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The use of infrared thermography and behavioural and physiological responses as early disease indicators in calves

A thesis submitted in fulfilment
of the requirements for the degree

of

Doctor of Philosophy in Biological Sciences

at

The University of Waikato

by

Gemma Louise Lowe



THE UNIVERSITY OF
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This thesis is dedicated to my supervisors
Dr. Mairi Stewart, Dr. Mhairi Sutherland and Prof. Joe Waas
as an acknowledgement of all the support, encouragement and advice
you offered throughout the course of my PhD

Abstract

Disease is widely acknowledged to be detrimental to an animal's welfare and generates significant economic losses. To minimise the impact of disease on animal welfare and consequently reduce economic costs, early disease detection is essential for promoting earlier diagnosis, treatment and isolation to prevent the spread of disease. As automation increases in the livestock industries, there is a need to develop and integrate early disease indicators into automated systems for remote monitoring of animal health and welfare. The aims of this thesis were to firstly, investigate the behavioural (milk feeding, lying and water drinking) and physiological (infrared temperatures and respiration rate) responses which occur during the onset of neonatal calf diarrhoea (NCD), and to assess their suitability as early disease indicators. Additionally, due to an increasing trend towards providing calves with greater milk allowances, the influence of milk allowance on indicator suitability was assessed. Secondly, to determine the optimum anatomical location (eye, cheek, side, shoulder and back) for infrared image collection for early disease detection. Thirdly, to investigate the water intake and drinking behaviour of calves and how these behaviours are affected by disease onset and milk allowance. Further, to develop and validate an algorithm for the automated detection and analysis of the eye and cheek regions from infrared images and further validate the use of infrared thermography as an automated method of recording respiration rate (RR) in young calves. Finally, the thesis aimed to develop and validate an automated water system for the purpose of recording drinking behaviour and further assessed the suitability of setting up an infrared camera at a water station for monitoring calf health and welfare.

Calves were defined as being clinically ill when they were observed as being diarrhoeic. Milk feeding, lying and water drinking behaviours changed prior to clinical signs of disease. Changes in milk feeding and lying behaviours prior to clinical signs were thought to occur due to calves experiencing a reduction in appetite, becoming lethargic and attempting to conserve energy in response to the onset of disease. Infrared temperatures of the eye, cheek and shoulder decreased and side (situated over the rumen fossa) temperature increased prior to clinical signs. The decrease in infrared temperatures was attributed to the animal generating a fever to fight against the infection by restricting blood flow to the extremities, in order to maintain homeostasis. Further, the decrease in infrared temperature was likely a result of a reduction in feed and metabolic activity during the onset of disease. The side was identified as the optimum anatomical location for early disease detection. The increase in side temperature was attributed to the proximity of the area to the underlying site of infection and localised inflammation of the intestines. Prior to clinical signs, there was little change in water drinking behaviour (number and duration of visits to the water trough), which was considered to be due to the young age of the calves at the time of diagnosis. Additionally, a lack of a response in RR prior to clinical signs was thought to be due to sampling rate and NCD being an enteric disease as opposed to a respiratory disease.

Milk allowance influenced the suitability of behavioural and physiological measures as early disease indicators. When provided with either a high (10 L/d) or low (5 L/d) milk allowance, milk feeding behaviours typically only changed for calves on a high milk allowance and infrared temperatures (eye and cheek) only changed for calves on a low milk allowance. Additionally, as opposed to being used individually, combining behavioural and physiological measures provided stronger

composite indicators of disease. Combinations of milk feeding and lying behaviours, and additionally infrared temperatures (eye and cheek) in the case of calves on a low milk allowance, provided the strongest composite indicators of disease.

