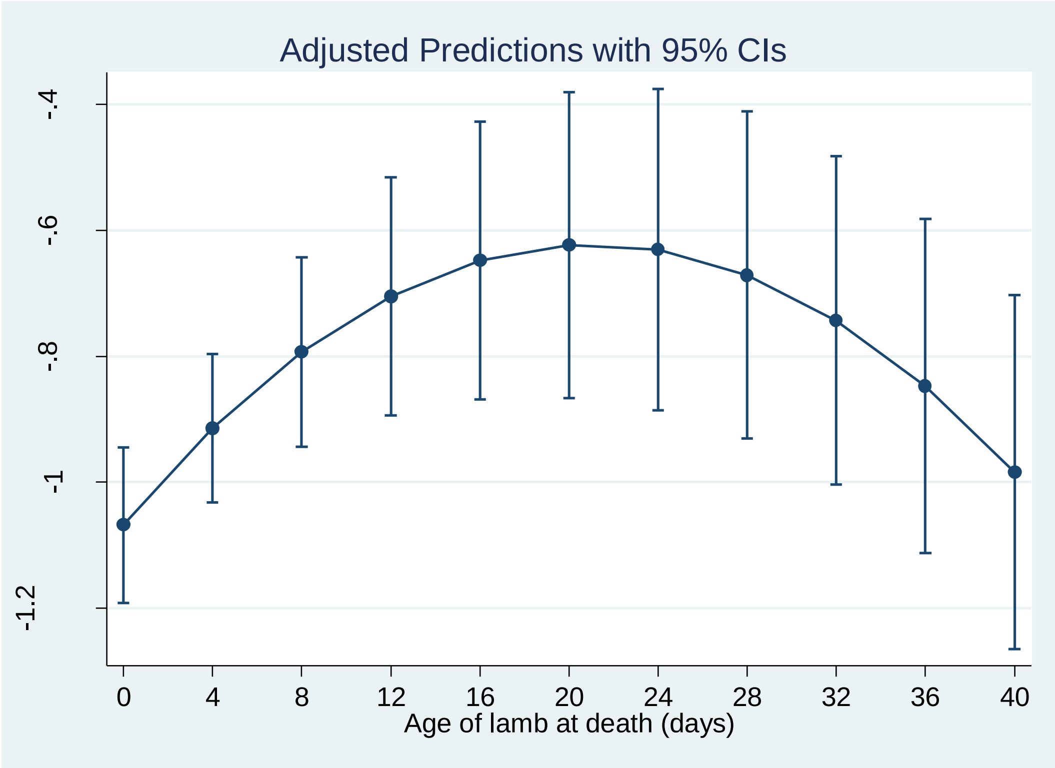


Figure 4.3 Predicted effects of age at death on lamb liver selenium concentration (natural logarithm transformed) in all lambs, in Prince Edward Island in 2014 to 2015.

CHAPTER 5

EVALUATION OF THE PRECISION XTRA® METER FOR MONITORING BLOOD β-HYDROXYBUTYRATE CONCENTRATIONS IN LATE-GESTATION EWES

5.1 Abstract

The most common nutritionally-related disease during the last trimester of pregnancy in sheep is pregnancy toxemia, which is commonly diagnosed by an elevated β-hydroxybutyrate (BHBA) concentration. Using an animal-side test is an alternative technique to determine the BHBA concentration for disease control. However, accuracy of the test to measure ewe blood BHBA concentration has to be evaluated before it is introduced into practice. Blood samples were collected from 384 late-gestation ewes. The fresh blood samples were immediately tested with an animal-side test, Precision Xtra® meter, whereas the serum samples were analyzed with a standard laboratory method to determine the BHBA concentration. The concentrations obtained from the two methods were analyzed with various statistical analyses to evaluate the accuracy of the Precision Xtra® meter compared to the standard laboratory method. Scatter plots of BHBA concentrations from the two tests revealed that the Precision Xtra® meter had a very good accuracy. The concentrations from the 2 tests were not statistically different, based on the paired t-test ($p = 0.117$). The Pearson's correlation coefficient, intra-class correlation coefficient and Lin's concordance correlation coefficient were 0.98 ($p < 0.001$), 0.97 and 0.98, respectively. The intercept and slope of a linear mixed model, containing the standard method results as an outcome and the meter results as a predictor, were 18.30 (95% CI: 0.30, 36.30) and 0.98 (95% CI: 0.96, 1.00), respectively. When the samples were classified into 3 classes of ketosis based on BHBA concentrations obtained from each method, the Cohen's Kappa statistic was 0.71 ($p < 0.001$). The ketotic classification was in agreement in 94.54% of samples. The optimal cut-off for the Precision Xtra® meter to detect samples having a laboratory BHBA

concentration ≥ 800 µmol/l was 800 µmol/l. The sensitivity and negative predictive value at this cut-off were 100%. The Precision Xtra® meter provided excellent diagnostic correlation and substantial agreement with the standard laboratory technique for measuring blood BHBA in late-gestation ewes.

5.2 Introduction

Ketosis is a common metabolic disorder of late-gestation ewes. The increasing energy requirement of ewes due to the rapid growth of fetuses in the last month of pregnancy contributes to an energy deficit (Brozos et al., 2011). Hormonal regulation of fat and carbohydrate metabolism during this period of energy deficit can lead to ketonemia and, in more advanced cases, clinical signs of ketosis (Andrew, 1997). Provision of rations which are either too low or too high in energy during late gestation have been reported as common risk factors for the disorder (Edmondson et al., 2012). Ewes carrying more than one fetus are more susceptible to ketosis than ewes carrying just one fetus (Schlumbohm and Harmeyer, 2008; Moallem et al., 2012). Stress and concurrent diseases are also predisposing factors of ketosis, likely because they suppress appetite (Cal-Pereyra et al., 2012). Clinical signs associated with ketonemia are mostly referable to the neurological system; however, it is likely that other systems are affected as well. For instance, ketotic ewes were exposed to higher oxidative stress (Al-Qudah, 2011; Gurdogan et al., 2014), and the immune response of affected ewes was suppressed (Lacetera et al., 2002). Placental transfer of ketones from ewes to fetal lambs was confirmed by Miodovnik et al. (1982) - while the significance of this finding is not known, small and weak lambs, non-viable lambs, and poor milk availability are reported consequences of maternal ketosis in lambs (Cal-Pereyra et al., 2012).

Andrew (1997) classified ketonemia in ewes into two classes, moderate and severe. The concentration of blood β-hydroxybutyrate (BHBA), 1 of 3 ketones produced during the metabolism of fat, is used for classification, with 800 to < 1600 µmol/l, and ≥ 1600 µmol/l, reflecting moderate and severe ketonemia, respectively. Monitoring the concentration of BHBA in blood or urine of late-gestation ewes is effective in predicting clinical ketosis during the peri-partum period (Fthenakis et al., 2012). Although laboratory analysis of blood BHBA concentrations is considered the gold standard, such testing is time consuming (Oetzel, 2007) and expensive at the flock-level. Thus, there is a need for an accurate, less expensive on-farm test for successful disease control (Brozos et al., 2011). Animal-side tests used for the purposes of measuring blood BHBA concentration have been evaluated in other domestic ruminants in the field, and have generally been found to be efficacious (Iwersen et al., 2009; Voyvoda and Erdogan, 2010; Dore et al., 2013; Kanz et al., 2015; Tatone et al., 2015). Precision Xtra® meter is an example of an animal-side test used to monitor blood concentrations of glucose and BHBA in diabetic human patients, but also BHBA in cattle. As an added benefit, measurement of blood BHBA concentration by this test is less invasive than traditional venipuncture because only one drop of blood is required (Pichler et al., 2014).

Two previous studies have examined the usefulness of the Precision Xceed® meter, an animal-side test from the same company as the Precision Xtra® meter, in measuring blood concentrations of glucose and ketones of sheep (Panousis et al., 2012; Pichler et al., 2014). The values obtained using the test were strongly correlated with the results from traditional laboratory analysis; however, the target group of those studies included both late-gestation and early lactation ewes. The objective of this study was to evaluate the accuracy and practicality of the Precision Xtra® meter for measuring blood BHBA in late-gestation ewes at risk of ketosis in commercial flocks.

5.3 Materials and methods

This study was conducted between September 2014 and May 2015 in 48 breeding groups from 34 commercial sheep flocks that represented the approximately 100 flocks located in Prince Edward Island (PEI), Canada. These lambing groups were visited one to three weeks before the expected first lambing date. The number of bred ewes comprising each breeding group ranged from 12 to 172. In each group, eight late-gestation ewes were conveniently selected for blood sample collection as illustrated in Chapter 3. A drop of unclotted blood was immediately taken from the tube and tested by the primary author (NR) at ambient barn temperature (between -20 to 27 °C) for BHBA concentration using the Precision Xtra® meter (Abbott Diabetes Care, Saint-Laurent, Canada). This device displays a one decimal point result for BHBA concentration in mmol/l, which was converted to µmol/l for statistical analysis. Once the samples arrived at the laboratory, the remaining blood samples were processed as described in Chapter 3. The degree of hemolysis of serum samples was classified as negative, mild and moderate. The results received from the laboratory were reported in µmol/l. Serum BHBA was determined by Veterinary Laboratory Services, Animal Health Laboratory, University of Guelph, using the Cobas 6000 c501 biochemistry analyzer (Roche Diagnostics, Laval, QC, Canada), which is a photometric system-based machine. The results for each of the two tests were obtained without prior knowledge of the results of the other test. Prior consent for the study was obtained for all participating producers, and the study protocols were reviewed and approved by the University of PEI Animal

Care Committee. Approximate temperatures at the time of sampling were obtained from a repository (Time and Date Website, 2017).

Statistical analyses were performed using Stata (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, Tx: StataCorp LP). Blood BHBA concentrations obtained from the laboratory analysis and the Precision Xtra® meter were first analyzed on their continuous scale, and compared using descriptive statistics. The difference between the values from the two methods was calculated - the result from the Precision Xtra® meter minus the result from the laboratory test. Percent difference was calculated by dividing the difference by the laboratory concentration. Pearson's correlation coefficient between percent difference and approximate temperature at the time of sampling was calculated. Limits of agreement were calculated and plotted, along with the differences and the means of each sample, to evaluate agreement of the two tests, as recommended by Bland and Altman (1986).

For analytical statistics, paired t-test, Pearson's correlation coefficient, intra-class correlation coefficient, Lin's concordance correlation coefficient and bias correction factor (Lin, 1989) were determined to compare the results from the two tests. A linear mixed model, with herd and lambing group as random effects, was also fitted to determine the intercept and slope (and 95% confidence intervals – CI) of the relationship between the Precision Xtra® results (predictor variable) and the laboratory test results (outcome variable).

For each method, ewes were classified as having a physiologic, moderate or severe ketotic state if their BHBA concentrations were < 800 μmol/l, 800 to < 1600 μmol/l, and ≥ 1600 μmol/l, respectively. The differences in average blood BHBA concentrations between the two tests, based on the three classifications by the laboratory method, were compared using the Kruskal-Wallis test. Inter-rater agreement of ketotic classification was carried out using the Cohen's Kappa statistic based on the three categories and two sets of test results.

Sensitivity, specificity, positive predictive value and negative predictive value were calculated, based on an 800 μmol/l cut-off from the laboratory BHBA concentration, to determine which of 3 different cut-offs for the Precision Xtra® concentration would be best: 700, 800 or 900 μmol/l. Area under the receiver operating characteristic (ROC) curve was also analyzed to assist in identifying the most appropriate cut-off for the Precision Xtra® meter to detect ovine ketosis. Cohen's Kappa statistic was again computed to determine an inter-rater agreement of dichotomous ketosis detection, based on the optimal Precision Xtra® meter cut-off and standard test cut-off. The McNemar's test was carried out to determine the dependence of dichotomous ketosis detection on methods of testing, in order to understand the Kappa statistic properly. For all statistical analyses, a p-value < 0.05 was considered statistically significant.

5.4 Results

Mean flock size was 93 (13-250) for breeding ewes. Three flocks (8.8%) had only 1 breed (Hampshire, Suffolk and Black Welsh Mountain). The other 31 flocks incorporated 2 or more of the following breeds: Suffolk, Dorset, Canadian Arcott, Rideau, Texel, Cheviot, Hampshire, Ile de France, Border Leicester, Corriedale, Finnsheep, Romanov, Shetland and Southdown.

Blood samples were collected from 384 ewes. However, 16 samples from 2 lambing groups could not be analyzed with the Precision Xtra® meter because of the low ambient temperature (≤ 0 °C). Moreover, one sample clotted before the test was performed, and for two samples, there was insufficient serum for the laboratory test (Fig. 5.1). The proportion of negative, mild and moderate hemolysis samples were 81.15%, 18.06% and 0.79%, respectively.

Minimum, median, maximum, mean, and standard deviation from 2 BHBA measuring methods are shown in Table 5.1, along with the differences and percent differences between test results. In general, the descriptive statistics of blood BHBA concentrations from the two methods were very similar. A few samples had substantial differences (e.g. 695 µmol/l) between test results, as shown by the minimum and maximum differences and percent differences. Eighty-eight samples (24.04%) had a difference of > 100 µmol/l. The concentrations obtained from the 2 tests were identical in 5 samples (1.37%). Four of these samples had 400 µmol/l, while another sample had 300 µmol/l.

Scatter plots of results from the 2 tests (Fig. 5.2) demonstrate diagrammatically the very good accuracy of the Precision Xtra® meter versus the laboratory test; all points are very close to the reduced major axis and the line of perfect concordance, and these 2 lines are overlapping. The proportions of samples having higher results from the laboratory test and the Precision Xtra® meter was similar at 57.38% and 41.26%, respectively. Also, no evidence of bias was found in the Bland-Altman plot (Fig. 5.3). The 95% confidence limits of agreement were -211.65 to +194.63 µmol/l. In Fig. 5.3, there were 2 observations which clearly deviated from other observations; the laboratory concentrations of these samples were higher than the concentrations obtained by the Precision Xtra® meter, but these samples had high BHBA concentrations and, therefore, would still be categorized as severely ketotic by the Precision Xtra® meter.

The concentrations from the laboratory test and the Precision Xtra® meter were not statistically different based on the paired t-test ($p = 0.117$). However, when only hemolyzed samples were taken into account, the results obtained from the 2 tests were significantly different ($p = 0.02$). The Pearson's correlation coefficient was 0.98 ($p < 0.001$). The intra-class correlation coefficient was 0.97 (95% CI: 0.97, 0.98). Lin's concordance correlation coefficient and bias correction factor were 0.98 and 1.00, respectively. The scale shift and location shift relative to the scale were 1.01 and -0.02, respectively. The intercept and slope of the linear mixed model were 18.30 (95% CI: 0.30, 36.30) and 0.98 (95% CI: 0.96, 1.00), respectively.

Based on the laboratory testing, most ewes were classified as having a physiologic ketosis (Fig. 5.1). The ketotic ewes were spread out among the lambing groups; 20 lambing groups (41.67%) had at least 1 ketotic ewe. The number of affected ewes in groups ranged from 0 to 5 out of 8 (0% to 62.50%). The mean number of ketotic ewes in affected groups was 1.5.

The mean concentration differences between the 2 tests in ewes with physiologic, moderate and severe ketosis (according to the standard laboratory test) were -10.45, +41.43 and -57.88 μmol/l, respectively, which were not equal based on the Kruskal-Wallis test ($p = 0.037$). The Cohen's Kappa statistic for ketotic classification was 0.71 (95% CI: 0.63, 0.80; $p < 0.001$), indicating a substantial agreement in ketotic classifications beyond that expected due to chance alone. The ketosis classification was in agreement in 94.54% of the samples.

Sensitivity, specificity, positive predictive value, negative predictive value and area under the ROC curve of each cut-off are shown in Table 5.2. The optimum cut-off of the Precision Xtra® meter for detecting ewes with at least a moderate level of ketosis was 800 µmol/l, based on the best area under the ROC curve, while maintaining 100% sensitivity and negative predictive value. Using this optimal Precision Xtra® meter cut-off, there was a substantial agreement with the standard laboratory test in detecting ketotic ewes, based on the Cohen's Kappa statistic of 0.74 (95% CI: 0.64, 0.84; $p < 0.001$). The tests agreed on the detection of ketosis in 95.08% of the samples. However, according to the McNemar's test, the dichotomous detection of ketosis was dependent on the method of testing ($p < 0.001$); the proportion considered ketotic by the standard test (7.85%) was significantly different from that detected by the meter test (12.81%), suggesting that reasons for the residual disagreement between the tests should be explored further. The Pearson's correlation coefficient between percent difference and approximate temperature at sampling time was low, but significant ($r = 0.14$, $p = 0.008$).

5.5 Discussion

For successful treatment and prevention of ketosis, early and accurate diagnostic detection of elevated ketones is required (Brozos et al., 2011). The highest blood BHBA concentrations in late-gestation ewes obtained from the laboratory test in the present study (6720 µmol/l) was greater than the highest concentrations reported in 2 previous ovine studies of Panousis et al. (2012) and Pichler et al. (2014), of 5100 µmol/l and

4500 µmol/l, respectively. However, the mean blood BHBA obtained from the laboratory test reported in the present study (546 µmol/l) was lower than the means reported in the previous studies (1200 and 700 µmol/l). These differences might, in part, be due to different breeds, nutritional management practices and environmental conditions in study populations.

The mean concentrations of BHBA for the two measurement methods were very close, which is consistent with results found by Panousis et al. (2012), in which an animal-side test from the same company as the Precision Xtra® meter was evaluated. The results obtained from the Precision Xtra® meter were mostly lower than the results obtained from the laboratory test. The mean difference (8.5 µmol/l) between the 2 results in the present study was lower than that found in a previous study (150 µmol/l), which was reported using an animal-side test from the same company as the Precision Xtra® meter (Pichler et al., 2014). The highest between-method difference was > 1000 µmol/l in that study while it was only 695 µmol/l in the present study, and the sample with the highest percent difference (-58.85%) had a lower BHBA concentration (486 µmol/l). There were 2 samples in the present study where large between-method differences were noted (620 and 695 µmol/l); both of these samples had high mean blood BHBA concentrations (6410 and 2148 µmol/l, respectively). The differences corresponded to -9.23% and -27.86% of the laboratory test value. However, these two samples were classified in the same severe ketosis category, regardless of measurement techniques. Ninety-five percent of differences ranged between -211.65 to +194.63 µmol/l. These findings indicate a good correlation between results of the two measurement methods.

Animal-side tests from the same company as the Precision Xtra® meter provided underestimated results compared to the laboratory-derived results, when it was used in dairy cows (Iwersen et al., 2009) and in sheep (Pichler et al., 2014) elsewhere. This trend was also noticed in the present study; the intercept of the linear mixed model was positive and significantly different from 0 ($p = 0.046$). Nevertheless, slightly lower Precision Xtra® results than laboratory results found in the present study might not be an actual underestimation, because an overestimation of standard laboratory-based BHBA concentrations in hemolyzed blood samples was reported in a previous experimental study. The adverse effect of hemolysis on results obtained from photometric-based methods is due to the interfered background absorbance of hemolyzed samples (Jacobs et al., 1992). Additionally, in the present study the slightly lower Precision Xtra® result was not found when hemolyzed samples were not included in analysis. The 95% CI of the intercept of the mixed model based only on non-hemolyzed samples included 0 (-6.00, 30.97). Thus the slightly lower Precision Xtra® results found when all samples were taken into account might be an overestimation of the standard laboratory test instead of an underestimation of the Precision Xtra® meter. Hemolyzed bovine serum samples were not included in a test evaluation study of the Precision Xtra® meter (Tatone et al., 2015). However, in the present study, other parameters determining accuracy of the device, such as Pearson's correlation coefficient, intra-class correlation coefficient, and Lin's concordance correlation coefficient, were not affected by hemolysis; therefore, hemolyzed serum samples were still included in our evaluation.

