Colostrum feeding and calf welfare assessment

the first steps to a better future

John Barry

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Thesis

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ABSTRACT

Dairy calf welfare is influenced by a wide range of factors. Following removal of the European milk quota system in 2015, Irish dairy herds have expanded in an effort to increase production and profitability. This expansion coupled with pre-existing standards of colostrum management, previously described as sub-optimum, could negatively impact on calf welfare, and particularly so given the short calving period applied among seasonal calving systems which operate in Ireland. The aim of this thesis was to evaluate the effect of colostrum quality and management on calf health and immunity, but also to assess calf welfare on commercial farms and identify improvement options. The effect of feeding single dam and pooled colostrum on calf passive immunity was assessed in a controlled experiment. The results showed that calves which received pooled colostrum had reduced absorption efficiency for IgG as well as lower 24 h serum IgG concentrations, compared to calves fed single dam colostrum. Having developed a welfare assessment protocol for dairy calves, which was feasible and reliable, this was then applied on commercial dairy farms. Findings indicate colostrum management on Irish dairy farms is of a high standard, with over 90 % of calves receiving adequate transfer of passive immunity. While mean calf serum IgG concentration was higher among smaller size herds, the mean calf serum IgG concentration across all herd sizes exceeded the threshold (>10 mg/ml IgG) for adequate passive immunity. Colostrum quality in Irish dairy herds was good; however large variation exists, both within and between herds. Based on collected data, associations between pre-weaning calf management and calf welfare and mortality on commercial dairy farms were investigated. Calf mortality was not associated with either herd size, space allowance per calf or post-colostrum feeding practices. Higher calf mortality was identified among herds which reported experiencing an on-set of calf pneumonia during weeks 8-10 of the calving season. Behavioural observations indicate that smaller group sizes could promote expression of positive behaviours, potentially resulting from an overall improvement in welfare. Space allowance per calf was not associated with observed behaviour frequencies. It was also identified that calf mortality was not associated with herd size. Improvements in the area of colostrum management, as described in this thesis, combined with regular assessment of calf welfare, using reliable protocols, can provide the future basis for enhanced calf welfare on commercial dairy farm.

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Chapter 1

General Introduction

Historically, agriculture and the systems of production employed have evolved in line with technological developments, all the while increasing levels of production and efficiency. The dairy sector is no different, and while many adaptations have successfully enhanced productivity, some have also had negative implications for animal welfare. Genetic selection for high production has been shown to result in cows having lower body condition scores, and lower reproductive performance, while also experiencing increased rates of metabolic disorders and reduced immune function in early lactation (Dillon et al., 2006, Roche et al., 2009). In 2015, the European milk quota systems which restricted production levels of European dairy farms for over 30 years was removed. As a result dairy farmers are increasing milk production levels by expanding their herds with the aim of enhancing the profitability of their system. Increasing herd size can also create welfare issues, for example, if a low stockperson-to-animal ratio is maintained this reduces time for individual care (Bewley et al., 2001). Another herd size related risk factor to welfare occurs when animals have to walk long distances to and from paddocks for milking, which increases the risk of lameness (Arkins, 1981). In Ireland removal of the milk quota system also has resulted in substantial expansion among Irish dairy herds (Zeng et al., 2017). This focus on herd expansion and achieving higher production levels could further increase animal welfare issues experienced within the dairy sector.

Simultaneously to developments in the dairy sector, there has been a considerable change in consumer attitudes towards animal production and animal welfare (Verbeke, 2009). This became apparent in a study which investigated attitudes of European citizens towards animal welfare (European Commission, 2016). Within this study it was identified that over 90 % of Europeans believe protecting animal welfare is important, 82 % believe welfare of farmed animals should be better protected than it is currently, and 64 % state that they would like more information about conditions under which farmed animals are treated in their respective countries. A particular point of interest from this study was that concerns for animal welfare were identified as being highest among Irish consumers. Combined, the increase in production at farm level and heightened concerns among consumers towards animal welfare has created a need for the development and application of mechanisms to assess and monitor animal welfare. Information gathered using such mechanisms could then be provided to consumers and all relevant stakeholders on developments surrounding farm animal welfare. Before describing the steps taken to assess and improve dairy calf welfare, the topic of this

thesis, it is first important to address recent developments surrounding management of calves on commercial dairy farms and the implications for calf welfare.

Dairy calf management in Ireland; recent developments

In Ireland, temperate climate conditions and fertile soils create ideal conditions for producing large quantities of herbage (Shalloo et al., 2004). To capitalise on the availability of this low cost feed (Finneran et al., 2012), the majority of Irish dairy farmers operate seasonal calving, pasture-based systems of production. These systems aim to synchronise herd feed demands with grass growth (Horan et al., 2004) by achieving a compact calving pattern at the on-set of grass growth (February-April) (Berry et al., 2013). This requires a target calving spread of twelve weeks to maximise financial performance (Ramsbottom et al., 2015). The nature of seasonal calving results in large numbers of calves being born in a short period of time, which can create issues with labour requirements. Recent reports have shown that in Ireland six week calving rate of the national herd has increased by over 10 % in the last nine years (ICBF, 2019), resulting in a more compact calving pattern, which could further compound any existing issues surrounding calf management. This combined with increased herd sizes would result in a greater number of calves being born in a short period of time and an even greater pressure regarding labour. Observation, and assistance when required, at calving (Mee, 2008), colostrum collection, prompt feeding of the new-born calf, navel sepsis and regular health checks (Lorenz et al., 2011) are just a number of management practices required at birth and during the first hours and days of life for neonatal calves. Where protocols are not in place, or sufficient labour is unavailable to deal with a large volume of young calves, this could negatively impact calf welfare.

Traditionally, Irish dairy farms are family operated enterprises, dependent on family labour (O'Donnell et al., 2008, Chen and Holden, 2017). Recently the Irish dairy industry has changed significantly and the average dairy herd has increased from 45 cows in 2005 to 76 cows in 2016 (Dillon, 2017). Such increases in herd size, however, do result in increased labour requirements and ultimately a shift away from the traditional family labour model. These developments could have trade-offs as increases in herd size, for example, are associated with increased calf mortality (Gulliksen et al., 2009). It has also been identified that calf mortality is considerably lower when the owners care for the animals within a farm

system (Martin et al., 1975). Prior to removal of the quota system, there was evidence of potential calf welfare issues on commercial farms, such as higher mortality among male calves (AIM, 2014, 2015), insufficient disease control management (Kennedy et al., 2014), and sub-standard colostrum management (Cummins et al., 2016). These issues could potentially be further compounded by herd expansion if correct measures are not applied. To protect and improve welfare of dairy calves, a clear understanding of influencing factors is first required.

Dairy calf welfare

Although the exact definition of welfare continues to be debated by experts in different fields (e.g., ethologists and veterinarians), it is widely accepted that welfare refers to physiological state, affective state (the experience of feeling or emotion) and the ability to perform natural behaviours (Fraser et al., 1997). Dairy calves are physically and immunological weak immediately after birth, making them particularly vulnerable to welfare issues in early life. Such issues can arise from a wide range of factors, some of which include colostrum management (Conneely et al., 2013), housing conditions (Vasseur et al., 2010), hygiene practices (Klein-Jöbstl et al., 2014), health treatments (Jorgensen et al., 2017), post-colostrum feeding, weaning and transport (Weary et al., 2008, Godden, 2009). While numerous factors can affect calf welfare, one of the most critical is the management and feeding of colostrum immediately after birth, which is one of the main focus points of this thesis. Colostrum is the first bovine mammary secretion produced post-parturition. The importance of colostrum management to calf health arises as no immune transfer occurs between cow and calf *in-utero*. The calf must instead acquire immunity passively after birth from colostrum, which contains an abundance of immunoglobulins (Ig), and particularly IgG (Furman-Fratczak et al., 2011). Immunity provided through colostrum feeding can be measured by assessing calf serum IgG concentrations, and research has shown a concentration of >10 mg/ml at 24 hours of age is associated with lower rates of morbidity and mortality, as well as improved performance (Donovan et al., 1986, Robison et al., 1988, Wells et al., 1996). Achieving a calf serum IgG concentration of >10 mg/ml at 24 hours is referred to as adequate transfer of passive immunity. Attaining this desired level of calf immunity is influenced by a number of

factors, such as timing of colostrum ingestion (Stott, 1979, Morin et al., 1997), IgG content and the volume of colostrum ingested (Besser et al., 1991).

Colostrum and calf immunity

Research has shown that colostrum IgG concentrations can vary among herds and countries. An Australian study found that approximately half of colostrum samples collected from dairy farms contained less than 50 mg/ml IgG, which is the threshold for good quality colostrum (Phipps et al., 2016, Abuelo et al., 2019), while a study in North-America found that approximately 30 % of colostrum samples (n=827) had IgG concentrations below the quality threshold (Morrill et al., 2012). In Ireland, however, there is evidence to suggest colostrum quality is particularly high, with Conneely et al. (2013) reporting a mean IgG concentration of 112 mg/ml from 704 cows, and just 4 % of samples containing < 50 mg/ml IgG. This could potentially be due to the relatively low production levels per cow in Ireland, compared with production levels per cow in North America, as (colostrum) yield is negatively associated with IgG concentration (Baumrucker et al., 2010, Conneely et al., 2013). High colostrum IgG concentrations can result in elevated levels of immunity among calves, however, it could also negatively impact vaccine efficacy (Ellis et al., 2001). Most Igs have a half-life of between 16 and 28 days and depletion rates vary based on the animal, but also the level of passive immunity achieved (Chase et al., 2008). Studies have found that no increase in vaccine derived antibody titres occurs when vaccines are administered in the presence of maternally derived antibodies, to the same disease (Ellis et al., 1996, Kirkpatrick et al., 2001). Previous studies which examined maternally derived antibody depletion in calves have focused on specific diseases (Howard et al., 1992, Taylor et al., 1995). Fulton et al. (2004) examined the depletion rate of a number of viral diseases in calves, however this study used beef calves, and the exact age of calves was unknown. Limited information is therefore available on the exact timing of antibody depletion (i.e., half-life) for a wide range of diseases in dairy heifer calves.

A knowledge gap also exists in the depletion rate of maternally derived antibodies in situations where high quality colostrum (> 100mg/ml IgG) is available, as is the case in Ireland (Conneely et al., 2013). By identifying the rate of maternal antibody depletion in dairy heifers, when high quality colostrum is provided, future protocols for vaccination and potential stress inducing practices (e.g. dis-budding, transport, turn out to pasture) can be revised and improved to prevent infectious disease. To bring about better welfare,

information on the current welfare status of calves, which includes physiological health information, is required. This information could be used as a benchmark for future studies, but also to evaluate welfare related technological innovations, when applied at farm level.

Assessing dairy calf welfare on commercial farms

Information regarding calf welfare, including physiological health information, on commercial Irish dairy farms is limited. Recent management data, however, suggest there are factors present which could negatively affect calf welfare. Cummins et al. (2016), assessed colostrum management practices through an interview with Irish dairy farmers, concluded that colostrum management practices were sub-optimal. Such practices included 66 % of farmers allowing calves to receive colostrum by suckling the dam, while 29 % described storing colostrum at room temperature for < 4 days. Both of these practices are associated with increased risk of failed transfer of passive immunity occurring (Weaver et al., 2000). Following the recent phase of expansion among Irish dairy herds, standards of colostrum management practices could have declined further. Alternatively, colostrum management practices may have been adapted in an effort to reduce labour requirements associated with feeding greater numbers of new-born calves. For example, pooled colostrum feeding (i.e. combining colostrum from a number of cows which is then fed to new-born calves) or allowing calves to drink directly from the cow could become more widely practiced, and the effects of such management adaptations on calf welfare warrant investigation. Although cryptosporidium is known to be one of the most common causes of enteritis among Irish calves (DAFM, 2018), and its occurrence can be associated with sub-optimum hygiene standards (Barrington et al., 2002, Delafosse et al., 2015), limited information is currently available on calf related hygiene practices on Irish dairy farms. Calf related hygiene practices on commercial dairy farms could potentially be having a negative impact on calf welfare, and a knowledge gap thereon currently exists; investigation on this subject is therefore warranted. The impact of hygiene practices on calf welfare must be investigated by assessing physiological health, but also by assessing affective state, through for example behaviour observations, which can provide valuable information when establishing welfare status.

To gain an insight into calf welfare, methods and protocols must be developed to accurately assess the welfare of calves on commercial dairy farms. For farm animals, different methods

of assessing welfare have been developed and investigated. The first protocols developed for on-farm application mainly used resource-based measurements. Resource-based measurements focus on management practices and housing conditions to assess animal welfare (Bartussek, 1999), and do not entail any physical or visual assessment of the animal. Resultantly, an accurate evaluation of welfare is not possible, nor is it possible to differentiate welfare of animals between herds when they are subject to similar management or housing conditions (Mullan et al., 2009, Phythian et al., 2011). More recent protocols, such as those developed as part of the Welfare Quality® project, however, tend to use animal-based measurements. Animal-based measurements involve direct assessment of the animal using physiological measurements (e.g., temperature, respiration rate etc.), visual assessment (e.g., demeanour, cleanliness, mobility etc.), as well as behaviour observations. While animal-based measurements provided comprehensive information when determining welfare status, such measurements can have a large time and financial requirement.

Outside of the Welfare Quality® project, methods to simplify the collection of welfare related information have been investigated, and include the use of routinely collected data, such as information on production and reproductive performance to predict welfare of dairy cattle (De Vries et al., 2014). Use of technological applications has also been investigated, and included use of sensors to measure activity or rumination (Lopreiato et al., 2018), infrared thermography for detecting mastitis (Colak et al., 2008) and hoof lesions (Alsaad and Büscher, 2012), but also the use of 3D imagery to monitor body weight (Song et al., 2018) or body condition (Song et al., 2019).

While the use of routinely collected farm data and technological applications have merit, they tend to focus on a limit number of areas and, so far, often fail to provide a complete assessment of welfare. The most feasible and all-encompassing method of welfare assessment therefore remains on-farm human application of animal-based measurements. This information can then be assessed together with routinely collected farm data, using for example morbidity and mortality information to identify potential risk factors to welfare.

While assessment protocols for several farm animals were produced during the Welfare Quality® project, a protocol for dairy calves has not yet been developed. The existing protocols for dairy calves tend to focus on female calves, using indicators from a single-category (animal, environment or management-based indicators) and have been developed for year round calving systems (Whay et al. 2003; Vasseur et al. 2012). Excluding male dairy

calves from welfare assessments is unjustified as it ignores the fact that, for example in Ireland, male dairy calf mortality rates are between 30-40 % higher than that found in female calves (AIM, 2013, 14, 15). While high calf mortality rates are not directly related to welfare, it does reaffirm the vulnerability surrounding calves. Limited information is available to differentiate between the management and welfare of male and female calves. Seasonal calving systems, which predominate in Ireland, have large numbers of calves being born in a short period of time. This results in challenges for both calf management and calf welfare which would not be experienced in year round calving systems. Such challenges can include ensuring each calf receives colostrum immediately after birth, providing sufficient space allowance per calf, but also maintaining good hygiene standards in pens where cows are calving as well as pens where calves are accommodated in groups. The contrast in calf issues which may be experienced in seasonal versus year round calving systems necessitates the need for welfare assessment protocol specific to each system type. Currently there is no protocol available which has been designed to assess calf welfare in seasonal calving systems, and such a protocol would provide an insight into current welfare standards among calves on Irish dairy farms. To protect and improve calf welfare in seasonal calving systems, a welfare assessment protocol designed specifically for pre-weaned male and female calves in seasonal calving herds is required.

Figure 1. Summary chart of the research process applied for this thesis

General aim and outline of the thesis

The general aim of this thesis was to i) evaluate the effect of colostrum quality and management on calf health and immunity, and ii) to assess calf welfare on commercial farms and identify improvement options.

The outline of the thesis is visualized in Figure 1. In chapter 2, the depletion rate of disease specific maternally derived antibodies in dairy heifer calves is studied, and the effect of feeding colostrum pooled from a number of cows versus colostrum from a single cow. In chapter 3, a welfare assessment protocol is developed for dairy calves using animal-based indicators, but that also includes resource based indicators (i.e., management and environmental conditions) to identify risk factors. The protocol differentiates between welfare and management of male and female calves and was developed for seasonal calving

systems and would allow for risk factor identification. Having developed the protocol it was then applied on-commercial dairy farms during the calving season (Feb-April). Based on collected information, chapter 4 evaluates associations between colostrum management, calf related hygiene practices and rates of mortality in pre-weaned dairy calves. In chapter 5, pre-weaning management of calves is investigated, and its association with calf welfare and mortality on commercial dairy farms is assessed. In chapter 6 results from the previous four chapters are discussed in relation to each other and wider implications of these studies for science and the dairy sector are postulated.

Chapter 2

Effect of feeding single dam or pooled colostrum on maternally derived immunity in dairy calves

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ABSTRACT

The role of colostrum management in providing adequate immunological protection to neonatal calves has been widely investigated. Thresholds for colostrum quality, as well as optimum volume and timing for colostrum feeding have been established. Limited information, however, is available on the effect of colostrum source (single dam or pooled) on passive immunity. This study aimed to assess the effect of feeding single dam colostrum (own and other dam) or pooled colostrum on transfer of passive immunity. In total, 320 cows and 119 dairy heifer calves were enrolled in the study which was conducted over a period of two years. Calves were blood sampled immediately after birth and received either own dam, other dam or pooled colostrum. Calves were blood sampled again at 24 h to assess serum immunoglobulin G (IgG) concentrations. Mean colostrum IgG concentration was higher for calves fed colostrum from another dam, whereas the IgG concentration was similar for calves fed own dam and pooled colostrum. For all these treatment groups, however, the mean IgG concentration in colostrum was > 80 mg/ml. Mean calf serum IgG concentration was 44 mg/ml, and was lower for calves fed pooled colostrum compared to those fed colostrum from their own dam or a single other dam. We also found that calves with a higher birth bodyweight (> 30 kg) had a lower serum IgG concentration at 24 h than calves with a lower birth bodyweight. We, therefore, conclude that feeding pooled colostrum can negatively impact calf immunity while continuing to present a risk of disease transmission within a herd.

Keywords: colostrum management, health, immunoglobulins, heifers, birth-weight

INTRODUCTION

Neonatal bovines are dependent on transfer of passive immunity (TPI) to provide protection from infectious diseases in early life (Godden, 2008). Passive immunity can be acquired from colostrum, the first mammary secretion post-parturition, which contains a wide range of immunological factors, such as immunoglobulins (Ig), cytokines and antimicrobials (e.g. lactoferrin and lactoperoxidase) (Stelwagen et al., 2009, McGrath et al., 2016). Immunoglobulins, also known as antibodies, are produced in the maternal bloodstream by lymphocytes in response to foreign antigens (Conneely et al., 2013) and account for over 70 % of the total protein content in colostrum (Larson, 1992). Immunoglobulins present in colostrum can be divided into three subclasses, i.e., IgG, IgA and IgM which account for 88 %, 5 % and 7 % of the total colostrum Ig content, respectively (Butler, 1983). Not only is IgG the most abundant Ig subclass in colostrum, but it also has the greatest specificity and affinity for target pathogens (Hurley, 2003). As a result, colostrum quality is determined mainly by IgG concentration, and a concentration of ≥ 50 mg/ml is considered as good quality (Weaver et al., 2000).

In calves, failure to achieve an adequate level of passive immunity is identified as a serum IgG concentration of < 10 mg/ml at 24 h old, resulting in increased susceptibility to disease as well as an increased risk of mortality (Weaver et al., 2000, Stilwell and Carvalho, 2011, Chigerwe et al., 2015). Achieving adequate TPI depends predominately on three main factors; 1) the volume of colostrum provided to the calf (Godden, 2009, Conneely et al., 2013), 2) the timing within which it is received by the calf after birth (Michanek et al., 1989, Morin et al., 1997), and 3) the quality of the colostrum (Beam et al., 2009). Conneely et al. (2014) found that providing a volume of colostrum equivalent to 8.5 % of the calves birth bodyweight achieved greater levels of TPI compared with feeding a volume equivalent to 7 % or 10 % of birth bodyweight. The ability of calves to passively absorb Igs is finite, with highest absorption capabilities existing within the first two hours of life (Weaver et al., 2000). As a result, providing colostrum within two hours of birth is recommended to ensure adequate TPI is achieved. Regardless of timing and volume, good quality colostrum is required to achieve adequate TPI. In Ireland, colostrum quality has been found to be particularly high (Conneely et al., 2013, Barry et al., 2019), which can be explained by the diet, which is predominantly grazed grass, the relatively low milk yields achieved, but also the genetic profile of the national herd (Conneely et al., 2013).

When feeding colostrum, the recommended practice is to provide each calf with colostrum from their own dam, once the colostrum is of sufficient quality (Godden, 2008). In Ireland, however, studies have found that feeding of non-maternal colostrum is commonly practiced. Over 30 % of farmers provide calves with colostrum which has been pooled from a number of cows, while almost 10 % of farms provide colostrum produced by another cow (Barry et al., 2019). Direct comparisons between passive immunity of calves fed colostrum from maternal colostrum (MC) and non-maternal colostrum (NMC) have yet to be made. Feeding pooled colostrum (PC), but also single dam colostrum, can have negative implications for TPI when colostrum quality is low (Weaver et al., 2000). There is limited information, however, identifying if these negative implications continue to apply in situations where colostrum quality is relatively high. It is unknown, for example, if pooling colostrum still elicit a reduction in colostrum quality and present a risk to TPI within a herd which has a high mean value for colostrum IgG.