Integrated into automated systems, behavioural and physiological measures could be utilised for early disease detection. In support of the future development of integrating infrared thermography (IRT) into automated systems, an algorithm was developed and validated for detection and analysis of the eye and cheek regions from infrared images. Further, the use of IRT as a method of recording respiration rate was validated for use in young calves. In addition, an automated water system was validated for the purpose of recording drinking behaviour (number and duration of visits to the water trough). Based on the findings that calves visited the water trough on average three times per day and began consuming water during the first week of life a water system was considered as a suitable and more affordable alternative to an automated calf feeder for setting up an infrared system to monitor calf health and welfare.

Although these measures have been assessed in relation to the detection of NCD, they have the potential to be applied further for the detection of other diseases and in other species. The integration of these measures into an automated on-farm system could enable earlier treatment and isolation of diseased animals to prevent the spread of disease, by alerting farmers to diseased animals earlier than is currently possible based on overt clinical signs. In addition to facilitating decision-making abilities for the farmer, the development of such a system would reduce costs on-farm and to the industry as a whole and would ultimately improve animal health and welfare.

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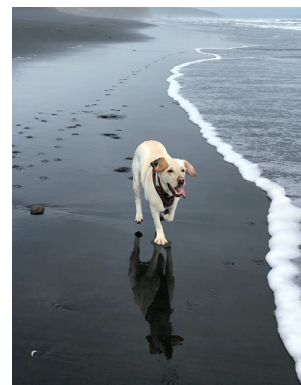
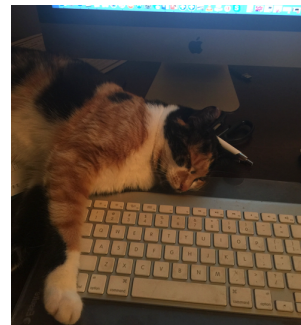


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CO-AUTHORSHIP FORMS

Declaration

As a thesis by publication, Chapters 3-7 are presented as papers following the style and formatting requirements of the journals in which they have been published, are under review, or are in the process of being submitted to at the time of thesis submission. Due to the different journals with which papers have been published or submitted to, there are some inconsistencies in the style and format between chapters. It is acknowledged that there is some repetition particularly in the introduction and method sections comprising the different chapters, however, the level of repetition has been reduced as and where possible. Whilst contributions to this research have been made by co-authors as indicated within each chapter and in Appendix 4, my input was greatest having carried out the research including the fieldwork, data analysis and manuscript preparation.

Chapter One

General Introduction



Disease has detrimental impacts on animal welfare and early disease detection promotes earlier diagnosis and treatment, which can significantly reduce the amount of time an animal suffers and prevents the spread of disease. Providing context for the research contained in this thesis, this introductory chapter presents an overview of animal welfare and discusses how animal welfare is affected by the incidence of disease. An outline of the thesis structure is included to highlight the main objectives of each chapter.

Assessing animal welfare

Animal welfare is a multi-dimensional concept, concerning both the mental and physical states of an animal as it experiences, and attempts to cope with, the conditions experienced within its environment (OIE, 2018). Depending on an animal's ability to cope in its environment, levels of welfare can range from being very good to very poor. When animals are subjected to situations in which they undergo difficulties or fail to cope in their environment, poor levels of welfare will be experienced (Broom, 1991). In contrast, animals will experience higher levels of welfare in situations where they are able to successfully cope with the conditions of their environment (Broom, 1991).

Over time, the level of public awareness regarding the treatment and well-being of animals has increased (Bennett and Blaney, 2003; McEachern et al., 2007). The promotion of good levels of animal welfare is not only a moral obligation towards animals, but is also essential in the sustainability of practices and the success of production systems which rely on animals (Broom, 2010, 2011). In relation to production animals, public awareness and perceptions around the ways in which these animals are farmed are increasingly driving a demand for high quality

products (Napolitano et al., 2010). This is largely due to the understanding that high quality products are acquired when high levels of animal welfare have been achieved. For example, consumer perceptions around food quality are based not only on the nature and safety of the end product, but also the welfare of the animals from which those products have been produced (Blokhuis et al., 2003). In addition, the occurrence of practices which are deemed detrimental to an animal's welfare can affect a producer's social license to operate (Hampton and Teh-White, 2019).