Based on the analytical statistics, blood BHBA concentrations provided by the Precision Xtra® meter were not different from those received from the laboratory test.

There was a strong positive correlation between the results obtained from the two methods. The Pearson's correlation coefficient in this study (0.98) was close to those reported by Panousis et al. (2012) and Pichler et al. (2014) using an animal-side test from the same company as the Precision Xtra® meter (0.99 and 0.96, respectively). A high Pearson's correlation coefficient and indifferent means are meaningful for comparison. The Pearson's correlation coefficient indicates good correlation of the test (Lin, 1989). Nevertheless, good correlation cannot be assumed based only on these two findings (Indrayan and Chawla, 1994). The intra-class correlation coefficient (0.97) indicated excellent correlation of the 2 measurement methods as well. Lin's concordance correlation coefficient, which is more appropriate than Pearson's correlation coefficient in evaluating correlation between 2 continuous scale tests (Tatone et al., 2015), was also very high (0.98).

The prevalence of late-gestation ewes in the present study with blood BHBA concentrations suggesting ketonemia was lower than that reported earlier by Panousis et al. (2012) and Pichler et al. (2014) (7.9% vs 32.2% and 17.5%, respectively). Body condition score of late-gestation ewes and number of fetuses are well-known factors influencing occurrence of ketosis (Olfati et al., 2013; Raoofi et al., 2013). However, comparisons of these factors are not possible because of unavailability of the data from previous studies. It was possible that, on average, proportion of ewes with appropriate body condition scores was higher, and litter size of the study population in the present study was lower compared to the two previous studies (Panousis et al., 2012; Pichler et al., 2014). An appropriate nutritional management based on number of fetuses and body condition score is useful practice to avoid ketosis (Fthenakis et al., 2012). The lower prevalence of ketosis in the present study might indicate that many of the producers were aware of the importance of feeding late-gestation ewes well, based on nutrition education efforts provided in the region.

Our results suggest that the optimal cut-off for using the Precision Xtra® meter to detect ewes having laboratory BHBA concentrations ≥ 800 µmol/l is ≥ 800 µmol/l. The two methods had substantial agreement in detecting and classifying ketotic late-gestation ewes based on the Cohen's kappa statistic. Nevertheless, the McNemar's test indicated that the prevalence of ketosis detected by the Precision Xtra® meter was overestimated compared to the prevalence detected by the laboratory test (12.8% vs 7.9%). Out of 18 false positive samples reported by the Precision Xtra® meter, 14 and 4 were 800 µmol/l and 900 µmol/l, which were clearly very close to being negative on the meter. Furthermore, the lowest laboratory BHBA concentration of the meter false positive samples was 524 µmol/l, with most of these false positive samples having > 600 µmol/l laboratory BHBA, also indicating that the continuous scale results from the 2 tests did not disagree by much when they disagreed on ketosis categories. Incompatibility between the Cohen's kappa statistic and the McNemar's test for agreement is not uncommon, especially when a large number of observations are assessed (Mecham, 2013).

Although some ewes diagnosed as positive using the meter device were not truly positive (low positive predictive value), the sensitivity and negative predictive value at this cut-off were excellent at 100%. The implications of false negatives (do not get fed better when really need it) are worse than false positives (get fed better when do not really need it), therefore, the optimal cut-off that has 100% sensitivity, and no false negatives, makes sense from a veterinarian's and producer's perspective.

The relatively low positive predictive value in the present study resulted primarily from the low prevalence of ketosis in the study population, a common finding within test evaluations (Dohoo et al., 2009). The practical consequence of a low positive predictive value is potentially wasted resources from unnecessary treatment of false positive animals – in our case, just extra feeding, which is likely not a waste. There are 3 approaches to enhance the positive predictive value: 1) using the test only in animals considered at risk; 2) increasing the cut-off in order to improve specificity; and 3) using multiple tests with series interpretation (Dohoo et al., 2009). Based on results found in the present study, when the cut-off was increased to 900 µmol/l in order to improve specificity and positive predictive value, there was a modest decrease in negative predictive value observed, accompanied by a substantial reduction in sensitivity from 100% to 83% (Table 5.2). For ketosis screening in late-gestation ewes, a maximal negative predictive value is probably more important than a high positive predictive value, because the treatment is inexpensive, and failing to treat an affected animal can lead to losses of both the ewe and lambs. Using multiple tests would likely be impractical and expensive. Therefore, testing only animals considered at risk (i.e. ewes found to have multiple fetuses at pregnancy diagnosis, or ewes in modest body condition) may be a better option to increase prevalence within the test population, and increase positive predictive value.

Sargison et al. (1994) reported that concentrations of BHBA > 3000 µmol/l were detected in most ewes demonstrating clinical signs of pregnancy toxemia. There were two ewes having a blood BHBA concentration greater than this level in the present study.

However, no clinical sign of ketosis were observed in these ewes during the blood collection. Therefore, other factors are likely necessary for clinical ketonemia to manifest.

In an earlier study, the performance of an animal-side test developed to measure milk BHBA concentration was not affected by temperature (Shire et al., 2013). However, the recommended temperature range for operating the Precision Xtra® meter is 10 °C to 50 °C, and using the device at very low temperatures resulted in an error reading in our hands. In most cases this problem could be overcome by storing the device and test strips in coverall pockets. However, variation due to ambient temperature may have occurred, therefore, the differences between the field and laboratory tests (i.e., percent difference using the laboratory concentration as a denominator) compared to the recorded daytime temperature at the time of sampling were examined. The percent difference was significantly and positively correlated to daytime temperature ($r = 0.14$, $p = 0.008$), suggesting that our observation of a mean of 2.35% underestimation of BHBA concentration by the Precision Xtra® meter when compared to the laboratory test (Table 5.1) may, in part, be due to operation at a colder than recommended ambient temperature. Therefore, perhaps ambient temperature when conducting the meter test should be standardized.

In addition to very good accuracy, there are a number of additional benefits of the Precision Xtra® meter. Only 1.5 µl of blood is required for testing. A drop of blood from the ear vein was sufficient for sample analysis using a similar device in an ovine study (Pichler et al., 2014). The cost of the BHBA animal-side test is also cheaper than a laboratory test (Panousis et al., 2012), and the cost of collection and transport of the sample to a laboratory is not required. Considering that a tube and/or syringe are unnecessary for testing with the Precision Xtra® meter (if blood is collected from the ear vein), the total cost of the Precision Xtra® meter is lower. A precision Xtra® meter and a ketone test strip were available online (http://www.google.ca) at < 50 and < 5 Canadian dollars, respectively. Some distributors offered a free test meter when purchasing a specified number of test strips. Additionally, waste produced by performing the field test is lower. The device provides a result within 10 seconds, which is much faster than results obtained from the traditional laboratory method. Although short turnaround time analyzers have been available, time for sample transportation is unavoidable for a laboratory method. Perhaps most importantly, treatment can be instituted immediately after measuring with the Precision Xtra® meter, thereby reducing the risk of ewes developing clinical ketosis or pregnancy toxemia (Panousis et al., 2012). Animal-side tests have been used for measuring BHBA concentrations in the urine and milk of cows; however, the results have been shown to be less accurate when compared to blood analysis (Oetzel, 2004; Iwersen et al., 2009).

A limitation of the present study should be considered when interpreting the results. The use of an intra-class correlation coefficient to evaluate test reliability assumes that the diagnostic methods are randomly selected from available methods, which is not true in the present study. Nevertheless, the assumption may have some validity in studies comparing two diagnostic tests (Indrayan, 2013).

5.6 Conclusions

In commercial ewe flocks managed under conditions typical of eastern Canada, the handheld Precision Xtra® meter provided excellent diagnostic correlation and substantial agreement with the standard laboratory technique for measuring blood BHBA concentration and detecting ketosis in late-gestation ewes. Use of the Precision Xtra® meter is likely to result in more rapid diagnosis and treatment of ketonemia, and better nutritional management of late-gestation ewes.

5.7 References

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Table 5.1 Descriptive statistics of β-hydroxybutyrate concentrations in 382 blood samples of late-gestation ewes in Prince Edward Island, between September 2014 and August 2015, tested with the standard laboratory method and handheld Precision Xtra® meter.

Parameters	Standard Laboratory Test (µmol/l)	Precision Xtra® Meter (µmol/l)	Difference (µmol/l) (Precision Xtra® - Laboratory Test)	Percent difference of the Precision Xtra® from the Laboratory Test
Minimum	107	100	-695	-58.8
Median	466	400	-16	-3.6
Maximum	6720	6100	292	52.7
Mean	546	537	-8.5	-2.4
Standard deviation	453	460	103.6	18.3
Number of animals tested	382	367	366	366

Table 5.2 Sensitivity, specificity, positive predictive value, negative predictive value and area under the Receiver Operating Characteristic Curve of different Precision Xtra® blood β-hydroxybutyrate cut-off concentrations for the detection of ketosis (Gold Standard: laboratory β-hydroxybutyrate concentration ≥ 800 μmol/l), using 366 blood samples of late-gestation ewes in Prince Edward Island, between September 2014 and August 2015.

Parameter	700 μmol/l	800 μmol/l	900 μmol/l
Sensitivity (%)	100.0	100.0	82.8
Specificity (%)	83.7	94.7	98.8
Positive predictive value (%)	34.5	61.7	85.7
Negative predictive value (%)	100.0	100.0	98.5
Area under the ROC curve (95% CI)	0.92 (0.90, 0.94)	0.97 (0.96, 0.98)	0.91 (0.84, 0.98)

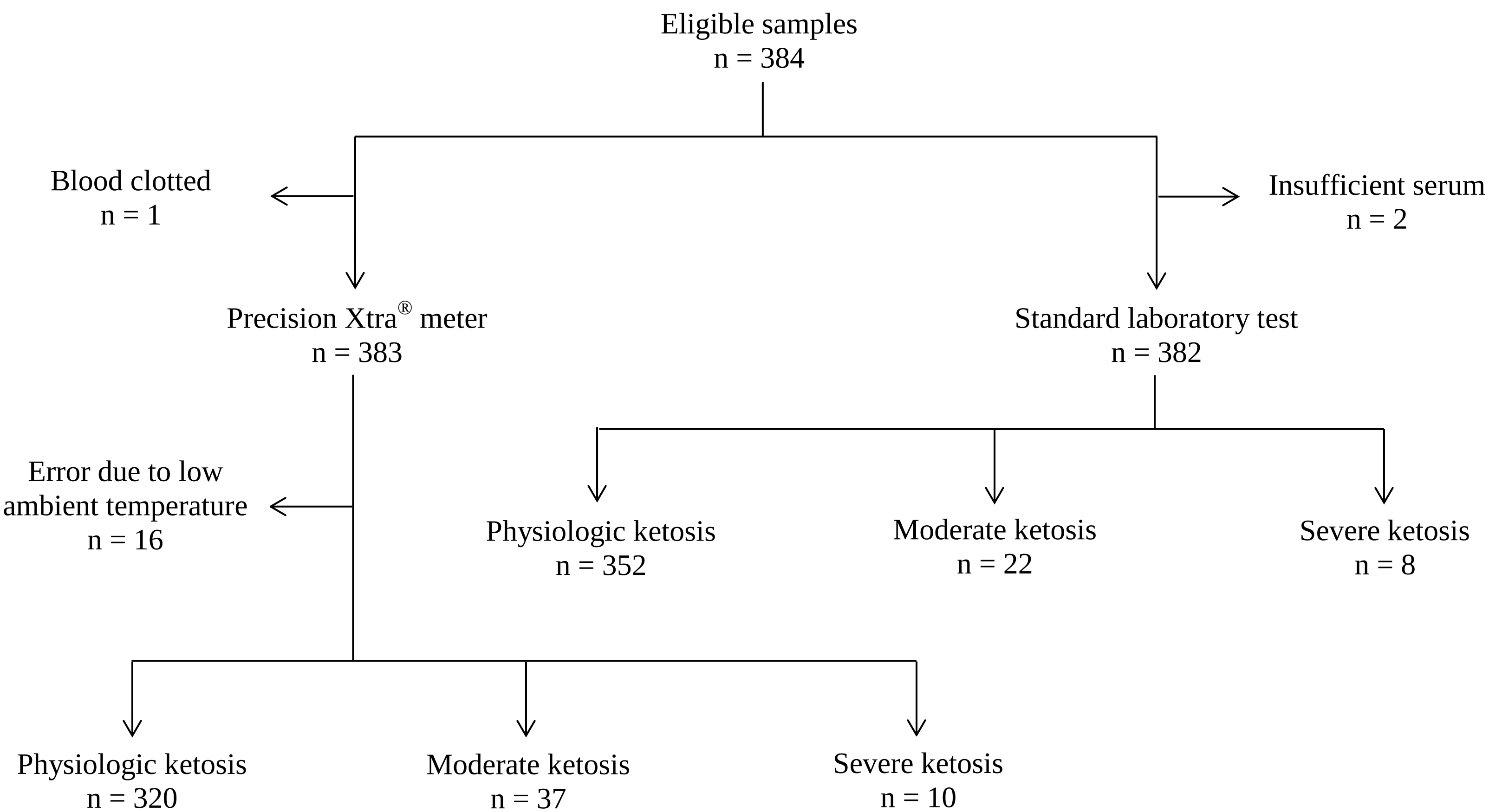

Figure 5.1 Diagram of numbers of samples analyzed with the standard laboratory test and Precision Xtra® meter, and number of samples classified into three ketosis categories by each method.

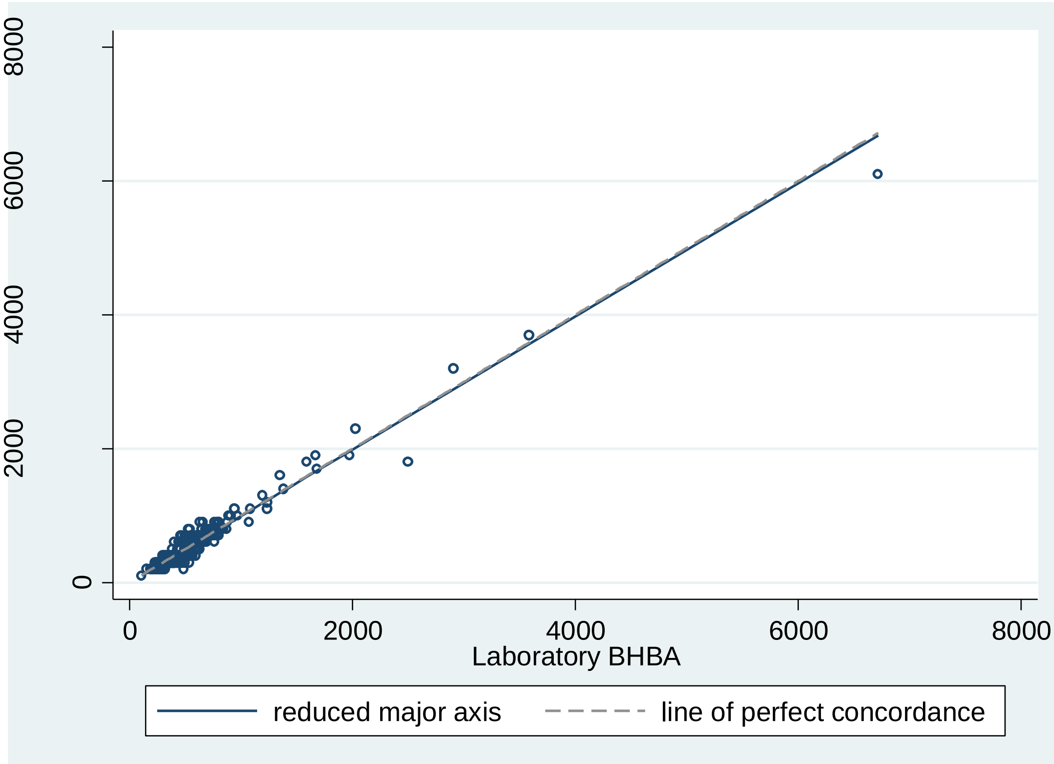

Figure 5.2 Scatter plots of blood β-hydroxybutyrate concentrations (μmol/l) in 366 blood samples of late-gestation ewes in Prince Edward Island, between September 2014 and August 2015, comparing the standard laboratory method and Precision Xtra® meter.

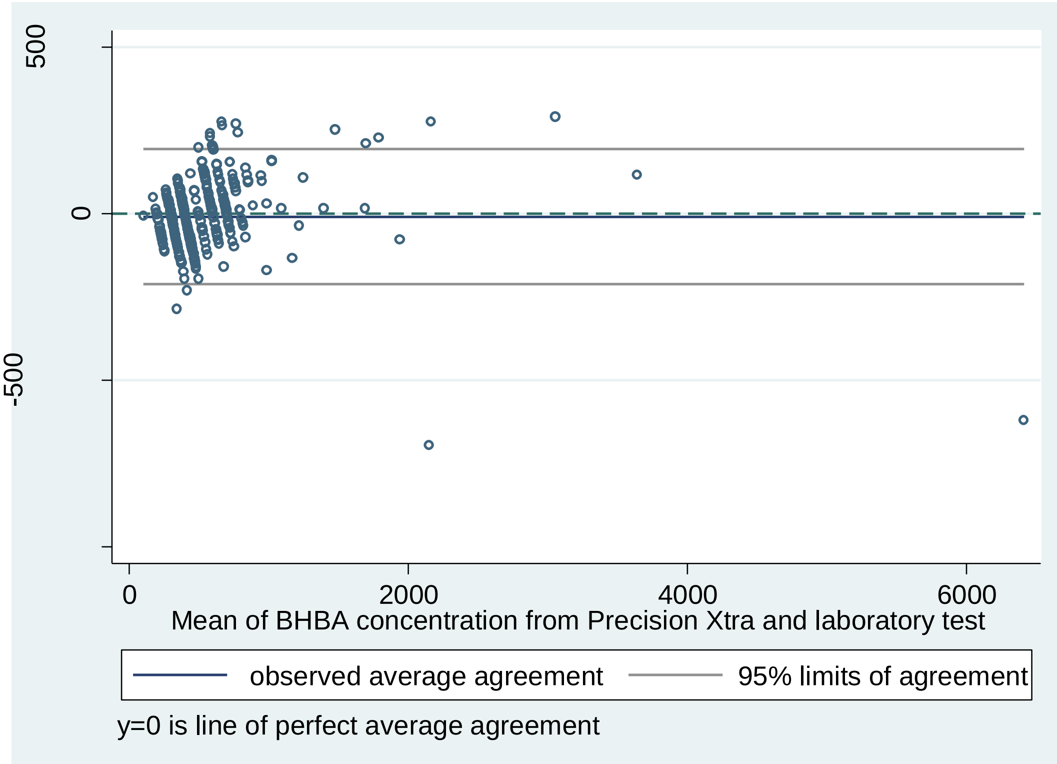

Figure 5.3 Bland-Altman plot of differences between β-hydroxybutyrate concentrations (μmol/l) from the standard laboratory method and Precision Xtra® meter against their mean β-hydroxybutyrate concentrations in 366 blood samples of late-gestation ewes in Prince Edward Island, between September 2014 and August 2015. The middle solid line displays the mean; the other two solid lines display the mean ±2SD.