By investigating the effects of feeding pooled or single dam colostrum (MC or NMC) and assessing passive immunity, improved protocols for colostrum feeding could be developed which could enhance calf immunity, ultimately contributing to improved welfare among calves.

This study aimed to assess the effect of feeding single dam colostrum (MC, NMC) or colostrum pooled for multiple cows on TPI among dairy calves.

MATERIALS AND METHOD

This study was carried out at Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland between January and October of 2016 and 2018. Ethical approval was received from the Teagasc Animal Ethics Committee (TAEC) (TAEC101/2015) and procedure authorisation was granted by the Irish Health Products Regulatory Authority (HPRA) (AE19132/P044). Experiments were undertaken in accordance with the Cruelty to Animals Act (Ireland 1876, as amended by European Communities regulations 2002 and 2005) and the European Community Directive 86/609/EC.

Aiming for 120 heifer calves, 320 cows were enrolled in the study. This was based on a power calculation and replication of the study over two years. In both January 2016 and

January 2018, 160 cows (120 multiparous, 40 primiparous, each year) were enrolled. Of these cows, 119 produced heifer calves and colostrum which was used as part of the study. The parity of these 119 cows was first (n= 46), second (n= 25), third (n= 25) and fourth or greater (n= 23).

A complete randomised block design was applied, with a total of 119 heifer calves (59 in 2016, and 60 in 2018) enrolled in the study: 88 Holstein Friesians (HF) and 31 HF × Jersey. Calves were balanced based on birth weight, breed and date of birth, and were assigned to one of the three treatments where they received i) MC, ii) NMC, or iii) PC. Pooled colostrum was prepared by combining colostrum at equal ratios from a number of cows (two cows in 2016, and from four cows in 2018). For colostrum IgG concentration, efforts were made to ensure similar quality colostrum was provided across each of the treatment groups. The indirect nature of IgG quantification by Brix refractometry, as well as the necessity to provide calves with colostrum in a timely manner, however, results in unavoidable variation in the IgG concentration of colostrum provided to the three treatment groups.

Calf management

All calving events were supervised by trained and experienced personnel. Following birth, calves were immediately separated from the dam as a standard biosecurity measure. Two identically numbered plastic ear tags were applied to each calf following birth, as required by the Department of Agriculture, Food and the Marine. Each calf was weighted (TruTest XR3000, Tru-test Limited, Auckland, New Zealand) and 10 % iodine was applied to the remnants of the umbilical cord, and the navel area. Calves were then placed in individual pens (0.8 m x 1.2 m) located indoors. Colostrum was collected either immediately post calving using a single portable milking unit, or in the parlour when calving occurred within 1 h of a scheduled milking (07:00 h or 14:30 h). Each calf received 8.5 % of its birth BW in colostrum within 2 h of birth via bottle and teat or an oesophageal tube feeder in the event the calf would not drink voluntarily. Colostrum quality was initially measured using a digital brix refractometer (Milwaukee, MA871 Refractometer; Milwaukee Electronics Kft, Alsóikikótor 11, H-6726 Szeged, Hungary) and a sample (100 ml) collected for laboratory analysis to directly determine IgG concentration (as described in *Colostrum and serum IgG analyses* section). Only colostrum with a brix value of ≥ 22 % was used (Bielmann et al., 2010). Colostrum that failed to satisfy the brix value requirement was immediately disposed of. When pooling colostrum, a sample was taken of all individual components prior to pooling,

as well as a sample once the pool had been formed. Colostrum fed to calves was sampled and stored at -20°C until analysis. Colostrum was either fed immediately or stored in refrigeration (4°C) for a maximum of 48 h or a freezer (-20°C) for use at a later date (Cummins et al., 2016a). Colostrum which had been frozen or refrigerated was defrosted/warmed using tepid water and fed immediately at a temperature of approximately 37°C, as determined by visual thermometer.

In 2016, following a single feed of colostrum all calves received milk replacer (MR) (26 % crude protein: Heiferlac Instant, Volac, Ireland) thereafter. In 2018, calves received colostrum and five feeds of transition milk from the same origin (i.e., a calf which received MC then received five feeds of transition milk from their own dam, in the case of PC, transition milk from the same cows was pooled and fed). Following the fifth feed of transition milk, calves received MR (26 % crude protein) thereafter. At four days of age, calves were transferred from their individual pen to a group pen (indoors; 7.5 × 4.7 m area with straw bedding) containing up to 15 calves. Calves from each treatment group were evenly distributed across the group pens, and once a group was created, calves remained in their allocated group until after weaning; the maximum difference in birthdate within groups was two weeks.

Automatic feeding stations (Vario Smart Powder, TAP5-VS1-50: Förster-Technik GmbH, Gerwigstrasse 25, D – 78234 Engen, Germany) were located in each group pen and provided MR according to a predesigned feeding program. Calves entering the group pen commenced an individual feeding programme, initially receiving 4 L/MR/day which increased gradually to 6 L/MR/day over a seven day period. This volume of MR per day remained constant for the duration of time taken to reach the minimum target weaning weight (95 kg for HF, 80 kg for HF × Jersey). At which point, the feeding program was modified to achieve gradual weaning over a period of 14 days. At all stages of the feeding program the daily MR allowance was delivered over three equal feeds, prepared freshly at 37°C in one litre portions at a concentration of 15 %. During the housing period fresh clean drinking water was available through drinking bowls; hay and concentrates (18 % crude protein, 10.4 % crude fibre, 6.2 % crude ash: Roches Feeds, Limerick, Ireland) were offered *ad libitum*. Post-weaning, calves were given full-time access to pasture and offered 1 kg of supplementary concentrate feed per day.

Blood sample collection

Within one hour of birth, and prior to colostrum feeding, one 10 ml blood sample was taken from each calf via the jugular vein using a plain serum tube (10 ml BD Vacutainer®, BD, Belleriver Industrial Estate, Plymouth PL6 7BP, United Kingdom). A second blood sample was taken from each calf 24 h post colostrum feeding. Blood samples were refrigerated at 4°C for 24 h prior to serum separation by centrifugation ($3,000\text{ g} \times 30\text{ min}$) at 4°C. Following centrifugation, serum samples were collected and frozen at -20°C until IgG concentration determination.

Colostrum and serum IgG analyses

Calf serum samples (0 and 24 h) and colostrum samples were defrosted at 4°C and analysed using radial immunodiffusion (RID) kits (Triple J Farms, Bellingham, Washington, USA). Each kit contained a 24 well test plate and three reference sera samples. Kits were stored in refrigeration at 4°C and removed 30 min prior to use. Colostrum samples were prepared at a 1:3 dilution with distilled water while 24 h serum samples were prepared at a 1:2 dilution using the same diluent. Zero h serum samples did not require any dilution prior to analysis, as IgG concentrations at this time point would be negligible (Stott, 1979). On each test plate, wells one to three contained the reference sera (196, 1402, 1976 mg/dl), well four was blank, and the remaining wells contained serum or colostrum samples, all of which were tested in duplicate. Plates were then incubated at room temperature (20-24°C) for 24 h. Diameters of the precipitate rings surrounding each well were measured, to the nearest 0.01 mm, using digital Vernier callipers. Ring diameter values of the three reference sera samples were squared and plotted against their known concentrations, producing a standard reference curve. This was then used to determine the IgG concentration of the serum/colostrum samples present on the plate. Samples which produced a value beyond the range of the reference curve were prepared at an increased dilution factor and reanalysed.

Apparent Efficiency of Absorption

The ratio of IgG absorbed into the neonatal circulatory system relative to the IgG mass ingested, apparent efficiency of absorption (AEA), was calculated using the following formula:

$$AEA = \frac{\text{Serum IgG(g/L)} \times \text{Plasma volume(L)}}{\text{Total IgG intake (g)}} \times 100$$

Plasma volume was calculated as follows: plasma volume = 0.089 x birth BW (kg) (Quigley et al., 1998). Total IgG intake was calculated as follows: IgG concentration of colostrum received × (Birth bodyweight × 0.085). Birth bodyweight by 0.085 is used to calculate the volume of colostrum received, as a feeding rate of 8.5 % of birth body weight was applied (Conneely et al., 2014).

Data editing

When calculating AEA, samples (colostrum and 24 h calf serum) for four calves produced a value of > 100 %. As this would not be biologically possible, and indicated an error existed in either the colostrum IgG concentration or 24 h calf serum IgG concentration value, these values (four values for colostrum IgG concentration and four serum IgG concentration values) were omitted from the data set.

Statistical analyses

Statistical analyses were conducted using SAS (version 9.4; SAS Institute Inc., Cary, NC). Normality of dependent variables was assessed using PROC UNIVARIATE through an assessment of residual skewness combined with a visual examination of the distribution pattern. All dependent variables were normally distributed. Significant associations were confirmed when $P < 0.05$; in both models, interactions were examined between significant variables and least square means assessed. Factors associated with colostrum and calf serum IgG concentration, were assessed using linear mixed models in PROC MIXED. Factors considered in the model investigating colostrum IgG included cow breed, year, and parity. For calf 24 h serum IgG concentration, factors considered in the model included colostrum feeding treatment (MC, NMC, PC), dam parity, calf breed, birth bodyweight, IgG concentration of colostrum fed and year. Birth bodyweight was included in the model as a continuous variable, but also in a separate model as a categorical variable based on quartiles of the data set. Birth month nested within year was also included in the model, and interactions between treatment and year were investigated.

RESULTS

Colostrum IgG

For PC, a preliminary analysis was conducted which examined the IgG concentration of the PC but also the mean value for IgG concentration of all individual samples used in the pool. No difference was detected between IgG concentration of PC and the mean IgG concentration of individual samples used in the pool.

Mean colostrum IgG concentration was 84.2 mg/ml, and ranged from 52.5 to 164.9 mg/ml. No interaction existed between treatment and year for colostrum IgG concentration. Treatment, year, calving month and parity were all associated ($P < 0.05$) with colostrum IgG concentration. Mean colostrum IgG concentration was higher for the NMC treatment group, while MC and PC were similar (Table 1). For all treatment groups, however, the mean IgG concentration was > 80 mg/ml, exceeding the quality threshold of 50 mg/ml. In 2016, mean colostrum IgG concentration was 71.2 mg/ml, ranging from 34.9 to 108.9 mg/ml, while in 2018, mean colostrum IgG concentration was 97.0 mg/ml, ranging from 54.8 to 164.92 mg/ml. Cows which calved in the month of March produced colostrum with the highest IgG concentration (95.1 mg/ml), compared to those which calved in January and February (87.2 and 85.5 mg/ml), which were similar. First and second parity cows produced colostrum with a lower mean IgG concentration than cows of third parity or greater. Across all parity groups, however, mean colostrum IgG concentration was > 80 mg/ml. No association was observed between cow breed ($P > 0.05$) and colostrum IgG concentration.

Table 1. Mean colostrum IgG concentration and calf serum IgG concentration at 24 h, as determined by radial immunodiffusion assay, for calves fed maternal, non-maternal and pooled colostrum. Superscript letters indicate statistically significant difference.

	Treatment			SEM	P-value
	Maternal	Non-maternal	Pooled		
Colostrum IgG	88.55 ^a	94.18 ^b	87.41 ^a	1.66	<0.01
24 h serum IgG	44.53 ^a	43.84 ^a	40.50 ^b	0.64	<0.01
AEA* (%)	55.39 ^a	54.07 ^a	51.52 ^b	0.76	<0.01

*AEA is apparent efficiency of absorption

Calf serum IgG

Mean calf serum IgG concentration was 43.7 mg/ml, and ranged from 18.7 to 105.4 mg/ml. Treatment ($P < 0.001$), year ($P < 0.001$), dam parity ($P < 0.05$), birth bodyweight ($P < 0.001$) and IgG concentration of colostrum fed ($P < 0.001$) were all associated with calf serum IgG concentration at 24 h. Calves which received PC had a lower mean 24 h serum IgG concentration than calves fed MC or NMC, which were similar (Table 1). In 2016, mean serum IgG concentration was 32.6 mg/ml, ranging from 18.7 to 59.8 mg/ml, while in 2018, mean serum IgG concentration was 58.4 mg/ml, ranging from 31.3 to 105.3 mg/ml. Serum IgG at 24 h was highest among calves born to second parity cows, however, calves born to cows across all parities had a mean serum IgG concentration of > 40 mg/ml at 24 h. For bodyweight, heavier calves had lower serum IgG concentrations at 24 h (Figure 1). Calves which received highest quality colostrum (i.e., containing > 100 mg/ml of IgG) had the highest serum IgG concentration at 24 h. When calf serum IgG concentration at 24 h was plotted against IgG concentration of colostrum received (Figure 2), this produced a regression equation of $y = 0.54x - 0.38$, with a coefficient of determination of 0.38.

Figure 1. Least square means ($\pm$ SE) for 24 h calf serum IgG concentration based on birth bodyweight (when included as categorical variable). Bars with different letters (a, b, c,) indicate a significant difference ($P < 0.05$) in means.

Figure 2. Immunoglobulin G (IgG) concentration of colostrum received by 119 dairy calves, plotted against the 24 h serum IgG concentration of the same calves, as determined by radial immunodiffusion assay.

DISCUSSION

The objective of this study was to assess the effect of feeding MC, NMC and PC on passive immunity in neonatal calves. Mean IgG concentration of colostrum used in the study was high, well exceeding the 50 mg/ml threshold for good quality, but also similar to that found during a recent study on commercial Irish dairy farms (85 mg/ml) (Barry et al., 2019). This indicates that findings from the present study could be reflective of expected outcomes for passive immunity which would be experienced on commercial dairy farms, when the same management practices are applied. Large variation existed in colostrum quality both between and within years. Based on existing literature, such variation would be expected as colostrum quality is influenced by a large number of factors including breed, parity, pre-partum nutrition, dry period length and time of colostrum collection (Godden 2008). Breed and parity of cows from which colostrum was used was similar in both years, and strict guidelines were applied for timing of colostrum collection. Pre-partum nutrition and dry period length, however, were not controlled, and could potentially be sources of variation. In addition, factors such as cow health (Dardillat et al., 1978) and mineral status (Weiss et al., 1990) can also influence colostrum quality and therefore be potential sources of variation both between and within years. Previous studies have found similar variation in colostrum quality; however, few studies have examined colostrum quality within a herd across a number of different years. The present study demonstrates that colostrum quality within a herd can vary

between years, which further emphasises the need for year on year assessment of colostrum quality, to avoid any negative implications of such variation on for example failed TPI.

A common misconception exists that colostrum produced by first parity cows is of poor quality and therefore would not be suitable for feeding. While the present study found the IgG concentration of colostrum produced by first and second parity cows was below that of cows of greater parity, it was above the 50 mg/ml threshold and therefore suitable for first feeding. This difference between parities has also been identified in previous studies (Shearer et al., 1992, Moore et al., 2005, Gulliksen et al., 2008). As is the case with any colostrum, but particularly for that of first and second parity cows, the quality should be assessed first, for example with a Brix refractometer, to determine if the colostrum is suitable for feeding (Gulliksen et al., 2008, Quigley et al., 2013).

In our study, IgG concentration was similar between MC and PC treatment groups. We, however, only pooled colostrum which produced a value of ≥ 22 % on a Brix refractometer. In situations where colostrum quality is not assessed prior to pooling and mixed at different proportions this could negatively affect overall IgG concentration (Weaver et al., 2000), and increase the risk of failed TPI occurring. A recent study by Barry et al. (2019) described how over 30 % of Irish dairy farmers reported feeding PC. In the same study it was also identified that among Irish dairy farmers fewer than 15 % actively measure colostrum quality. Results from the present study demonstrate that when strict procedures are followed (i.e., using only good quality colostrum, combining in equal proportions, storing correctly), PC can contain an IgG concentration capable of providing adequate TPI. This study, however, was conducted within a research herd where high herd health standards are achieved through comprehensive vaccination programmes, regular disease screening, prompt isolation or culling of any animals suspected of harbouring infectious disease, as well as meticulous hygiene standards. The associated risk of disease transfer within a herd when pooling colostrum, however, is extremely difficult to overcome (Godden, 2009). Pooling colostrum, therefore, should not be practiced in situations where diseases transmissible through colostrum, such as Johnes disease, are present (Nielsen et al., 2008).

The difference in calf 24 h serum IgG concentration between treatments indicate that colostrum from a single dam (MC, NMC) results in higher levels of TPI. Colostrum quality, however, for the PC treatment group was similar to that of the MC treatment group. This suggests that while the pooling process did not affect IgG concentration of colostrum, it does

have an impact on antibody uptake when fed to calves. This is further supported as AEA was lower among calves which received PC, compared to single dam colostrum (MC and NMC). The absorption of IgG, as well as other Igs, occurs in the small intestine of the calf by an active process, pinocytosis, whereby Igs are transferred across the intestinal epithelium (Quigley et al., 2002). Differences in AEA in the present study indicate that colostrum source can influence the efficiency with which this process occurs. This finding however is in contrast to that of Besser et al. (1985) who reported no difference in AEA between calves receiving MC and PC. When investigating colostrum source, the study by Besser et al. (1985) used a total of 23 calves, with eleven receiving MC and twelve receiving PC. This was on a much smaller scale to the present study which involved 119 calves and an additional treatment (NMC). Immunological differences in neonates based on colostrum source has been demonstrated previously among pigs. Tuboly and Bernath (2002) reported leukocytes are selectively absorbed by piglets from MC, while leukocytes in colostrum from NMC may not be absorbed. Interestingly, a study by Riedel-Caspari (1993) reported that in dairy calves, leukocytes are positively correlated with passive immunity. A hypothesis for the lower 24 h serum IgG concentration among calves fed PC in the present study could be that pooling colostrum has a negative impact on the concentration of leukocytes in colostrum or absorption of leukocytes, which in turn reduces the rate of Ig absorption by the calf. Further investigation is therefore warranted among calves to assess potential interactions between colostrum source, leukocyte concentration and absorption and TPI.

When pooling colostrum, the process increases the risk of bacterial contamination as colostrum is first collected individually before being mixed together. This could potentially result in pathogenic bacteria being present but also affect TPI once fed, and studies have shown a decline in calf serum IgG as bacterial content of colostrum increases (Gelsinger et al., 2015, Cummins et al., 2016b). In situations where pooling of colostrum will be carried out, it is imperative that good hygiene standards must be achieved to prevent any impact on TPI as a result of contamination.

The positive linear relationship between calf serum IgG concentration at 24 h and IgG concentration of colostrum fed was as expected. A study by Osaka et al. (2014) identified a positive linear relationship between IgG intake and calf serum IgG concentration at 24 h, but also across different first feeding time points from birth. This indicates that providing a high quantity of IgG will result in a high calf serum IgG concentration, compared to feeding colostrum with a lower IgG concentration. The association identified in the present study

between calf serum IgG concentration at 24 h and birth bodyweight, suggests that calves < 30 kg at birth have better antibody absorption capabilities. Lower calf bodyweight at birth is associated with lower rates of dystocia (Berry et al., 2007), which has been found to negatively impact on TPI (Barrier et al., 2013). This occurs as corticosteroid levels in the neonatal calf are directly related to antibody absorption from colostrum, and following a difficult birth cortisol release in the calf is inhibited resulting in lower levels of antibody absorption (Chase et al., 2008). Dystocia induced acidosis could also be a factor, as studies have demonstrated lower IgG absorption among calves diagnosed as acidotic (Boyd, 1989, Besser et al., 1990). Greater vigour among calves with a lower birth bodyweight could also be a factor, and to the authors' knowledge this has not been previously investigated. Murray et al. (2015) reported lower AEA among calves that did not achieve sternal recumbency within 15 minutes of birth. This supports the hypothesis that calf vigour is a determining factor for AEA, and further investigation is warranted on AEA and interactions between birth weight and calf vigour. Findings from the present study indicates that particular attention must be given to large calves, but also those which experience difficult births, to ensure they achieve adequate TPI. Based on findings from the present study, feeding PC to calves which experience a difficult calving could increase the risk of failed TPI occurring.

CONCLUSION

Our results show that feeding colostrum from a single cow (MC or NMC) resulted in higher passive immunity among calves, relative to those which received PC. For calves fed single dam or PC, mean 24 h IgG concentration exceeded the adequate TPI threshold of 10 mg/ml. In this study, however, assessment of colostrum ensured that only high quality was used for feeding. Calf bodyweight was associated with TPI, and lighter calves achieved a higher serum IgG concentration at 24 h.

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Chapter 3

Development of a welfare assessment protocol for dairy calves from birth through to weaning

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ABSTRACT

The aim of this study was to develop a welfare assessment protocol using different indicators, for pre-weaned dairy calves, that is feasible and time efficient. To this end, the protocol had to combine animal-based indicators (measurements on physiology, general appearance, and behaviour) providing the basis for welfare assessment, with resource-based indicators (measurements on management and the environment) providing the basis for identifying risk factors. Indicators, both animal and resource-based, were selected by a review of existing literature and a process of expert consultation. Following the formulation phase, the protocol was then applied on five Irish dairy farms to develop further for completeness and on-farm feasibility. After each on-farm application, the protocol was critically evaluated, and modifications were made accordingly. Upon completion of the on-farm application phase, a feasible, reliable and time efficient protocol was produced.