In 1965, stemming from such public awareness and concern, an investigation was commissioned by the British Government whom appointed a committee to look into the welfare of intensively farmed livestock (McCulloch, 2013). The resulting 'Brambell report' put forth two key concepts surrounding the welfare of farmed livestock: 1) that suffering should be the primary consideration when conducting evaluations of animal welfare, and 2) that animals have natural behaviours that they need the opportunity to perform to ensure their welfare is not negatively impacted (Mench, 1998). As would later become known as 'Brambell's Five Freedoms', the report made the following suggestion:

"An animal should have at least sufficient freedom of movement to be able without difficulty, to turn round, groom itself, get up, lie down and stretch its limbs". – Brambell, 1965.

The Brambell report further recommended the development of a farm animal advisory committee; this was acted upon in 1967 through the establishment of the Farm Animal Welfare Advisory Committee (FAWAC), which disbanded in 1979 upon the establishment of the Farm Animal Welfare Council (FAWC) (McCulloch, 2013). In 1979, in response to the Brambell report, FAWC developed and

formalised the concept of the “Five Freedoms”. As presented in Figure 1, the five freedoms were developed to reflect the ideals of animal welfare.



Figure 1. The five freedoms concept illustrates ideals of animal welfare which together are designed to provide a logical, comprehensive framework for assessing animal welfare (diagram generated based on the five freedoms as outlined by the FAWC, 2013).

Each of the five freedoms are comprised of two parts, i) the *freedom* and ii) the *provision*, with four of the five freedoms (freedoms 1-3, and freedom 5) denoting a freedom *from* and freedom four denoting a freedom *to* (Figure 1). Those denoting a freedom *from*, relate to an animal not being in the state or condition outlined in the freedom, with the freedom *to*, relating to an animal having the ability to express

normal behaviour (McCulloch, 2013). Collectively, the five freedoms highlight nine conditions, to enforce ideals whereby an animal should be free from, hunger, thirst, discomfort, pain, injury, disease, fear and stress and should have the ability to express normal behaviour (McCulloch, 2013). The five freedoms are still widely recognised today, acting as a framework contributing in the development of animal welfare legislation and codes of welfare (Green and Mellor, 2011). However, the five freedoms concept is not exempt from criticism. One particular criticism being that the freedoms are focussed on poor welfare and suffering, as opposed to focusing on an animal's quality of life, which perpetuates the negative image of farming and production (FAWC, 2009).

Since the development of the five freedoms, a number of additional concepts have been put forth to illustrate what constitutes animal welfare, and how welfare can be assessed. Furthermore, advances in animal welfare since the development of the five freedoms has led to the suggestion that instead of focussing on poor welfare and suffering, ideals which would act to ensure good welfare and prevent suffering should also be given consideration (McCulloch, 2013). As a means of refining the five freedoms and providing a framework for determining an animal's overall quality of life, in 1994, the "Five Domains" model was developed (Webster, 2016; Mellor, 2017). The model is designed to support a structured and comprehensive approach to assessing animal welfare, focussing not only on factors which can compromise welfare, but additionally those which can ultimately improve welfare (Mellor, 2017). In the five domains model (Figure 2), the first four domains consider the nutrition, environment, physical health and behaviour of an animal collectively (considered the 'physical/functional domain') and are individually classed as inputs which can be both positive and negative (Webster, 2016). The fifth

domain, 'the affective experience domain' is concerned with an animal's mental state in terms of its affective state and psychological well-being, and consists of a series of outcome indicators which again, can be both positive or negative (Webster, 2016). The five domains aim to identify the inputs acting upon an animal and in turn how those inputs impact the outcome indicators (Webster, 2016). The outcome indicators, which reflect the animal's mental state, can be used collectively to provide an overall measure of welfare status (Webster, 2016). Based on the five domains, welfare may be considered good when an animal's nutritional, environmental, health, behavioural and mental needs are met; these needs can be met when animals are managed in such a manner as to enable the avoidance of negative mental states, whilst allowing and encouraging the promotion of positive mental states (Green and Mellor, 2011). The model has been applied to assess the impacts of both current and proposed approaches for managing and interacting with animals (Mellor, 2017).