CHAPTER 6

THE ASSOCIATION OF
SERUM β-HYDRPXYBUTYRATE CONCENTRATION WITH
FETAL NUMBER AND HEALTH INDICATORS IN
LATE-GESTATION EWES IN COMMERCIAL MEAT FLOCKS IN
PRINCE EDWARD ISLAND

6.1 Abstract

Late-gestation ewes are susceptible to ketonemia resulting from high energy requirement for fetal growth during the last few weeks of pregnancy. High lamb mortality is a possible consequence of effects of ketonemia on both ewes and lambs. Determining risk factors for ketonemia is a fundamental step in identifying ewes at risk in order to avoid losses caused by ketonemia. Serum β-hydroxybutyrate (BHBA) concentrations of 384 late-gestation ewe samples were determined. Physical examinations, including body condition, FAMACHA© and hygiene scoring were performed. Udders and teeth were also examined. Fecal floatation was performed to detect gastrointestinal helminth eggs of the ewe fecal samples. General feeding management practices and season at sampling were recorded. Litter sizes were retrieved from lambing records. Factors associated with the log serum BHBA concentration were determined using a linear mixed model, with flock and lambing groups as random effects. The mean serum BHBA concentration was 545.8 (±453.3) μmol/l. Ewes with a body condition score (BCS) of 2.5 to 3.5 had significantly lower BHBA concentrations than ewes with a BCS of ≤ 2.0, by 19.7% ($p = 0.035$). Ewes with a BCS of > 3.5 had a trend toward higher BHBA concentrations compared to ewes with a BCS of 2.5 to 3.5. Ewes with a FAMACHA© score of 3 had significantly higher BHBA concentrations than ewes with a FAMACHA© score of 1 or 2, by 12.1% ($p = 0.049$). Ewes in which gastrointestinal helminth eggs were detected had significantly higher BHBA concentrations than ewes in which helminth eggs were not detected, by 12.3% ($p = 0.040$). An increased litter size was associated with higher BHBA concentrations, with the BHBA concentrations of ewes having twins, triplets, and quadruplets or quintuplets

being higher than those of ewes having singletons by 19.2%, 30.4%, and 85.2%, respectively ($p \leq 0.003$). Season at sampling confounded the association between BHBA concentration and FAMACHA© score and, therefore, was retained in the final model even though it was not statistically significant. Intra-class correlation coefficients at the flock and lambing group levels were 0.14 and 0.32, respectively.

6.2 Introduction

Ketonemia is a metabolic disorder that occurs during the final weeks of pregnancy in ewes. The disease associated with ketonemia in late-gestation ewes is most commonly called pregnancy toxemia or ovine ketosis. Ketonemia is a consequence of negative energy balance as a result of an elevated energy requirement for fetal growth in the face of a suppression of feed intake (Brozos et al., 2011). Hormonal and metabolic changes that occur as a result of pregnancy, including a higher insulin level, and lower growth hormone, progesterone and prolactin levels, also play a role in the onset of this disorder (Vernon et al., 1981).

Ketonemia can present in clinical or sub-clinical forms; where it is diagnosed clinically, it should be considered a flock-level problem rather than a disorder of individual ewes (Rook, 2000; Mavrogianni and Brozos, 2008) because factors leading to high β-hydroxybutyrate (BHBA) concentration often operate at the flock level. In addition to the detrimental effects of the disorder on ewe health and productivity, lambs of affected ewes tend to be less viable than lambs of non-affected ewes. Perinatal mortality of lambs born to ewes in a negative energy balance is greater than lambs born to ewes without the problem (Barbagianni et al., 2015c). Early detection of sub-clinical ketonemia is the goal to allow for timely management interventions (Andrews, 1997)

The energy requirement of ewes during late gestation increases with fetal number (NRC, 2007). Forage is the major part of the ration of ewes and this, combined with rapid expansion of the uterus in late-gestation ewes carrying multiple fetuses, can lead to a maternal constraint on dry matter intake (Harmeyer and Schlumbohm, 2006). Provision of grain to late-gestation ewes is a common management practice adopted by sheep producers to avoid insufficient energy intake (Olfati et al., 2013).

Aside from fetal number, nutritional, health and environmental factors can influence feed intake and energy balance in the late-gestation ewe. These factors include body condition, dental health, gastrointestinal parasitism, energy density of the ration, environmental conditions, and genetics (Rook, 2000; Radostits et al., 2007; Mavrogianni and Brozos, 2008; Brozos et al., 2011; Alidadi et al., 2012).

The overall health status of ewes can be assessed by physical examination and measurement of body condition score (BCS). Monitoring and maintaining BCS is an effective strategy to prevent development of pregnancy toxemia during late gestation (Rook, 2002; Olfati et al., 2013). Intestinal parasitism is often assessed by conducting fecal tests, and by monitoring fecal consistency and conjunctival pallor (Miller et al., 2012). Nutritional and environmental management is best performed by on-farm assessment of facilities and management.

The BHBA is a useful biochemical indicator of energy balance in late-gestation ewes (Lynch and Jackson, 1983). Andrews (1997) reported that a BHBA concentration of ≥ 800 µmol/l indicates undernourishment. Previous studies using this cut-off value for a standard laboratory test found that between 18% and 32% of late-gestation ewes were classified as undernourished (Panousis et al., 2012; Pichler et al., 2014).

It can be inferred from published experimental studies (Lynch and Jackson, 1983; Papadopoulos et al., 2013; Barbagianni et al., 2015a; Barbagianni et al., 2015c) that health, nutrition, and fetal number influence plasma BHBA concentration in ewes; however, there are no study that examine the relative importance of these factors under field conditions. The objective of this study was to determine factors associated with serum BHBA concentration in late-gestation ewes under conditions typical of commercial meat flocks in Prince Edward Island (PEI).

6.3 Materials and Methods

6.3.1 Animals and samples collection

Forty-eight breeding groups from 34 sheep flocks in PEI, having a predicted lambing period between September 2014 and August 2015, were included in the present study. They were visited approximately one to three weeks before expected commencement of lambing.

In each group, eight ewes were restrained using convenience sampling for physical examination and sample collection as described in Chapter 3. Laboratory procedures for BHBA concentrations were conducted on serum by Veterinary Laboratory Services, Animal Health Laboratory, University of Guelph using the Cobas® 6000 c501 analyzer.

Feeding management data, including average weight of grain fed per ewe per day for each of the six weeks leading up to the beginning of lambing, management system, type of forages, and forage feeding space per gestating ewe were retrieved from the questionnaire mentioned in Chapter 3. Participants were asked to keep lambing records, including litter size for each lambing ewe.

All study procedures were approved by the University of PEI Animal Care Committee and were accepted by participants.

6.3.2 Statistical analysis

Descriptive statistics were calculated for serum BHBA concentration, which was then subjected to natural logarithm transformation for further analyses. Descriptive statistics were also calculated for all independent variables. Variables with negligible variation were not used for further analyses. FAMACHA© score was re-categorized into 3 categories, score 1 to 2 (normal), score 3 (intermediate), and score 4 to 5 (anaemic). BCS was also re-categorized into 3 categories, score ≤ 2.0 (under-conditioned), score 2.5 to 3.5 (proper-conditioned), and score ≥ 4.0 (over-conditioned). Litter size was categorized into four categories, singleton, twins, triplets, and quadruplets to quintuplets. All continuous variables were centered. Unconditional analysis was performed for each variable to evaluate their associations with the log serum BHBA concentration. In addition to linear effects, quadratic and cubic effects were evaluated for all continuous variables. The effect having the lowest p-value for each variable was selected for building a linear mixed model.

Due to the hierarchical data structure, flock and lambing group effects were entered as random effects, and the remaining variables were entered into the models as fixed effects. The mixed command of Stata (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, Tx: StataCorp LP) with the REML option and backward elimination was used for modelling. Variables with an overall p-value ≥ 0.05 were removed from the model, except BCS which was a main factor of interest.

Fixed effects were assessed for suspected confounding in the candidate model, including season at sampling and amount of grain fed. Suspected two-way interactions were tested and kept in the final model if they were significant and made biological sense. Random slope and contextual effects of all continuous variables in the candidate model were also assessed and were retained in the final model if the model was improved by inclusion. Intra-class correlation coefficients at lambing group and flock levels were calculated. Assumptions of the model were checked, based on linear mixed model diagnostics, including linearity, normality and homoscedasticity of residuals.

6.4 Results

6.4.1 Observational data

A total of 384 ewes were subjected to physical examination and sample collection. For two ewes, there was not enough serum to permit BHBA analysis. The mean and median of serum BHBA concentrations of samples analyzed were 545.8 (±453.3) and 466.5 µmol/l, respectively. The lowest and highest concentrations detected were 107 and 6720 µmol/l, respectively. Thirty samples (7.9%) had serum BHBA ≥ 800 µmol/l.

A BCS of 2.5-3.5 was assigned to 83.3% of the ewes examined, whereas, 10.2% and 6.5% were assigned a BCS of ≤ 2.0 and ≥ 4.0, respectively. More than half of the study ewes had clean udders and legs (57.0%; hygiene score of 1 or 2). For the FAMACHA© scores, 32.0% of ewes examined were classified as 1 or 2. Forty-five percent of ewes scored 3, and 22.7% scored 4 or 5. Only 1 ewe had an abnormal udder on palpation, and 14 ewes (3.7%) had broken or lost incisors. Udder health was not used for further analysis because of low variation. A positive fecal floatation test was identified in 52.8% of fecal samples.

Proportions of lambing groups visited in autumn, winter and spring were 35.4%, 50.0% and 14.6%, respectively. Six groups (12.5%) were fed only hay and 42 groups (87.5%) were fed ensiled forage. Ewes in 16 lambing groups (33.3%) did not receive any grain at 6 weeks before lambing. The mean and median daily amount of grain fed at 6 weeks before lambing was 0.3 (±0.3) and 0.2 kg, respectively, ranging from 0.0 to 1.0 kg. All lambing groups received grain at two weeks before lambing until the week of lambing. The mean and median daily amount of grain fed at the week of lambing was 0.6 (±0.3) and 0.5 kg, respectively, ranging from 0.2 to 1.4 kg. The amounts of grain fed each week were significantly correlated (p-values < 0.005). The amount of grain fed at six weeks before lambing was used for further analyses because it had the greatest variation and lowest p-value on the unconditional analysis with log BHBA concentration. Approximately one-third (31.25%) of ewes were in flocks in which a total confinement system was employed. Both mean and median forage feeding space were 37 (± 13) cm, ranging from 10 to 64 cm. Litter sizes of 222 ewes were recorded. Approximately one-fourth (25.7%) of ewes for which litter sizes were recorded had single lambs, whereas more than half (55.9%) had twins. The proportions of triplets, quadruplets and quintuplets were 14.4%, 3.2% and 0.9%, respectively.

6.4.2 Associated factor analysis

Based on the unconditional linear regression analyses, there were 2 out of 11 variables significantly associated with the log BHBA concentration (Table 6.1). However, all 11 variables were tested in the linear mixed model because a backward regression analysis process was used. Five fixed effects were retained in the final model built from 213 ewes in 37 lambing groups (79% of lambing groups) within 29 flocks (85% of flocks) for which complete data for BCS, FAMACHA© score, fecal flotation, and litter size was available (Table 6.2).

Season was a confounding factor in the association between FAMACHA© score and log serum BHBA concentration. The coefficient of FAMACHA© score 4 or 5 changed from -0.001 to -0.026 when season was included in the model. Ewes having a BCS of 2.5 to 3.5 had significantly lower BHBA concentrations than ewes having a score of ≤ 2.0 by 19.7%, while greater BCS ewes had a trend toward higher BHBA concentrations compared to ewes with a BCS of 2.5 to 3.5 based on coefficients of these 2 BCS categories. Ewes with a FAMACHA© score of 3 had significantly higher BHBA concentrations than ewes with FAMACHA© scores of 1 or 2 by 12.1%. However, the BHBA concentrations of ewes with FAMACHA© scores of 4 or 5 were not different from ewes with a scores of 1 or 2. Ewes in which gastrointestinal helminth eggs were detected had significantly higher BHBA concentrations than ewes in which helminth eggs were not detected by 12.3%.

The BHBA concentration increased with increasing litter size. The BHBA concentrations of ewes having twins were significantly higher than ewes having singleton by 19.2%. Ewes having triplets and ewes having quadruplets or quintuplets had 30.4% and 85.2% higher BHBA concentrations compared to ewes having singleton, respectively.

The final model did not violate the model assumptions, and no significant collinearity was detected between independent variables in the final model. No interactions between main effects remained significant in the final model. Intra-class correlation coefficients at the flock and lambing group levels were 0.14 and 0.32, respectively.

6.5 Discussion

There were four significant variables (BCS, FAMACHA© score, helminth presence, and litter size), and one confounding factor (season) retained in the final linear mixed model. Controlling BCS in pregnant ewes is an important strategy to limit the occurrence of ketonemia (Rook, 2000; Olfati et al., 2013). Andrews (1997) reported that ketonemia was common in under-conditioned ewes, whereas Marteniuk and Herdt (1988) and Van Saun (2000) reported that over-weight ewes were most susceptible to clinical ketonemia. It is theorized that fat deposits in over-conditioned ewes restrict gastrointestinal capacity (Schulz and Riese, 1983). In the present study, only the difference between ewes having BCS $\leq$ 2.0 and ewes having BCS 2.5 to 3.5 was significant. However, with the coefficient of BCS $\geq$ 4.0 being negative relative to the baseline BCS of ≤ 2.0, but higher than the coefficient of BCS 2.5 to 3.5, our findings support that under- and over conditioning during the late gestation should be avoided.

The FAMACHA© scoring system, an animal-side test used as a tool to diagnose infection with *Haemonchus contortus* in small ruminants, is a simple, commonly employed method of assessing the degree of anemia due to the parasite by comparing color of the lower eyelid conjunctivae to a previously set color-guided scorecard standard (van Wyk and Bath, 2002). According to the results of the present study, the presence of any intestinal helminth eggs was not significantly related to the FAMACHA© score. Nonetheless, the association between log serum BHBA concentration and eye mucus membrane color was significant. Ewes with FAMACHA© scores of 1 or 2 (pink mucus membranes) had lower log BHBA concentrations than ewes with FAMACHA© scores of 3 (borderline pale mucus membranes).

The association between presence of gastrointestinal helminth eggs in fecal samples and log BHBA concentration was significant. The log serum BHBA concentration of ewes in which gastrointestinal helminth eggs were identified on fecal flotation was higher than ewes in which no eggs were identified. Detrimental effects of parasitism on the BHBA concentration and development of pregnancy toxemia have been previously reported (Rook, 2000; Papadopoulos et al., 2013; Barbagianni et al., 2015a). Intestinal parasites can have a negative influence on the heavy energy metabolism demands during the last trimester of pregnancy (Dakkak, 1990). According to the associations between the log BHBA concentration and both FAMACHA© score and presence of gastrointestinal helminth eggs, effective parasite control strategies should be implemented in order to avoid the occurrence of ketonemia. In this study population, the significant association between accessibility to pasture any time of the year and presence of helminth eggs in fecal samples was detected (Chi-square p-value < 0.001). Ewes in a total confinement system would be less likely to have a positive result on the fecal floatation test. Thus, a total confinement system is one of the control strategies which can be used for preventing pregnancy toxemia.

The log BHBA concentration in the present study population was significantly associated with litter size. The log BHBA concentration of ewes increased with increasing number of fetuses. Many studies have also reported that the BHBA concentration of late-gestation ewes is dependent on the number of fetuses (Lynch and Jackson, 1983; Raoofi et al., 2013; Barbagianni et al., 2015c). The increased energy requirement of ewes as a result of increased fetal demands, and the restricted dry matter intake resulting from uterine expansion in ewes bearing multiple fetuses contribute to a negative energy balance during the late gestation period (Cal-Pereyra et al., 2012; Raoofi et al., 2013). Thus, the number of fetuses should be considered when determining rations for late-gestation ewes (Rook, 2000; Fthenakis et al., 2012).

Other than grouping by number of fetuses, ewes should also be separately fed based on BCS, age and stage of pregnancy (Brozos et al., 2011; Fthenakis et al., 2012). Evaluation of the concentration of BHBA in 10-15% of animals is a statistically valid strategy to identify ketonemia and prevent pregnancy toxemia, and groups of ewes with results $\geq$ 800 μmol/l of BHBA require special attention (Brozos et al., 2011). An alternative approach to avoid high BHBA concentrations resulting from large litter sizes is to limit the number of fetuses through genetic selection. This approach, however, is unlikely to be economically feasible in commercial meat flocks in PEI where the number of lambs should be maximized in order to gain highest benefits.

Gupta et al. (2007) reported that the incidence of ketonemia in pregnant goats was higher in January and February compared to July and August, and attributed this to low seasonal temperature. Grain supplementation reduced the blood BHBA concentration in ewes close to parturition (Kerslake et al., 2010). In the present study, both season and amount of grain fed at 6 weeks before parturition were not significant in the final model. Based on assessment of the final model, only season was a confounder in the association between log serum BHBA concentration and FAMACHA© score.

An association between high BHBA concentration and mastitis was found by Barbagianni et al. (2015b). Because there was only one ewe having an unhealthy udder in the present study, the effect of udder health on log BHBA concentration could not be evaluated.

The proportion of variance was highest at the ewe level, compared to the flock and lambing group levels. This indicates that factors at the ewe level are the most important ones in the control of the serum BHBA concentration of late-gestation ewes compared to flock and lambing group levels. However, based on the substantial proportion of variance at the lambing group level, this level also deserves attention.

6.6 Conclusions

In conclusion, under conditions typical of commercial meat flocks in PEI, increased BHBA concentration was associated with a BCS $\leq$ 2.0, FAMACHA© score $\geq$ 3, gastrointestinal helminth infection and increased fetal number. Flock managers and their advisors should take steps to monitor BHBA concentration and identify ewes having high BHBA concentrations, especially where any of these risk factors exist in the flock.

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Table 6.1 Characteristics and p-values from unconditional mixed linear regression analyses of independent variables for log BHBA concentration in 382 late-gestation ewes in 34 Prince Edward Island sheep flocks, sampled during September 2014 to May 2015.

Variable	Level	Type of variable	Number of observations	p-value from unconditional analysis
BCS	Individual	3 categories	382	0.909
Hygiene score	Individual	4 categories	382	0.375
FAMACHA© score	Individual	3 categories	382	0.153
Incisor abnormality	Individual	2 categories	382	0.238
Fecal helminth present	Individual	2 categories	375	0.026
Type of management system	Lambing group	2 categories	382	0.361
Type of forage	Lambing group	2 categories	382	0.553
Season	Lambing group	3 categories	382	0.173
Amount of grain fed at 6 weeks before lambing (kg)	Lambing group	Quadratic association	382	0.362
Forage feeding trough space	Lambing group	Quadratic association	376	0.188
Litter size	Individual	4 categories	222	< 0.001

Table 6.2 Final linear mixed model* for log BHBA concentration, based on 213 late-gestation ewes in 29 Prince Edward Island sheep flocks, sampled during September 2014 to May 2015.