Keywords Animal welfare, dairy calves, behaviour, housing, health, management

INTRODUCTION

Animal production systems are continually evolving in an effort to improve production efficiency and meet increasing demand for animal-derived protein (Thornton 2010; Boland et al. 2013). Evolution of animal production, however, has not always been in sync with animal welfare. In the dairy industry, for example, intense selection for milk production resulted in declining fertility, and increasing rates of lameness and metabolic disorders (Webster 2005; Rauw 2009; Oltenacu and Broom 2010). Welfare assessments are required to identify potential risk factors of diminished animal welfare (Leruste et al. 2015; Brscic et al. 2012; Lundborg et al. 2005) in evolving production systems. These can be carried out on-farm, through human application and technological applications using sensor data or vision technology. Potential technological applications include using 3D imagery to detect lameness, or thermal imagery to detect mastitis (Hovinen et al. 2008; Song et al. 2008; Rushen et al. 2012; Viazzi et al. 2014). Evaluation of routinely collected herd data, such as mortality, productivity, and fertility parameters, can also be used to assess welfare. Mortality rates provide a good starting point for identification of physiological health issues as these rates can be reflective of basic herd-level health and functioning (Ortiz-Pelaez et al. 2008; De Vries et al. 2011), however mortality has limited meaning with respect to animal welfare (Ortiz-Pelaez et al. 2008). In North America and some European countries, calf mortality rates of $\geq 10\%$ have been identified (Compton et al. 2017). Differences have also been identified in mortality rates between sexes, with bull calf mortality approximately 40% higher than that of female calves in commercial Irish dairy herds (DAFM 2013; 2014; 2015).

Although using routinely collected data to estimate welfare has some merit (Sandgren et al. 2009; De Vries et al. 2014; Parker Gaddis et al. 2016), on-farm assessments remain necessary for confirmatory purposes, for conducting other welfare measurements that would not be routinely collected (e.g. behavioural observations), and also due to discrepancies in data recording. Such discrepancies could exist as a result of unintentional errors in the recorded data, or manipulation of data, in an attempt to conceal welfare issues, enhance breeding values, or to avoid inspections, or potential financial penalties to subsidies received. Such penalties may arise from having elevated values for parameters such as calving difficulty or mortality rates. Routinely collected data often focus on performance traits, such as herd fertility or herd productivity, and as welfare is determined by factors beyond that of physical performance, this limits the capabilities of routine herd data to provide an indication of welfare. Traditionally, on-farm welfare assessments for dairy cattle, such as the 'Animal

Needs Index', have used mainly resource-based (management and environment) indicators to assess welfare (Bartussek et al. 2000). These assessments cannot accurately differentiate welfare of animals exposed to similar management and environments and therefore do not provide an adequate evaluation of welfare status. To assess welfare more accurately, animal-based indicators, such as physiological health parameters, general appearance assessment, and behavioural measurements should represent the majority of the protocol (Webster et al. 2004; Fraser 2008). The EU funded Welfare Quality® project commenced in 2004 with the aim of developing standardised systems for assessing animal welfare using mainly animal-based indicators. In this project an assessment protocol for dairy cows was developed that requires a considerable amount of time to implement (6 h for one person to apply to a 60 cow herd) due to a large number of animal-based indicators, 43 in total, in the protocol (De Vries et al. 2013). Hence, the Welfare Quality® protocol for dairy cows lacked feasibility and therefore has limited practical use (Knierim & Winckler 2009; Blokhuis et al. 2010; De Vries et al. 2014). It is therefore imperative that any protocols developed and implemented to assess animal welfare be balanced for application time, feasibility and assessment accuracy.

While assessment protocols for a wide range of animals were produced during the Welfare Quality® project, a protocol for dairy calves (male and female) was not developed. Existing protocols for dairy calves tend to focus on female calves, using indicators from a single-category (animal, environment or management-based indicators) (Whay et al. 2003; Vasseur et al. 2012). Single-category assessments can create analysis constraints; for example, assessment focusing solely on animal-based indicators do not allow for risk factor analysis. By combining indicators from all three categories valid assessments can be made, which, when applied on a large number of farms, could also allow for identification of potential risk factors.

The objective of this study, therefore, was to develop a reliable, feasible and time-efficient welfare assessment protocol, using different indicators, for male and female dairy calves during the pre-weaning and weaning phases (defined as beginning on day one of life to the point when milk feeding has ceased).

Protocol development consisted of two phases. Phase 1, the formulation phase, included the following steps: i) formulation and structuring of an interview for the calf manager, to assess management practices, ii) selection of environmental measurements and observations, and iii) selection of animal-based welfare indicators and appropriate scoring systems. Phase 2

included on-farm evaluation and further development of the protocol, conducted on commercial Irish dairy farms.

FORMULATION PHASE

Interview: self-reports of management practices

Management is a key determinant of welfare status (Fraser 2008; Cummins et al. 2016). To identify management practices already applied within the herd, an interview was developed to be carried out face-to-face with the principal calf manager. Key management areas directly related to calf welfare were selected based on a comprehensive review of literature in this field, and a process of consultation with experts in the fields of animal welfare and welfare assessment, and a number of farm managers. These areas included colostrum management and post-colostrum feeding (Godden et al. 2009), morbidity and mortality (Uetake 2013), health treatments administered, hygiene (Lehenbauer 2014), and weaning methods (Weary et al. 2008). In addition, a postal survey by Cummins et al. (2016) investigating management practices on spring calving dairy farms was reviewed and a number of questions, relevant to the interview were selected and adapted to an interview style. The interview consisted of 60 questions, which followed the natural sequence of processes in a calf management system from birth through to weaning. Where relevant, questions differentiated between management of male and female calves, and between calves born within the hours of a working day (06:00-20:00 h) and at night (20:00-06:00 h). Subjects covered during the interview, and justification for their inclusion, are given in Table 1.

Table 1. Subjects, and topics, addressed during the interview with the principal calf manager and justification for addressing these areas.

Subject	Topic	Justification
General Information	Herd size	Gulliksen et al., 2009
	Herd health	Mallard et al., 1998
	Vaccinations	Meganck et al. 2015
Colostrum Management	Timing of colostrum collection	Connecly et al., 2013, Moore et al., 2005
	Timing of colostrum feeding	Jaster, 2005
	Volume of colostrum provided	Jaster, 2005, Beam et al., 2009, Godden et al. 2009
	Colostrum feeding method	Besser et al., 1991, Godden et al. 2009
Post-colostrum feeding	Transition milk feeding	Godden et al. 2009
	Volume of milk provided	Jasper and Weary, 2002, Vieira et al., 2008, Quigley et al., 2006, Soberon et al., 2012
	Grouping of calves	Vieira et al. 2008
	Feeding method	Hammell et al., 1988, Jensen and Budde, 2006
Health and treatments	Morbidity	Johnson et al., 2017, Jorgensen et al., 2017
	Mortality	Windeyer et al., 2014
	Treatments	Sayers et al. 2016
Hygiene	Cleaning procedures	Phipps et al., 2016
	Cleaning frequency	Klein-Jöbstl et al., 2014
Weaning	Weaning method	Weary et al. 2008
	Weaning age	Eckert et al., 2015

In addition to data collected by interviewing the calf manager further information was available on farmer's consent from the Irish Cattle Breeding Federation (ICBF) HerdPlus®; this provided information on calf mortality rates and still births, calf date of birth, breed, sex, sire and economic breeding index value.

Assessment of the environment

Housing facilities and the environment calves experience can have a pronounced effect on welfare (Peña et al. 2016). For this reason, assessment of environmental conditions was an essential part of this protocol, and could be used to identify risk factors. Environmental measurements pertaining to space allowance (Jensen & Kyhn 2000; Tapki et al. 2006), temperature and wind speeds within and outside the calf house (Bokkers & Koene 2001; Peña et al. 2016), hygiene conditions (Ridge et al. 2005; De Waele et al. 2010) and feed and water supply (Coverdale et al. 2004; Porter et al. 2007), were included in the protocol. Selection of indicators was based upon i) correlation with health and welfare outcomes in the literature and ii) feasibility and reliability of recording.

To gain an insight into space allowance per calf, dimensions of all group and individual pens, and number of animals present per pen were included in the protocol. Adequate ventilation and air capacity in a calf house is important to prevent respiratory issues (Bryson 1985, Sivula et al. 1996). Measurements of length and breadth of the calf house and height at the roof eaves and ridge were included to allow calculation of cubic air capacity of the house. Type of bedding material, and average depth of bedding in each pen were incorporated into the environmental assessment, as this can influence welfare, by affecting physiological health and cow comfort (Panivivat et al. 2004). To assess cleanliness and dryness of bedding material a scoring system adapted from Lundborg et al. (2005) was used (Table 2).

Table 2. Assessment of bedding material on hygiene and moisture content, using a scoring system of zero to three, zero representing clean/dry and three representing soiled/wet.

Score	Hygiene Description	Moisture Description
0	Fresh /Clean	Dry/fresh
0.5	75 % Clean	Wet at perimeters
1	Slightly dirty	Slightly damp
1.5	Moderately dirty	Wet in spots
2	Dirty	Wet
2.5	Very Dirty	Very wet
3	Extremely dirty/wet	Extremely wet

Ammonia gas concentrations at elevated levels in animal housing facilities can cause ocular and respiratory tract irritation in animals (Urbain et al. 1996; Lundborg et al. 2005). Ammonia gas concentrations at bedding surface level were incorporated (Gastec ammonia tubes and the Gastec GV-100S pump; Envirosafe Ireland, Wicklow, Ireland). To maintain feasibility, measurements would be taken in at least two pens with representative bedding conditions observed on the farm and at least at one location per pen (e.g. centre of the pen). High ammonia concentrations occur as a result of the presence of faeces and urine, and can therefore be used to assess bedding hygiene in combination with other measurements.

Climatic conditions, such as temperature and wind velocity, can affect thermoregulation of calves and can therefore influence calf welfare (Lago et al. 2006; Norton et al. 2010). To assess the microclimate to which calves were exposed, ambient temperature and wind speed were included. Although ambient temperature and wind velocity might differ between pens, it would be measured at least once in a central location of the calf house which is deemed representative of that experienced in the house, and also outdoors in a clear, open area, as a reference. Temperature data loggers are ideal for this purpose. Wind speed would be measured at calf level (0.75 m from ground level) in at least one location within the housing facility using an anemometer (Airflow TA-2 anemometer; Airflow Developments Ltd, High Wycombe, England). A wind velocity measurement would also be recorded externally, as a reference.

In calf rearing systems, hygiene can have an impact on physiological health and therefore on welfare (Dewes & Goodall 1995; Al Mawly et al. 2015). To assess cleaning routines, and verify answers given during the interview, an assessment of hygiene of feeding implements, such as stomach tubes used for feeding new born calves, and colostrum collection containers, was included. Hygiene would be assessed using commercial test kits, such as 3M™ Clean-Trace surface protein plus test kits (3M™ Minnesota, USA). These kits measure the presence of proteins, such as milk residues and biological contaminants (bacteria, fungi), to verify effectiveness of cleaning routines. This is a semi-quantitative assay where colour formation indicates surface hygiene on a four point reference scale, providing a result within 15 minutes of testing. This provides an assessment of cleaning routines, which is validated (Simpson et al. 2006), quick, feasible and easy to use. This assessment will then allow for differentiation between farms, cleaning methods (e.g. use of cold versus hot water, as determined during the interview), and feeding implements.

Solid feed intake rates begin to increase from 14 days of age, when offered, and promote rumen development through anaerobic fermentation of feed particles, and development of absorption mechanisms (see Khan et al. 2011). Availability of solid feed is particularly important at weaning to address the energy deficit arising from milk removal. Offering solid feed pre-weaning can therefore influence calf welfare by reducing weight loss and stress experienced in this dietary transition period (see Khan et al. 2011). Availability of concentrates and roughage, such as hay or straw, in each pen was recorded. Provision of fresh drinking water and method by which it is provided in each pen was also recorded. Calving pen use, and design, can influence calf welfare (Vasseur et al. 2010), therefore, observations on calving pens were included: floor type classification (solid concrete, concrete slats etc.), number of calving pens, distance from calving pens to calf house, and if air space is shared between calving pens and calf houses. In the calf house, observations regarding how animals are penned (number of pens, number of calves per pen, mixed sex or single sex groups etc.), and highest and lowest identification number of calves in each group should be recorded to determine maximum age differences within groups, once date of birth is accurately recorded, as is a mandatory legislative requirement in Ireland.

Assessment of animals

Assessment of clinical health, physiology and behaviour, requires animal-based measurements, some of which are directly related to management and environmental

conditions (Cummins et al. 2016). Clinical health is one of the major aspects of welfare, and can be quickly assessed using visual indicators, such as demeanour, faecal consistency and respiration rate. Clinical health of all calves would be assessed using a visual health scoring system which was developed as part of this protocol by combining and modifying systems developed by the School of Veterinary Medicine, University of Wisconsin-Madison (https://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf) and Teagasc (Sayers et al. 2016) (Table 3). Preferably all calves in the herd should be assessed with this clinical health protocol, for a more definitive assessment of welfare.

Table 3. Definition of, and scoring levels, used in the health scoring system, adapted from the School of Veterinary Medicine, University of Wisconsin-Madison and Teagasc (Sayers et al., 2016) to assess dairy calf health in the welfare protocol.

Indicator	Definition	Scoring	Score levels
Demeanour	Combined evaluation of behaviour and responsiveness.	3 point scale of 0 to 2	0= Bright, alert, responsive 1= Dull possibly depressed, less responsive 2= Dull, apathetic, unresponsive
Mobility	Ability to stand unassisted and move freely.	3 point scale of 0 to 2	0= Stands unassisted, actively mobile 1= Slow to stand, limited mobility 2= Assistance required to stand, no mobility
Cleanliness	Appearance and condition of each calf's coat of hair.	3 point scale of 0 to 2	0= Clean, dry, shiny coat 1= Slightly dirty, dull coat 2= Dirty, matted, dull coat
Lesions/Wounds	Any damage or abrasions to skin surfaces	Yes or No – If Yes, location of lesions/wounds recorded.	Yes - Visible Lesions/wounds No - Absence of lesions/wounds
Ears	Positioning and activity of ears	3 point scale of 0 to 2	0= upright, alert and mobile 1= Slightly drooped, active 2= Drooped and limp
Nasal	Presence of any mucous discharge from nasal passages	3 point scale of 0-2	0= Clear, discharge free 1= Small amount of cloudy mucous visible 2= Excessive bilateral mucous discharge
Eye	Position, appearance and presence of ocular discharge	3 point scale of 0-2	0= Bright, pronounced 1= Slightly dull, presence of discharge 2= Dull, Sunken, discharge visible
Cough	Presence of a cough, increased respiration rate	3 point scale of 0-2	0= Normal breathing 1= Spontaneous cough 2= Continuous cough, increased respiration
Navel	Evidence of navel infection	3 point scale 0-2	0= Normal, pain free to handle 1= Slightly swollen, tender 2= Swelling, inflammation of navel area
Faecal consistency	Evaluation of faecal density/viscosity	3 point scale of 0-2	0= Normal, solid 1= Semi formed, paste like 2= Watery, fluid
External parasites	Presence of external parasites such as lice, mites	Yes or No	Yes – Parasite visible on skin/ in coat No – Skin/hair free from external parasites
Ringworm	Evidence of fungal infection on skin	Yes or No	Yes – Hair loss, skin lesions No – Normal hair coat & skin

Colostrum provides a source of passive immunity to the immunologically naïve calf, and the importance of colostrum quality and its association with calf health have been well documented (Robison et al. 1988; Godden et al. 2009; Furman-Fratczak et al. 2011). Fresh colostrum samples, from ≥ 6 cows, should be collected where possible, to investigate the quality of colostrum produced by cows in the herd. In studies investigating colostrum quality on commercial farms, the number of samples collected per farm varies. A survey assessing colostrum quality on dairy farms in Pennsylvania collected a single colostrum sample (produced by a single cow) on 55 different farms (Kehoe et al. 2007). A larger study investigating colostrum quality in the United States collected 827 colostrum samples from 67 dairy farms, equating to approximately 12 samples per farm (Morrill et al. 2012). In this study, however, herd sizes ranged from 70 to 500 cows. Given the relatively small average herd size in Ireland, (70 cows; ICBF, personal correspondence, March 27, 2018) it was decided that a minimum of 6 samples from 6 individual cows should be collected. In situations where large herd sizes are being assessed, the minimum sample requirement should be increased, to approximately 10 % of the herd. These can then be analysed in duplicate, to determine immunoglobulin G (IgG) concentration, using radial immunodiffusion kits (Triple J Farms, Bellingham, Washington, USA), which are considered the gold standard for quantifying IgG in bovine serum and colostrum (Weaver et al. 2000; Biemann et al. 2010).

Calf blood serum IgG concentrations are indicative of colostrum management, passive immunity, and potential risk of morbidity and mortality (Godden et al. 2009). The collection of blood samples from calves' ≤ 6 days old (Tyler et al. 1996) will indicate if calves have achieved adequate passive transfer of immunity, and also if practices relating to colostrum management outlined in the interview are being implemented. The number of calves' ≤ 6 days of age will vary depending on factors such as herd size, timing of visit within the calving season, and compactness of calving, therefore a minimum sample number is not described. To collect blood samples the jugular vein can be used as other sampling sites such as the coccygeal artery can be small and difficult to isolate in calves (Adams et al. 1991). The collected blood samples should be kept on ice until refrigerated at 4°C for 24 hours prior to serum separation by centrifugation (3000 g x 30 mins) at 4°C. Following centrifugation, serum samples can be frozen at -20°C until IgG concentration determination.

The collection of faecal samples will allow for the investigation of the presence of pathogens commonly associated with calf enteritis, such as *E.coli*, *Cryptosporidium parvum*, rotavirus and coronavirus (Meganck et al. 2015). Fresh faecal samples were collected using faecal

containers (Sarstedt AG & Co, Germany). Faecal sample analysis can be conducted using vertical flow immunochromatography test kits (Bio-X Diagnostics S.A, Belgium).

Behaviour is an important indicator of welfare: for example play behaviour is generally associated with good welfare, while high levels of oral manipulation of pen mates, or the pen structure, can be indicative of a welfare issue (Margerison et al. 2003; Krachun et al. 2010). To minimise time required on-farm, and maintain assessment feasibility, indirect behavioural observations can be made from video recordings taken during the farm visit. Based on consultation with experts in the field of animal welfare, a recording period of 60 minutes was deemed sufficient to have an impression for normal and abnormal behavioural patterns and to be able to compare behaviour of calves between farms. Recordings of two group pens, of contrasting composition (i.e. male and female calves), or if male and female calves were grouped together, then a group of older calves (>3 weeks old) and a younger group (<3 weeks old) would be assessed, to provide a reflection of the situation on that particular farm. To ensure consistency across behavioural observation conditions, recordings should be made during daylight hours, as was carried out during the on-farm development phase.

Once the groups were selected, video cameras (GoPro Inc., California,) were set-up to capture a clear and unobstructed image of the entire pen area, within which the activities of calves could be identified. Prior to recording, any lighting available must be switched on to ensure maximum visibility in the recordings. Recordings can then be scored at a later date using the ethogram as outlined in Table 4, which is adapted from De Wilt (1985). Behaviours would be observed by scan sampling at five minute intervals ($\pm$ 30 seconds) for the duration of the 60 minute recording. During the interview, feeding times would be determined in order to relate behaviour to feeding times. Observations would be limited to two groups due to availability of recording equipment; however, observations on a greater number of groups should be conducted where possible. This should not influence assessment feasibility as setting up of recording equipment has a relatively small time requirement.

Table 4. Ethogram, adapted from de Wilt (1985), which categorises and defines various behaviours, used for behavioural observations in the welfare protocol.

Category of behaviour	Behaviour	Definition
Posture	Standing	Calf is standing
	Lying	Calf is lying/resting
General	Walking	Calf is walking
	Not visible	Behaviour of the calf is not visible
	Defecating/Urinating	Calf defecates or urinates
Feeding behaviour	Drinking milk	Calf is drinking from a bucket, trough, teat or automatic feeding station.
	Drinking water	Calf is drinking water
	Eating	Calf eats concentrates or roughage, or other solid feed (proximity of head to feed)
	Ruminating/chewing	Calf is chewing
Comfort behaviour	Grooming	Calf licks itself, including snout/nose licking.
	Scratching/Rubbing/Stretching	Calf scratches itself with one of the legs (generally hind legs).
		Calf rubs itself on pen structure Calf stretches itself
Abnormal behaviour	Tongue playing/rolling	Calf makes repeated movements with its tongue inside or outside its mouth
	Urine drinking / oral manipulate prepuce	Calf drinks the urine of another calf Calf attempts to suck the navel area of another calf.
	Orally manipulating pen structure	Calf licks, nibbles, sucks, or bites at the pen structure (barriers, walls, buckets, troughs etc.)
Play behaviour	Play behaviour/Mounting/ Head butting	Calf runs, jumps, changes direction suddenly, bucks, kicks hind legs, twists or rotates body. Calf mounts, or attempts to mount, a pen mate. Calf is engaged in head to head pushing with another calf.
Social behaviour	Social interaction	Calf licks another calf in the same area multiple times
		Calf nibbles, sucks or bites at another calf

Observers

Application of the protocol requires two persons. This is due to the inclusion of blood sampling and heart girth measurements, during which one person is responsible for restraining the animal while the second person collects the sample/measurement. The remaining components of the protocol (interview, observations, etc.) can be distributed between the two persons to reduce the time requirement of the protocol. A trained and experienced individual should conduct the interview, health scoring and environmental observations (e.g. bedding assessment) on each protocol application, while the second person, who has received basic training, organises the video recording and carries out the environmental measurements (wind speed, ammonia measurements). This separation of tasks could serve to eliminate variation in measurements (e.g. bedding assessment, health scores, and interview application) due to differences between observers.