Physical/Functional Domain			
1. Nutrition	2. Environment	3. Physical Health	4. Behaviour
<u>Negative</u> Deprivation of food Deprivation of water Malnutrition	<u>Negative</u> Environmental change	<u>Negative</u> Disease Injury	<u>Negative</u> Behavioural restriction
<u>Positive</u> Appropriate nutrition Available food	<u>Positive</u> Environmental change and opportunity	<u>Positive</u> Fitness Ableness	<u>Positive</u> Behavioural expression



Affective Experience Domain			
5. Mental State			
<u>Negative</u>		<u>Positive</u>	
Pain	Breathlessness	Safety	Vitality
Fear	Boredom	Reward	Calmness
Distress	Frustration	Goal directed	Security
Discomfort	Anger	Engagement	Contentment
Debility	Dizziness	Playfulness	Affectionate
Weakness	Etc...	Curiosity	Companionability Etc...



Animal Welfare Status

Figure 2. Five domains model as an assessment for animal welfare status (diagram adapted from “Extending the ‘Five Domains’ model for animal welfare assessment to incorporate positive welfare states”, by Mellor, D. J., and N. J. Beausoleil. 2015. Anim Welf. 24:241-253).

As public awareness continues to increase, the understanding of what animal welfare is, and what therefore constitutes good welfare, continues to be influenced by public perceptions concerning what are considered acceptable and unacceptable ways of treating animals (Green and Mellor, 2011); these perceptions differ amongst individuals and are shaped by ethical, social, cultural, economic and religious values (Green and Mellor, 2011). Based on three key public perceptions the ‘Three circles’ concept of animal welfare was introduced in 1997 (Fraser et al., 1997). As opposed to being predominantly focussed on affective state, the three circles concept suggests that an animal’s quality of life be evaluated based on three key ethical concerns: 1) basic health and functioning, 2) affective state, and 3) natural living (Fraser et al., 1997) (Figure 3).

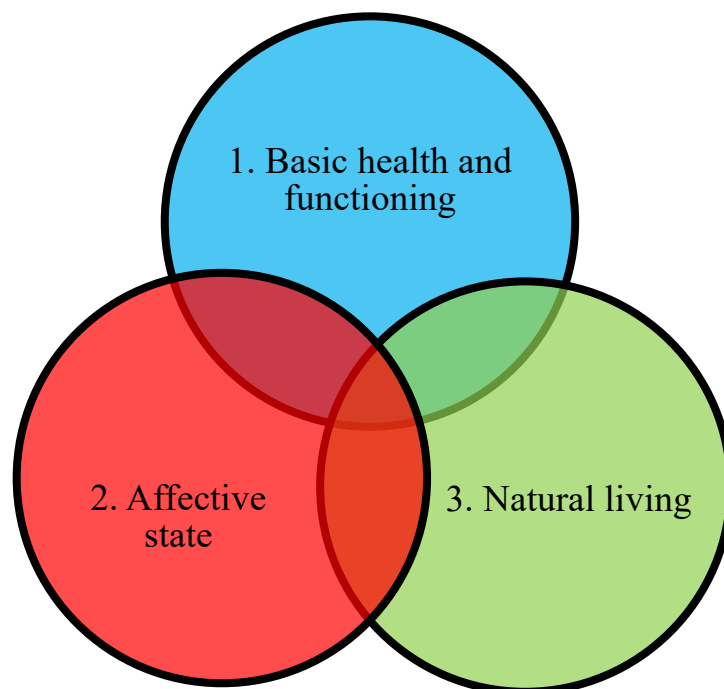


Figure 3. The three circles concept of animal welfare illustrating the three key overlapping concepts that based on public perceptions should be considered when evaluating an animals quality of life (diagram adapted from “Understanding animal welfare: The science in its cultural context”, by Fraser, D. 2008b. Oxford: Wiley-Blackwell).