Variable	Coefficient	p-value	95% confidence interval	
BCS (≤ 2.0 as reference category)				
2.5-3.5	-0.219	0.035	-0.423	-0.015
≥ 4.0	-0.104	0.543	-0.440	0.232
FAMACHA© score (1-2 as reference category)				
3	0.114	0.049	0.000	0.228
4-5	-0.026	0.731	-0.173	0.122
Helminth (negative as reference category)				
Positive	0.116	0.040	0.005	0.227
Litter size (1 as reference category)				
2	0 .175	0.003	0.061	0.289
3	0.266	0.001	0.107	0.424
4-5	0.616	< 0.001	0.368	0.865
Season (winter as reference category)				
Spring	0.127	0.167	-0.053	0.307
Autumn	-0.072	0.636	-0.372	0.227
Constant	6.020	< 0.001	5.752	6.288

*Log restricted-likelihood = -92.58

CHAPTER 7

MANAGEMENT FACTORS INFLUENCING LAMB MORTALITY IN PRINCE EDWARD ISLAND FLOCKS

7.1 Abstract

We aimed to identify influential management factors associated with lamb mortality risk for sheep flocks in Prince Edward Island, Canada. Data were collected for 51 lambing groups from 37 sheep flocks. Variables of interest included flock management practices, ewe health indicators, ewe and lamb nutrition, litter size and lamb birth weight. Principal component analysis was performed and resulting scores were used for further analysis using a mixed Poisson regression model with lamb mortality risk as the outcome. The mean group-level lamb mortality was 11.14% (± 6.64%), with 25 groups having lamb mortality greater than 10%, which is considered higher than acceptable. Four principal component scores were retained in the final model identifying factors associated with lamb mortality, Flock1, Genhealth4, Forage1 and Lambhealth1. These 4 principal components comprised 13 original variables each having a high contribution to these scores. Specifically, the following management factors contributed to lower lamb mortality: using goal setting; seeking veterinary advice for animal treatment; using benzimidazole-derivative anthelminthics; feeding forage with higher crude protein, digestible energy, net energy for maintenance, and lower acid detergent fiber to late-gestation ewes, applying visual lamb identification methods, feeding a coccidiostat to lambs, administering clostridial vaccination to lambs, and avoiding separation of hypothermic lambs from their dams. A history of neurological problems in ewes contributed to a higher mortality in lambs. These results should help sheep producers and researchers direct attention to management variables that could reduce lamb mortality in sheep flocks.

7.2 Introduction

Intensive management practices have been adopted in sheep farming to increase return on investment. For instance, selecting for high prolificacy leads to a greater number of progeny whereas total confinement systems prevent loss from predators. However, additional knowledge and skills are required if such intensive management practices are adopted, because these practices usually predispose animals to other management challenges (Cronin et al., 2014; Vannier et al., 2014), one of the most important being a higher risk of lamb mortality (Binns et al., 2002).

Lamb mortality risk is an important index determining efficacy of sheep flocks. Sheep producers gain more return on investment from sheep production by minimizing lamb mortality risk. Pre-weaning lamb mortality risk > 10% should be considered excessive, and improvements should be made to address this issue (Kennedy, 2012). Numerous factors are associated with lamb mortality risk, such as weather conditions, hygiene, feeding and health management practices of the dam and offspring, birth weight, and birth type (Chaarani et al., 1991; Southey et al., 2001; Binns et al., 2002; Sawalha et al., 2007).

Although lamb-level factors, especially birth weight, are key factors associated with lamb mortality risk (Owens et al., 1985; Upreti, 1989), flock-level factors should not be overlooked. The performance of lambs is affected by environmental conditions and flock-level management practices. For instance, birth weights of lambs are conditional on feeding management of the pregnant ewes (Christley et al., 2003; Mandal et al., 2007; Rooke et al., 2015). Additionally, some lamb-level factors associated with lamb mortality risk are difficult to manipulate, such as gender, whereas it is easier to modify management practices at the flock-level to minimize lamb mortality risk. Identifying flock-level factors associated with lamb mortality risk is a fundamental step towards optimizing lamb survival.

Because many flock-level factors can influence lamb mortality risk, evaluation of factors affecting risk can be complicated. Moreover, flock-level factors are likely to be interrelated. Principal component analysis (PCA) is a statistical technique employed to manipulate datasets containing large numbers of interrelated variables. PCA has proven useful to reduce the number of variables for further statistical analyses (Hair, 2010). Regular PCA is used where continuous variables are being considered, whereas in a dataset containing categorical variables, polychoric correlation is integrated with PCA in order to provide more accurate results (Kolenikov and Angeles, 2004).

The sheep industry in Prince Edward Island (PEI) has expanded during the last decade. In 2014, 2400 lambs were produced, which is 2.7 times the production in 2004 (Department of Agriculture and Fisheries, PEI, 2015). High lamb mortality is commonly experienced by PEI sheep producers. The present study was conducted to identify the most influential flock-level factors associated with lamb mortality risk.

7.3 Materials and methods

7.3.1 Flocks

Thirty-seven sheep flocks in PEI agreed to participate in the project supported by the PEI Growing Forward II Program. Participant recruitment was previously described in Chapter 3. Fifty-one groups of ewes which were bred within the same breeding period in these flocks, and gave birth during September 2014 to August 2015, were incorporated into the present study. Each lambing group was visited twice; the first visit was at one to three weeks before the expected first lambing date, and the second visit was at two to four weeks after the first lambing date.

7.3.2 Data collection

An in-person questionnaire and a flock visit record were completed during each visit. Detail of the questionnaires and flock visit records are explained in Chapter 3. Each producer received a farm logbook to record lambing events, including gender, type of birth and birth weight of lambs and lamb mortality risk during the first 56 days. The farm logbook was collected after all lambs in the flock reached 8 weeks of age.

7.3.3 Sample collection

Eight pre-partum ewes in each group were selected through convenience sampling for blood and fecal sample collection. These eight ewes were also physically examined to evaluate body condition score, anemia score (FAMACHA©), cleanliness score, incisors and udder abnormalities. In groups containing more than 80 pre-partum ewes, additional ewes were physically examined in order to get at least 10% of ewes examined, but blood and fecal samples were not taken from these ewes. An electric powered core sampler was used to take pre- and post-partum forage samples. More information on sample collection methods was described in Chapter 3.

7.3.4 Laboratory analysis

All questionnaires, flock visit records, physical examination records and sample collection were completed by the principal investigator and two trained research assistants. More details of laboratory analyses are available in Chapter 3. The study was conducted under the protocol reviewed and approved by the University of PEI Animal Care Committee (14-005 (6005670)).

7.3.5 Data analysis

7.3.5.1 Descriptive and unconditional analysis

Data from questionnaires and flock visit records were entered into Epi Info™ 7, and data from other sources were entered into Microsoft Excel 2010. All data were then imported to Stata 14 (StataCorp LP, College Station TX, US). Descriptive statistics of all variables were evaluated. Original independent variables having ≥ 10% missing values were not considered for further analyses. Some categorical variables were re-categorized as appropriate for further analyses. For instance, categories with low response rates were combined with related categories, and results from related questions were combined to form another single question. For example, use of a coccidiostat in the flock is a combination of the use in ewes, rams and/or lambs. Categorical variables with low variation, i.e. > 90% of animal groups were in one category, were also not considered for further analyses. All continuous variables were standardized to have mean and standard deviation of zero and one, respectively.

Unconditional mixed Poisson regression analysis was conducted for individual factors associated with the outcome of interest: number of dead lambs, with flock random effects, and the population-at-risk was an offset for all original independent variables. Dummy coding was used for categorical variables, whereas only linear associations were evaluated for continuous variables. Variables with p-value < 0.30 in unconditional analyses were retained for further analyses.

7.3.5.2 Principal component analysis (PCA)

Independent variables with p-value < 0.30 in unconditional analysis were grouped into 8 domains containing biological- and practical-related variables. Because a domain containing highly correlated original categorical variables cannot be analyzed with polychoric PCA, in such cases, only the variable which most biologically related to lamb mortality risk among the associated variables was kept for the analysis. Each domain was analyzed with the polychoricpca command in Stata 14. Number of principal components retained to represent the underlying construction of each domain for multivariate modelling was based on the latent root and percentage of variance criteria (Hair et al., 2010). All principal components with eigenvalues greater than 1.0 were retained. Additional principal components were also retained until 80% of the total variance of the domain was accounted for. Principal component scores of the selected principal components were computed for each observation to use in mixed Poisson regression model building.

7.3.5.3 Mixed Poisson regression model building

With lamb mortality risk as the outcome, unconditional mixed Poisson regression analysis, including the population-at-risk as an offset, was carried out using linear, quadratic and cubic functions for all selected principal components, with flock random effects. Linear associations were initially examined for significance, with quadratic and then cubic terms, in turn, being examined where linear terms were not significant. Principal components with p-values < 0.20 in unconditional analysis were selected to be included in the initial mixed Poisson regression model. Season of lambing was also included in the initial model. The model was firstly built with complete case analysis, in which only all non-missing observations were included. Because two lambing groups belonging to a flock having less than one year sheep farming experience contained many missing values, these two groups were excluded from the mixed model building. The backwards elimination procedure was performed manually. Variables with p-values ≥ 0.05 were excluded from the model until all remaining variables had p-values < 0.05. All possible two-way interactions between variables were explored for significance in the model, and possible confounding effects of season were evaluated. Interactions with p-values < 0.05, and confounding factors producing changes > 20% in coefficients would be retained in the model. Random slopes and contextual effects for significant variables were also evaluated, using the same significance level (Dohoo et al., 2009). Assumptions of mixed Poisson regression model were assessed for the final model.

Finally, to determine if missing data had a substantial impact on the final model, missing values of original variables associated with principal components retained in the final model were replaced by the medians of the original variables. The principal component scores of observations with missing values were computed. The model was then rebuilt with the replaced principal component scores, and compared with the model without the missing data imputations.

7.4 Results

One group of ewes was lost to follow up after the first visit; thus data from this group were not considered for analyses. Therefore, there were 50 lambing groups from 36 flocks in the final dataset.

There were 242 original independent variables which were considered for evaluation of their effects on lamb mortality risk. Ten variables were not further analyzed because they contained $\geq$ 10% missing values. Seven categorical variables were not further analyzed because of low variability. Descriptive statistics of data from lambing records are shown in Table 7.1. Proportions of both male and female lambs were close to 50%. The means for number of lambs per lambing and for birth weight of lambs were 1.85 lambs and 4.32 kg, respectively. Half of the 50 lambing groups had more than 10% lamb mortality risk during the first 8 weeks of life. Descriptive statistics of other variables are displayed in Chapter 3.

Eighty-four original variables had p-values < 0.30 in unconditional analyses. They were divided into eight domains for analysis with polychoric PCA. Ten original variables were highly correlated with other original variables in the same domains and were excluded from the PCA. Descriptive statistics of 74 variables included in the PCA are displayed in Tables 7.2 and 7.3. Based on the latent root and percentage of variance criterions, 33 principal components were selected for further analyses. Twenty of these 33 principal components had p-values < 0.20 in unconditional analysis of principal components and were considered eligible for mixed Poisson regression model building, including: two components each from flock characteristics, ewe feeding management, ewe health management and lamb feeding management, and pre-partum ewe forage quality; three components each from biosecurity and breeding management, lamb health management, general health management and pre-partum ewe health.

Four of the eligible PCA variables were significant and retained in the final model (Table 7.4). Lamb mortality risk was lower in groups having high principal component scores of Flock1, Genhealth4 and Forage1, with the components of these PCA variables shown in Table 7.5. Main original variables positively influencing these principal component scores were: no neurological problems in ewes (Flock1), setting goals (Flock1), using benzimidazole in flocks (Genhealth4), treating sick animals based on veterinary advice (Genhealth4), crude protein (Forage1), digestible energy (Forage1), and net energy for maintenance (Forage1). Conversely, no wasting problems in ewes (Flock1), and acid detergent fiber (Forage1) negatively influenced these scores (Table 7.5). The lamb mortality risk ratios associated with increasing one unit of principal component score of Flock1, Genhealth4 and Forage1 in a flock, when other principal component scores were stable, were 0.83, 0.71 and 0.90, respectively. These ratios indicate that having neurological problems in ewes, not having wasting problems in ewes, not setting goals, not using benzimidazole in flocks, treating sick animals without veterinary advice, using forages with low crude protein, low digestible energy, low net energy for maintenance and high acid detergent fiber contributed to high lamb mortality.

The association between principal component Lambhealth1 and lamb mortality risk (Table 7.4) was best described by a cubic polynomial (Fig. 7.1). Original variables having positive high contributions to this principal component score were: providing visual identification, using coccidiostats and clostridial vaccine in lambs, and keeping hypothermic lambs in barns. As seen in the graph, lamb mortality risk was highest in groups with principal component score of Lambhealth1 ranging from -1.25 to -1.00 and lowest in groups with principal component score of Lambhealth1 ranging from 0.75 to 1.00.

There were no significant random slopes, contextual effects or interactions in the final model, and the model met its assumptions to a satisfactory degree.

Means of the four principal component scores in the final model were close to zero. The ranges and standard deviations of these principal component scores were close to four and one unit(s), except for principal component Forage1, which had greater variation (Table 7.6). Based on real observations of principal component Flockhealth1, which contained only categorical variables, only one unit difference was detected between group 40 and 45. In group 40, clostridial vaccine and benzimidazole were not used, while routine hoof trimming was performed. Conversely, in group 45, the vaccine and benzimidazole were used, but routine hoof trimming was not performed in this group.

The model that was built with missing data imputations had very similar results to those reported in Table 7.4 and, therefore, these results are not shown. The reason for the similar results was that the variables with missing values contributed very little to the principal component scores -0.06 and 0.27 for percent ewes died each year and percent lambs needing lambing assistance, respectively.

7.5 Discussion

This study has been able to identify significant groups of factors associated with lamb mortality through the PCA variables, and specific management practices within these variables that could be targeted to reduce lamb mortality. PCA has the advantage of assessing associations between an outcome and combinations of factors when there are many factors to analyze. The PCA was able to combine the 74 eligible variables into 20 eligible principal components for statistical analysis, which was helpful for determining significant factors in the relatively small dataset of 50 observations of lambing groups. Regarding the 4 principal components in the final model, there were 13 original variables (Table 7.5) which highly contributed to lamb mortality risk (PCA loading $\geq$ 0.35 or $\leq$ -0.35), and these are discussed below.

7.5.1 Lambhealth1 Principal Component

Hypothermia has previously been reported as a common cause of perinatal lamb mortality (Woolliams et al., 1983; Mellor and Stafford, 2004). Allowing hypothermic lambs to stay with their dams contributed to increased survival risk in the present study. Moving lambs away from their dams to warm places might interfere with mother-young bonding, leading to mis-mothering, which is another major cause of lamb mortality (Woolliams et al., 1983). Early detection and providing an additional energy source to hypothermic lambs before they are warmed is important to recover hypothermic lambs (Eales et al., 1983; Martin, 2010). On the other hand, allowing hypothermic lambs to generally stay with their dams may simply suggest that such flocks have a less severe problem with hypothermia due to a range of other factors that support lamb survival.

In the present study, applying visual identification to lambs made a valuable contribution to lamb survival. Visual identification allows producers to track their lambs easily; thus mis-mothering can be earlier detected and lambs requiring assistance can also be identified. Consequently, lambs have a better chance to survive. The contribution of this factor to lamb mortality has not been noted elsewhere. On the other hand, visual identification of lambs may not be causal, but may reflect good overall management and be correlated with other factors that support lamb survival.

Eimeria spp. is a common pathogen affecting the gastrointestinal system of lambs; however, the pathogen itself is usually not the primary cause of lamb death (Dohoo et al., 1985; Mandal et al., 2007). According to our final model, provision of coccidiostats to lambs contributed to decreased lamb mortality risk. The finding indicates that coccidiosis might be a common problem in participant flocks, which is consistent with a considerable proportion (15.8%) of lesions associated with coccidia found in dead lambs submitted for post-mortem examination to the Atlantic Veterinary College Diagnostic Services during 2005 to 2014 (Chapter 2). However, Binns et al. (2002) reported that flocks in which a coccidiostat was routinely administered had greater stillborn lambs compared to other flocks. The authors of that report proposed that coccidiostats were mostly used in intensive management flocks, which had a higher risk of infection with the pathogen. It remains unclear whether coccidiostat use is associated with an increased risk of stillborn lambs.

Sheep are generally considered quite susceptible to clostridial diseases. The case fatality risk of sheep with pulpy kidney disease, which is caused by *Clostridium perfringens* type D, is high (Dohoo et al., 1985). Thus, clostridial vaccination is considered necessary for sheep flocks (Eales et al., 1983). A previous study reported that clostridial vaccination in lambs up to 30 days of age had no benefit on lamb survival (Hoefler and Hallford, 1985). On the other hand, Costa et al. (2012) found that when ewes received a polyvalent clostridial vaccine a month before lambing they could transfer adequate immunity against the pathogens to their lambs. It has been suggested that lambs born to pre-partum vaccinated ewes should not be vaccinated before 6 weeks of age in order to avoid interference by maternal antibodies (Rosa et al., 1997; Badia et al., 2011). In the present study, a lower lamb mortality risk was a contribution of practicing lamb clostridial vaccination. Most producers who provided the vaccine to lambs also vaccinated their ewes. The timing of vaccination in both ewes and lambs in our study groups was mostly as recommended by the product label and by the studies mentioned above (after 6 weeks). Thus, in the first 56 days, lamb mortalities reported by producers were not directly related to clostridial vaccination in this group of lambs. It might be inferred that providing a clostridial vaccine in lambs reflected a higher level of flock management with attention to correct vaccination schedules, indirectly leading to better clostridial flock immunity and passive transfer, and higher lamb survival risk. Good management leading to lower pathogen contamination within groups using the vaccine in lambs might be responsible for the lower mortality risk observed as well.

Birth weight of lambs is considered the most important factor associated with lamb mortality risk (Owens et al., 1985; Upreti, 1989; Gama et al., 1991). Both too small and too large lambs have higher risk of mortality than lambs with intermediate birth weight. Small lambs are susceptible to hypothermia and hypoglycemia, while large lambs are more likely to die from dystocia-related problems (Gama et al., 1991; Sawalha et al., 2007). Birth weight is affected by various factors including breed, genetics, age of ewes, nutrition of ewes during breeding and pregnancy, body condition score of ewes at mating, ewe health status, lambing system, litter size, gender of lambs, season and environmental condition (Kleemann et al., 1990; Sormunen-Cristian and Suvela, 1999; Christley et al., 2003; Fisher, 2004; Thomson et al., 2004). The flock mean birth weight in the study of Christley et al. (2003) ranged from 3.8 to 5.1 kg, whereas in the present study the range was 3.0 to 5.4 kg, which is very close to the 3.0 to 5.5 kg optimal birth weight for maximum lamb survival risk (Nowak and Poindron, 2006). In the present study, the median birth weight of lambing group was not eligible for PCA because the p-value from the unconditional analysis was 0.55.