ON-FARM DEVELOPMENT PHASE

Ethical approval was received from the Teagasc Animal Ethics Committee (TAEC) (TAEC102/2015) and procedure authorisation (AE191132/P053) was granted by the Health Products Regulatory Authority (HPRA) of Ireland.

The on-farm development phase was carried out on 5 commercial Irish dairy farms between December 1 and 16, 2016 to evaluate the processes involved and feasibility of the assessment. The number of farms in the on-farm development phase was based on 10 percent of a sample size required for a large scale study (Lackey and Wingate 1997), determined by a power calculation. Farms were selected on the criteria that they had a minimum of 50 autumn (September to December) calving cows, to ensure a minimum of 20 calves would be available when applying the protocol. Participation was on a voluntary basis and each farm was visited once. Herd owners were given 3 days' notice, in advance of the visit, informed of the tasks that would be carried out (interview, environment and animal based measurements) and the approximate duration of the visit (2.5 h).

Average herd size of the five farms was 302 (SD $\pm$ 115.6) cows. One farm was 100 % autumn calving, while the remaining four operated split calving systems (a proportion of the

herd calving in the spring 44.2 (SD $\pm$ 25) % and the autumn 55.8 (SD $\pm$ 25) %. The average autumn calving herd size was 137 cows, with an average calving season length of 12.8 (SD $\pm$ 5.7) weeks.

The on-farm development phase allowed for identification of protocol strengths and weaknesses, which were then modified accordingly. These improved protocol clarity and allowed for a more accurate evaluation of calf welfare on commercial dairy farms.

Modifications to Interview Questions

In the interview process the following modifications were made during the course of the on-farm development phase to capture additional information and enhance the quality of information. In some instances farmers provided valuable information which was outside the scope of the original question. Therefore to ensure such information was collected each time the interview was conducted, additional relevant questions were included.

If disease testing of cows was conducted through bulk milk sample analysis, a follow-up question was included to determine which milk processor, or laboratory, was providing the service. Once known, the range of diseases screened for could be identified. Failure to conduct disease screening within a herd, and take appropriate action based on findings (e.g. culling infected cows etc.), could result in an increased risk of calves being exposed to diseases early in life, but also in-utero (Jawor et al. 2007). Contract rearing involves the transfer of animals from the owner's farm to that of another party for rearing under contractual agreement. A question was also included in the general information section to investigate the use of contract rearing for heifer calves and the age at which calves move to contract rearing systems. This question was included to achieve a clear understanding of management systems on each farm and identify any movement of calves during the pre-weaning period.

On the topic of calf management and colostrum management, further information was required on a number of specific areas. A question was included to determine the amount of time per day spent feeding and observing calves. This allowed for correlations between time dedicated to calves and calf welfare to be investigated, and also provided information on the labour requirements associated with calf rearing. Regular monitoring of cows approaching parturition is essential if difficult calving's are to be lessened and prompt provision of colostrum achieved. Use of calving cameras and frequency of physical observation of cows at

night was therefore investigated to allow risk factors for poor calf welfare to be assessed. When revising the assessment protocol one of the weaknesses identified was the lack of information on factors affecting adequate passive transfer (APT) of immunity in calves. The importance of good colostrum management in achieving APT is well established, however evidence exists that in Ireland for example, on-farm management is sub-optimum (All Island Disease Surveillance Report 2016; Cummins et al. 2016). In light of this, a particular emphasis was placed on assessment of colostrum management practices. In the originally formulated interview, colostrum management areas addressed included timing from parturition to first milking of the cow, method and timing for feeding colostrum, and if and how colostrum was heated prior to feeding. During the pilot study however, it was decided to include additional questions to determine if and how colostrum quality was assessed prior to feeding, if colostrum was pooled for feeding, if freshly calved cows were grouped together and milking frequency applied. This information would allow for risk factors associated with achieving APT in calves to be identified, both within and between herds, in conjunction with collection and analysis of blood samples.

On occasions during the on-farm development phase, additional information of value was voluntarily provided by interviewees in the areas of post colostrum feeding and management. To ensure this additional information was collected in future interviews, specific questions related to these areas were formulated and included in the interview. These questions addressed the source of transitional milk used (e.g. calves' own dam, or combined from a number of cows), use of individual calf pens, duration spent in these pens and method of feeding calves in these pens. If computerised feeding systems were used, follow-up questions were included to determine the age at which calves were introduced to the feeder, and details of the feeding program applied. When investigating the frequency with which feeding equipment was cleaned, an additional question was included to determine how thoroughly the equipment was cleaned (e.g. using cold water only, or warm water and a detergent) on each occasion, as this could influence the spread of infectious disease.

When collecting information on health and treatments a number of questions were redesigned to improve clarity and ensure there was no ambiguity surrounding any questions. Also, the prevalence, cause and method of diagnosis of calf pneumonia were investigated through the inclusion of additional questions. For sick calves placed in isolation, a follow-up question was included to determine where they were re-located to once recovered, as re-introduction to

their original group could be a potential risk factor to calf health (Lundborg et al. 2005), however such calves should enter a group to allow for social interaction.

Carry-over of infectious agents from one year to the next can occur if facilities are not sufficiently cleaned in the intervening period. On the subject of hygiene practices, a question was therefore included to determine how (e.g. add additional bedding material, or clean out all bedding material and re-bed) and when calving pens and calf pens were cleaned once the calving season was completed.

To gain a clear understanding of weaning practices additional information was sought on weaning thresholds applied (e.g. age, bodyweight, concentrate intake etc.). A question was also included to determine the age at which calves move outdoors to grass (e.g. prior to weaning, post-weaning, calendar date etc.). When calves were being sold, a follow-up question was included to determine which specific types of calves are sold. This information allowed for differentiation of management, and health and welfare, of calves prior to sale to be compared to those remaining on the farm.

Following these modifications, the interview yielded clear and comprehensive information on how calves were managed, at all times differentiating between male and female calves and those born during the day and at night. Furthermore, verifying the accuracy of the information given during the interview was possible using results from environment and animal-based measurements. Environmental measurements and observations proved successful, allowing within-farm evaluations to be made (e.g. bedding conditions of different pens) and also between farm comparisons.

Following application of the protocol and analysis of the data collected, herd owners should be furnished with a report in order to identify improvements which could be made on their farms. This should contain results on individual animal-measurements (e.g. colostrum quality for cows, serum IgG concentrations for calves, faecal sample results etc.), together with the average figure within their herd, and recommended threshold values (e.g. colostrum IgG ≥ 50 mg/ml). Information should also be provided on the environmental-assessment (e.g. bedding score, ammonia concentration, space allowance etc.) and recommended levels for each measurement. If the protocol is applied to a group of farms (e.g. in a large scale study), group average figures could also be included in the report to allow farmers to compare the performance of their system to that of the group. Finally, the report should include expert-derived recommendations on improvement options. These farm specific improvement options

should be practical, feasible and must be communicated in a manner that is easy to follow, to allow for successful implementation.

During the on-farm development phase, the interview revealed contrasting management practices across the five farms indicating that the protocol is able to detect variation amongst farms. For example, colostrum from two out of the five farms was frozen, while colostrum quality was only measured on one of the five farms. The mean mortality rate across the five farms (excluding stillbirths), identified through individual Herdplus® accounts, was 8.5 %, which is similar to the national average (6.1 %: DAFM, 2015), and ranged from 3.4 to 11.9 %. From calf manager statements, pneumonia and scours were identified as the main causes of calf illness, and four out of five farms reported experiencing issues with both on an annual basis. The specific cause of pneumonia was unknown in four out of five farms, while the cause of scours was known, from veterinary diagnosis, on three of the four farms that had experienced this health issue. Among the farms, feeding times, feeding method and volume of colostrum provided to bull and heifer calves were similar. For post-colostrum feeding, bull and heifer calves were subject to different management strategies, on four of the five farms. These included reduced feeding frequency and lower volumes provided to bull calves, and also differences in the type of milk provided. This demonstrates the importance and effectiveness of differentiating between sexes, and ages, as applied throughout the protocol. The results illustrate that within a small study population (n=5), large variation existed in management practices applied to which calves were exposed.

Behavioural observations made through on-farm video recordings allowed for investigation of the presence of abnormal behaviours (e.g. tongue rolling, cross-suckling, etc.), and comparisons of observed general behaviour patterns with those of expected behavioural patterns, based on existing literature. Using the ethogram, the types and frequencies of behaviours displayed by the observed groups were identified by scan sampling (Table 5), allowing for behaviour at group level to be assessed.

Table 5: Proportion of behaviours (average and range) observed at five minute intervals ($\pm$ thirty seconds) during a sixty minute observation period of five pens of older, pre-weaned, calves (> 21 days; group 1) and five pens of younger calves (< 21 days; group 2).

Behaviour	Group 1		Group 2	
	Observation (%)	Range (%)	Observation (%)	Range (%)
Standing	35.50	21.70 - 58.40	34.21	33.30 - 48.30
Lying	52.72	33.40 - 77.98	59.25	50.00 - 82.10
Walking	0.83	*	0.26	
Eating	6.37	1.70 - 13.50	4.37	1.20 - 6.26
Drinking water	0.43		**	
Ruminating	1.08	1.10 - 3.20	0.71	1.04 - 1.80
Grooming	0.70	1.10 - 1.70		
Oral manipulating pen structure	0.42			
Scratching	1.25	1.70 - 2.70		
Chewing	0.15			
Social licking	0.30			
Tongue rolling			0.26	
Not visible	0.28		0.42	
Other			0.26	

*Behaviour observed in a single pen only, therefore, behaviour range is not available

**Behaviour was not observed

During the on-farm development phase the ethogram was altered slightly. The category 'urinating/defecating' was removed, and replaced with 'other behaviour', to account for actions and bodily functions which are not related to welfare status. Lying was the most commonly observed behaviour and was witnessed more frequently among younger compared to older calf groups. This result is similar to the findings of Hänninen et al. (2003) who noted it is the natural behaviour of neonatal calves to have a high resting frequency (20 h/day) which decreases with age. On two occasions humans were in the area where calves were contained for part of the recording period. Increased activity was witnessed in behavioural observations of groups on these farms, which could potentially be due to human presence. Based on this, it was established that to prevent calf behaviour being influenced in any way, recordings must not commence until all individuals have vacated the area. This was successfully achieved by communicating clearly too all persons present on the farm to refrain from entering the area until recording had ceased.

Blood sampling proved an important physiological measurement, providing information on animal health and colostrum management, and with a relatively small time requirement. As venepuncture sampling is invasive, approval must be granted from relevant authorities, and also from farmers, prior to application of the protocol. Internationally, this may not always be possible due to legal restrictions and cultural attitudes.

DISCUSSION

By combining management, environmental and animal based indicators, a welfare assessment protocol for dairy calves was successfully developed. On one occasion during the on-farm development phase, it became apparent that answers provided during the interview were reflective of best practice, and not applied practice. This was only identified when animal-based and environmental indicators were undertaken, which illustrates the importance of on-farm assessment, and the potential pitfalls associated with the exclusive use of self-reports on management practices. The combination of indicators used in the protocol, therefore, ensures that an assessment of calf welfare, based on existing literature and previously validated measurements, can be made in a feasible and time efficient manner, with little variation in application time based on herd size. Further improvements could be made to this protocol by validating measurements that were relevant for calf welfare but for which limited evidence

was found in literature, for example ammonia concentrations, and bedding material assessment.

To achieve protocol feasibility and time efficiency, the duration of the observation period per pen and the number of pens per farm was chosen to be limited; therefore, conclusions which can be made from the displayed behaviour are also limited. Although a longer observation period, for example 8 or 24 h (Jensen & Kyhn 2000; Jensen et al. 1998), would provide more conclusive information on behaviour, it would be less feasible and time efficient.

The observation period chosen for this protocol, however, does provide an impression of group behaviour which can be referenced to expected behaviour, relative to proximity of feeding times. It can also be used to benchmark a group against other groups (male versus female) when enough farms are included in a study: and one farm against other farms. Behaviour observations can also be compared to other information collected; for example, feeding levels can be compared to frequency with which oral behaviours are displayed (cross suckling, manipulating pen structure). By including the one hour observation period, an impression of behaviour is achieved, without any negative impact on protocol feasibility.

To produce reliable assessments, measurements must be precise and accurate. For human observations, variation can exist in measurements, both within (intra) and between (inter) observers. To quantify, and minimise measurement variation with training, intra and inter observer reliability must be tested. During the on-farm development stage, observations were made by a single observer. Prior to this, the observer carried out bedding quality assessments, and clinical health assessments in two calf groups, twice per day, on three separate occasions, at Teagasc Moorepark, research farm. From these measurements intra-observer reliability was high (>0.90 ; Cronbach's alpha). In cases where the protocol will be applied on a large number of farms, and more observers are needed to collect on-farm data, training would be required for all observers, and intra and inter-observer reliability assessments needed to ensure consistent scoring.

This protocol draws on some aspects of the work of the Welfare Quality® project and could potentially contribute to the recommendation that development and refinement of welfare assessment systems continue to be undertaken in the wake of the Welfare Quality® project (Blokhuys et al. 2010). While this protocol was developed in Ireland, where seasonal calving is in operation, the indicators used are generic to calf welfare. By applying alterations, this protocol could be used internationally; for example a number of questions in the interview

would have to be re-designed to account for year-round calving systems. In the European Union, of which Ireland is a member state, directives exist on farm animal welfare. Each member state must incorporate these directives into national legislation and ensure they are enforced. Invasive procedures for calves, such as disbudding, castration etc., are controlled under such legislation, and therefore were not assessed as part of this protocol. For non-E.U member states and other international regions where invasive procedures are not regulated, it would be imperative that an assessment of such procedures is included as part of the welfare assessment. Areas of international application could include i) in research to assess and compare production systems, ii) to improve dairy welfare standards, iii) in quality assurance schemes, and iv) as a basis for determining legislative compliance.

Protocol application required approximately two and a half hours, and as two persons were required, this equates to approximately five hours in total spent on-farm. Given that a large quantity of data was collected in this period of time, five hours was not overly excessive, and is less than that generally required for on-farm welfare assessments which include animal based measurements (Knierim & Winckler 2009).

The combined use of previously validated animal, management and environmental measurements, a welfare assessment protocol for dairy calves was successfully developed. Following an on-farm application phase, the protocol proved feasible, providing a time and cost effective method of assessing welfare on commercial farms, particularly during periods of high labour demand. To achieve assessment feasibility, certain constraints were required which created assessment limitations. A sixty minute observation period provided an indication of behaviour; however a longer period of repeated observations would provide more conclusive information. The protocol is applied in a single visit, and while heart girths are used to estimate bodyweight, average daily gain (ADG) cannot be determined. Average daily gain would provide valuable information on animal performance and welfare; however it would necessitate a minimum of two measurements at different time points. To evaluate the success with which risk factors can be identified having applied the protocol, a large scale study would be required along with the associated statistical analysis of the data. This study was conducted as part of a larger project which aims to apply the protocol on a large number of farms at a later date.

In welfare assessments, classification systems are strongly desired by stakeholders, such as consumers, processors and government agencies, as it would allow them to rate and compare animal welfare standards among producers. This would indeed be invaluable, however it is

imperative that such systems are reliable and precise, to safeguard animal welfare and ensure all producers receive a result which is representative of welfare standards achieved. Attempts to reduce the associated subjectivity of overall welfare assessments have included development of ranking and weighting systems. Such systems would be transparent and standardised, and while the development of these systems proved successful, limitations to these systems have been identified.

Ranking systems involve positioning herds based on individual measurements, followed by producing an overall ranking (e.g. from best to worst) based on the sum of the individual measurements. This system has been considered by Whay et al. (2003) and Webster (2005), and while it is clear and easy to understand, particularly for farmers, it is limited to standards within the observed population. For example, in a population where overall welfare standards are poor, some will inevitably rank highly and could be perceived as having good welfare standards.

A weighting system, which assigns values to measurements based on the associated impact on welfare, calculates an overall score as the sum of the weighted values. Such systems have been developed for animal welfare assessment (Bartussek, 1999; Bracke et al. 2002). A considerable limitation to this system is that compensation can occur as high scores in certain areas can offset low scores in others, giving an overall value which can be indicative of good to moderate welfare, which may not be the case. This has been identified in a number of studies evaluating welfare classification (Botreau et al. 2009; De Vries et al. 2013; Sandøe et al. 2017). Where a welfare classification system was developed for pregnant sows (Bracke et al. 2002), the authors stated that it could not be determined at which point a change in “welfare score” would equate to a definite change in welfare. This, along with other studies, highlights the level of deviation better welfare classification and actual welfare status. The consensus among welfare classification studies is that compensation is extremely difficult to overcome in such systems, which not only results in inaccurate classifications but can also mask existing issues.

We believe that these limitations are due to the complex nature, and level of subjectivity associated with welfare appraisal, and not due to errors or shortcomings on the part of those who have developed existing ranking/weighting systems. Therefore, we decided not to proceed with the development of a classification system, and instead focused on the individual measurements. This manuscript therefore describes how a feasible and reliable

protocol was developed to assess different indicators of calf welfare; however the scope of this study does not extend to that of classification of welfare based on protocol application. Any parties who wish to apply this protocol with a view to classifying welfare, should familiarise themselves with the advantages and limitations of different overall assessment methods, and then select, and develop, the most suitable system to use in conjunction with this protocol, based on their overall goal (i.e. ranking herds or comparing herds).

CONCLUSION

Through the combined use of management, environment, and animal indicators, a reliable, feasible, and time efficient dairy calf welfare assessment protocol was developed. By completing validation on a number of measurements, this protocol could be used for large scale welfare assessments and for identifying risk factors associated with dairy calf welfare.

Chapter 4

Associations between colostrum management, passive immunity, calf-related hygiene practices and rates of mortality in pre-weaned dairy calves

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ABSTRACT

Pre-weaned calves are particularly vulnerable to health issues and experience high rates of mortality. Poor colostrum quality, or sub-standard colostrum management, combined with poor hygiene can increase disease susceptibility contributing to elevated mortality rates. This study aimed to assess colostrum and calf management together with subsequent mortality rates in pre-weaned calves. Forty-seven Irish spring-calving, pasture-based dairy herds were enrolled in the study. To investigate if colostrum and hygiene practices change as the calving season progresses, each farm was visited in both the first and last six weeks of the calving season. Concentration of IgG in 250 colostrum samples and 580 calf serum samples were determined by radial immunodiffusion assay. Mean colostrum IgG concentration was 85 mg/mL, while mean calf serum IgG concentration in the first and last six weeks of the calving season was 30.9 mg/mL and 27.1 mg/mL, respectively. Smaller herd size, and younger age at sampling, was associated with higher calf serum IgG concentration. Dairy breed calves were associated with higher serum IgG concentrations; no association was detected based on sex. For feeding equipment hygiene, presence of protein residues was assessed, and hygiene levels tended ($P = 0.07$) to worsen from the first to the final six weeks of the calving season. No association was found between feeding equipment hygiene and herd size or 28-d calf mortality rate. Colostrum and calf management practices were not associated ($P > 0.05$) with either calf serum IgG concentration or 28-d calf mortality rate. This study found IgG concentration in colostrum produced in Irish dairy herds was generally good, although, large variation existed, emphasizing the need for assessment of colostrum prior to feeding. Results also suggest that hygiene practices associated with calf rearing can be improved, particularly in the latter half of the calving season.

Key words: health, welfare, bull calves, Serum IgG, Commercial farms

INTRODUCTION

Seasonal, pasture-based dairy cow systems predominate in Ireland. These systems target a compact calving pattern in spring (February-April), where the objective is to have all cows calve in a twelve-week period (Berry et al., 2013). This enables herd energy requirements to be synchronized with grass growth (Horan et al., 2004), but also results in a large number of calves being born in a short period of time. Such a peak in calf births can create challenges for animal management, health and welfare.

Pre-weaned calves are particularly vulnerable to health issues such as enteritis, and are generally the age group within which bovine mortality rates are greatest (Murray et al., 2016; Ring et al., 2018). Colostrum quality and management, as well as calf-related hygiene practices (e.g. cleaning routine for feeding equipment, calving pens, group calf pens), can have a pronounced effect on dairy calf health and mortality (Godden, 2008; Uetake, 2013; Renaud et al., 2018).