The first category (Figure 3), basic health and functioning, relates to the physical fitness of an animal, regarding the animals' health, and their ability to function, grow and develop (Fraser et al., 1997). The second category (Figure 3), affective state, relates to how the animal is feeling in terms of pain, suffering, distress and other emotions. Affective states can be positive or negative where, respectively, they are experienced as being rewarding or pleasurable, or aversive and punishing (Mellor, 2015). Based on the ethical concern of affective state, animals should be free from prolonged negative states and have the ability to experience normal pleasures (Fraser et al., 1997). The third category (Figure 3), natural living, refers to the ability of an animal to live as naturally as possible within an environment where they have the ability to carry out natural behaviours (Fraser, 2003). As illustrated in Figure 3, although regarded as individual categories, overlap between categories highlights the potential that they may be affected simultaneously. Furthermore, it is possible that focusing solely on one category could lead to poor levels of welfare from the perspective of the other categories (Fraser, 2008). Therefore, it is considered highly beneficial to consider all three categories when evaluating an animal's quality of life and addressing animal welfare concerns.

Considered one of the most comprehensive biological sciences (Dawkins, 2006), animal welfare is a multi-disciplinary science involving numerous fields including, ethology, physiology, pathology, epidemiology, biochemistry, genetics, nutrition, evolution, and neuroscience (Dawkins, 2006; Green and Mellor, 2007). As animal welfare encompasses a number of different scientific fields, it is acknowledged that there is no single measure which alone can determine an animal's level of welfare (Dawkins, 2006). Instead, the fields listed above contribute a number of different

measures which can be implemented as indicators for the purpose of evaluating animal welfare.

Welfare indicators can be divided into two categories: those which are resource-based and those which are animal-based. Resource-based indicators are measured from the environment instead of the animals themselves (e.g., space allowance, provision of food and water, and environmental enrichment (Salas and Manteca, 2016; Manteca and Salas, 2015)). Animal-based indicators in comparison are those which are measured directly from the animals themselves (e.g., their physiology, behaviour, appearance, and health) (Salas and Manteca, 2016). With a number of physiological measures available (e.g., blood parameters, heart rate, respiration rate and body temperature) an animal's physiology can provide valuable insights into an animal's welfare status. Some of the most commonly used physiological indicators relate to the hypothalamic-pituitary-adrenal (HPA) axis, which is activated by stress and results in a subsequent secretion of glucocorticoids. Assessed through the measurement of cortisol and corticosterone, for example, the stress response can be assessed to evaluate welfare (Salas and Manteca, 2016). Another measure for evaluating animal welfare, animal behaviour, looks not only into abnormal behaviours (e.g., stereotypies and apathy) but also the frequency, duration and intensity of normal behaviours (e.g., changes in feeding patterns, play, aggression, affiliative and maternal behaviours) (Salas and Manteca, 2016). Further, an animal's appearance (e.g., body condition, coat condition and posture) can also provide an insight into the level of welfare it is experiencing (Salas and Manteca, 2016). For example, poor body condition can be indicative of malnutrition, disease or chronic hunger (Salas and Manteca, 2016). In contrast, high body condition can be a reflection of overfeeding or inadequate space and as a

consequence can negatively impact an animal's health and increase their susceptibility to other issues (e.g., lameness) (Salas and Manteca, 2016).