Lambs born into a large litter size are more susceptible to mortality primarily because of litter size's effect on the birth weight (Gama et al., 1991; Demirören et al., 1995; Binns et al., 2002; Chniter et al., 2013). Litter size is influenced by various factors. McHugh et al. (2016) found that the average numbers of lambs per lambing in crossbred and purebred ewes were 2.01 and 1.87, respectively. Ewes delivering in the natural lambing season have larger litter sizes than ewes delivering out of season (Fisher, 2004). Litter size increases with age of ewes (Maria and Ascaso, 1999). In flocks where the accelerated lambing system is used, litter sizes are smaller than annually lambing flocks (Sormunen-Cristian and Suvela, 1999). Litter size also depends on peri-mating nutrition (West et al., 1991). Time of mating also affects litter size via follicular maturity (Khalifa et al., 2013). The average litter size at the group-level was 1.86 in the present study, and most participating flocks were crossbred flocks lambing in the natural lambing season. In the present study, because the p-value from the unconditional analysis of the average litter size of lambing group was 0.41, this variable was not eligible for PCA.

Gender of the lamb is another known factor related to lamb mortality risk. Many studies have reported a higher survival risk in female lambs compared to male lambs

(Southey et al., 2004; Mandal et al., 2007; McHugh et al., 2016). Male-to-female lamb ratios in our participating lambing groups were varied, ranging between 36 to 64 and 73 to 28, and is somewhat susceptible to alterations with certain management practices. Sex ratio was affected by the level of omega-6 fatty acids in pre- and post-conception diets elsewhere (Gulliver et al., 2013). The ratio was also influenced by insemination timing, which was a result of alteration of acidity in the reproductive tract of ewes. Mating at 15 and 30 hours after the onset of estrus led to high proportions of female and male lambs, respectively (Khalifa et al., 2013). The variable proportion of male lambs resulted in a p-value of 0.75 in the unconditional analysis, thus this variable was also not eligible for PCA. Overall, based on our findings, lamb-level factors, including birth weight, litter size and gender, might not be appropriate to be used as flock level factors for evaluating their associations with lamb mortality.

7.5.2 Flock1 Principal Component

A history of neurological problems in ewes in the last three years contributed to increased lamb mortality risk in the present study. In Germany, metabolic and toxic diseases were the most common causes of neurological problems in sheep, and another main problem was infection (Schenk et al., 2008). Pregnancy toxemia, thiamine deficiency and hypocalcemia are common neurological-related metabolic diseases in sheep, generally related to improper feeding management. Optimal nutritional management is one of the key determinants of lamb survival risk (Chaarani et al., 1991; Atashi et al., 2013; Fisher, 2014) because of its effects on various lamb mortality-related factors, such as litter size, birth weight, thermoregulation of lambs and colostrum and milk production (Huffman et al., 1985; Ojha et al., 2013; Dwyer et al., 2016).

Listeriosis and Maedi-visna diseases are sporadic infectious diseases that can also produce neurological signs in sheep. Ensiled forage is a common predisposing factor of listeriosis (Edmondson et al., 2012); however, in the present study, the association between using ensiled forage and having neurological problems in ewes was not significant (Chi-square p-value = 0.563), partly due to low number of lambing groups (n=8 of 48 responses) reporting neurological problems in ewes. Besides neurological effects of listeriosis, the pathogen can also cause abortion, stillbirth or weak lambs (Edmondson et al., 2012). Although most sheep carrying Maedi-visna virus do not manifest specific clinical signs other than suboptimal health, Arsenault et al. (2003) found greater mortality risk in lambs born to seropositive ewes. Moreover, lambs born to ewes of suboptimal health are lighter and are more likely to get sick and/or be abandoned than lambs born to healthy ewes (Hamilton Prime Lamb Breeders, 2005; Gokce et al., 2013) making them more susceptible to mortality. Within-flock seroprevalence of Maedi-visna disease in Quebec, Canada, ranged between 3% and 70%, with an overall seroprevalence of 32% (Arsenault et al., 2003).

In conclusion, the most likely cause of neurologic disease in our study flocks is pregnancy toxaemia, and its influence in the final model likely reflects a variety of factors relating to nutritional management of ewes.

In the present study, a history of wasting problem in ewes contributed to decreased lamb mortality risk. This biologically implausible outcome might be a result of different acceptable body condition by participating producers because it is a subjective assessment (Evans, 1978). Knowledgeable experienced producers probably preferred sheep with greater condition, and might report that they had wasting problems when the sheep did not reach their desired body conditions. Lamb mortality risk of these flocks might be lower due to better management practices implemented by the experienced producers. Emaciation can be attributed to clinical or subclinical diseases. Old age sheep with teeth abnormalities are also likely to have poor body condition (Britt and Baker, 1990). Dwyer (2003) reported that ewes with poor body condition during breeding and gestating periods tended to produce lighter lambs than ewes with acceptable body condition. Additionally, inadequate maternal behaviors are not uncommon in thin ewes.

Having predetermined flock goals contributed to decreased lamb mortality risk in the study population. Setting a goal, such as reducing lamb mortality to below 10%, might lead to increased attention given by a producer to factors affecting lamb survival. Elliott et al. (2011) reported that producers concerned about their flock productivity were more likely to accept new interventions that could improve their productivity.

It was interesting that in the present study, pneumonia, mastitis and helminth problems did not pass unconditional analysis, and were not included in further steps of identifying factors associated with lamb mortality risk. Helminth problems will be discussed below under benzimidazole use.

7.5.3 Genhealth4 Principal Component

Helminthiasis is considered one of the main causes of lamb mortality, especially in older lambs (Dohoo et al., 1985; Britt and Baker, 1990; Green and Morgan, 1994). In the present study, decreased lamb mortality risk was a contribution of benzimidazole use. Benzimidazole was the second most common anthelminthic used in participant groups following ivermectin. Fenbendazole and albendazole were benzimidazole derivatives used in these groups. Resistances to ivermectin and fenbendazole, as well as multiple-drug resistance, are very commonly reported in Ontario sheep flocks (Falzon et al., 2013), and are likely to be widespread in PEI flocks also. Although some of our participating producers had suspected or confirmed (through fecal egg count reduction tests) an anthelminthic resistance problem in their flocks, not all flocks had data on anthelmintic efficacy from their own flock. It is likely that those flocks where benzimidazole is still used have no noticeable anthelmintic resistance and/or are flocks with lower overall helminth challenge, perhaps explaining the positive effect on ewe health and lamb survivability; however, further research is required for confirmation.

In the present study, treating sick animals under veterinary advisement contributed to decreased lamb mortality. An immediate treatment based on veterinary guidance is recommended by Eales et al. (1983). In addition to treatment guidance, veterinarians are also an important source of knowledge to maximize flock productivity, including lamb survival risk (Dwyer et al., 2016). Following veterinary advice might reflect a good working relationship between producers and veterinarians. Thus, the reduced lamb mortality probably resulted from a combination of direct effects of treating sick lambs and indirect effects from a closer relationship between veterinarian and flock manager.

7.5.4 Forage1 Principal Component

Energy and protein availability for ewes a few weeks pre-partum are determining factors of colostrum and milk quantity and quality (Banchero et al., 2015). Inadequate colostrum consumption is one of the major underlying causes of lamb mortality (Dwyer et al., 2016). Not only birth weight is affected by energy and protein restriction during the second half of gestation, but mother-lamb recognition was also reported to be interfered (Fenochio et al., 2013). These effects of the two main nutrients in feed were consistent with results found in the present study, where providing forage containing higher energy and/or higher protein for late-gestation ewes contributed to decreased lamb mortality risk. Significant associations between type of forage fed to late-gestation ewes and crude protein and net energy for maintenance in forage were detected in the present study; the levels were higher in ensiled forage compared to hay. However, type of forage was not included in the model building due to a high p-value for this variable in the unconditional analysis (p-value = 0.918).

7.5.5 Study limitations

With only 50 lambing groups and a large number of management variables to examine for associations with lamb mortality, PCA was determined to be a preferable methodology in order to maximize the ability to identify important management factors of lamb mortality. The disadvantage of using PCA is that we do not have a coefficient from that final model attributable to specific management factors, and controlled for confounding by other factors in the model, in order to quantify the possible effect of that factor. Therefore, the variables within the significant principal components in the final model should be further investigated.

It is recommended that at least five variables should be included in each PCA (Hair et al., 2010), therefore, different areas of original variables passing unconditional analysis were combined into larger domains, sometimes with somewhat weak rationale. For instance, biosecurity-related variables were combined with breeding-related and ram management variables, because there were insufficient eligible ram management variables to create a ram domain. Numbers of variables in the domains ranged from 7 to 11.

All variables not considered for analyses because of ≥ 10% missing values were continuous variable. Except for three variables missing by design, other variables were difficult to be estimated or recalled by producers, including weight of ewes at first breeding, duration of new animal's isolation, weight of lambs at weaning, frequency of checking the flock after lambing, proportion of male lambs, litter size and lamb birth weight. All of these variables were not significant (p-values > 0.05) when non-missing observations were evaluated with unconditional analysis. Additionally, when the seven original variables were individually included in the final mixed Poisson regression model, they were not significant. Therefore, the missing data were not considered a major problem for interpreting the study results.

The pre-weaning mortality should be limited at 10% (Kennedy, 2012). The range of weaning age of participating flocks in the present study was large, ranging from 4 to 22 weeks. Thus, lamb mortality risk within a specific duration might be more appropriate for investigation. There was 1 lambing group in which all lambs survived longer than 56 days. The greatest mortality risk in participating lambing groups was almost one-third of lambs born. The mean lamb mortality risk in our study groups was 11% whereas a study conducted in the US reported a 25% lamb mortality risk during the first 56 days (Nash et al., 1996).

7.6 Conclusions

Lamb mortality was a substantial problem in some PEI sheep flocks, and several flock-level factors were found to be associated with lamb mortality risk. Setting flock goals, treating sick animals based on veterinary advice, using benzimidazole-derivative for helminth control seemed to be protective factors of lamb mortality. Feeding high quality forage (high protein, high energy, and low fiber) was also beneficial to lamb survival. Various lamb management practices were more likely to reduce lamb mortality risk, including applying a visual identification, offering a coccidiostat, administering clostridial vaccination, and avoiding separation of hypothermic lambs from their dams. Neurological problems in ewes tend to have some detrimental effects on lamb survival. Based on these findings, in order to improve flock profits by maximizing lamb survival, producers have to ensure that high quality forages of late-gestation ewes, appropriate control strategies of clostridial, helminth and coccidiosis, suitable lamb management practices, and intimate relationship with veterinarians are provided.

7.7 References

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Table 7.1 Descriptive statistics of data from lambing records based on 50 lambing groups of 36 sheep flocks in Prince Edward Island in 2014-2015.

Variable	Minimum	Median	Maximum	Mean	Standard deviation
Proportion of male lambs (%; n = 42)	35.7	52.6	73.1	53.0	7.0
Average number of lambs per lambing (lambs; n = 44)	1.08	1.85	2.75	1.86	0.35
Median birth weight of lambing group (kg; n = 44)	3.00	4.32	5.35	4.28	0.54
Lamb mortality risk (%, n = 50)	0.00	9.95	30.30	11.14	6.64

Table 7.2 Descriptive statistics of categorical variables included in Principal Component Analysis based on 50 lambing groups of 36 sheep flocks in Prince Edward Island in 2014-2015.

Original variable	Number of Observations	Category	Percent
Domain 1: Biosecurity and breeding management			
Remove manure from lambing area	50	Yes	60
Isolate every sick sheep	50	Yes	30
Dog contact	50	Yes	80
Cat contact	50	Yes	58
Mechanical ventilation in barn	50	Yes	20
Ram clostridial vaccination	50	Yes	50
Estrous synchronization	50	Yes	20
Breeding nulliparous ewes together with other ewes	50	Yes	48
Domain 2: Flock characteristics			
Producer experience	50	< 1 year	4
		1-2 years	6
		3-5 years	24
		5-10 ears	18
		> 10 years	48
Neurological problem in ewes	48	Yes	17
Wasting problem in ewes	48	Yes	10
Goal setting	48	Yes	60
Record keeping	47	Yes	79
Domain 3: Ewe feeding management			
Number of forage types offered to pre-partum ewes	50	1	84
		2	16

Original variable	Number of Observations	Category	Percent
Feeding ewes based on body condition score	50	Yes	22
Offering hay to post-partum ewes	50	Yes	26
Offering commercial grain to post-partum ewes	50	Yes	40
Offering blue salt to post-partum ewes	50	Yes	30
Domain 4: Ewe health management and lamb feeding management			
Ewe clostridial vaccination	50	Yes	72
Ewe ivermectin treatment	50	Yes	56
Ewe benzimidazole treatment	50	Yes	30
Grouping pre-partum ewes by ram	50	Yes	20
Manually check udder at weaning	50	Yes	42
Colostrum supplementation	50	Yes	90
Offering creep feed free-choice	50	Yes	82
Offering commercial creep feed	50	Yes	82
Offering blue salt to lambs	50	Yes	20
Domain 5: Lamb health management			
Navel dipping for every lamb	50	Yes	46
Lamb identification	50	Yes	78
Lamb castration	50	Yes	22
Lamb coccidiostat supplementation	50	Yes	48
Lamb clostridial vaccination	50	Yes	48
Lamb benzimidazole treatment	50	Yes	28
Keep hypothermic lambs in barn	50	Yes	52
Wean on weight	50	Yes	48

Original variable	Number of Observations	Category	Percent
Domain 6: General flock health management			
Use clostridial vaccine	50	Yes	76
Use ivermectin	50	Yes	56
Use benzimidazole	50	Yes	40
Use coccidiostat	50	Yes	50
Routine hoof-trimming	48	Yes	71
Use foot bath	50	Yes	16
Animal treatment based on veterinary advice	50	Yes	14
Domain 8: Pre-partum ewe forage quality			
Acid detergent insoluble protein < 12%	50	Yes	64
Season	50	Autumn	16
		Winter	44
		Spring	40

Table 7.3 Descriptive statistics of continuous variables included in Principal Component Analysis based on 50 lambing groups of 36 sheep flocks in Prince Edward Island in 2014-2015.

Variable	Minimum	Median	Maximum	Mean	Standard deviation
Domain 1: Biosecurity and breeding management					
Total ewes bred (heads; n = 50)	10	44	180	62	47
Breeding period (days; n = 50)	6	60	150	81	49
Domain 2: Flock characteristics					
Number of ewes (heads; n = 50)	13	97	250	102	65
Number of rams (heads; n = 50)	1	3	10	4	3
Ewes died per year (%; n = 47)	0	2	25	4	5
Domain 3: Ewe feeding management					
Amount of grain fed at 6 weeks pre-partum (kg; n = 50)	0.00	0.23	1.00	0.30	0.30
Amount of grain fed at 5 weeks pre-partum (kg; n = 50)	0.00	0.23	1.00	0.30	0.29
Amount of grain fed at 4 weeks pre-partum (kg; n = 50)	0.00	0.34	1.14	0.38	0.28
Amount of grain fed at 3 weeks pre-partum (kg; n = 50)	0.00	0.45	1.14	0.41	0.28

Variable	Minimum	Median	Maximum	Mean	Standard deviation
Linear bunk space (cm, n = 48)	10	37	64	37	13
Domain 4: Ewe health management and lamb feeding management					
Frequency of checking ewes close to lambing (times per day; n = 50)	3	7	24	8	6
Domain 5: Lamb health management					
Lambs need lambing assistance (%; n = 47)	0	15	40	16	13
Domain 7: Pre-partum ewe health					
Body condition score (n = 48)	2.0	3.0	4.0	2.9	0.4
Serum total protein (g/l; n = 48)	57.25	61.62	68.88	62.34	3.44
Serum glucose (mmol/l; n = 48)	2.32	3.31	4.35	3.30	0.42
Serum beta-hydroxybutyrate (µmol/l; n = 48)	287.00	496.44	1767.63	546.11	242.02
Serum gamma-glutamyl transferase (Unit/l; n = 48)	39.88	54.38	83.00	55.25	9.07
Serum phosphorus (mmol/l; n = 48)	1.07	1.48	1.92	1.50	0.20
Serum potassium (mmol/l; n = 48)	3.70	4.92	5.36	4.91	0.27
Serum selenium (µg/ml; n = 48)	0.005	0.077	0.203	0.081	0.041
Serum vitamin E (µmol/l; n = 48)	1.09	2.29	4.90	2.37	0.72

Variable	Minimum	Median	Maximum	Mean	Standard deviation
Domain 8: Pre-partum ewe forage quality					
Crude protein (%; n = 50)	6.52	12.10	19.34	12.71	3.09
Acid detergent fiber (%; n = 50)	25.32	34.79	40.88	34.14	3.39
Digestible energy (Mcal/kg; n = 50)	2.28	2.62	3.17	2.66	0.19
Calcium (%; n = 50)	0.22	0.66	1.41	0.73	0.31
Phosphorus (%; n = 50)	0.18	0.26	0.39	0.26	0.05
Magnesium (%; n =50)	0.12	0.23	0.36	0.23	0.06
Potassium (%; n = 50)	0.91	1.82	2.93	1.85	0.43
Copper (ppm; n = 50)	1.52	5.09	9.70	5.32	1.68
Zinc (ppm; n = 50)	9.57	19.18	40.29	19.82	5.89
Net energy for maintenance (Mcal/kg; n = 50)	1.14	1.32	1.60	1.34	0.10

Table 7.4 Parameters in the final mixed Poisson model identifying factors associated with lamb mortality risk, based on 48 lambing groups of 35 sheep flocks in Prince Edward Island in 2014-2015**.**

Parameter	Coefficient	Standard error	P-value	95% confidence interval	
Flock1	-0.19	0.06	0.002	-0.31	-0.07
Genhealth4	-0.34	0.10	0.001	-0.54	-0.15
Forage1	-0.11	0.02	<0.001	-0.16	-0.06
Lambhealth1	-0.47	0.13	<0.001	-0.73	-0.22
Lambhealth1 (quadratic term)	-0.08	0.06	0.154	-0.03	0.19
Lambhealth1 (cubic term)	0.16	0.05	0.001	0.07	0.26
Constant	-2.46	0.09		-2.63	-2.28

Log likelihood = -116.29

Between-flock variance estimate = 0.022 (0.003, 0.176)

Table 7.5 Loadings of original variables in principal components found to be significant in the final mixed Poisson model to identify factors associated with lamb mortality risk, based on 50 lambing groups of 36 sheep flocks in Prince Edward Island (Letters in bold indicate factors having high contribution to the principal component scores; loading ≥ 0.35 or ≤ -0.35) in 2014-2015.