The importance of colostrum management for bovines is derived from the lack of transfer of immunity from the maternal circulatory system to the fetus, owing to the structure of the placental barrier (Chucuri et al., 2010). Immunity must therefore be acquired passively from good quality colostrum (IgG concentration ≥ 50 mg/mL; Weaver et al., 2000; McGuirk and Collins, 2004), the first milk produced post-parturition (Barrington and Parish, 2001). Failure to achieve adequate transfer of passive immunity (TPI), measured as a serum IgG concentration < 10 mg/mL during the age of ≥ 24 h and ≤ 7 d, increases the risk of disease (Weaver et al., 2000; Beam et al., 2009; Stilwell and Carvalho, 2011). Acute enteritis, one of the most common causes of calf mortality, is associated with colostrum management, but also calf-related hygiene practices (Svensson et al., 2006; Klein-Jöbstl et al., 2014; Hotchkiss et al., 2015).

Failure to achieve adequate passive immunity can also affect performance later in life, through a reduction in growth rate (Furman-Fratczak et al., 2011) or first lactation yield (DeNise et al., 1989). Understanding factors associated with successful TPI, therefore, is important. Three factors will ultimately determine the success of TPI: 1) colostrum quality, commonly measured as IgG concentration (Garry et al., 1996), 2) colostrum management (e.g., timing of collection, timing of feeding, feeding method, storage (Weaver et al., 2000; Conneely et al., 2014)), and 3) the volume of colostrum provided (Weaver et al., 2000). While previous studies have assessed colostrum quality in research herds, limited information

is available on colostrum quality in commercial dairy herds in Ireland. Recent studies which investigated management of colostrum on commercial Irish dairy farms have described it as sub-optimum (Kennedy et al., 2014; Cummins et al., 2016). These studies, however, did not investigate associations between colostrum management practices and subsequent mortality rates. The objective of the present study was to assess colostrum and calf management practices on commercial Irish dairy farms, and associated calf mortality rates.

MATERIALS AND METHODS

This study was carried out between 4 February and 13 April, 2017. Approval was received for the ethical nature of the study from the Teagasc animal ethics committee (TAEC; TAEC 102/2015), while procedure authorisation (AE191132/P053) was granted by the Health Products Regulatory Authority of Ireland (HPRA). Experiments were undertaken in accordance with the Cruelty to Animals Act (Ireland 1876, as amended by European Communities regulations 2002 and 2005) and the European Community Directive 86/609/EC.

Herd Selection

Dairy herds recruited onto the study were located within an 80 km radius of Teagasc Moorepark (the area most densely populated with dairy herds in Ireland), to minimize travel time and facilitate sample processing. Herds had to be subscribed to HerdPlus, a data management and reporting system operated by the Irish Cattle Breeding Federation (ICBF) (Bandon, Co. Cork, Ireland). In Ireland, pasture-based, spring-calving production systems predominate, with the average herd containing 80 cows. Selected herds, therefore, fulfilled the criteria of operating a spring-calving, pasture-based system, with a minimum herd size of 70 cows. A hardcopy letter inviting 188 identified herds was sent to each herd owner in an attempt to recruit them onto the study. This letter outlined the objectives of the project, as well as an overview of the work involved, the expected outcomes, and the feedback participants would receive. Expressions of interest for inclusion in the study were collected by responding to a telephone number or email address, as outlined in the invitation letter. After 14 d, a reminder letter was sent to those who had not yet responded, requesting they provide a response. Forty seven herd owners agreed to participate (25 % response rate) and

were contacted by telephone for details regarding expected calving start date, specific farm location, a suitable visit time (i.e., am or pm) and date.

To investigate if changes in colostrum and calf management occur as the calving season progresses, each farm was visited twice. Based on expected calving start date provided by each herd, a visit was made once early in their calving season (i.e., within 6 wk of expected calving start date), and again in the latter half of the calving season (i.e., between wk 6 and 12 of expected calving start date). The first visits occurred between 4 February and 11 March, while the second visits occurred between 13 March and 13 April. Full consent was granted by participants to allow access to data on their herd through the HerdPlus database.

For each farm visit, two persons were present; an individual experienced in assessment of animal health, accompanied by an individual who had received basic training in animal handling, as well as conducting environmental measurements, as required for this study.

Calf and Colostrum Management

A calf welfare assessment protocol (Barry et al., 2019), which included an assessment of animal health, a management survey, and environmental measurements was conducted during each farm visit. An interview was conducted with the principal calf manager to collect information on: 1) calf management, such as timing of removal from the cow, timing of the first feed, feeding method used, and volume given, 2) colostrum management, which addressed timing of colostrum collection, colostrum storage, quality assessment as well as use of externally sourced, or manufactured, colostrum.

In the second visit, the interview instead focused on potential areas where management practices might have changed (e.g., timing of calf removal from the cow after birth) as the number of calves present on farm increased and the associated workload intensified.

Hygiene Practices

The interview also contained questions on calf-related hygiene practices to determine cleaning routines for feeding equipment, calving areas, and calf pens (individual and groups pens). In each visit, trained personnel physically assessed hygiene to determine the effectiveness of such routines while also verifying answers given during the interview. The presence of surface protein residues on two feeding implements, for example, esophageal

tube feeders and group feeders, was assessed using 3M Clean-Trace surface protein plus test kits (3M, Minnesota, USA). Samples were collected by placing the swab at the center point of the feeding implement, or center point of liquid holding pouch for esophageal tube holders, and moving the swab once around the inner surface of the feeding implement. For automatic feeders, the swab was passed over the outer surface at the center point of the drinking teat. This is a semi-quantitative assay that involves collecting a swab sample from the implement of interest. The swab is then placed in a chemical solution, which creates a chemical reaction resulting in color formation. Color formation is related to the amount of proteins present, such as milk residues or biological contaminants (e.g., bacteria, fungi), which is measured on a four-point reference scale, with one describing a low level of surface residue (i.e., good hygiene) and four describing a high level of surface residue (poor hygiene) (Simpson et al., 2006).

Colostrum Sample Collection

Participants were given six empty sampling containers (120 mL polythene container; SGR Scientific, Unit 1B, Metropoint Business Park, Kettles Lane, Swords, Co. Dublin) during the first visit and asked to collect six colostrum samples at random from freshly calved cows. This number of samples per farm was based on a review of studies that investigated colostrum quality in commercial herds (Kehoe et al., 2007; Morrill et al., 2012) while also considering the relatively small dairy herd sizes in Ireland. Participants were required to record the identification number of each cow from which a colostrum sample was collected. This enabled information such as breed, parity etc. to be collected on each animal via the HerdPlus database. Participants were asked to immediately freeze the samples (-18°C) until collection during the second visit. Samples remained frozen up to the point of analysis for IgG concentration.

Serum Sample Collection

Any calf ≥ 24 h and ≤ 6 d of age, inclusive at the time of visit was blood sampled to assess serum IgG concentration. This was undertaken to indicate if adequate TPI had been achieved but also examine the level of success of feeding practices, as answered in the interview. Blood samples were taken via the jugular vein using a plain serum tube (10 mL BD Vacutainer, BD, Belliver Industrial Estate, Plymouth PL6 7BP. UK). The identification

number of each sampled calf was recorded, which allowed information on its breed and date of birth to be acquired from the ICBF database. Blood samples were kept on ice until return to the research center, where they were refrigerated at 4°C for 24 h prior to serum separation by centrifugation (3000 g x 30 min) at 4°C. Following centrifugation, serum samples were collected and frozen at -20°C until IgG concentration determination in a laboratory located at Teagasc Moorepark.

Colostrum and Serum Sample Analysis

Colostrum and serum samples were defrosted at 4°C for 24 h prior to analysis by radial immunodiffusion (RID) assay (Triple J Farms, Bellingham, Washington, USA). Each RID kit contained a 24-well test plate and three reference sera samples. Kits were stored at 4°C and removed 30 min prior to use. Colostrum samples were prepared at a 1:3 dilution using de-ionized water while serum samples were prepared at a 1:2 dilution using the same diluent. On each test plate, wells one to three contained the reference sera (196, 1402, 1976 mg/dL), well four was blank, while remaining wells contained serum or colostrum samples, all of which were tested in duplicate. Plates were incubated at room temperature (20-24°C) for 24 h. Diameters of the precipitate rings surrounding each well were measured to the nearest 0.01 mm using digital Vernier calipers. Ring diameter values of the three reference sera samples were squared and plotted against their known concentration, producing a standard reference curve. This was then used to determine the IgG concentration of the serum/colostrum samples present on the plate. Samples which produced a value beyond the range of the reference curve were prepared with a greater dilution factor and re-analyzed.

Calf Mortality & Individual Cow 305-d Milk

Mortality rate of calves born in participating herds in the year 2017 were provided by the ICBF. Also provided was the status (i.e., alive or deceased) of calves from which serum samples had been collected, at the end of the calendar year in which they were born. For all deceased calves, the age at which mortality occurred was provided. Mean milk yield and 305-d geometric mean somatic cell count (SCC), for the calendar year 2016 were provided by the ICBF for cows from which a colostrum sample was taken.

Data Editing

To investigate statistical differences categories were created using dataset quartiles for the following variables: herd size, birth week of year for calves, age of calf at blood sampling, mean milk yield and 305-d geometric mean SCC. Participant herd sizes were categorized as: <110, 110 – 139, 140 – 189 and ≥ 190 cows. The extent of assistance required at each calving was subjectively scored by farmers on a scale of 1 to 4; 1 = no assistance provided, 2 = manual assistance provided, without mechanical aid, 3 = assistance provided, including use of mechanical aid, and 4 = veterinary assisted calving. Animals from which samples were collected were assigned a single breed descriptor. This was breed-specific for cows and sire breed specific for calves (i.e., dairy or beef breed). For cows, if breed composition consisted of Holstein and Friesian only, then the animal was described as being Holstein-Friesian (HF). If HF represented the majority of breed composition of an animal, together with any another dairy breed, for example Jersey or Norwegian Red, then the animal was defined as a HF cross (HFX). For calves, those sired by a dairy bull were classified as ‘Dairy’, while a ‘Beef’ classification was assigned to those sired by a beef breed. Calves were categorized based on the calendar wk of year when born as; within the first 6 wk, wk 6-7.9, wk 8-9.9, and during or after wk 10 of 2017. Age of the calf at blood sampling was categorized as 1-2 d, 3-4 d, 5 d, and 6 d old. Mean yield for the 2016 milking season was categorized, based on quartiles of the complete dataset, as: < 5000 L, 5000-6000 L, > 6000-7000 L and, > 7000 L. Similarly, individual cow 305-d geometric mean SCC for the calendar year 2016 was categorized as: < 50,000 cells/mL, 50,000-74,999 cells/mL, 75,000-150,000 cells/mL and > 150,000 cells/mL.

Statistical Analyses

All dependent variables followed a normal distribution pattern. Significant associations were confirmed at $P < 0.05$, and in each model, interactions were examined between significant variables, and least square means assessed. Risk factors associated with colostrum IgG concentration, calf serum IgG concentration were assessed using linear mixed models in PROC MIXED (SAS version 9.4; SAS Institute Inc., Cary, NC), while risk factors associated with calf mortality rates (excluding stillbirths) were assessed using fixed effects regression models in PROC GLM (SAS version 9.4). The logit of the probability of a calf dying when serum IgG concentration was below the recommended threshold (i.e., < 10 mg/mL) was

determined using logistic regression in PROC GENMOD (SAS version 9.4), assuming binomial distribution of the error term.

For colostrum IgG concentration, fixed effects considered in the model were herd size, calving early or late in the season, cow breed, parity, milk yield and SCC. Herd was treated as a random effect with a covariance structure assumed among records within herd. When investigating associations with the dependent variable of serum IgG concentration, fixed effects considered were herd size, hygiene of feeding equipment, calf and colostrum management practices, calf breed, sex, birth week of year, age at blood sampling and the extent of assistance required at calving; visit number was treated as a repeated effect, and a covariance structure assumed among records within visit. Calf serum IgG concentration, herd size, hygiene of calf feeding equipment, and management practices were all tested for association with the dependent variable 28-d mortality rate.

RESULTS

Colostrum IgG Concentration

Mean colostrum IgG concentration, as determined by RID assay, was 85.1 (SD = 51.6) mg/mL. In total, 21 % of the samples had an IgG concentration less than the recommended threshold of 50 mg/mL. No association was observed with the independent variables of herd size, calving week of year, breed, parity, milk yield or SCC.

Serum IgG Concentration and Calf Mortality

Mean calf serum IgG concentration in visit one and two were 30.9 (SD = 13.4) mg/mL and 27.1 (SD = 14.0) mg/mL, respectively, although not different from each other. Mean serum IgG concentration for both visits was 29.3 (SD = 13.3) mg/mL for males and 30.3 (SD = 14.1) mg/mL for female calves, albeit not different from each other. Herd size ($P < 0.01$), breed ($P < 0.05$) and age at blood sampling ($P < 0.01$) were all associated with serum IgG concentration. An inverse relationship existed between serum IgG concentration with both herd size (Fig 1) and age at blood sampling (Fig 2).

Figure 1. Least square means (one standard error each side represented as error bars) for serum IgG concentration in calves from one to six days old, determined by radial immunodiffusion assay, by herd size (number of cows)

^{ab} Superscripts indicate a significant difference ($P < 0.05$) in means

Figure 2. Least square means (one standard error each side represented as error bars) for serum IgG concentration in calves between one and six days of age, determined by radial immunodiffusion assay, located across forty seven commercial Irish dairy farms

^{ab} Superscripts indicate a significant difference ($P < 0.05$) in means

A significant interaction between calf breed group and serum IgG concentration existed based on calf sex. Mean serum IgG concentration was highest in female dairy calves (31.2 mg/mL), and lowest in female beef calves (26.0 mg/mL). Mean calf mortality rate at different age time points are summarized in Table 1. Mortality among calves with a serum IgG concentration <10 mg/mL was not different ($P = 0.18$) to that of calves with a serum IgG concentration ≥ 10 mg/mL.

Table 1. Mean mortality rate, from birth to six different time points during the first year of life, of all calves born in 2017 on forty seven commercial Irish dairy farms (standard deviations in parentheses)

Time point from birth	Cumulative mortality rate (%)
Three d	3.57 (2.45)
Seven d	4.43 (2.48)
Twenty eight d	5.86 (3.12)
Three mo	7.34 (3.44)
Six mo	8.28 (3.72)
Twelve mo	9.31 (4.29)

Hygiene Practices

The median hygiene score of the feeding implements in visit one and visit two are reported in Table 2.

Table 2. Mean hygiene score for calf feeding implements, determined using surface residues test kits, and scored on a four point scale, with one representing low residue levels and four representing high residue levels, assessed in the first (visit one) and last (visit two) six weeks of the calving season

Feeding Implement	Visit one mean hygiene	Visit two mean hygiene
Automatic feeder	1.0 (n=3)	1.0 (n=3)
Bottle and teat feeder	2.5 (n=2)	2.3 (n=2)
Multi-teat feeder	2.3 (n=64)	2.9 (n=61)
Individual teat feeder	2.4 (n=13)	2.1 (n=7)
Esophageal tube feeder	4.0 (n=1)	4.0 (n=1)

Hygiene tended to decline from visit one to visit two ($P = 0.07$), while no association was identified between hygiene score and herd size ($P = 0.19$). Better hygiene (i.e., low hygiene score) tended to associated with higher calf serum IgG concentration ($P = 0.07$). No association ($P = 0.26$) was detected between hygiene score and 28-d mortality rate. Responses for method and frequency hygiene practices were applied during each visit are outlined in Table 3. Hygiene practices were not associated with serum IgG concentration or 28-d calf mortality rate.

Table 3. Responses from calf managers across forty seven commercial Irish dairy farms on preferred frequency and method for specific calf related hygiene practices during the first (visit one) and last (visit two) six weeks of the calving season

Hygiene practice	Description	Visit one (%)	Visit two
Frequency for cleaning feeding	Daily	21.3	10.5
	Every 2 nd day	46.8	53.2
	Once a week	17.0	27.7
	Every 2 nd week	12.8	4.3
	Once a month	2.1	4.3
Preferred method of cleaning	Hot water only	41.3	25.5
	Hot water & detergent	13.0	23.5
	Cold water only	34.8	40.4
	Cold water & detergent	10.9	10.6
Frequency for cleaning calving	After each cow	6.4	4.3
	Once a day	6.4	4.3
	Every 2 nd day	10.6	4.3
	Once a week	19.2	27.7
	Every 2 nd week	14.9	12.7
	Once a month	17.0	12.7
	<Once a month	25.5	34.0
Preferred method of cleaning	Clean out, wash,	42.5	44.7
	Clean out, wash	19.1	19.1
	Clean out, re-bed	38.4	36.2
Frequency for cleaning calf pens	Daily	4.3	-
	Once a week	23.4	12.8
	Every 2 nd week	23.4	27.7
	Once a month	10.6	14.9
	<Once a month	38.3	44.6
Preferred method of cleaning calf	Clean out, wash,	44.5	53.2
	Clean out, wash	12.8	-
	Clean out, re-bed	38.4	46.8
	Top-up bedding	4.3	-

Calf Management and Colostrum Feeding

Participant responses on management practices related to collection, storage, source and quality assessment of colostrum are reported in Table 4.

Table 4. Responses from calf managers across forty seven commercial Irish dairy farms on management practices related to collection, storage, source and quality assessment of colostrum

Colostrum Management	Description	Response (%)
Colostrum collection timing	<2h	33.0
	Next scheduled milking	63.8
	<24h	1.1
	Not collected	2.1
Is colostrum stored	Yes	91.5
	No	8.5
Where is the storage location	Fridge	12.8
	Freezer	63.8
	Room temperature	23.4
Time from collection to feeding	<2h	59.6
	2-3h	10.6
	<4h	4.3
	4-6h	2.1
	>6h	2.1
	Not collected/stored	21.3
Testing colostrum quality	Yes	12.8
	No	87.2
Source colostrum externally	Yes	14.9
	No	85.1
Using colostrum replacement	Yes	12.8
	No	87.2

No association was identified for colostrum management practices and calf serum IgG concentration or 28-d mortality rate. Responses on colostrum feeding and calf management during the first and last six weeks of the calving season, for male and female calves, are reported in Table 5.

Table 5. Responses from calf managers across forty seven commercial Irish dairy herds on colostrum feeding and calf management practices, in the first (visit one) and last (visit two) six weeks of the calving season, and for female and male calves

Calf management and feeding practices	Description	Visit One (%)	Visit Two (%)	Female (%)	Male (%)
Timing of calf removal from cows after birth	0h	30.3	33.5	35.6	28.2
	<6h	24.5	14.9	19.7	19.7
	>6h	36.7	32.4	30.8	38.3
	>12h	8.5	19.2	13.9	13.8
Source of first feed	Calf drinks cow	23.4	32.5	27.1	28.8
	Other dam	10.1	9.6	9.6	10.1
	Own dam	29.3	29.2	30.9	27.6
	Pooled 1 st milking's	37.2	28.7	32.4	33.5
Timing of first feed	<2h	80.3	72.3	77.6	75.0
	<4h	6.4	2.1	4.3	4.3
	<6h	10.1	14.4	11.7	12.8
	>6h	3.2	-	1.6	1.6
	Calf drinks cow	-	11.2	4.8	6.4
Method for providing first feed	Bottle & teat	49.5	52.7	55.3	46.9
	Bucket	0.5	-	-	0.5
	Bucket & teat	9.0	7.5	5.3	11.2
	Stomach tube	7.5	17.0	12.8	11.7
	Drinks cows	33.5	22.8	26.6	29.7
Volume of colostrum provided	≤2L	2.6	3.2	3.2	2.6
	≤3L	55.3	59.0	58.5	55.8
	≤4L	13.8	17.0	15.4	15.4
	>4L	2.1	2.1	2.1	2.1
	Unknown	26.2	18.7	20.8	24.1

Similarly, colostrum feeding and calf management responses were not associated with either calf serum IgG concentration or 28-d mortality rate.

DISCUSSION

The importance of colostrum management for calf health has been well established (Jaster, 2005; Godden, 2009; Erickson, 2015), with much work conducted internationally assessing colostrum quality on commercial dairy farms (Gulliksen et al., 2008; Morrill et al., 2012; Phipps et al., 2017). Limited information, however, is available on colostrum quality but also calf related hygiene practices on commercial dairy farms in Ireland. This study aimed to assess colostrum and calf management practices, as well as passive immunity levels on commercial Irish dairy farms, while also investigating associations with calf mortality.

Colostrum IgG Concentration

Of colostrum samples collected in this study, 21 % were below the recommended threshold of 50 mg/mL (Weaver et al., 2000). This is greater than values reported in previous studies assessing colostrum quality in dairy cows. Conneely et al. (2013), for example, reported that 4 % of samples collected from four Irish dairy research herds were below the threshold, while Quigley et al. (2013) found that 16 % of samples, collected from seven commercial US dairy farms, were below the threshold. In a Norwegian study, similar to the present study, where 1250 colostrum samples were collected by dairy farmers across 119 herds, Gulliksen et al. (2008) reported that 57.8 % of these samples were below the threshold. Some of the colostrum samples were, however, collected from cows that had already nursed their calf, in which case a lower IgG concentration would be expected (Foley and Otterby, 1978; Godden, 2008). In the present study, if the proportion of calf serum samples below the threshold for adequate TPI was similar to that of colostrum samples identified as being poor quality (IgG concentration of <50g/L), this could indicate colostrum quality was influencing passive immunity. As the percentage of calf serum samples below the recommended threshold was lower (8 %) than that of colostrum samples (21 %), this supports the hypothesis that the sampling procedure, carried out by participants, could in some cases have potentially influenced colostrum IgG concentration (Hopkins and Quigley, 1997).