Impact of disease on animal welfare

Given an animal's welfare is influenced by its ability to cope within its environment, it is understandable that the presence of pathogens in the environment may impact an animal's health and therefore, their level of welfare (Broom and Corke, 2002). It is acknowledged that when an animal is diseased, welfare will always be poorer than when there is no incidence of disease (Broom and Corke, 2002). Disease is frequently determined based on an animal displaying a series of 'sickness behaviours' (Hart, 1988; Millman, 2007). Sickness behaviours include symptoms such as lethargy, depression, fever, anorexia, and decreased exploratory and social behaviours (Hart 1988). Sickness behaviours are associated with various physiological and behavioural changes, and act as adaptive strategies, increasing the ability of the immune response, thus enabling an animal to make use of its energy resources to fight against the disease. Disease is detrimental to welfare, not solely because it can cause significant pain and discomfort, but also because it can weaken an animal's ability to obtain resources necessary for survival. Furthermore, disease negatively impacts welfare by hindering the animal's ability to express natural behaviours and their likelihood of being able to experience positive mental states (Salas and Manteca, 2016). Finally, poor welfare itself usually leads to a greater susceptibility to disease because of the impact on the immune system as the animal attempts to cope under difficult conditions (Broom and Corke, 2002).

Caused by pathogens such as rotavirus, *Cryptosporidium*, *Salmonella* and coronavirus (Rai et al., 2011), neonatal calf diarrhoea (NCD) is an enteric disease

which significantly impacts the welfare and productivity of young calves. Recognised as an issue for livestock industries worldwide, NCD causes severe diarrhoea, resulting in dehydration, weight loss, and in severe cases, death (Schroeder et al., 2012). In the long term, NCD also has implications for growth and productivity as a result of reduced weight gain and stunted growth (De Graaf et al., 1999). A major issue with NCD is that by the time an animal presents overt clinical signs of disease (e.g., diarrhoea) much of the damage to the internal organs has already occurred (intestinal damage leads to calves becoming diarrhoeic). In order to improve welfare and prevent unnecessary suffering, measures which can be used as early indicators of disease need to be identified and implemented to enable earlier diagnosis and treatment. Furthermore, with the increasing level of automation in the livestock industry (Hamadani and Kahn, 2015) it is important that indicators of disease which have the potential to be incorporated into automated systems are developed. As a shift towards automation often leads to a less “hands-on” approach to farming, the incorporation of indicators of disease into automated, on-farm systems are essential to support remote and reliable monitoring of animal health and welfare, and to ensure that cases of NCD do not go undiagnosed.

Thesis structure

This thesis is comprised of a series of studies which have been conducted to meet the aims of this thesis as outlined above. Chapter 2 forms a literature review of disease in livestock with a focus on NCD, the physiological and behavioural responses that occur during disease and automated methods (e.g., infrared thermography (IRT), automated calf feeders, accelerometers and automated water systems) for disease detection. Chapters 3-5 have been published in international

journals. Chapter 6 has been submitted for publication in an international journal and is currently under review. Chapter 7 is being finalised in preparation for submission to an international journal. Appendix 1 presents the validation of an automated water system for monitoring the individual water intake and drinking behaviour of calves as published in the 2017 proceedings of the New Zealand Society of Animal Production (NZSAP). Appendix 2 presents an abstract published in the 2018 proceedings of the 52nd Congress of the International Society for Applied Ethology (ISAE) and the related poster presentation. Appendix 3 presents an abstract published in the 2019 proceedings of the ISAE Australasia-Africa regional meeting and the related poster presentation. Appendix 4 contains the University of Waikato thesis co-authorship forms which indicate the individual contributions made by co-authors involved in this research.

The first research chapter (Chapter 3), focussed on identifying behavioural and physiological responses associated with the early onset of disease in calves experimentally infected with NCD. Also, by assessing thermal fluctuations of different anatomical regions, the study assessed the suitability of IRT as a non-invasive method for early disease detection and aimed to define the optimal anatomical location for image collection.