Original variables	Principal component 1	Principal component 2	Principal component 3	Principal component 4
	Flock1*	Flock2	Flock3	Flock4
Producer experience	0.24	0.15	**-0.38**	**0.71**
Number of ewe	0.05	**-0.55**	0.30	0.13
Number of ram	0.15	**-0.47**	0.34	0.27
No neurological problem in ewes	**0.54**	0.20	-0.11	0.08
No wasting problem in ewes	**-0.52**	0.13	**0.36**	0.34
Goal setting	**0.49**	-0.22	0.13	-0.33
Record keeping	0.32	0.32	**0.58**	0.29
Percent ewes died per year	0.06	**0.50**	**0.39**	-0.28
Eigenvalue	2.98	2.28	1.28	1.01
Proportion of variance explained	0.37	0.28	0.16	0.13
	Lambhealth1*	Lambhealth2	Lambhealth3	Lambhealth4
Percent lambs need lambing assistance	0.27	**-0.54**	0.34	-0.15
Navel dipping for every lamb	-0.11	**-0.43**	-0.06	-0.31
No lamb identification	**-0.45**	0.22	-0.19	0.21
Lamb castration	0.13	**0.37**	**0.35**	**-0.60**

Original variables	Principal component 1	Principal component 2	Principal component 3	Principal component 4
Lamb coccidiostat supplementation	**0.38**	0.16	**0.47**	**0.44**
Lamb clostridial vaccination	**0.48**	0.03	-0.05	0.07
Lamb benzimidazole treatment	0.22	**0.53**	-0.09	-0.04
Keeping hypothermia lamb in the barn	**0.38**	-0.18	**-0.43**	0.34
Deciding to wean on weight	-0.34	-0.06	**0.56**	**0.40**
Eigenvalue	2.67	2.04	1.50	1.23
Proportion of variance explained	0.30	0.23	0.17	0.14
	Genhealth1	Genhealth2	Genhealth3	Genhealth4*
Use clostridial vaccine	**0.37**	-0.03	**0.63**	-0.21
Use ivermectin	**0.51**	0.23	-0.23	-0.25
Use benzimidazole	**0.37**	-0.01	-0.28	**0.71**
Use coccidiostat	**0.45**	-0.28	**0.45**	0.11
Routine hoof trimming	-0.23	**0.63**	0.25	-0.14
Foot bath	0.28	**0.68**	0.01	0.20
Animal treatment based on veterinary advices	**-0.35**	0.07	**0.45**	**0.57**
Eigenvalue	2.62	1.48	1.38	0.96
Proportion of variance explained	0.37	0.21	0.20	0.14

Original variables	Principal component 1	Principal component 2	Principal component 3	Principal component 4
	Forage1*	Forage2	Forage3	
Crude protein	**0.37**	0.27	-0.09	
Acid detergent fiber	**-0.36**	0.30	0.11	
Digestible energy	**0.37**	-0.30	-0.10	
Calcium	0.25	**0.36**	**-0.45**	
Phosphorus	0.32	0.07	**0.42**	
Magnesium	0.27	0.32	-0.30	
Potassium	0.29	0.05	-0.05	
Copper	0.26	0.34	**0.37**	
Zinc	0.25	-0.05	**0.58**	
Acid detergent insoluble protein < 12%	0.12	**-0.55**	-0.11	
Net energy for maintenance	**0.36**	-0.29	-0.11	
Eigenvalue	5.45	2.45	1.13	
Proportion of variance explained	0.50	0.22	0.10	

*Principal components retained in the final model

Table 7.6 Descriptive statistics of principal component scores remaining in the final mode to identify factors associated with lamb mortality risk, based on 50 lambing groups of 36 sheep flocks in Prince Edward Island in 2014-2015.

Principal component	Minimum	Median	Maximum	Mean	Standard deviation
Flock1	-2.50	0.11	1.64	-0.10	0.93
Genhealth4	-1.07	-0.11	2.41	-0.01	0.73
Forage1	-4.18	-0.26	4.79	0.00	2.32
Lambhealth1	-2.25	-0.05	1.75	-0.04	1.14

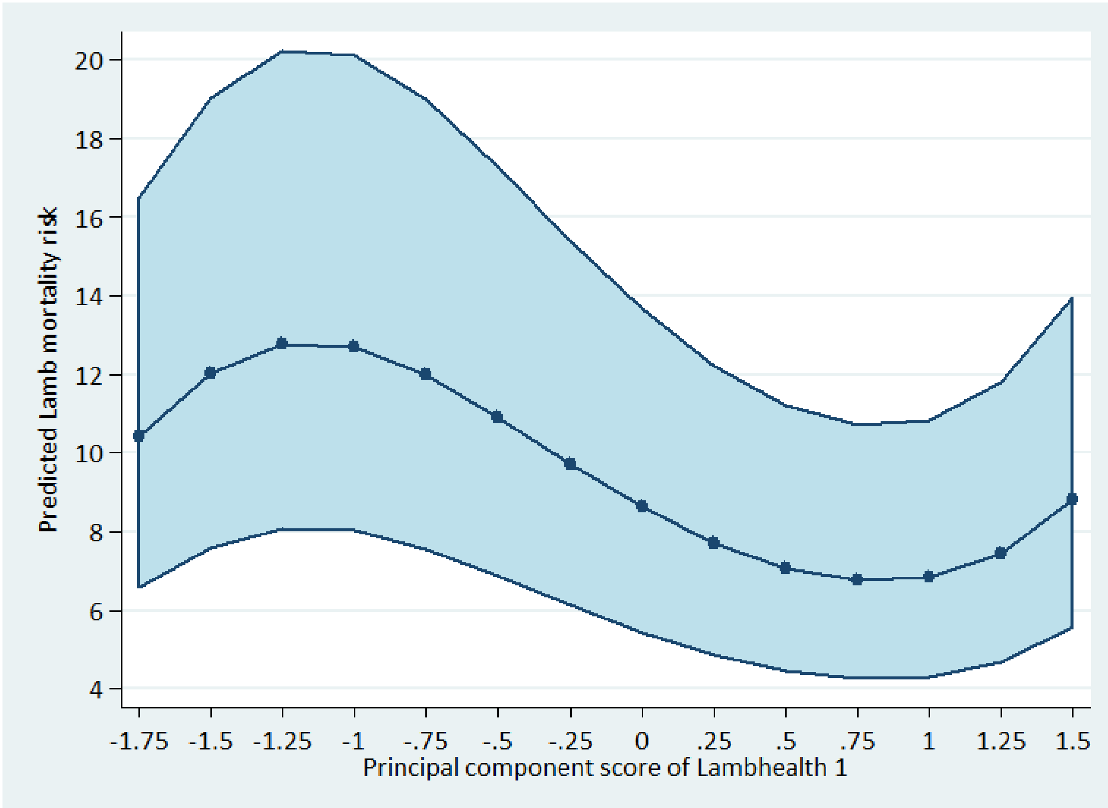

Figure 7.1 Estimated association between the principal component scores of Lambhealth1 and lamb mortality risk, based on 48 lambing groups of 35 sheep flocks in Prince Edward Island in 2014-2015, when other principal component scores in the final model are set at 0.

CHAPTER 8

SUMMARY AND GENERAL DISCUSSION

8.1 Introduction

Sheep are a multi-purpose livestock species widely distributed across the world; their meat, milk, wool, and skin can be used to support the livelihood of sheep producers with sheep production contributing significantly to the economies of many regions. Regardless of the production system employed, lamb production is essential for flock profitability, thus maximizing lamb survival is a fundamental goal of all sheep producers.

Studies examining risk factors for lamb mortality have been conducted in several livestock-raising regions around the world. The identified risks varied widely in type and importance among these studies. These differences can be attributed to the variety of environmental conditions and types of feed, flock characteristics and breeds, management practices, and follow-up periods utilized in the studies. The highest reported average lamb mortality, measured from birth to weaning, was 25.9% in a flock at the University of Minnesota Agricultural Experimental Station (Yapi et al., 1990). In contrast, only 4% average pre-weaning mortality was reported in Mehraban sheep flocks in Iran (Atashi et al., 2013).

Most lamb mortalities occur during the first few days of life (Binns et al., 2002; Brien et al., 2009), with hypothermia, hypoglycaemia, and dystocia being the most common causes of neonatal lamb losses (Woolliams et al., 1983; Mellor and Stafford, 2004). Infectious causes of mortality are generally found in older lambs (Rowland et al., 1992; Green and Morgan, 1994). Identifying common underlying causes of lamb mortality within flocks is a worthwhile practice to identify management interventions that can help avoid further unacceptable lamb losses (Rowland et al., 1992; Nash et al., 1996).

Lamb mortality is influenced by various lamb-level factors, with weight at birth being the most important determinant of lamb mortality (Huffman et al., 1985; Upreti, 1989). A quadratic association between birth weight and lamb mortality has been reported, with the highest survivability found in lambs with intermediate birth weights (Piwczynski et al., 2012). For type of birth, non-singleton lambs are more susceptible to mortality than singleton lambs (Piwczynski et al., 2012; Chniter et al., 2013). Although effects of gender on lamb mortality are not consistent among studies, most studies reported lower survival in male lambs than female lambs (Binns et al., 2002; Mandal et al., 2007; Brien et al., 2009). Not surprisingly, inactive lambs have lower survivability than active lambs (Nowak and Poindron, 2006).

In addition to individual-level factors of lambs, some individual-level factors of ewes have been reported associated with lamb mortality, such as age and body condition of ewes at mating and lambing (Carson et al., 2001; Sawalha et al., 2007; Thompson, 2011; Piwczynski et al., 2012). Due to the importance of colostrum to lamb survivability, lamb mortality is also influenced by maternal behavior, teat and udder conformation, and colostrum and milk production of ewes (Berger et al., 1989; Nash et al., 1997; Everett-Hincks and Dodds, 2008). Some individual-level factors of lambs and ewes can partly be manipulated by management practices, such as birth weight of lambs, and body condition of ewes, but some are difficult to manipulate.

Several flock-level factors, including environmental conditions and management practices, have impacts on lamb survival. Detrimental effects of poor environmental conditions on lamb mortality can be minimized by appropriate housing, and management practices (Thompson, 2011; Chniter et al., 2013). A positive association between flock size and lamb mortality has been reported by Thompson (2011). The adverse effect of large flocks on lamb mortality can be alleviated by split-flock lambing (Andrewes and Taylor, 1986; Hawkins et al., 1989). Selection of breeding stock for desirable traits is a possible approach to manipulate lambing difficulty, litter size, maternal behavior, and cold resistance (Demirören et al., 1995; Carson et al., 2001; Dwyer, 2008; Cloete et al., 2009; Elliott et al., 2011).

Lamb mortality is affected by management of feeding practices of both ewes and lambs. Well-fed late-gestation ewes produce active large lambs, which are more likely to survive (Bloomfield et al., 2003; Redmer et al., 2004); these ewes are better producers of colostrum and milk (Elliott et al., 2011). Metabolic diseases in late-gestation ewes interfere with survivability of lambs. Nutritional muscular dystrophy, which is a metabolic disorder causing lamb mortality, can be prevented by appropriate selenium and/or vitamin E management practices in ewes during the last trimester of pregnancy (Kott et al., 1983; Munoz et al., 2006). Colostrum is not only an important source of nutrient supply, but it is also a major source of passive immunity. Lamb mortality due to starvation and infectious diseases can be reduced in lambs receiving sufficient colostrum (Mandal et al., 2007), and weak lambs receiving suckling assistance have a higher chance of survival than non-assisted lambs (Holmøy et al., 2012).

Lamb survival is influenced by flock disease control practices, including biosecurity. Ewes with suboptimal health or chronic diseases are more likely to produce small, weak lambs and usually cannot provide sufficient care to their lambs (Hamilton Prime Lamb Breeders, 2005). Vaccination against infectious abortion diseases diminishes lamb loss by reducing abortion, stillbirth, and lambs born weak (Thompson, 2011). Clostridial vaccines are among the most widely used vaccines for sheep, as they provide good protection from lamb losses due to several clostridial diseases (Eales et al., 1983). Internal helminths and coccidia are common gastrointestinal pathogens of sheep, especially lambs (Dohoo et al., 1985); thus, practices which reduce the severity of these infections are beneficial to overall ewe and lamb health.

Litter size is partly determined by breeding management practices. In intensive management systems where large litters can lead to higher mortality, appropriate management of ewes and rams around breeding time can moderate litter size and improve survival (Christley et al., 2003; Mandal et al., 2007). Time of lambing can be selected to prevent adverse effects of weather and environment on lamb mortality (Nash et al., 1997; Sormunen-Cristian and Suvela, 1999). For maximal lamb survival, lambing should be closely supervised, and assistance should be immediately provided to ewes and lambs requiring interventions (Rowland et al., 1992; Cloete et al., 1993).

Various aspects related to lamb mortality were explored in the series of studies presented in this thesis. The overall objective of this thesis was to determine underlying flock-level factors associated with lamb mortality in Prince Edward Island (PEI) flocks. This line of research was pursued because factors associated with lamb mortality had never been examined before in PEI flocks, and there was anecdotal information suggesting that many flocks were experiencing lamb mortality risks in excess of the industry standard goal (< 10% pre-weaning mortality), which was of great concern to the industry.

Chapter 1 provided a literature review on lamb mortality, including literature from all major lamb-raising regions of the world. In Chapter 2, common causes of lamb mortality in PEI were identified, drawing on retrospective data from 10 years of post-mortem submissions to the Atlantic Veterinary College Diagnostic Services, University of PEI. Flock characteristics, management practices, and health status of late-gestation ewes in PEI sheep flocks were described in Chapter 3, with a focus on those factors that were known from the literature to be related to lamb survival. In Chapter 4, factors specifically associated with liver selenium and vitamin E concentrations of dead lambs were identified, because these micronutrients have been identified as likely to be deficient in other livestock populations in PEI. The efficacy of an electronic device, used as an animal-side test for determining the blood concentration of β-hydroxybutyrate (BHBA), was assessed in late-gestation ewes, and factors associated with BHBA concentration of late-gestation ewes were identified in Chapters 5 and 6, respectively. Finally, flock-level factors associated with lamb mortality were examined comprehensively in Chapter 7.

The results of these thesis chapters will lead to a better understanding of characteristics of PEI sheep flocks, management practices, and factors associated with lamb mortality in these flocks. Consequently, appropriate management practices, and interventions to minimize lamb mortality can be identified and applied in sheep flocks.

8.2 General discussion and limitations

Flock-level determinants of lamb mortality were examined in order to help PEI sheep producers identify appropriate management practices and interventions to improve lamb survival, and increase profits.

8.2.1 Chapter 2

In Chapter 2, we reviewed post-mortem records of 385 lambs (≤ 90 days of age) submitted to the Atlantic Veterinary College Diagnostic Services, University of PEI between January 2005 and May 2014. The natural breeding season in PEI might partially explain the large proportion of lambs submitted in the first half of each year. Hypothermia and hypoglycemia due to unfavourable environmental conditions during the first few months of the years (Chniter et al., 2011) might be another cause of the higher submissions during this period. Out of 385 lambs, 3.4%, and 1.3% were aborted fetuses and stillborn lambs, respectively, whereas greater proportions of stillbirth out of total lamb mortalities (22%-30%) were reported in previous population-based studies (Berger, 1997; Kennedy, 2010). According to previous studies in Peru and Canada, 1.2%-1.7% of ewes experienced abortion (Ameghino et al., 1984; Dohoo et al., 1985). The median age at death in our study population was 19 days. By contrast, a previous prospective study in Quebec, Canada, reported that most pre-weaning lamb deaths occurred within the first few days (Arsenault et al., 2003). These differences among studies are attributed to different environmental conditions, endemic diseases and management practices in study populations (Nash et al., 1996).

More than half of the pot-mortem submissions in the present study had lesions in the respiratory system, but the gastrointestinal system was the most common body system accounting for death (25.1%). Lesions in the respiratory system are typical in dead lambs regardless of the primary cause of death (Matthews and Ogden, 1957). However, according to Khan et al. (2006), abnormalities in both the gastrointestinal and respiratory systems are common causes of lamb mortality. In the present study, death related to the gastrointestinal system was more frequent in lambs ≥ 3 days of age than in younger lambs.

The most frequent primary cause of death in the present study was infection, which is consistent with a previous study conducted in Canada (Dohoo et al., 1985). Bacteria were the most common pathogens responsible for infectious lamb mortality in the present study. The importance of infectious diseases in older lambs found in the present study agrees with a previous study (Arsenault, 2002). Primary cause of death could not be identified in 29% of the submissions, which was more frequent in lambs ≤ 2 days of age than older lambs. Based on our findings, management changes to reduce pathogen exposure and increase diseases resistance, including passive transfer of immunoglobulins, are required to avoid infectious mortality.

The main limitation of this retrospective study was that lambs submitted for post-mortem examination were not randomly selected; the submissions might not properly represent the source population, which was PEI lambs dying by 90 days of age. Due to incompleteness of laboratory-based data, the results should be interpreted with caution (Janiec et al., 2012). Selection bias is a typical potential bias in a retrospective laboratory-based post-mortem study, and bias adjustment is possible only during the data analysis process (Geneletti et al., 2009). However, approximate ranking of causes of lamb mortality from bias unadjusted results from laboratory-based data can be useful to prevent further losses and suggest valuable areas of additional studies (Dohoo et al., 1985; Dorea et al., 2013).

8.2.2 Chapter 3

Flock characteristics, current management practices, and basic health statuses of late-gestation ewes in 37 PEI sheep flocks holding 51 lambing groups were examined in Chapter 3. The majority of PEI sheep flocks were small, crossbred, meat sheep flocks. Thus, high lamb mortality resulting from inadequate supervision because of large flock size (Binns et al., 2002) should not be a problem in our study flocks. These flocks might also gain some advantages in vigor and disease resistance due to the large proportion of crossbred flocks (Yapi et al., 1990; Nash et al., 1996; McHugh et al., 2016). A total confinement system was employed in one-third of study flocks, which was three times greater than the proportion previously reported in Canadian flocks (Dohoo et al., 1985). Sheep in most of our study flocks were fed ensiled forages, along with concentrate and mineral supplementation, with increasing concentrate feed being offered for late-gestation ewes to support their rising requirements during this period (NRC, 2007). However, the amounts of grain supplement in some study flocks were lower than recommended for late-gestation ewes (Rankins and Pugh, 2012).

The concentration of vitamin E is typically low in stored forages and grains (Menzies et al., 2004; Liu et al., 2014), and these were the main feeds provided to the late-gestation ewes by all participating producers. In addition, soil in Ontario, Canada, contains low concentrations of selenium (Beauchamp et al., 1969). As a result, there is a high risk of selenium and vitamin E deficiencies in our study flocks. Although not many producers supplemented their ewes with a selenium and vitamin E injection, more than two-thirds of producers gave injectable selenium and vitamin E to their lambs within the first week of life.

Vaccination against clostridial diseases, which are common in sheep, was administered to over half of study flocks. Coccidiosis was reported as a common health problem in lambs in some study flocks. Many participating producers added coccidiostats in lamb creep feeds to alleviate the problem; however, the addition of coccidiostats in late-gestation ewe feeds, which is recommended by some veterinarians (Rankins and Pugh, 2012), was rare. Deworming was widely adopted in the present study; nevertheless, the proportion of flocks in which an anthelminthic drug was not used was much higher than the proportion reported by Dohoo et al. (1985). A total confinement housing system, which is one of the strategies that can be employed for gastrointestinal parasite control, was implemented in most flocks that did not receive any anthelminthic. In grazed flocks where anthelmintic was not used, producers may have intended to avoid anthelminthic use in order to slow down development of drug resistance. Macrocyclic lactone anthelminthic was the most frequent anthelminthic group used in our study flocks, while benzimidazole derivatives were also used in a considerable proportion. Biosecurity measures to prevent disease transmission from external sources and within a flock were adopted by our participating producers at varying levels. However, sheep in most flocks were allowed contact with other animals that can transmit diseases, especially dogs and cats.