In the present study, a relatively high mean IgG concentration in colostrum was identified; however, considerable variation existed therein, ranging from 4 to 324 mg/mL. Previous studies have reported smaller variability in colostrum IgG concentration (7-159 mg/mL: Quigley et al. 2013; 4-235 mg/mL: Gulliksen et al. 2008; 13-256 mg/mL: Conneely et al. 2013). While sampling procedure would contribute to some of the variation identified, much

of this would be naturally occurring due to variation in immune system strength and functionality (Hurley, 2003), as well as the known heritable genetic variation in colostrum IgG concentration in dairy cows (Conneely et al., 2013). The extent of the variation identified in Irish dairy herds highlights the need for assessment of colostrum quality prior to feeding, a practice currently applied on less than 15 % of farms (Table 4).

Serum IgG Concentration

Mean calf serum IgG concentration in both visit one (30.9 mg/mL) and visit two (27.1 mg/mL), were almost three times that of the threshold for adequate TPI, with less than 10 % of calf serum samples being below the threshold. Given the intense labor requirement during the calving period in seasonal-calving systems, a reduction in serum IgG concentration in calves born later in the season would have been expected. This study, however, found no difference in IgG concentration in calf serum samples collected early or late in the calving season. While this study is unique in that herds were visited on two separate occasions, studies which collected serum samples at single time points reported a lower mean serum IgG concentration (21.6 mg/mL: Lombard et al. 2016; 22.0 mg/mL: Chigerwe et al. 2015). The relatively high serum IgG concentration in the present study could be attributed to the high mean IgG content of colostrum, which would facilitate the achievement of high levels of passive immunity (Weaver et al., 2000).

While a statistical difference was identified between mean serum calf IgG concentration in dairy and beef calves, both breed groups had mean values over two times that of the recommended threshold. Breed and serum IgG were also statistically different based on sex. Female dairy calves, which would be considered to have the highest monetary value, had the highest serum IgG concentration, while female beef calves which would be of a lower monetary value had the lowest serum IgG concentration. Both groups had a serum IgG concentration over twice that of the threshold for passive immunity, however, perceived monetary value could be influencing actual management received. In terms of dairy calf management, previous studies have demonstrated preferential treatment can be provided to female calves (Fecteau et al., 2002; Shivley et al., 2016; Shivley et al., 2018). The difference in mortality rates between male and female dairy calves observed nationally in Ireland (DAFM 2015, 2016), suggests management practices could differ depending on calf sex. Although management practices may vary based on sex and breed, serum IgG concentration

indicates no difference in successful TPI based on sex. This suggests that on commercial Irish dairy farms, all calves are managed in a way that facilitates achievement of adequate TPI, regardless of sex.

Although a negative association between serum IgG concentration and herd size was identified, biologically similar rates of passive immunity were achieved. Previous studies investigating associations between calf health and herd size have also identified negative associations (Lance et al., 1992; Del Río et al., 2007; Gulliksen et al., 2009). The negative association identified in the present study, could potentially be attributable to the use of labor-saving measures in larger herds. One such measure is pooled colostrum feeding which was practiced by over 30 % of participants in the present study. Pooling colostrum of unknown quality can reduce the overall IgG concentration (Weaver et al., 2000), and increase the risk of calf exposure to colostrum-borne pathogens (Godden, 2009). Given the high frequency with which pooled colostrum feeding is practiced, further investigation is warranted on pooled colostrum to determine the level and extent of immunity achieved when using this feeding strategy.

Following colostrum feeding, serum IgG concentration peaks at approximately 24 h and declined gradually thereafter (Shivley et al., 2016; Tóthová et al., 2016). The association identified in this study between serum IgG concentration and age at blood sampling was therefore as expected. A reduction of approximately 20 %, or 1.28 mg/mL per d, over a five d period was identified in serum IgG concentration in calves from 1 d to 6 d of age (31.9 vs 25.5 mg/mL IgG). This rate of antibody depletion is similar to that identified in dairy calves by Shivley et al. (2018), who reported a depletion rate of 0.71 mg/mL IgG per d in early life. This equates to a reduction rate of approximately 16 % over a five d period, given the mean reported value of 21.6 mg/mL IgG. Knowing the rate at which antibody depletion occurs is important for herd owners and veterinarians alike, as it can enable back calculations to be made retrospectively to determine if adequate TPI was likely achieved.

Failure to find a difference in mortality based on the calf serum IgG threshold of 10 mg/ml indicates that this threshold may need to be revised, particularly for Irish dairy systems. The 10 mg/ml IgG threshold is widely accepted as the cut-off point for passive immunity, and applied in many research studies. Some studies, however, suggest that this threshold may need to be adjusted for different countries or regions, but also different production system types (i.e., seasonal calving systems). Wittum and Perino (1995) found that for calves raised in a beef production environment the risk of mortality was greater when calf serum IgG

concentration was <8 mg/mL, compared to those which had serum IgG concentration >16 mg/ml. In Holstein heifer calves, Robison et al. (1988) reported a two fold increase in mortality among calves with serum IgG concentration <12 mg/ml. These studies, along with the present study, suggest a study is required to re-evaluate cut-off points for passive immunity among calves in seasonal calving systems.

Hygiene Practices

No association was detected in the present study between serum IgG concentration and calf mortality. Similarly, no association was detected between serum IgG concentration and either hygiene scores or hygiene practices. Poor hygiene practices for feeding equipment can result in bacterial contamination of colostrum, and numerous studies have reported a negative association between colostrum bacterial content and TPI (James et al., 1981; Staley and Bush, 1985). Godden (2009) reported that feeding colostrum with low bacterial levels resulted in higher serum IgG concentration, and reduced rates of morbidity in calves; however no reference was made to the effect on calf mortality. The mean colostrum IgG concentration in the present study was greater than that of Godden (2009) (85.1 vs 61.1 mg/mL), and thus could potentially be counteracting any effect of contamination, through antigen binding (Indyk et al., 2008).

Calf Management and Colostrum Feeding

No associations were found between calf management and either serum IgG concentration or mortality rate. Similarly for colostrum feeding practices, no association was detected with either serum IgG concentration or mortality rate. While the likelihood of mortality occurring in calves with a serum IgG concentration < 10 mg/mL was not significantly different to that of calves with a serum IgG concentration ≥ 10 mg/mL, numerically the risk was almost twice. Similar studies, however, have reported associations between management practices and mortality rates (Shivley et al., 2018), as well as associations between serum IgG concentration and mortality rates (Lora et al., 2018). Failure to detect such associations in the present study could potentially be due to management data referring to general practices, and

not specific management received by individual calves from which a serum sample was collected.

While adjustments to management and feeding practices were identified as the calving season progressed, this did not impact on the level of passive immunity achieved in the calves. Failure to measure colostrum quality on the majority of farms is an issue of concern, which could contribute to the occurrence of failed TPI. Pooled colostrum feeding and allowing calves to drink directly from the cow, which are both commonly practiced on Irish dairy farms could have negative implications for calf health (Weaver et al., 2000) but also disease transfer within herds.

Study limitation

Field studies, and particularly those conducted on-farm, generally operate with certain limitation. In this study, the number of samples collected, as well as collection method used, was influenced by the nature of the study. Prior to commencement of the study, a power calculation was conducted to determine subset required per herd, and age criteria for blood sampled calves was selected (i.e., ≥ 1 d and ≤ 6 d old). This limited the availability of suitable calves, particularly during the second visit when the number and frequency of cows calving had declined. For collection of colostrum samples, farmers were instructed to collect samples from freshly calved cows at random, however a potential selection bias could exist. This was given careful consideration prior to commencement of the experiment and as there was no recent data for colostrum quality on commercial dairy farms it was considered important that an effort was made to benchmark current colostrum quality. Ideally to exercise greater control over cow selection and the sampling procedure would have been preferred; this, however, this would require a prolonged presence on-farm, which would not be feasible. Also, it was important that colostrum samples were collected from freshly calved cows, a point emphasized to participants, and pre-selecting cows to be sampled could have influenced results, for example in cases where a pre-selected cow had calved unsupervised and the participant proceeded to collect a sample without knowing the time since parturition.

Overcoming such issues would prove difficult given the number of restrictive factors associated with large scale studies on calf health in seasonal calving systems (i.e., herds calving simultaneously, short calving season length, age restrictions for sampled calves, relatively small herd sizes). The present study was conducted in a single calving season and

covered a limited geographical area. Future studies could be conducted over a number of calving seasons and limit enrollment to larger herds or cover a larger geographical area.

CONCLUSION

The results show that colostrum management on commercial Irish dairy farms is of a high standard, with > 90 % success rate for achieving adequate TPI. Serum IgG concentration was higher in calves born in smaller herds (< 110 cows), but mean serum IgG concentration across all herd sizes exceeded the recommended threshold (≥ 10 mg/mL). Colostrum quality in Irish dairy herds is generally good; however, considerable variation exists in colostrum IgG concentration. Negative implications associated with such variation could be overcome by assessing colostrum quality, a practice currently conducted on less than 15 % of Irish dairy farms. Hygiene practices related to calf feeding equipment can be improved, and particularly during the final six weeks of the calving season. This could reduce the risk of bacterial contamination of colostrum, and further increase the rate of passive immunity achieved on commercial dairy farms.

Chapter 5

Pre-weaning management of calves on commercial dairy farms and its influence on calf welfare and mortality

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ABSTRACT

Welfare and management of calves is of increasing interest, and also influences performance of these animals in later life. The aim of this study was to assess pre-weaning management, space allowance, group size and associated behaviour, as well as the presence of enteric pathogens and calf mortality on commercial Irish dairy farms. We included 47 spring-calving, pasture-based herds in this study. Herd and animal-specific data, such as mortality rate, age and breed, were gathered from all participants via the HerdPlus® database. Information pertaining to management practices was collected by conducting an interview with the principal calf rearer, while an assessment of calf housing facilities was conducted to identify conditions calves were reared in. The environmental assessment included measurements of space allowance per calf, as well as feeding equipment hygiene. We used video observations to assess calf behaviour based on the number of calves present in a group and the amount of space available per calf (pen size (m²) divided by the number of calves present). Faecal samples were also collected to determine the presence of enteric pathogens among calves. To establish if changes to management practices, space allowance, as well as calf health occur as the calving season progresses, each farm was visited twice.

Calf mortality was not associated with either herd size, space allowance per calf or post-colostrum feeding practices. Higher calf mortality was identified among herds which reported experiencing an on-set of calf pneumonia during weeks 8-10 of the calving season. This study demonstrates that factors associated with calf welfare on commercial Irish dairy farms (e.g., passive immunity, space allowance, mortality rate) are independent of herd size. Some management practices however, such as methods used for treating health issues can affect rates of calf mortality experienced. Calf mortality, for example, was lower in herds which treated scour cases by administering electrolytes, while milk was offered as normal. Behavioural observations indicate that smaller group sizes could promote expression of positive behaviours, potentially resulting from an overall improvement in welfare. Space allowance per calf was not associated with observed behaviour frequencies. We also identified that similar rates of calf mortality are experienced across herds of different sizes.

Keywords Space allowance, health treatments, hygiene, post-colostrum feeding, transition milk

IMPLICATIONS

This study demonstrates that factors associated with calf welfare on commercial Irish dairy farms (e.g., passive immunity, space allowance, mortality rate) are independent of herd size. Some management practices however, such as methods used for treating health issues can affect rates of calf mortality experienced. Behavioural observations indicate that accommodating calves in groups of 10, or less, could promote expression of positive behaviours, potentially resulting from an overall improvement in welfare.

INTRODUCTION

The dairy industry has undergone considerable changes in the last century, and production intensification has resulted in growing public concerns about health and welfare of animals in the sector (Barkema *et al.*, 2015). Establishing and improving current welfare standards would not only address consumer concerns, but could also enhance production efficiency (Wilson *et al.*, 1997; Warnick *et al.*, 2001). For dairy calves, research has shown that welfare during the pre-weaned period can influence future health and welfare, and hence performance levels in later life (DeNise *et al.*, 1989; Gelsinger *et al.*, 2016). Pre-weaning nutrition and environmental conditions can have a pronounced effect on physiological health of calves, as well as calf behaviour (Brscic *et al.*, 2011; Webb *et al.*, 2013; Leruste *et al.*, 2014). Colostrum, the first milk produced post-parturition, for example, is an essential element of pre-weaning nutrition. It has a significant impact on calf welfare when a sufficient quantity of good quality colostrum (IgG concentration $\geq 50\text{mg/ml}$) is not received quickly after birth (Weaver *et al.*, 2000, Chigerwe *et al.*, 2015). Colostrum contains immunoglobulins (Igs) which can provide the immunologically naïve calf with passive immunity. Transition milk, produced in milking two to six post-parturition (Godden, 2008), also contains Igs. The calf's ability to absorb Igs into the circulatory system thereby achieving passive immunity ceases 24 hours after birth. When transition milk is fed to calves >24 hours old, the Igs therein can however provide local immunity in the gut and reduce susceptibility to enteric pathogens (Berge *et al.*, 2009). Given that enteritis, or diarrhoea, is the most common cause of morbidity in Irish dairy calves (DAFM, 2017), transition milk feeding could contribute to

improved calf health. In Ireland, the recommended milk feeding rate for calves is a volume equivalent to 15 % of bodyweight per day (e.g. 6L per day for a 40 kg calf) (Conneely *et al.*, 2014). Solid feed availability can also influence calf welfare by establishing rumination capabilities, but also reducing stress and weight loss at weaning (Khan *et al.*, 2011). A dairy calf welfare assessment protocol for seasonal calving systems has been developed recently (Barry *et al.*, 2019a), and used to assess colostrum management on commercial dairy farms (Barry *et al.*, 2019b). Limited information, however, is available on transition milk usage, feeding rates applied, as well as the prevalence of enteric pathogens in calves on commercial Irish dairy farms.

In addition, seasonal calving dairy production systems, especially in combination with increased herd sizes resulting from milk quota abolishment in the EU (DAFM, 2015), could create challenges for accommodation and general management of calves, as large numbers of calves are born in a relatively short period of time. This could impact calf welfare, as best practices for calf management often have high labour requirements.

Providing adequate space allowance is important for calf welfare to minimise disease transfer (Svensson and Liberg, 2006), promote locomotor function, but also to facilitate expression of natural behaviours (Jensen *et al.*, 1998). In the EU, providing a minimum space allowance of 1.5 m²/calf is a legal requirement. For expanding herds, failure to install additional housing facilities in-line with increases in cow numbers could result in larger group sizes and overcrowding of calves (space allowance <1.5 m²/calf), which could impact welfare. Svensson and Liberg (2006) reported an increased risk of respiratory disease for calves in larger groups (12-18 calves), relative to that of smaller groups (6-9 calves), however space allowance per calf across all group sizes was not reported. Observation of calf behaviour can provide valuable information on interactions between calves and their environment, as well as the management practices they are subject to (Duve *et al.*, 2012, Rushen and de Passillé, 2012). Play behaviour, for example, is considered an indicator of good welfare (Held and Špinka, 2011; Jensen *et al.*, 1998) and could potentially be used to assess the effect of group size on calf welfare.

Currently limited information is available on pre-weaning management of calves in commercial Irish dairy herds, and the associated effect on calf welfare and mortality.

The objective of this study, therefore, was to investigate associations between pre-weaning calf management and calf welfare and mortality on commercial dairy farms.

MATERIALS AND METHODS

This study was carried out between 4 February and 13 April, 2017, on 47 commercial Irish dairy farms. Ethical approval was received from the Teagasc animal ethics committee (TAEC; TAEC 102/2015), while procedure authorisation (AE191132/P053) was granted by the Health Products Regulatory Authority of Ireland (HPRA). Experiments were undertaken in accordance with the Cruelty to Animals Act (Ireland 1876, as amended by European Communities regulations 2002 and 2005) and the European Community Directive 86/609/EC.

Data collection for the present study was conducted simultaneously to that of Barry *et al.* (2019b), using a welfare assessment protocol developed by Barry *et al.* (2019a). As the materials and methods have been described previously in great detail (Barry *et al.*, 2019a), a more concise description of data collection will be provided for the present study.

Herd selection

To facilitate data collection and sample processing, all enrolled herds were located within an 80 km radius of Teagasc Moorepark (Fermoy, Co. Cork, Ireland). All herds were subscribed to Herdplus®, a central database system which reports on animal and herd related performance, operated by the Irish Cattle Breeding Federation (ICBF) (Bandon, Co. Cork, Ireland). To ensure the cohort of herds in the study was representative of dairy systems nationally (average herd size 80 cows, operating spring-calving (Feb-April) production systems), herds were stratified on system (i.e., spring-calving, pasture-based production) and herd size; cut-off point for herd size was 70 cows.

Suitable herds (n=188) were contacted requesting their participation in the study; outlined in the communication were the background and objectives of the study, as well as feedback each participant would receive. Forty seven herd owners agreed to participate (25 % response rate) and information was collected to arrange suitable visit times (i.e., expected calving start date, specific location information, as well as preferred visit dates).

To assess management practices applied during different stages of the calving season each farm was visited twice, once early in the calving season (weeks 1 to 6 of calving), and once in

the latter half (weeks 6 to 12 of calving). To avoid coinciding with important farm tasks (i.e., milking and calf feeding times), visits were conducted between 10:00 and 16:00 h. Two persons were present for each farm visit, an individual trained and experienced in the assessment of animal welfare, as well as an individual who had received basic training in animal handling, and conducting environmental measurements required for this study.

Feeding equipment hygiene

Hygiene of two feeding implements (e.g., oesophageal tube feeders and group feeders) was physically assessed using 3M™ Clean-Trace surface protein plus test kits (3M™, Minnesota, USA). This is a semi-quantitative assay which provides a result based on the amount of surface proteins present, such as milk residues and biological contaminants (bacteria, fungi). For further details see Barry *et al.* (2019a).

Post-colostrum feeding, health management and faecal sampling

Management practices related to post-colostrum feeding, as well as calf health, were identified by conducting an interview with the principal calf manager, as described by Barry *et al.* (2019b). During the interview, information was collected on the source and number of transition milk feeds provided, post-transition milk feeding, feeding method as well as volume provided and source. Questions were also included on the formation of calf groups, timing of calf health issues, vaccine use, as well as treatment procedure for calf enteritis. Faecal samples were collected to determine the presence of pathogens commonly associated with calf enteritis, such as *E.coli*, *Cryptosporidium parvum*, rotavirus and coronavirus. Random sampling was carried out from any calves seen defecating, using faecal containers (Sarstedt AG & Co, Germany). Samples were frozen (-20°C) upon return to the research centre, where they remained frozen up to the point of analysis using vertical flow immunochromatography test kits (Bio-X Diagnostics S.A, Belgium).

Space allowance, behaviour observations and mortality

Space allowance per calf was determined by measuring the dimensions of the pen area, and recording the number of calves present. Pen dimensions were recorded using a laser distance measure (Spectra Precision QM 95, Nikon & Trimble, Japan). Behaviour observations were

made in-directly using video recordings. We selected two contrasting calf groups (e.g., male and female or older and younger), and recorded pen activity for 1 h. Types and frequencies of behaviours displayed were subsequently identified by scan sampling of these recordings at 5 min intervals (± 30 s). Classification of behaviours was carried out using an ethogram as described by Barry *et al.* (2019a). Information on mortality of calves born in participating herds in 2017 was provided by the ICBF, through the Herdplus® database.

Data editing & statistical analyses

To determine statistical differences for the continuous variables herd size, space allowance, calf mortality and group size, categories were created for these variables using quartiles of the dataset. Enrolled herd sizes were categorised as: < 110, 110-139, 140-189, and ≥ 190 cows. Space allowance was categorised as: < 2.07, 2.08-2.55, 2.56-3.83, and > 3.83 m²/calf. Mortality for 2017 was categorised as: ≤ 5.93 , 5.93-9.14, 9.14-11.35, > 11.35 % of calves deceased within 12 months of birth. Calf group sizes were categorised as: ≤ 7 , 8-10, 11-14, > 14 calves. When used as dependent variables, herd size, space allowance calf mortality and group size remained in the original continuous format. Statistical analyses were conducted using SAS (SAS version 9.4; SAS Institute Inc., Cary, NC). Normality of dependent variables was assessed using PROC UNIVARIATE, and significant associations were confirmed at $P < 0.05$. Mixed model analyses (PROC MIXED) were used to investigate factors associated with the frequency of observed behaviours. Behaviour observations from visit one and two were combined for analysis. This was carried out as observations were not repeated on the same calf groups in both visits; however, herd was included as a random effect. Fixed effects regression models in PROC GLM, as well as mixed models (PROC MIXED) were used to investigate factors affecting calf mortality. Probability of enteric pathogen presence, as well as likelihood of reduced mortality rates where vaccines were administered was analysed using a logistic regression in PROC GENMOD, assuming a binominal distribution of the error term.