Due to the shift towards automation occurring in many farming systems, it is important that automated methods are developed to provide a remote and reliable means of monitoring health and welfare. As such, the second research chapter (Chapter 4) reports on an algorithm which has been developed, enabling automated detection and analysis of the eye and cheek regions from infrared images collected from calves. This development is a necessary step towards the integration of

infrared technology into automated systems, where IRT could be implemented to support animal health and welfare monitoring.

The research reported in Chapter 5 was conducted with the purpose of validating the use of IRT as an alternative method for recording respiration rate (RR) in calves. This validation study was an important preliminary step required to determine whether IRT is a suitable method for recording RR prior to further development of algorithms.

Following on from the study presented in Chapter 3, the research presented in Chapter 6 aimed to further assess the behavioural and physiological responses which occur prior to overt clinical signs, and the use of IRT as a method for early disease detection. Infrared images collected during this study were analysed automatically for the eye and cheek regions using the automated eye and cheek detection algorithm developed in Chapter 4. Additionally, with a trend towards increasing milk allowances, this study was focussed on investigating the effect of different milk feeding allowances on the suitability of these behavioural and physiological responses individually and in combination as early disease indicators. As opposed to being experimentally infected with NCD, in this study, calves were monitored for naturally occurring incidences of NCD.

The purpose of the research described in Chapter 7 was to investigate the drinking behaviour and water intake of young, preweaned, group housed calves provided high and low milk allowances. There is a current lack of information regarding the water intake and drinking behaviour of young calves, so this study aimed to investigate the age at which calves start to consume water, how much they consume, and how frequently they visit the water trough. Additionally, the study assessed the

influence of water provision on body weight and milk feeding, hay and meal feeding, and lying behaviours. Finally, for the purpose of monitoring calf health and welfare, this study aimed to determine the suitability of setting up an infrared camera system at a water station as an alternative, more affordable option for farmers than an automated calf feeder. In comparison to previous chapters (Chapters 3 and 6) where drinking behaviour had been investigated in relation to the onset of NCD, the aim of this study was to gain an insight into the drinking behaviour and water intake of healthy calves.

Chapter 8 forms a summary of the results contained in this thesis, collectively highlighting the overall conclusions and implications arising from the research conducted. This final chapter also discusses the practical considerations, limitations and areas for future investigation which have become evident as a result of this research. Finally, this chapter highlights the animal welfare and economic implications of this research.

Ethical statement

As this research required the participation of animals, applications for ethical approval were submitted to the University of Waikato Animal Ethics Committee (WAEC) and the Ruakura Animal Ethics Committee (RAEC) for consideration and approval prior to commencing each trial. Trials were approved by the WAEC under protocols #955, 985 and 1017, with protocol 1017 jointly approved by the RAEC under protocol #14089. Due to the nature of this research, it was necessary to use live animals. Power analyses were conducted to determine the minimum number of animals required to ensure significant differences could still be observed. Once

identified as being clinically ill, animals were treated accordingly with electrolytes, antibiotics and anti-inflammatories as required to help them overcome disease.