The lambing period is another factor influencing lamb mortality (Fisher, 2004). In the present study, the typical lambing period was the natural lambing season, and most ewes were bred without estrus induction. Shelter provision to ewes and lambs prevents lamb losses from hypothermia, hypoglycemia, and predator attack (Upreti, 1989; Dwyer, 2008). As all flocks in our study lambed indoors, they benefited from shelters. Navel disinfection was not routinely performed by half of participating producers.

To ensure optimal neonatal nutrition and passive transfer, at least 10% of body weight of good quality colostrum should be ingested within the first 12 hours of life (Rankins and Pugh, 2012). In the present study, where neonatal lambs were fed colostrum by the producer, on average less than 100 ml of colostrum (less than 2.5% live weight) was fed. Because creep feeds stimulate development of the rumen, it should be offered to lambs as soon as possible (Rankins and Pugh, 2012). In the present study, the mean age of lambs at first creep feed was 11 days of age, which was earlier than was reported by Dohoo et al. (1985). Most producers allowed free-choice access to creep feeds, and also provided mineral supplements to their lambs. The ewe-lamb bond is properly formed during a period of close proximity, which is consequently beneficial to lamb survival (Nowak and Poindron, 2006; Dwyer et al., 2016). In the present study, almost all producers allowed ewes and their lambs to stay in claiming pens for a period of time.

The variations of nutrient compositions of pre- and post-partum forages in participating flocks were considerable. Fourteen percent of the samples contained less than 9% crude protein, and a considerable proportion of ensiled forages were not properly fermented. The poorly ensiled forage contained high proportion of bound protein (Schroeder, 2012) and could predispose animals to some diseases such as listeriosis (Vilar et al., 2007).

Thompson (2011) found that lamb survival was associated with body condition of ewes at lambing. In the present study, almost half of late-gestation ewes examined were not in ideal body condition. Two-thirds of late-gestation ewes examined had some degree of pale conjunctiva, which was consistent with the high proportion of late-gestation ewe fecal samples in which gastrointestinal helminth eggs were detected. Helminth eggs were found in almost all flocks; however, most fecal samples contained fewer than 1000 epg, which is one threshold that is used to determine if nematode treatment is likely to be beneficial (Gonzalez-Garduno et al., 2014).

Most late-gestation ewe blood samples contained concentrations of total protein, calcium, selenium, and vitamin E that were below our laboratory reference ranges. Hypoproteinemia found in our study population might result from under-nutrition (either low dry matter intake or low available protein), helminth infection, or other chronic disease (e.g. Johne's disease). As the calcium levels in forage samples were in an acceptable range, low ewe serum calcium concentrations might be attributed to low availability of calcium, resulting from interactions with other minerals, and/or the high calcium requirement for fetal growth in late-gestation ewes (NRC, 2007). Given that the majority of ewes in our study carried multiple fetuses, the latter is more likely to be the explanation for this observation.

The limited selenium content in soil (Beauchamp et al., 1969) was the most probable reason for inadequate serum selenium concentrations found in the present study. Vitamin E deficiency in the study population was probably due to the increased vitamin E requirement in the last trimester of pregnancy (Goff and Stabel, 1990). Additionally, late-gestation ewes mainly depended on forage which is harvested and stored for weeks or months, leading to vitamin E degradation (Kostadinovic et al., 2013).

The main limitation of the work presented in this chapter was the excessively long first visit questionnaire, which took up to an hour to complete. Although this questionnaire was tested with a small group of producers prior to use and found to be effective, length of a questionnaire can affect both unit and item response rates of a postal survey (Sahlqvist et al., 2011). Additionally, response rates to surveys are influenced by the difficulty of questions (Dillman et al., 1993). The questionnaire used in the present study contains some difficult-to-answer questions, such as average abortion risk in the last three years and frequency of checking ewes close to lambing, and many missing data were found for these questions. Thus, non-response biases might have occurred in these difficult questions. Quality of responses of a long questionnaire can also be adversely affected, specifically for questions close to the end of the questionnaire (Galesic and Bosnjak, 2009). Although there was no unit non-response in the present study, item non-responses and response quality should be considered. A possible solution of this limitation is splitting the questionnaire into shorter questionnaires, and interviewing more than once.

Another study limitation was a non-probability sampling of late-gestation ewes, so sampled ewes might not properly represent health statuses of all ewes in the flocks. A simple random sampling was impossible in most PEI sheep flocks because a complete list of the population in the flocks was not available. Although systematic random sampling was feasible in our study flocks, it required more time and some farm equipment to arrange animals sequentially, and on most flocks, due to the small flock sizes in PEI, there were no dedicated sheep handling facilities.

8.2.3 Chapter 4

In chapter 4, factors associated with selenium and vitamin E concentrations in liver tissue of dead lambs were identified. The proportions of lambs with liver selenium and vitamin E concentrations in the deficient range were higher in the present study than the proportions reported in a study conducted in Ontario, Canada, using the same cut-offs (Menzies et al., 2003). Serum selenium and vitamin E concentrations in late-gestation ewes were not significantly associated with each other, but the liver concentrations of selenium and vitamin E in lambs were positively correlated. These findings contradict a previous study, in which a significant association between concentrations of selenium and vitamin E was detected in ewe liver samples, but no association was found in lamb liver samples (Menzies et al., 2003). Differences in group's serum selenium concentrations in late-gestation ewes, untreated and treated with injectable selenium and vitamin E, were significant, but the difference of vitamin E concentrations was not significant. However, treated lambs had both liver selenium and vitamin E concentrations higher than untreated lambs.

In untreated lambs, log liver selenium concentrations varied by age at death, and an interaction between serum concentration of late-gestation ewes and type of death. The highest log selenium concentration was detected in lambs dying at 20-24 days of age. The large amounts of selenium in colostrum (Pehrson et al., 1990) might be responsible for a marked increase of lamb liver concentration during the early period of life. The effect of ewe serum concentration found in the present study is consistent with a previous study reporting that amount of selenium transfer from ewes to lambs in utero depended on the status of ewes (Bostedt and Schramel, 1990). Based on the significant interaction, the strength of association between serum concentration of late-gestation ewes and liver concentration of lambs was higher in aborted fetuses and stillborn lambs than in lambs dying after birth.

The log lamb liver vitamin E concentrations also depended on age at death, which peaked in the third week. As colostrum is a vitamin E-rich source (Pehrson et al., 1990; Liu et al., 2014), a marked increase during the early stage of life was not unexpected. The concentrations were lower in aborted fetuses and stillborn lambs than lambs born alive, which indicate the importance of vitamin E sources for lambs other than the transplacental transfer (Rooke et al., 2004).

In selenium and vitamin E injected lambs, both log selenium and log vitamin E concentrations constantly decreased over time after supplementation in an individual lamb, but increased with a higher group mean time between lamb treatment and age of lamb at death. Different dosages and times of selenium and vitamin E supplementation used in each lambing group might partly be responsible for the latter finding. Further studies are required to determine an appropriate dosage and timing of the injectable supplementation in lambs, and whether higher liver selenium and vitamin E concentrations in lambs might protect them against mortality during the early stage of life.

Although there were some study limitations, results from this chapter illustrate the selenium and vitamin E status of lambs in PEI, and indicate management practices which can improve that status. The results would be more reliable if the exact treatment status of each lamb was known, instead of presuming based on age at the time of treatment and age at death, which might lead to information bias. The dosage of treatment should be included in an analysis to evaluate the effect of dosage variation, and identify a proper dosage. It would be better to include the concentrations of their own dams in the analyses instead of the average flock concentrations.

Lambs included in the present study were not randomly selected and all of them were dead lambs; therefore, selection bias might be another limitation. To improve accuracy of the results, bias analysis is required to adjust for both information and selection biases. Moreover, additional studies conducted in live lambs may be required because factors associated with liver selenium and vitamin E concentrations of dead and live lambs may not be exactly the same.

8.2.4 Chapter 5

We assessed the efficacy of the Precision Xtra© meter (Abbott Diabetes Care, Saint-Laurent, Canada), a device used to measure blood glucose and BHBA concentrations in diabetic patients, for measuring blood BHBA concentrations of late-gestation ewes. The correlation, and agreement of the Precision Xtra© meter when compared to a standard laboratory test, were satisfactory. Underestimation of BHBA concentrations obtained from handheld electronic devices was reported in previous studies conducted in dairy cattle and sheep (Iwersen et al., 2009; Pichler et al., 2014). In the present study, the Precision Xtra© meter provided slightly lower results than the standard laboratory results. This finding might not be an actual underestimation, but could be caused by an overestimation of standard laboratory BHBA concentrations due to hemolysis of blood samples (Jacobs et al., 1992). Another possible explanation for the lower Precision Xtra® results was unsuitable temperatures when the device was operated in the cold sheep barns, as the temperature range recommended for operating the Precision Xtra© meter is 10 °C to 50 °C. A significant but weak positive correlation between the difference in results from the two methods (Precision Xtra© meter minus standard laboratory test) and environmental temperatures at the time of testing was identified in our data.

According to our findings, the 800 µmol/l blood BHBA concentration was recommended as a cut-off to determine ketotic ewes with the Precision Xtra© meter, which provided 100% sensitivity. Although false positives were found at this cut-off, the cost of treatment is inexpensive (eg. feed more concentrate, preferably in a separate group of other ketosis-positive ewes), and over-treatment is considered of less concern than leaving an affected ewe untreated, which can lead to losses of both ewes and their lambs.

The main limitation of chapter 5 was that environmental temperatures when the Precision Xtra© meter was operated were out of the recommended temperature range. This problem is unavoidable under field use in many PEI sheep flocks, because the typical lambing period in this area is winter and autumn. However, results of the present study suggest acceptable performance of the meter when it was applied under field conditions. The temperature data (from a Charlottetown weather station) used to assess the impact of weather on the results obtained from the Precision Xtra© meter will not have matched exactly the temperature in the barns where each test was conducted. To evaluate an actual effect of temperature on efficacy of the Precision Xtra© meter, temperatures would need to be measured on-site at the time of testing.

8.2.5 Chapter 6

In chapter 6, body condition score (BCS), color of conjunctiva (FAMACHA© score), presence of helminth eggs in fecal samples, and litter size were identified as factors related to ewe log serum BHBA concentration. Ewes with BCS of 2.5 to 3.5 had the lowest log BHBA concentrations, which is in agreement with previous studies reporting that both under- (Andrews, 1997) and over-conditioned late-gestation ewes (Marteniuk and Herdt, 1988; Van Saun, 2000) had a greater risk of developing ketosis than ewes in good body condition.

In the present study, ewes with quite pale mucus membranes (FAMACHA© score of 3) had greater log BHBA concentrations than ewes with pink mucus membranes (score of 1 or 2) and white mucus membrane (score of 4 or 5). Ewes with intestinal helminth eggs detected in fecal samples had greater log BHBA concentrations than ewes in which eggs were not detected. The FAMACHA© system was developed to identify sheep affected by *Haemonchus contortus* based on degree of anemia (van Wyk and Bath, 2002). Because intestinal helminth challenge can influence energy utilization during the last trimester of pregnancy (Dakkak, 1990), significant associations between log BHBA concentration and the presence of helminth eggs and FAMACHA© score in the present study were not unexpected. A concomitant correlation between BHBA concentration and litter size found in our study population was likely a result of increased energy demand in the face of limited rumen capacity, due to the size of the conceptus (Cal-Pereyra et al., 2012; Raoofi et al., 2013; Barbagianni et al., 2015).

Based on all findings, appropriate feeding management practices of late-gestation ewes, including assessing litter size and using this information to adjust the ration in keeping with predicted nutrient demand, along with control of gastrointestinal helminth burden, are likely to be effective strategies to prevent ketosis in PEI flocks.

The number of missing data comprised the primary limitation of this analysis, as litter size was not recorded by producers in 42% of our study population. Because the complete case analysis approach was used for model building, these ewes could not be included in the data analysis. Selection bias could also be a problem if observations without a missing value are not representative of the study population as a whole (Dohoo et al., 2009). Bias analysis can be performed to minimize such an effect; however, to do so, bias parameters are required, and these were unavailable in our study.

8.2.6 Chapter 7

The mean lamb mortality by 56 days of age in the 50 lambing groups included in this dataset was 11%, with half of these groups having more than 10% mortality. Several flock-level factors were found to be related to lamb mortality.

A history of neurological problems in the flock within the last three years contributed to increased lamb mortality. Common causes of neurological manifestations in sheep are inappropriate nutritional management (causing, for instance, pregnancy toxaemia) and infectious diseases (Schenk et al., 2008). Nutritional status of ewes is a factor affecting several determinants of lamb mortality, such as birth weight of lambs and colostrum production of ewes (Ojha et al., 2013; Dwyer et al., 2016), while pathogens causing common neurological diseases in sheep, such as Maedi-visna disease and listeriosis, can also influence lamb survivability (Arsenault et al., 2003; Edmondson et al., 2012).

Suboptimal health of ewes may affect mothering behavior (Hamilton Prime Lamb Breeders, 2005). However, the present study found that experiencing a wasting problem contributed to decreased lamb mortality. Because an assessment of body condition is subjective (Evans, 1978), the judgement of thin body condition might reflect the level of attention producers paid to their flocks. Producers paying more attention to the flocks might be more likely to classify sheep as “thin” or “wasted”.

Elliott et al. (2011) reported that producers concerned about productivity of their flocks have a higher tendency to accept new, useful interventions and, not surprisingly, setting goals for the flock contributed to decreased lamb mortality in the present study.

The high energy and protein in forage provided to late-gestation ewes contributed to increased lamb survival. Greater lamb birth weight, better mothering behavior, and colostrum production were probably responsible for this finding (Fenochio et al., 2013, Banchero et al., 2015).

Seeking and using veterinary advice for management of illness was another factor that contributed to decreased lamb mortality in the present study. This finding is concordant with a review article suggesting prompt treatment of sick sheep based on veterinary advice (Eales et al., 1983). In addition to treatment advice provided by veterinarians, these flocks might also obtain other non-treatment-related advice, which could be beneficial to limit lamb mortality.

Benzimadazole anthelminthic use contributed to decreased lamb mortality. This finding might suggest that efficacy of this group of anthelminthics has remained generally acceptable in PEI sheep flocks; however, it is likely a proxy for good parasite control, because in flocks that practice suboptimal control, benzimidazoles are unlikely to be effective (Falzon et al., 2013) and would no longer be used.

Coccidiostat use in lambs also contributed to decreased lamb mortality. This finding indicates the importance of control of endemic coccidiosis in our study flocks, which is supported by a considerable proportion of lesions caused by *Eimeria* spp. found in post-mortem reports reviewed in chapter 2.

Sheep are susceptible to diseases caused by *Clostridium* spp., and clostridial vaccination is recommended in sheep flocks (Eales et al., 1983). An association between low lamb mortality and provision of this vaccine to lambs in the present study supports this recommendation. Because most flocks used the vaccine in lambs after 6 weeks of age, as recommended (Badia et al., 2011), the advantage of vaccination on lamb mortality within the first 8 weeks might not be a direct effect of the vaccine in vaccinated lambs. The appropriate use of vaccination programs may instead indicate superior management practices. Better passive transfer from ewes might also be responsible for greater disease resistance in flocks with a history of appropriate vaccination.

One of the most common causes of lamb mortality is hypothermia (Mellor and Stafford, 2004). Some producers in the present study provided warm places for hypothermic lambs, and, therefore, these lambs were separated from their dams. This practice contributed to increased lamb mortality, which might be a result of disturbance of the mother-young relationship. In addition, this finding may be a proxy for good overall lambing practices, because not separating mother and lamb may simply reflect the aptitude of the producer for observing and intervening with milk supplementation before hypothermia became so severe as to require separation.

Lamb visual identification was another factor that contributed to decreased lamb mortality in the present study. Several practices are more easily accomplished if lambs have visual identification, such as colostrum supplementation as needed and, therefore, survivability of these lambs was higher. In addition, this factor may be a proxy for good overall flock management.

The selection biases due to missing data and non-probability sampling, mentioned in chapter 3, were also the major limitations of this chapter. Poor quality of responses to the questionnaires could lead to information bias. Both selection and information biases can be adjusted by bias analyses, however, additional data that are required to use as bias parameters were not available. Another limitation was that effects of some practices on lamb mortality in the study population could not be assessed because of no or too small variation in the data. For instance, all flocks used an indoor lambing system and, thus, the influence of an outdoor lambing system on lamb mortality was unknown.

8.3 Conclusions and future research

Lamb mortality was found to be a considerable problem in our study flocks, with mortality above the industry goal of 10% in half of the lambing groups. The practical management strategies most likely to result in meaningful improvements relate to nutritional management of the late-gestation ewes; these include testing of forage quality, and balancing gestating rations with grains, and using forages of appropriate energy and protein content. Ensiled forage should be fermented properly to avoid nutrient loss and growth of pathogens.

Fetal number is an important factor influencing nutrient requirements of late-gestation ewes; determination of fetal number is a useful practice for dividing the flock into nutritional management groups and for feeding based on requirements. Nutritional status of sheep can be assessed by body condition scoring, which should be maintained between 2.5 and 3.5 during the last trimester of pregnancy. The Precision Xtra© meter can also be used to evaluate the energy balance of late-gestation ewes and initiate management interventions before clinical ketosis is encountered. Intestinal helminth infection, which was a widespread problem in our study flocks, disturbs nutrient availability and absorption, so parasite control strategies have a place in managing lamb survival.

Based on late-gestation ewe serum samples and post-mortem lamb liver samples, selenium and vitamin E deficiencies were common in PEI sheep flocks, although injectable selenium and vitamin E products were administered in many flocks. These findings indicate the importance of continuous dietary selenium and vitamin E supplemention in sheep flocks in this region. Colostrum is an important source of nutrients and disease resistance for lambs, including selenium and vitamin E, thus attention should be paid to quality and quantity of colostrum intake.

In order to achieve acceptable lamb survival, use of clostridial vaccines appears to be important, sick animals should be treated promptly, and advice from veterinarians should be sought. Hypothermic lambs should be assisted promptly and properly to avoid possible adverse effects attributed to prolonged separation from the dam, such as mismothering. Visual identification of lambs simplifies lamb management practices, and setting performance targets for flocks is a helpful approach to improve flock performance, which can consequently decrease lamb mortality.

As infection was a common cause of lamb mortality in PEI sheep flocks, based on a retrospective study of 10-year of records of dead lambs submitted to the Atlantic Veterinary College Diagnostic Services, University of PEI, appropriate practices to improve flock hygienic and immune statuses should be taken into consideration. However, many of the interventions suggested above will tend to improve resistance to pathogens.

In spite of the limitations in the present study, our findings suggest several points in sheep flock management that can be modified to improve lamb survival, as well as several aspects deserving further investigation. For instance, disease resistance and common pathogens causing lamb mortality in PEI require further exploration to minimize losses from infection, which was the most frequent cause in post-mortem examined lambs. Due to widespread selenium and vitamin E deficiencies in PEI sheep flocks, further research is needed to determine appropriate timing and dosage to deliver injectable and oral selenium and vitamin E supplementation. Given the positive relationships between lamb liver selenium and vitamin E concentrations, and group mean time between supplementation and death, further studies to assess protective effects of high liver selenium and vitamin E concentrations on early neonatal mortality should be considered. Identifying factors associated with live lamb liver selenium and vitamin E concentrations is another possible study to confirm our findings in dead lambs. Additionally, based on the association between use of benzimidazole and low lamb mortality, the antihelminthic efficacy of benzimidazole in PEI sheep populations should be further evaluated.