RESULTS

Calf mortality rates

Mean mortality rate in participating herds for calves born in 2017 was 9.3 %, and ranged from 2.3 to 26.7 %. When mortality figures experienced within different age time points of participating herds, were compared with total mortality figures (Table 1), it was identified that 64 % died within four weeks of birth. No association was detected ($P = 0.15$) between herd size and mortality rate. Space allowance was also not associated with mortality rates ($P = 0.33$).

Table 1. Number of deceased calves, and cumulative mortality relative to the total mortality, at different time points from birth for all calves born in 2017, across forty seven commercial Irish dairy farms

Time point	Number dead	Cumulative mortality relative to 2017 total mortality (%)
3 days	231	34.3
7 days	305	45.3
28 days	432	64.1
3 months	531	78.8
6 months	601	89.2
12 months	674	100

Group size, space allowance and observed behaviour

For visits one and two, mean space allowance provided was 2.9 (SD 1.53) and 3.1 (SD 1.89) m² per calf, respectively. Mean group size for both visit one and two was 11 (SD 7.2) calves. Behaviour types and frequencies identified through scan sampling video recordings are reported in Table 2. Group size was associated with the frequency of a number of observed behaviours. Calves kept in small size groups (≤ 7 calves) displayed the highest rates of play and grooming behaviour and orally manipulating pen structure. Grooming behaviour as well as scratching/rubbing behaviour was found less frequently among calves in large groups (> 14 calves). No association was found between observed behaviours and space allowance per calf.

Table 2. Frequency of behaviours observed from video recordings of calf groups on the 47 participating herds. Two separate calf groups were recorded on each farm during both the first and second visit.(Standard deviations in parentheses)

Behaviour	Significance		Frequency of behaviour (%) by group size			
	Group size	Space Allowance	≤ 7	8-10	11-14	>14
Lying	NS	NS	69.29	63.28	53.21	60.61
Playing	***	NS	4.34 ^a	2.31 ^b	1.95 ^b	1.52 ^b
Standing	*	NS	19.66 ^a	25.46 ^{ab}	34.48 ^b	29.35 ^b
Eating	NS	NS	7.10	8.47	5.22	7.76
Self-grooming	***	NS	2.88 ^a	2.51 ^a	2.70 ^a	1.22 ^b
Social-grooming	***	NS	1.89 ^a	1.35 ^b	1.57 ^{ab}	0.74 ^c
Orally manipulating pen structure	*	NS	3.26 ^a	1.88 ^b	1.78 ^b	0.97 ^b
Scratching/Rubbing	***	NS	1.60 ^a	1.20 ^b	1.35 ^{ab}	0.69 ^c

*P<0.05 ***P<0.001 NS- Non-Significant

Post-colostrum feeding, health management and faecal samples

Participant responses to post-colostrum feeding and health management practices are outlined in Table 3 and 4. No associations ($P > 0.05$) were identified between calf mortality and post-colostrum feeding practices. On calf health management, calf groups were dynamic on 61.7 % of farms; however, no association ($P > 0.05$) was identified between group formations (i.e., stable or dynamic) and calf mortality. Scour treatment method was associated ($P < 0.05$) with mortality rate, which was lowest (6.8 %) among herds who treated scour cases by administering electrolytes and offer milk as normal. Timing of pneumonia occurrence was associated ($P < 0.05$) with mortality rate, and those experiencing issues with calf pneumonia in weeks 8-10 of the calving season had the highest rate of mortality (18.1 %) in calves during the first 12 months of life. Table 5 shows findings of the faecal sample analyses. No association was identified between faecal sample results (i.e., proportion of samples per farm which test positive for each of the four pathogens) and hygiene practices or calf mortality.

Table 3. Responses from calf managers, across forty seven commercial Irish dairy farms, on post-colostrum feeding practices for male and female calves

Post-colostrum feeding	Description	Responses (%)	
		Bulls	Heifers
Number of transition milk feeds	0	0	2.1
	1-2	8.5	12.8
	3-5	12.8	17.0
	>5	78.7	68.1
Subsequent feed type used	Additional Transition Milk	2.1	2.1
	Whole Milk & Milk Replacer	4.3	4.3
	Whole Milk & Waste Milk	6.4	4.3
	Milk Replacer	4.3	38.3
	Waste Milk	65.9	36.2
	Whole Milk	14.9	12.7
	Yoghurt Milk*	2.1	2.1
Milk volume provided (L/day)	3-4L	10.6	10.6
	4-5L	22.7	25.5
	6L	34.0	38.3
	>6L	26.3	19.2
	Ad-lib	6.4	6.4

*Yoghurt milk refers to whole milk which has had a live bacteria culture added and allowed to ferment for a period before being fed to calves.

Table 4. Responses from calf managers across forty seven commercial Irish dairy farms on calf health management practices and the occurrence of calf health issues during the calving season

Health Information	Description	Responses (%)
Formation of calf groups	All in/ All out	38.3
	Dynamic	61.7
Sick calf housing	Isolated	47.8
	Remain in-situ	52.2
Timing calf health issues arise during calving season	2-4 weeks	19.6
	5-7 weeks	67.4
	8-10 weeks	13.0
Timing scour issues occur during calving season	2-4 weeks	19.2
	5-7 weeks	65.9
	8-10 weeks	14.9
Timing pneumonia issues occur during calving season	<2 weeks	5.6
	2-4 weeks	11.1
	5-7 weeks	72.2
	8-10 weeks	11.1
Scour treatment method	Electrolytes only	19.2
	Milk + antibiotics	2.1
	Milk + electrolytes	44.7
	Milk + electrolytes + antibiotics	2.1
	Reduced milk volume + electrolytes	29.8
	Reduced milk volume + electrolytes + antibiotics	2.1
Calf scour vaccine use	Yes	42.0
	No	58.0
Calf pneumonia vaccine use	Yes	21.0
	No	79.0

Table 5. Number of faecal samples collected from calves across forty seven commercial Irish dairy farms during the first (visit one) and last six weeks (visit two) of the calving season, and the number of samples which tested positive and negative for rotavirus, coronavirus, *E.coli* and *Cryptosporidium parvum*, respectively

Visit	Total Samples		Rotavirus	Coronavirus	<i>E.coli</i>	<i>C. parvum</i>
1	163					
		Positive	24	1	6	66
		Negative	139	162	157	97
2	171					
		Positive	30	1	18	103
		Negative	141	170	153	68

Weaning and calf sales

Participant responses for practices related to calf weaning and calf sales are outlined in Table 6. When weaning calves (i.e., removing milk from the diet), the majority of participants did so based on age, most commonly between six and eight weeks of age. Participants generally reported applying a gradual weaning process, most commonly over a period of between four and seven days. When offering calves for sale, the majority of participants described calves as being between two and four weeks of age when sold. No association was detected between calf mortality and weaning practices, or the age calves were sold at.

Table 6. Responses from calf managers on calf weaning practices and calf sales across forty seven commercial Irish dairy farms.

Weaning and sales information	Description	Responses (%)
Weaning metric	Age	55.3
	Concentrate intake	8.5
	Visually (size)	17.0
	Weight	19.2
If weaning by age, preferred weaning age	< 6 weeks	4.3
	6-8 weeks	47.8
	8-10 weeks	13.0
	> 10 weeks	34.7
Weaning method	Gradual	85.2
	Abrupt	14.8
Weaning period length	≤ 4 days	26.2
	>4 days ≤ 7 days	50.0
	> 7 days	23.8
Sale age of dairy bulls	0-2 weeks	34.1
	2-4 weeks	54.5
	> 4 weeks	11.4
Sale age of beef bulls	0-2 weeks	25.0
	2-4 weeks	52.5
	> 4 weeks	22.5
Sale age of beef heifers	0-2 weeks	24.4
	2-4 weeks	53.6
	> 4 weeks	22.0

DISCUSSION

Large variation existed in mortality rates of participating herds, with the majority (> 60 %) of mortality cases occurring in the first four weeks of life. Failure to identify an association between herd size and mortality rate is not unexpected as numerous factors influence mortality. Some of these factors are herd health status, managerial skill levels, facilities, rate of expansion and stockperson-to-animal ratio (Barkema *et al.*, 2015). Previous research indicates that there is no consistent relationship between herd size and mortality. Gulliksen *et al.* (2009) and Del Río *et al.* (2007), for example, identified a positive association, while Jago and Berry (2011) and Raboisson *et al.* (2013) reported negative associations between herd size and calf mortality. Findings from the present study indicate herd size is not a determining factor for calf mortality on Irish dairy farms, as similar rates of calf mortality were experienced across different herd sizes.

Space allowance provided was almost twice that of the legal requirement in both the first and second visit. The increase in space allowance between visit one and two could be attributed to a reduction in calf numbers on-farm due to the sale of male dairy calves, as well as male and female crossbreeds with a beef breed, such as Hereford or Aberdeen Angus. Having served the required number of cows to dairy sires, beef sires are then used to produce higher value calves, resulting in crossbred calves being present on dairy farms. Failure to detect associations between observed behaviours and space allowance suggests that space availability does not impair behavioural expression. In previous studies, however, effects of space allowance on behaviour were found. Jensen *et al.* (1998), for example, reported higher levels of play behaviour among calves kept at 4 and 3 m² per calf, compared with calves kept at 2.2 and 1.5 m² per calf. The study by Jensen *et al.* (1998) did observe the same calf groups on three different occasions for a total of 56 h. Conclusions which can be made from single, short observation periods, as applied in the current study, however, are limited (Barry *et al.*, 2019a). Positive behaviours, such as playing, are displayed at low frequencies, at specific times (e.g. shortly after feeding) (Dannenmann *et al.*, 1985), and resultantly the level of positive behaviour observed will be determined by the length of the observation period. Given the short observation period applied in the present study, detection of behavioural differences may only have been identified when large differences in space allowance existed.

No association was found between space allowance and calf mortality. Although space allowance has been shown to influence health (Mogensen *et al.*, 1997; 1.5 v 3 m²/calf) and

performance (Tapkı *et al.*, 2006; 1.5, 3 & 4 m²/calf), no definitive links between space allowance and calf mortality have been reported previously. Negative implications of low space allowance (<1.5 m²/calf) therefore, may not directly translate to higher mortality, however, its effect on health and behaviour is clearly demonstrated in the literature. The high space allowance provided in some participating herds, could contribute to good health and behaviour, and therefore have a positive influence on calf welfare.

Behaviours associated with positive welfare (e.g., playing, social-grooming) were more commonly displayed in smaller groups. This suggests that welfare, but also social development is enhanced when calves are accommodated in smaller size groups, with adequate space allowance. Health and growth are parameters which can be reflective of welfare, and a study by Svensson and Liberg (2006) concluded that housing calves in groups of <10 is preferable. Based on behaviour observations, this recommendation could be reiterated by the present study. Although displayed less frequently, positive behaviours were also witnessed among larger groups (>10 calves). Positive behaviours are accepted as a reflection of good welfare status, however, it is unknown if increased rates of positive behaviour are directly proportional to improved welfare status. Oral manipulation of the pen structure furthermore is considered an abnormal behaviour when shown extensively, and is used as coping mechanism when welfare is compromised (Broom and Fraser, 2007). Noteworthy, this behaviour was also more frequently observed in smaller group sizes in the present study. While potentially reflective of compromised welfare, this behaviour is generally attributed to management (i.e., feeding levels; Webb *et al.*, 2013) and less likely to the number of calves present in a group. Based on participant responses, feeding levels identified in the present study are sub-optimum with 34.7 % (average responses for male and female feeding rate) of participants providing calves with ≤ 5 L of milk per day. This low feeding level could be contributing to the establishment of abnormal behaviours; however, the length of the observation period limits the conclusions which can be made from frequencies of displayed behaviours.

In this study no associations were observed between post-colostrum feeding practices and calf mortality rates. Feeding transition milk was commonly practiced by participants, with approximately 73 % of calves receiving ≥ 5 feeds of transition milk. While Igs present in transition milk will not enter the circulatory system of calves more than 24 h old (Weaver *et al.*, 2000), health benefits have been reported when multiple feeds are provided to calves (Conneely *et al.*, 2014). This indicates that good colostrum management practices applied on

Irish dairy farms (Barry *et al.*, 2019a) could be limiting any negative effects which post-colostrum feeding practices could have on calf mortality.

Over half of participants feed waste milk (non-saleable) to their calves, a practice which has been shown to contribute to the development of antimicrobial resistance, when antibiotic residues are present (Aust *et al.*, 2013; Maynou *et al.*, 2017). While this practice is not prohibited and also reported in other countries (e.g. in the UK, Brunton *et al.*, 2012), its contribution to antimicrobial resistance on-farm should be communicated to herd owners in an effort to reduce the frequency of application within the national herd.

Enteritis, or scour, remains one of the main health issues experienced in pre-weaned dairy calves, and in this study an association was identified between scour treatment method and mortality rates. This not only highlights the continued prevalence of scour in calves, but also the importance of providing correct health treatments. Where participants treated calves with scour by administering electrolytes while continuing to offer milk, lower rates of mortality were experienced. Scour causes dehydration, an electrolyte imbalance but also metabolic acidosis (Smith and Berchtold, 2014). Recommended treatment consists of administering a suitable oral rehydration solution (Sayers *et al.*, 2016), while continuing to offer milk, to provide an energy source. Failing to provide electrolytes, but also withholding milk, will impede the recovery of calves with scour, and as identified in the present study, will ultimately contribute to higher mortality rates.

Pneumonia, or bovine respiratory disease, is one of the most prevalent diseases in calves, and in the present study an association was found between responses for timing of pneumonia occurrence, and mortality rate in calves during the first 12 months of life. Weaning, the process of removing milk from the calves' diet, generally occurs between six and eight weeks of age. In seasonal-calving systems, weaning is often followed immediately by turn-out to pasture, at which point calves enter larger groups than they would have been present in during the milk feeding period. Stress associated with weaning has been widely documented, and could potentially be further compounded if weaning coincides with turn-out to pasture, which generally coincides with introduction to unfamiliar calves. This culmination of events, and associated stress levels experienced, could predispose calves to respiratory infections, ultimately resulting in increased rates of mortality. Similar associations have been identified previously. Closs & Dechow (2017), for example, reported greater survival rates in calves which were not treated for pneumonia in the first six months of life. To minimise infectious

disease at weaning, a gradual process of weaning must be applied, and where possible should not coincide with other stress inducing practices, such as turn-out to pasture or dis-budding.

No association was identified between vaccine use, against scour or pneumonia, and mortality rate. Vaccine use in isolation will not eliminate health issues, or reduce rates of mortality, but instead must be used as part of a concerted effort to improve animal health. Such an effort must include measures to reduce infection pressure, as well as maximising levels of immunity (e.g. good colostrum management, minimising stress etc.). Studies investigating pneumonia vaccine use and mortality in US herds reported no association (Windeyer *et al.*, 2012), while Waltner-Toews *et al.* (1985) also found no association between scour vaccine use and calf mortality.

CONCLUSIONS

Post-colostrum feeding practices on commercial Irish dairy farms were not associated with calf mortality rates. Space allowance provided to calves is almost twice that of the minimum legal requirement and was not found to affect calf behaviour. Group size, however, was found to influence calf behaviour, and could potentially influence calf welfare in early life. Hygiene practices were not associated with the presence of faecal pathogens. Vaccine use was not associated with calf mortality on commercial Irish dairy farms. Correctly treating diarrheic calves, by administering an electrolyte solution while continuing to offer milk, is of critical importance, and is associated with calf mortality on Irish dairy farms.

Declaration of interest

The authors have no conflict of interest to declare.

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Chapter 6

General discussion

INTRODUCTION

Dairy calves are born without immunity due to inhibited transfer of immunity in-utero, and must acquire passive immunity from colostrum, produced by the cow immediately post-parturition (Godden, 2008). Failure to receive adequate transfer of passive immunity (TPI) can negatively impact calf welfare through an increased risk of morbidity or mortality (Donovan et al., 1986, Wittum and Perino, 1995). While substantial research has been conducted on factors affecting colostrum quality (Gulliksen et al., 2008, Conneely et al., 2013, Lombard et al., 2016) and TPI (Donovan et al., 1986, Hopkins and Quigley, 1997, Beam et al., 2009), limited information is available on the effect of the source of colostrum fed (single dam or pooled) as well as subsequent maternal antibody survival. Furthermore, little is known about colostrum management on commercial Irish dairy farms. Seasonal calving systems, targeting a compact calving spread, predominate in Ireland (Berry et al., 2013). The large numbers of calves being born in such systems in a short period of time can create challenges for the management in terms of cow and calf care. These challenges include supervising large numbers of cows calving simultaneously, ensuring each calf receives a sufficient quantity of colostrum immediately after birth, while also identifying and treating morbidity cases within large groups of calves. If these challenges are not overcome due to work pressure, calves run the risk of impaired welfare. Removal of the European milk quota system in 2015 has resulted in expansion among Irish dairy herds, which could further add (more calvings, more calves) to the existing challenges associated with calf welfare faced by seasonal calving systems.

The main aims of this thesis, therefore, were to evaluate the effect of colostrum quality and management on calf health and immunity, and to assess calf welfare on commercial Irish dairy farms operating seasonal calving systems. Prior to the assessment of calf welfare at farm level, a protocol was developed which also includes an assessment of management practices and environmental conditions under which calves are being reared. In this chapter, I will first discuss the impact of colostrum quality and management on calf health and immunity. Next, existing calf welfare on commercial Irish dairy farms and potential improvement options will be addressed. Finally, the main conclusions of this thesis are given.

The impact of colostrum quality and management on calf health and immunity

Good quality colostrum has an immunoglobulin (IgG) concentration of ≥ 50 mg/ml, a value that is required to achieve adequate TPI (Weaver et al., 2000, McGuirk and Collins, 2004). On commercial Irish dairy farms, a high mean colostrum IgG concentration of 85 (SD 51) mg/ml was identified. Similarly, a high mean colostrum IgG concentration of 71 and 96 mg/ml were identified in the experiment in 2016 and in 2018. While mean IgG concentration in both the commercial and experimental settings (Chapter 2 and Chapter 4) are indicative of good quality colostrum, large variation existed both between and within-herds, but also within a herd between different years. Twenty-one percent of colostrum samples collected on commercial farms, for example, had an IgG concentration of less than 50 mg/ml (Chapter 4). Research shows that variation in colostrum quality is inherent due to the large number of influencing factors, such as volume of colostrum produced, timing of collection from the cow, breeding (Moore et al., 2005, Conneely et al., 2013) and pre-partum diet (Mann et al., 2016). Le Cozler et al. (2016) even described how significant variation was identified in IgG concentration of colostrum produced in the hind quarter teats relative to the front quarter teats in Holstein dairy cows. To overcome negative implications of such variation, overall quality of colostrum should be assessed prior to feeding, using for example a brix refractometer (Silva-del-Río et al., 2017, Rayburn et al., 2019). In both years of the experiment (Chapter 2), all colostrum was screened using a brix refractometer prior to feeding, and as result only colostrum with an IgG concentration of > 50 mg/ml was fed. Mean 24 h calf serum IgG concentrations were 32.5 (SD 8.7) and 59.5 (SD 19.1) mg/ml, in 2016 and 2018 respectively, and all sampled calves had serum IgG concentrations of > 10 mg/ml, which indicated that adequate TPI was achieved (Weaver et al., 2000, Beam et al., 2009, Stilwell and Carvalho, 2011). Of all calves (n=543) blood sampled on commercial farms, 8.6 % had serum IgG concentrations of < 10 mg/ml (Chapter 4). On the same commercial farms, however, just 15 % of farmers were measuring colostrum quality (Chapter 4), and across all farms visited a mean colostrum IgG concentration of 85.1 (SD=51.6) mg/ml was identified. While mean colostrum quality was similar in both the experiment and on commercial farms, the low level of colostrum screening at farm level could be an important factor contributing to the occurrence of failed TPI, predisposing calves to an increased risk of morbidity and mortality.

The rate of mortality on commercial farms was numerically, but not statistically, greater among calves with failed TPI, relative to calves which had adequate TPI (Chapter 4).

In addition to colostrum quality, delayed timing from birth to receiving colostrum can also increase the risk of failed TPI (Morin et al., 1997), which can ultimately result in increased rates of mortality. During labour intensive periods of the calving season the ability of farmers to provide calves with colostrum in a timely manner could be challenged as large numbers of cows calve simultaneously. Currently on commercial dairy farms, over 75 % of calves receive colostrum within 2 h of birth (Chapter 4). This indicates that while the importance of measuring colostrum quality is not yet fully appreciated among farmers, providing colostrum immediately after birth is a factor which farmers understand has implications for calf health. In Norway, Gulliksen et al. (2009) described how 68 % of dairy farmers provided colostrum to calves within 2 h of birth. These farms, however, did not operate seasonal calving systems but instead have year round calving. Given the high labour requirement during the calving period on seasonal calving farms, for timing of colostrum feeding, Irish dairy farmers are achieving high standards which surpass that achieved in countries where year round calving systems operate; however, further improvements can be made to ensure all calves receive adequate TPI.

Another factor which could potentially impact on passive immunity is the source from which colostrum is fed. Ideally colostrum from the calf's dam should be provided, once it is of sufficient quality. Failure to test colostrum quality coupled with feeding colostrum from a source other than the calf's dam, which could occur during labour intensive periods, could increase the risk of failed TPI. The nature of seasonal dairy production systems creates a high labour requirement during calving, as large numbers of cows are calving simultaneously and the resultant calves have a high care requirement. In an effort to reduce the labour requirement associated with feeding new-born calves, farmers have deviated from the gold standard of feeding the calf colostrum from their own dam; with over 30 % of farmers now providing colostrum which has been pooled from a number of cows (Chapter 4). While biosecurity risks associated with this practice have previously been identified (Kennedy et al., 2014), experimental evidence now exists that compared to feeding colostrum from a single cow, pooled colostrum feeding resulted in a reduced serum IgG concentration (Chapter 2). It is important to note that in the experimental situation, high hygiene standards were applied when collecting colostrum, screening of colostrum quality was conducted, and correct colostrum storage conditions were adhered to (Cummins et al., 2017). Given the previously

stated low rate of application of colostrum screening, it is unlikely that the same hygiene and colostrum storage procedures would be adhered to on commercial farms. The associated impact of pooling colostrum on calf serum IgG concentrations could therefore be greater on commercial farms than witnessed under controlled conditions of an experiment.

Management of colostrum provision can have a profound effect on calf welfare, and data suggests management practices could potentially differ for calves based on their economic value (e.g., male and female dairy calves) (AIM, 2015, 16) or based on herd size (Lance et al., 1992, Gulliksen et al., 2009). Calf serum IgG concentrations on commercial Irish dairy farms indicate that similar rates of adequate TPI are achieved among male and female calves, and across different herd sizes (Chapter 4). While this in itself is a positive finding for dairy calves, it should not be taken as a definitive indication of welfare status. For this to be obtained, a more comprehensive approach is required to assess calf welfare on commercial dairy farms.

Calf welfare on commercial dairy farms and options for improvement

Amongst other factors, calf welfare on commercial Irish dairy farms could potentially be compromised by colostrum management practices (Cummins et al., 2016), but also by herd expansion arising from the removal of the milk quota system (Dillon, 2011). To protect and improve calf welfare, the current status and influencing factors had to be first assessed.

For this, a calf welfare assessment protocol was developed to suit commercial Irish dairy farms with seasonal calving (Chapter 3). Following a review of existing literature, measurements were selected on a number of welfare parameters, which were combined to produce a reliable and feasible protocol. As the protocol was composed of previously validated measurements, and applied by trained individuals, it could consistently produce an accurate assessment of welfare, as identified through its application in a pilot study (Chapter 3).

From the on-farm assessments we found that space allowance provided on Irish dairy farms was 3 m² per calf, which is twice that of the EU minimum requirement of 1.5 m² (Chapter 5). Where adequate space allowance has been provided, the number of calves per group could also be of importance for welfare. In Chapter 4, for example, we found higher rates of play

behaviour among smaller size calf groups, which supports the hypothesised relationship between group size and welfare. While no typical behaviour patterns were found among larger group sizes to suggest a negative impact on welfare, smaller group sizes could contribute to enhanced calf welfare. In addition to addressing individual space requirements, social contact and functional areas, such as designated spaces to rest, to feed and for activity are valuable for calves (Newberry, 1995, Bokkers et al., 2011, Mandel et al., 2016). The dairy sector must aim to optimise conditions under which calves are reared through continued research and the use of technological developments. If the Irish dairy sector is to take a progressive approach and aims to promote positive calf welfare, rather than the traditional approach of simply limiting any negative impact on welfare, optimising colostrum management and housing conditions are methods of doing so. This, however, would not be without challenges as colostrum management is dependent on labour pressure, and changes to the structural design of calf pens would require financial investments. Also, further research is required on the interactions between calf age, space allowance and group size, as even within seasonal calving systems, large variation in calf age within groups can exist, which has implications for disease transfer as well as competition for resources (e.g. feed, water, lying place) between calves (e.g., older versus younger, large versus small).

We found no association between herd size and calf mortality in Irish dairy herds (Chapter 5). The results, however, have to be interpreted with care. Reported figures for mortality can be subject to manipulation through underreporting at farm level or can be ambiguous about what is exactly included. Furthermore, in situations where high standards of welfare are achieved, an outbreak of infectious disease can result suddenly in an elevated mortality rate. Alternatively, situations can arise where welfare is severely compromised and mortality rates remain relatively low. This for example was the case with battery cages for laying hens, which are now banned in the EU. These cages offer a barren environment with restrictive space per bird resulting in compromised welfare. The mortality rates, however, appeared to be lower compared to other egg production system types (e.g., litter based housing or free-range systems) (Mollenhorst et al., 2006, Fossum et al., 2009). Mortality figures must therefore be viewed over a number of years and not taken in isolation when used in the context of welfare estimates. A broader assessment which examines animals, but also management and environmental conditions, is required.

While our results (Chapter 4 and 5) indicate that expansion among Irish herds has not resulted in an increase in calf mortality, the relationship with welfare in general is less clear.

Large herd size has been reported as a risk factor to welfare (Lance et al., 1992, Gulliksen et al., 2009) whereas others suggest that increased herd size can often result in improved animal welfare (Whitaker et al., 2004, Jago and Berry, 2011, Chapinal et al., 2013). The latter argue that welfare can be higher on larger farms through improved facilities, increased use of technological aids, continuous staff development in the area of animal husbandry, as well as a reduction in the stockperson to animal ratio. While some of these developments would be more accessible for large herds, due to economies of scale, these are not exclusive to expanding or large herds. Future research in this area must monitor developments in herd size and animal welfare, and identify practical methods of facilitating welfare improvements where a herd enters a significant phase of expansion.

Health treatments and hygiene practices have been cited as areas which require improvement on Irish dairy farms (Chapter 4 and 5). Providing correct health treatments during the early onset of diseases results in better outcomes (Sayers et al., 2016), while good hygiene standards can prevent the spread of infectious disease. The provision of incorrect treatments is resulting in increased calf mortality at farm level (Chapter 5). This must be addressed through training and advisory programmes to ensure farmers and all stockpersons are educated on the correct administration of health treatments. Such training programmes would also prove valuable as the dairy sector continues to address the issue of antimicrobial resistance (Duse et al., 2015). To provide the correct treatment, morbidity must first be identified and the cause correctly diagnosed. Limited information is currently available on sensitivity and specificity of calf disease identification among dairy farmers, and this should be addressed in future studies. For the dairy sector this is an issue which must be addressed to combat antimicrobial resistance and safeguard human health, but also to ensure safety and security in the food chain.

The aforementioned results were obtained using a protocol developed specifically to assess welfare within restricted financial resources and time availability. On the one hand, this is a distinct strength of the developed protocol (Chapter 3), but on the other hand, this also created constraints. Behaviour observations, for example, taken for a period of 1 h cannot capture the full behavioural repertoire of animals, let alone the variation and patterns over the day. Positive behaviours, such as playing, tend to be displayed during certain times of the day, for example in the morning or evening, but also immediately after feeding for group fed calves (Jensen et al., 1998). Also, when observing group housed calves on commercial farms the age of calves within and between groups can vary, and must be accounted for in any

subsequent analysis. This however can prove difficult as the normal behaviour pattern of calves relative to exact age is not yet fully known. Another important piece of information that is yet to be fully determined is the expected behavioural pattern of calves experiencing different health issues (e.g., enteritis, respiratory diseases, navel ill etc.), but also during different stages of infection. Presumably, the reason such detailed information has not been collected previously is that it requires constant human observation of a large number of calves over a prolonged period, as well as an in-depth analysis of behaviour and health data over time. In reality, such observations can only be achieved using modern technology. The use of accelerometers in the field of animal observations, for example, could provide a feasible method of measuring activity over long periods, and eliminate the need for some of the direct observations (Leos-Barajas et al., 2017). Wearable technology has also been used to measure rumination activity in pre-weaned calves, and could provide valuable information on feeding behaviours, rumen development as well as early detection of disease (Lopreiato et al., 2018).

An important issue to address, as the development of behaviour measuring technology continues, is what exactly constitutes 'normal' behaviour. Is this the behaviour of a calf which remains with its dam, and up to what age? Or is it the behaviour of a calf which is removed from its dam immediately after birth? Is it the behaviour of calves reared indoors or at pasture? Defining 'normal' behaviour will most likely be the greatest challenge faced by technological developers as this differs based on system type, geographical region, climatic conditions, culture as well as numerous other factors. Also, in many cases a deviation from 'normal' behaviour may occur, however, identifying the point at which this deviation has become a negative, or abnormal behaviour, could prove challenging. Correct interpretation of behaviour data could reduce rates of morbidity and mortality through early detection of disease (van Dixhoorn et al., 2018), remote monitoring of animals (Weary et al., 2009) and enhanced feeding programmes that improve performance while satisfy the needs of the animal (Hepola, 2003). While there are many challenges, these are minor when the potential benefits to animal welfare, agricultural systems internationally, as well as improved food safety for consumers are considered.

Another technological development more specific to calf rearing is the use of automatic milk feeding systems, which are expected to become more common on commercial dairy farms in the future (Medrano-Galarza et al., 2017). These systems not only reduce the labour requirement for calf feeding, but also provide a more natural feeding regime whereby calves

receive a number of milk feeds spread throughout the day (Medrano-Galarza et al., 2017). It has also been identified that such feeding systems can provide information on a number of feeding parameters which could be used to identify calves in the early stages of a health issue. These parameters include milk volume consumed per day, drinking speed but also number of rewarded and unrewarded visits to the feeding station (Svensson and Liberg, 2006, Knauer et al., 2017, Sutherland et al., 2018). This is a development that would be particularly beneficial in seasonal calving systems, as it would not only serve to address the high labour requirement associated with feeding large numbers of calves, but would also serve to improve calf welfare through early identification of health issues which would allow for more successful treatment outcomes. Future research however is required in this area to determine behaviour thresholds for the identification of specific diseases, but also to determine how data produced from different sensors (e.g., accelerometers, feeding stations, body temperature loggers, calf rumination monitors etc.) can be combined to provide accurate information on calf welfare. While many of these sensor derived measurements have value in relation to welfare, it is only when they are used in combination that the true benefit is realised. For example, automatic calf feeding stations fitted with 3 dimension capable cameras can monitor feeding behaviour and physical performance (i.e., bodyweight) (Song et al., 2014). Another example would be the combined use of body temperature loggers and accelerometers to track movement/behaviour. Fluctuations in movement/behaviour or body temperature in isolation would not necessarily indicate a health issue, however a simultaneous change in both parameters would warrant investigation.

Internationally, the use of technology to assess and improve animal welfare is relatively new and is under high attention from research institutes and commercial companies. The success of such developments will ultimately depend on successful collaboration between all relevant stakeholders, such as technological developers, experts in the areas of animal welfare, ethologists, veterinarians, farmers as well as consumer representatives. Many innovations are expected to emerge from this area in the future, and with successful collaboration and development could enhance animal-based systems of production internationally and the welfare of animals experiencing such systems.

Main conclusions

- Pooled colostrum feeding, compared to that of a single dam, was negatively associated with calf serum immunoglobulin G concentration at 24 hours. The risk of failed transfer of passive immunity can be overcome by assessing colostrum quality and only using colostrum with an immunoglobulin G concentration greater than or equal to 50 mg/ml.
- Using previously validated measurements, a welfare assessment protocol for dairy calves was developed, and could be applied in seasonal calving systems, while also differentiating between male and female calves.
- Colostrum quality is high among Irish dairy herds, however large variation exists and could be a potential risk factor to calf health as less than 15 % of farmers are measuring colostrum quality before feeding.
- Mean serum immunoglobulin G concentration at 24 hours was higher among calves born in smaller herds (less than 110 cows). The mean 24 hour calf serum immunoglobulin G concentration across all herd sizes, however, exceeded the threshold (greater than or equal to 10 mg/ml) for transfer of passive immunity.
- Providing correct health treatments was associated with calf mortality, and on farms where the recommended protocol when treating calves with diarrhoea was followed, reduced rates of calf mortality were experienced.
- Post-colostrum feeding practices were not associated with calf mortality on commercial dairy farms.
- Space allowance on Irish dairy farms, which was twice that of the minimum legal requirement (1.5 m²), was not associated with observed behaviours. Group size, however, was negatively associated with play behaviour frequency, and could potentially influence calf welfare in early life.

Summary

Dairy calf welfare is a complex status that is influenced by a wide range of factors. Inhibited transfer of immunity in-utero results in calves being born without immunity, which must be acquired passively through ingestion of colostrum immediately after birth. Management of colostrum is therefore a factor of significant importance to calf welfare, and when managed incorrectly can predispose calves to increased risk of morbidity and mortality. In Ireland, previous studies have described colostrum management as sub-optimum. In the intervening period, the landscape of the Irish dairy sector has changed substantially. Following removal of the European milk quota system in 2015, Irish dairy herds have expanded in an effort to increase production and profitability. This expansion coupled with pre-existing standards of colostrum management, which have been described as sub-optimum, could negatively impact on calf welfare, and particularly so given the short calving period which is applied among seasonal calving systems which operate in Ireland.

The aim of this thesis was to i) evaluate the effect of colostrum quality and management on calf health and immunity, and ii) to assess calf welfare on commercial farms and identify improvement options.

Pooled colostrum feeding is commonly practised on commercial dairy farms and could present a risk to passive immunity levels, while also facilitating the spread of infectious diseases within a herd. In **Chapter 2**, the effect of feeding single dam and pooled colostrum on calf passive immunity was assessed in a controlled experiment. In total, 119 calves were enrolled in the study. Calves were blood sampled immediately after birth and received either own dam, other dam or pooled colostrum. Calves were blood sampled again at 24 h to assess serum immunoglobulin G (IgG) concentrations. The results showed that calves which received pooled colostrum had reduced absorption efficiency for IgG as well as lower 24 h serum IgG concentrations, compared to calves fed single dam colostrum. A hypothesis for the lower 24 h serum IgG concentration among calves fed pooled colostrum could be that pooling colostrum has a negative impact on the concentration of leukocytes in colostrum or absorption of leukocytes, which in turn reduces the rate of Ig absorption by the calf. Further investigation is warranted to identify the exact processes which affect transfer of passive immunity among calves when feeding pooled colostrum. We also found calves which had a high birth bodyweight (> 30 kg) had a lower serum IgG concentration at 24 h than calves

with a lower birth bodyweight. This could potentially be occurring as high birth bodyweight is associated with dystocia, or calving difficulty, which is associated with reduced antibody absorption in calves. Calf vigour could also be a factor; however, further research is required to investigate associations between antibody absorption and calving difficulty and calf vigour, among dairy calves.

Expansion among dairy herds, but particularly in seasonal calving systems, could potentially have an impact on calf welfare. To protect and improve dairy calf welfare in seasonal calving systems welfare assessment protocols are necessary, and no such protocol has been previously developed. In **Chapter 3**, a welfare assessment protocol for (non-veal) dairy calves which allows for risk factor identification yet is feasible, time efficient and reliable was developed and a pilot study carried out. To this end, the protocol had to combine animal-based indicators (measurements on physiology, general appearance, and behaviour) providing the basis for welfare assessment, with resource-based indicators (measurements on management and the environment) providing the basis for identifying risk factors. Indicators, both animal and resource-based, were selected following a literature review and a process of expert consultation. Once the protocol was developed a pilot study was conducted on five Irish dairy farms in December 2016. During the pilot study the protocol was critically assessed, and modifications were made accordingly. Following completion of the pilot study, a robust, feasible, time efficient and reliable protocol was produced. With this protocol the welfare of calves on dairy farms can be assessed and when applied on a sufficient number of farms a risk factor analysis can be performed. The protocol was developed and piloted in Ireland, where seasonal calving systems operate. With minor alterations, however, the protocol would be suitable for international application.

Calves are particularly vulnerable to welfare issues as they are both physically and immunologically weak, and experience high rates of morbidity and mortality. On commercial farms, poor colostrum quality or substandard management, combined with poor calf related hygiene can increase disease susceptibility, culminating in a negative impact on calf welfare. In **Chapter 4**, colostrum quality and management, but also hygiene practices, on forty seven commercial Irish dairy farms was assessed, both in the first and last six weeks of the calving season. Findings indicate colostrum management on Irish dairy farms is of a high standard, with over 90 % of calves receiving adequate transfer of passive immunity. While mean calf serum IgG concentration was higher among smaller size herds, the mean calf serum IgG concentration across all herd sizes exceeded the threshold (>10 mg/ml IgG) for adequate

passive immunity. Colostrum quality in Irish dairy herds was good; however large variation exists, both within and between herds. Measuring colostrum quality would allow farmers overcome such variation; however assessment of colostrum quality prior to feeding is practised by fewer than 15 % of farmers. Hygiene standards for calf feeding equipment can be improved overall, and particularly during the final six weeks of the calving season. This would reduce the risk of bacterial contamination of colostrum, as well as disease transfer, and could further increase the rate of passive immunity achieved on commercial dairy farms.

Pre-weaning nutrition and environmental conditions can have a pronounced effect on physiological health of calves, as well as on calf behaviour. Following the removal of milk quota systems, expansion at farm level could result in changes to management, and ultimately the welfare of pre-weaned dairy calves. To protect and improve calf welfare an assessment of current management practices surrounding pre-weaned calves, and associated effects on calf welfare and mortality, on commercial dairy farms is warranted. In **Chapter 5**, therefore, associations between pre-weaning calf management and calf welfare and mortality on commercial dairy farms were investigated. An environmental assessment of conditions under which calves were accommodated included measurements of space allowance per calf, as well as general hygiene. Video observations were used to assess calf behaviour, while clinical health status of calves was determined by visual assessment. Faecal samples were also collected to determine the presence of enteric pathogens among calves. To establish if changes to management practices, space allowance, as well as calf health occur as the calving season progresses, each farm was visited twice. Calf mortality was not associated with either herd size, space allowance per calf or post-colostrum feeding practices. Higher calf mortality was identified among herds which reported experiencing an on-set of calf pneumonia during weeks 8-10 of the calving season. This study demonstrates that factors associated with calf welfare on commercial Irish dairy farms (e.g., passive immunity, space allowance, mortality rate) are independent of herd size. Some management practices however, such as methods used for treating health issues can affect rates of calf mortality experienced. Calf mortality, for example, was lower in herds which treated scour cases by administering electrolytes, while milk was offered as normal. Behavioural observations indicate that smaller group sizes could promote expression of positive behaviours, potentially resulting from an overall improvement in welfare. Space allowance per calf was not associated with observed behaviour frequencies. It was also identified that calf mortality was not associated with herd size.

In **Chapter 6**, I reflect on the impact of colostrum quality and management on calf health and immunity. The quality of colostrum produced on commercial Irish dairy farms is high, and could potentially be compensating for shortcomings in the area of colostrum management (i.e., timing and volume of feeding). High levels of adequate transfer of passive immunity were identified among male and female dairy calves, which indicate colostrum management practices do not deviate based on calf sex. This is a positive finding for the dairy sector and demonstrates that calves of low economic value are managed similarly to their higher value counterparts. Observation and assessment of animal behaviour can prove valuable when aiming to assess and improve calf welfare. In the past, however, this has proven difficult as there is a large time and financial burden associated with such measurement. Technological developments could present a breakthrough in the field of animal welfare, and can allow for early identification of issue which could negatively impact calf welfare.

In conclusion, improvements in the area of colostrum management, as described in this thesis, combined with regular assessment of calf welfare, using reliable protocols, can provide the future basis for enhanced calf welfare on commercial dairy farms.

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About the author

John Barry was born in Limerick, Ireland, and grew up on his parent's dairy farm. He obtained his Bachelor degree in Agricultural Science at Waterford Institute of Technology in 2015.

After graduation he proceeded as a Walsh Fellowship PhD student, working in conjunction with Teagasc Moorepark and the Animal Production System Group at Wageningen University. His research has focused on accurately assessing dairy calf welfare and identifying ways by which calf welfare can be improved in the future. He has presented his research at international conference in countries such as Canada and France, and has attended numerous courses in both Ireland and the Netherlands.

PUBLICATIONS

Refereed scientific journals

Barry, J., Bokkers, E. A. M., De Boer, I. J. M. and E. Kennedy. 2019. Associations between colostrum management, passive immunity, calf-related hygiene practises and rates of mortality in pre-weaned dairy calves. *Journal of Dairy Science*. 10;10266-10267.

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Fennell, R. and **J, Barry**. 2019. Optimising immunity in dairy-beef calves. Dairy Beef 2019. Tuesday 21st May, 2019. Available at; <https://www.teagasc.ie/media/website/publications/2019/Dairy-Beef-2019-Booklet.pdf>

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Education certificate

Completed training and supervision plan¹

The basic package (3 ECTS)

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- Course ‘Leadership for Agri-Food Researchers’, Ireland (2019)

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- Poster, EAAP 2016, Belfast, Northern Ireland (2016)
- Theatre, BSAS 2018, Dublin, Ireland (2018)
- Poster, 7th WAFL congress, Ede Wageningen, the Netherlands (2017)
- Poster, 27th EGF congress, Cork, Ireland (2018)
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¹Listed activities demonstrate that the PhD candidate has satisfied the educational requirements set by the Graduate School of Wageningen Institute of Animal Science (WIAS). One ECTS equals a study load of 28 hours.

Colophon

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