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APPENDIX A

Observational Study First Flock Visit

Questionnaire

To be filled out during interview of principal manager during first visit

PEI Tax Exempt ______________ **Farmer Name: _______________________**

Time of visit: ______________ **Date of visit: ____________**

LSD_____________

Please answer the following questions as it relates to your farm and flock for the 3 past years

Part 1 Characteristic of principal manager

1. Gender of principal manager
   □ Male □ Female
2. Age of principal manager <20 20-30 30-40 40-50 50-60 >60
3. Formal Education of principal manager
   □ Primary education □ Secondary education □ Post-secondary education
4. Degree or Diploma in Agriculture?
   □ Yes □ No
5. Experience in sheep farming (years)? <1 1-2 3-5 5-10 >10
6. Is farming income derived from another agricultural enterprise?
   □ Yes □ No
7. Do you hire any workers to assist with the sheep operation?
   □ Yes □ No If yes, FTE __________________

Part 2 Flock Characteristic

1. Ewes
   1.1 Number
   ____

   1.2 Breed(s)
   ____
2. Rams
   2.1 Number
   ____

   2.2 Breed(s)
   ____
3. Purpose of flock (check all that apply)
   □ Meat □ Fleece □ Milk
   □ Breeding stock □ Other ______
4. How many separate lambing periods did you have last year?
   ____
5. Month(s) in which your lambing period(s) began?
   ____

Part 3 Housing and waste management

1. Where do you feed your ewes?
   □ On pasture/drylot and barn □ Only barn
2. What kind of material is used as flooring?
   2.1 Where the ewes lamb ________________________
   2.2 In lambing jugs/claiming pens________________________
   2.3 After ewes/lambs come out of the jugs________________________
3. Do you clean and/or disinfect lambing area between lambing groups?
   □ Yes □ No If yes,
   3.1 Cleaning method
   □ Bedding added to manure pack □ Manure pack removal only
   □ Manure pack removal and addition of a disinfectant
   □ Manure pack removal and high pressure washing
   □ Manure pack removal and high pressure washing with a disinfectant
   3.2 Disinfectant
   ____
4. What kind of ventilation system is used in your barn?
   □ Only passive or natural ventilation
   □ Assisted with mechanical ventilation (e.g. fan)
5. Can ewes and lambs access to outdoors during the indoor period?
   □ Yes □ No
6. How often is manure cleaned from the pens, either partially or entire bedding pack removed?
   ____

Part 4 Feed and water management of ewes during the 6 weeks before expected lambing

1. What kind of roughage is used for late-gestation ewes?
   □ Grazing □ Hay □ Haylage □ Silage
2. How many times late-gestation ewes are fed roughage per day?
   □ 1 □ 2 □ >2 □ Free choice
3. What kind of grain ration is used for late-gestation ewes?
   □ Commercial complete feed □ Farm-grown grain
   □ Farm-grown grain with supplement pellet □ Other ______________
4. Do you feed a protein supplement to the late-gestation ewes anytime up to 6 weeks before lambing?
   □ Yes □ No
   If yes, amount per ewe per day ______________________________ lb
   Do you know if it contains bypass protein (e.g. extruded soybeans, roasted soybeans, corn gluten meal, fish meal)?
   □ Yes □ No □ Don't know
5. Allowance of grains fed to late-gestation ewes?

Weeks before lambing	Amount (lb/ewe/day)
6	
5	
4	
3	
2	
1	

0	

6. Do you adjust late-gestation ewe ration according to number of fetuses?
   □ Yes □ No □ Do not determine fetal number
7. Do you group late-gestation ewes and feed them according to body condition score?
   □ Yes □ No
8. What salt and/or mineral are offered to the sheep during 6 weeks before lambing (check all that apply)? And how are they offered?
   □ White salt block (no mineral added) ___________________________________
   Amount ____
   □ Blue salt block (cobalt and iodine added) _______________________________
   Amount ____
   □ Pink salt block (trace mineral added) __________________________________
   Amount ____
   □ Sheep mineral (loose)
   □ Free choice □ Fed daily □ Fed weekly
   □ Other ___________________________Amount _______________________
   □ Other livestock mineral
   □ Free choice □ Fed daily □ Fed weekly
   □ Other ___________________________Amount _______________________
   □ Nothing
9. Do you use rotational grazing for ewes during pregnancy?
   □ Yes □ No □ Do not graze pregnant ewes
10. How is water provided to late-gestation ewes?
   □ Bucket □ Water trough
   □ Nipple or other automated watering system
11. How often do you clean water containers?
   □ Once a day □ Once a week □ More than once a week
   □ Other __________________________________

Part 5 Health management of flock

1. Vaccination (diseases/time)
   1.1 Ewes ____
   1.2 Rams ____
   1.3 Lambs ____
2. Selenium and vitamin E injectable supplement (dosage/time)
   2.1 Ewes____
   2.2 Rams____
   2.3 Lambs ____
3. Other supplements in feed, dosed orally or injected (e.g. vitamin A, D, coccidiostat e.g. Rumensin or Bovatec, antibiotic) (product/route/dosage/time)
   3.1 Ewes ____
   3.2 Ewes during perinatal period ____
   3.3 Rams ____
   3.4 Newborn lambs ____
   3.5 Lambs ____
4. Deworming (drug/dosage/time/reason for deworming)
   4.1 Ewes ____
   4.2 Rams ____
   4.3 Lambs ____
   4.4 Do you suspect you have resistance to dewormers?
   □ Yes □ No
   4.4.1 If yes, which one(s)?
   ____

4.4.2 Why do you think this?
____

5. External parasite control (e.g. ticks and keds) (product/time)
   5.1 Ewes ____
   5.2 Rams ____
   5.3 Lambs ____
6. Routine hoof trimming (time)
   6.1 Ewes ____
7. Footrot preventions?
   7.1 Ewes ____
8. What health problems are common in your ewes?
   □ Pneumonia □ Wasting
   □ Parasitism due to gastrointestinal nematodes □ Neurological diseases
   □ Mastitis □ Other ________________
9. What health problems are common in your lambs?
   □ Pneumonia □ Diarrhea
   □ Parasitism due to gastrointestinal nematodes □ Coccidia
   □ Neurological diseases □ Other ________________
10. What do you most often do with sick animals?
   □ Call veterinarian □ Treat on your own
11. Do you keep records for individual ewes each year?
   □ Yes □ No □ Only lambing-time records
12. Do you analyze performance of your flock (e.g. "number of lambs weaned per ewe per year?")?
   □ Yes □ No
13. Do you set goals for performance of your flock?
   □ Yes □ No
14. Do you have sheep losses from predator attacks in the last 3 years?
   □ Yes □ No
   14.1 If yes, what kind of predator(s) were responsible for the attacks?
   □ Coyote □ Dog
   □ Fox □ Eagle □ Other ________________
   14.2 What measures do you use to mitigate predator attacks? (check all that apply)
   □ Guardian animals □ Fencing □ Hunting/trapping
   □ Other ___________________________
15. Culling ewes (In the last 3 years)
   15.1 Proportion of the ewe flock (%) ___________________ per year (Average)
   15.2 Common reasons (Check all that apply)
   □ Lamb performance/production □ Teat/udder problem
   □ Reproductive problem (e.g. prolapse) □ Infertility/failure to get pregnant
   □ Unacceptable habit (e.g. reject their lambs, difficult to handle)
   □ Genetic defects □ Old age
   □ Physical problem (e.g. lambing difficulty, poor conformation)
   □ Diseases (specify) ______________________________
16. Ewes died on farm (In the last 3 years)
   16.1 Proportion of the ewe flock (%)____________________per year (Average)
   16.2 Causes of death____

Part 6 Biosecurity

1. Have you introduced new animals from other farms in the last 3 years?
   □ Yes □ No □ Just rams
2. If you have, do you check the health status of the flock prior to purchase?
   □ Yes □ No

2.1 If yes, what diseases do you concern?
____

3. Do you isolate purchased animals from other sheep?
□ Yes □ No
3.1 If yes, how long? _________________________________
4. Are visitors allowed to enter the barn where the sheep are?
□ Yes □ No
4.1 If yes, please describe how this is done
____
5. Do you usually isolate sick sheep from the flock?
□ Every case □ No □ Depend on the reason for the illness
6. Do you remove placenta from the lambing area after each birth?
□ Yes □ No
7. Do you share pasture with other farms at any time during the grazing season (both at the same time and different times)?
□ Yes □ No
7.1 If yes, what species? ____
8. What kinds of animals have regular direct contact with your ewes?
□ None □ Dog □ Cat
□ Rodent □ Cattle □ Goats
□ Other ________________________

Part 7 Breeding and pre-partum management

1. Average age and weight of ewe lambs at first breeding
____________________________ months ______________________________ lb
2. Do you flush (improve ewes body condition score) ewes before breeding?
□ Yes □ No
3. Do you use an estrus synchronization program?
□ Yes □ No
3.1 If yes, when
□ During breeding season □ Transition period (Before normal breeding season
□ Out of breeding season
3.2 How
□ Light control □ Progesterone
□ Oral route (MGA) □ Vaginal route (CIDR)
□ Other ____________________________
4. How many ewes abort each year? (Average in the last 3 years) ___________________% of ewe flock
5. Causes of abortion as diagnosed by a veterinarian or diagnostic laboratory
____
6. Do you vaccinate or give any medicine to breeding ewes for preventing abortion?
□ Yes □ No
Detail (disease/time)____
7. How many ewes bred in this lambing season? _________________________________
8. How many ewes bred in next lambing season in the same year? ___________________
9. How many ewe lambs bred in this lambing season? _____________________________
10. How many ewe lambs bred in next lambing season in the same year? _______________
11. Ewes:ram ratio by this breeding period
Group 1 ______________________ Group 2 ________________________
Group 3 ______________________ Group 4 ________________________
12. How long are breeding ewes exposed to ram? ____________________________ days
13. Do you breed first-time lambers separately from older ewes?

□ Yes □ No

14. How many groups do you manage of gestating ewes? (describe)

____

____

15. Do you perform pregnancy diagnosis?

□ Yes □ No

15.1 If yes, typical pregnancy rate (Average in the last 3 years)____________ %

16. Do you determine fetal number of pregnant ewes?

□ Yes □ No

17. Do you shear ewes before lambing?

□ Yes □ No

17.1 If no, do you crutch the ewes?

□ Yes □ No

18. Mastitis control

18.1

Observation	Visual check of udder	Manual check of udder	Check for milk/colostrum
Before breeding			
At lambing			
At weaning			

18.2 Do you use dry treatment?

□ Yes □ No

19. Do you have a separate group for ewes close to lambing?

□ Yes □ No

Part 8 Lambing and lamb management

1. Where do ewes routinely lamb?

□ Outdoors □ Indoors

2. How long do you check the ewes to lamb? (frequency and hours/day)

____

3. What are the reasons you decide to intervene in the lambing?

____

3.1 Proportion of lambings that require lambing assistance

__________________________%

4. Do you have navel dipping practice?

□ Yes □ No □ Sometime

4.1 Do you use fresh dip on each lamb?

□ Yes □ No

4.2 Which disinfectant is used for navel dipping?

□ Iodine ________% □ Chlorhexidine ________%

5. Do you weigh lambs at birth?

□ Yes □ No

6. If a lamb is slow to begin breathing what do you do?

□ Rubbing □ Other______________ □ Nothing

7. If a lamb is not suckling what do you do?

□ Assist □ Leave alone

7.1 How long do you leave a lamb until assistance is given? ________ (minutes / hours)

8. Do you supplement colostrum to lambs?

□ Yes □ No

8.1 If yes, source of colostrum used (Please rank these items)

□ Mother □ Other sheep
□ Cow □ Commercial

8.2 How do you store colostrum?

□ Fridge Maximum time stored ___________________________________
□ Freezer Maximum time stored ___________________________________

8.3 How do you thaw colostrum?

□ Bench □ Fridge □ Microwave
□ Water bath Temperature / time _____________________________________
□ Other ____

8.4 How much colostrum do you feed at the first feeding?
_____________________________________ (ml / cc / oz)

8.5 How do you measure amount of the first fed colostrum?
____

8.6 How many lambs need colostrum supplementation?
_____________________________________%

9. For lambs that are artificially reared, how is colostrum/milk fed? (Check all that apply)
□ Bottle feeding □ Tube feeding □ Automatic milk feeder

10. Do you routinely cross-foster lambs?
□ Yes □ No
If yes, how do you cross-foster? (Check all that apply)
□ Use an odour mask on the lambs, such as placenta, skin of death lamb, commercial product
□ Restrain the ewes □ Hog tie lamb's legs
□ Other ____

11. Do you measure rectal temperature of lambs that appear weak or empty?
□ Yes □ No

12. For lambs that are identified by you as being chilled, how do you routinely warm them?
____

13. For lambs that are identified by you as being weak, starved, do you routinely perform any of the following practices? (Check all that apply)
□ Supplement with a bottle of milk □ Tube with warm colostrum
□ Tube with dextrose solution □ Inject a dextrose solution into the abdomen

14. When do you first offer creep feed to lambs (age in weeks)?
____

15. Describe your creep feeding program including type of creep feed and how fed?
____

16. Do you apply a type of visual identification to newborn lambs?
□ Yes □ No
If yes, when age (hours)____
If yes, what type of identification is used? ___________________________________

17. Do you use RFID tags on your lambs
□ Yes □ No

18. Do you tail-dock your lambs?
□ Yes □ No □ Not ram lambs for market
If yes, when (age days)/how ____

19. Do you routinely castrate your lambs?
□ Yes □ No
If yes, when (age days)/how ____

20. How long do you check on health of ewes and lambs daily? (frequency and hours/day)_____________________________________ hours

21. How long do ewes and lambs stay in lambing cubicles/claiming pens? (number of days or range) ____

22. At what age are lambs are typically weaned?
_____________________________________ weeks

23. How do you decide exactly when to wean?
 □ Weight __________________ lb □ Age ___________________ weeks
 □ Pasture availability □ Other ________________________

Part 9 Ram management

1. Do you share your ram with other farms?
 □ Yes □ No
2. Do you get a vet to perform breeding soundness examination before breeding season (e.g. visual inspection of testicles and prepuce, palpation and measurement of testicles and semen collection)?
 □ Yes □ No
3. Do you increase energy in the diet to rams before breeding season to gain their body condition?
 □ Yes □ No
4. Do you use a marking method for rams breeding ewes?
 □ Yes □ No
5. Which mating system do you use?
 □ Single-sire □ Group
6. How often do you rotate rams between pens during breeding season?
 ____

"Thank you for taking the time to complete this questionnaire"

APPENDIX B

Observational Study First Visit

Record

To be filled out through observation by investigator during first visit

PEI Tax Exempt:_____________ **Farmer Name:________________________**
Time of visit: _____________ **Date of visit: _________________________**
LSD____________

11. Stocking density in pens: Low / Medium / High
12. Grain trough linear space per ewe (pace out, in meters):____________________
13. Grain trough management
    13.1 Trough at time of visit: Full / Part-full / Empty
    13.2 Cleaned or spoiled feed: Cleaned/ Spoiled
14. Forages ***Forage1*** ***Forage2***
    14.1 Type(s):
    14.2 Stage of maturity 1 / 2 / 3 1 / 2 / 3
    14.3 Leaf content 1 / 2 / 3 1 / 2 / 3
    14.4 Legume content: (%) ____________ ____________
    14.5 Stem texture? 1 / 2 / 3 1 / 2 / 3
    14.6 Weed, mold, soil, pest (Y/N, specify): Y / N _______ Y / N _______
    14.7 Length of cut (cm, or not chopped): ____________ ____________
    14.8 Forage odor (acid, ammonia, vinegar, alcohol): Y / N _____ Y / N _____
    14.9 Estimate forage access: 100% / 75% / 50% / 25% / <25% of the day
15. Grain / concentrate quality (spoiled, abnormal color, foreign matter)
    Low / Medium / High
    15.1 Specify any problems: ____________________
16. Water quality (odor, color, contamination):
    Low / Medium / High
    16.1 Specify any problems: ____________________
17. Bedding quality, cleanliness (moisture, odor, ammonia, contamination, mold)
    Low / Medium / High
    17.1 Specify any problems: ____________________
18. Lambing facilities and practices:
    18.1 Overall shelter sufficient / insufficient / not present
    18.2 Lambing jugs sufficient / insufficient / not present
    18.3 Bedding of maternity areas: sufficient / insufficient / not present
    18.4 Dryness dry / wet / very wet
    18.5 Labour units to assist with lambing (specify full time equivalents) __________FTE
    18.6 Lambing supplies sufficient / insufficient / not present
19. Other comments
    ____

APPENDIX C

Observational Study Second Flock Visit

Questionnaire and Record

Farmer Name: _____________________ Date of visit: ____________________

1. What kind of forage is used for lactating ewes?
   □ Grazing □ Hay □ Haylage □ Silage
   1.1 Different forage than before lambing?
   □ Yes □ No (if so, test)
2. How many times lactating ewes are fed roughage per day?
   □ 1 □ 2 □ >2
   □ Free choice
3. a. What kind of grain ration is used for lactating ewes?
   □ Commercial complete feed □ Farm-grown grain
   □ Farm-grown grain with supplement pellet □ Other ____________________
   b. Allowance of grains fed to lactating ewes?

Group	Describe group	Amount (lb/ewe/day)
Group 1		
Group 2		
Group 3		
Group 4		

4. Do you offered salt and/or mineral to lactating ewes?
   □ Yes □ No
   4.1 If yes, how are they offered?
   □ Free choice □ Fed daily
   □ Fed weekly □ Other _________________
   Type of mineral ____
5. How many lambs need colostrum supplementation?
   ____
6. How many lambs have had to cross-foster?
   ____
7. What kind of creep feed is used for lambs?
   ____
8. How many times lambs are fed per day?
   □ 1 □ 2 □ >2
   □ Free choice
9. Do you offered salt and/or mineral to lambs?
   □ Yes □ No
   9.1 If yes, how are they offered?
   □ Free choice □ Fed daily
   □ Fed weekly □ Other _________________
   Type of mineral ____

10. Do you give a pain killer to ewes with dystocia?
□ Yes □ No
10.1 If yes, what kind of pain killer is used?
____

11. Have you been recording births?
□ Yes □ No

12. Have you been weighing all lambs within 24 hours?
□ Yes □ No

13. Have you been recording illnesses and treatments?
□ Yes □ No

14. Have all dead lambs been submitted to AVC?
□ Yes □ No

15. Grain / concentrate quality (spoiled, abnormal color, foreign matter)
Low / Medium / High
15.1 Specify any problems ____________________________________

16. Water quality (odor, color, contamination)
Low / Medium / High
16.1 Specify any problems ____________________________________

17. Bedding quality, cleanliness (moisture, odor, ammonia, contamination, mold)
Low / Medium / High
17.1 Specify any problems ____________________________________

18. Lambing facilities and practices:
18.1 Overall shelter sufficient / insufficient / not present
18.2 Lambing jugs sufficient / insufficient / not present
18.3 Bedding of maternity areas: sufficient / insufficient / not present
18.4 Dryness dry / wet / very wet
18.5 Labour units to assist with lambing (specify full time equivalents)
____________ FTE
18.6 Lambing supplies sufficient / insufficient / not present