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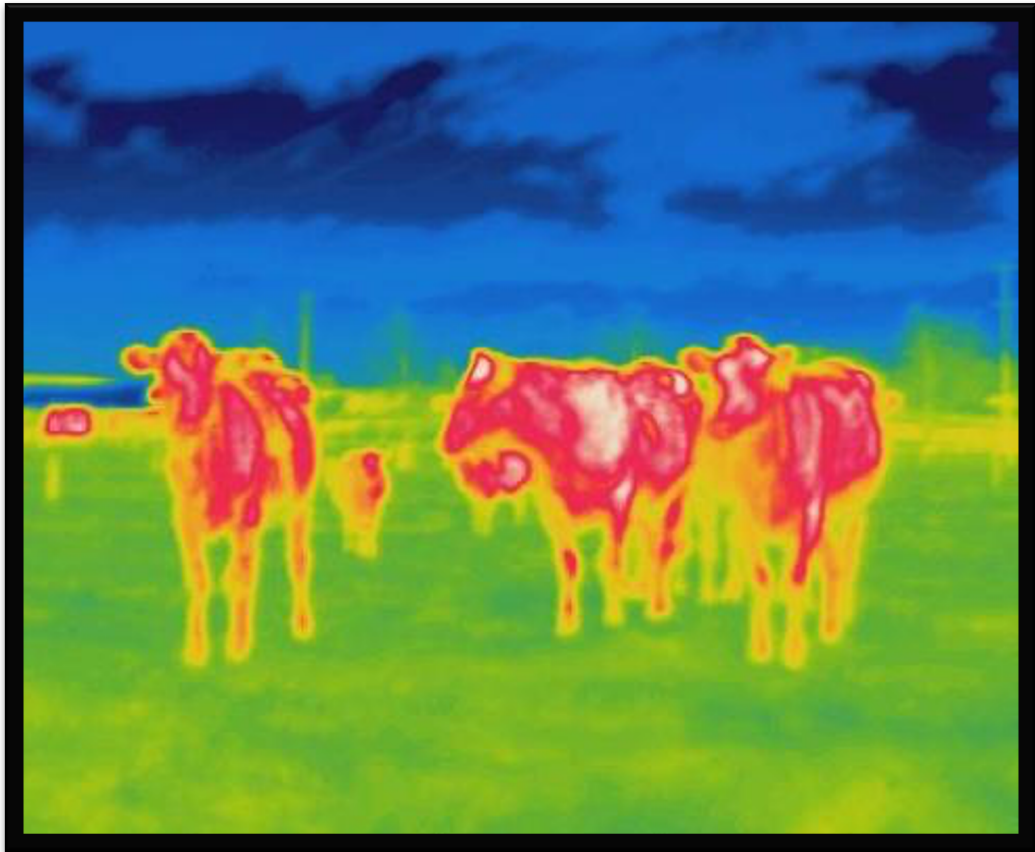
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Chapter Two

*Disease in livestock and the potential for automated systems to
promote early disease detection*



Disease in livestock

Disease can be described as being a physical or mental condition which detrimentally impacts the normal functioning of an animal (Cockram and Hughes, 2011). When an animal is diseased it is acknowledged that the level of welfare it experiences will always be poorer than when it is not suffering from disease (Broom and Corke, 2002), and “a freedom from pain, injury and disease, by prevention or rapid diagnosis and treatment”, forms one of the concepts of the five freedoms of animal welfare (FAWC, 2013). As outlined in Table 1, there are various ways in which disease can impact welfare. Briefly, these impacts are not limited to the pain and discomfort associated with disease, but further through hinderance of an animal’s ability to acquire the resources necessary to ensure its chances of survival (Salas and Manteca, 2016). The incidence of disease is also known to prevent animals from being able to perform natural behaviours (e.g., feeding, play and affiliative behaviours) and as such decreases the potential for diseased animals to experience positive mental states (Salas and Manteca, 2016).

Within the livestock industry, the incidence of disease is of worldwide concern, especially in relation to the detrimental impacts of disease on animal health and welfare (Tomley and Shirley, 2009; McElwain and Thumbi, 2017). In the case of zoonoses, there is also significant concern surrounding the threat of such diseases to human health (Bennett, 2003; McElwain and Thumbi, 2017). The incidence of disease is of further concern based on its economic impact relating to issues such as reduced production, decreased perceived or actual product quality and consumer demand, increased international trade restrictions, premature culling and the cost of disease prevention and control (Morris, 1988; Bennett, 2003; McElwain and Thumbi, 2017). Of the numerous diseases to which livestock are susceptible, some

common diseases include mastitis, lameness, respiratory disease and pestivirus-based diseases such as bovine viral diarrhoea (BVD), classical swine fever (CSF) and border disease (BD).

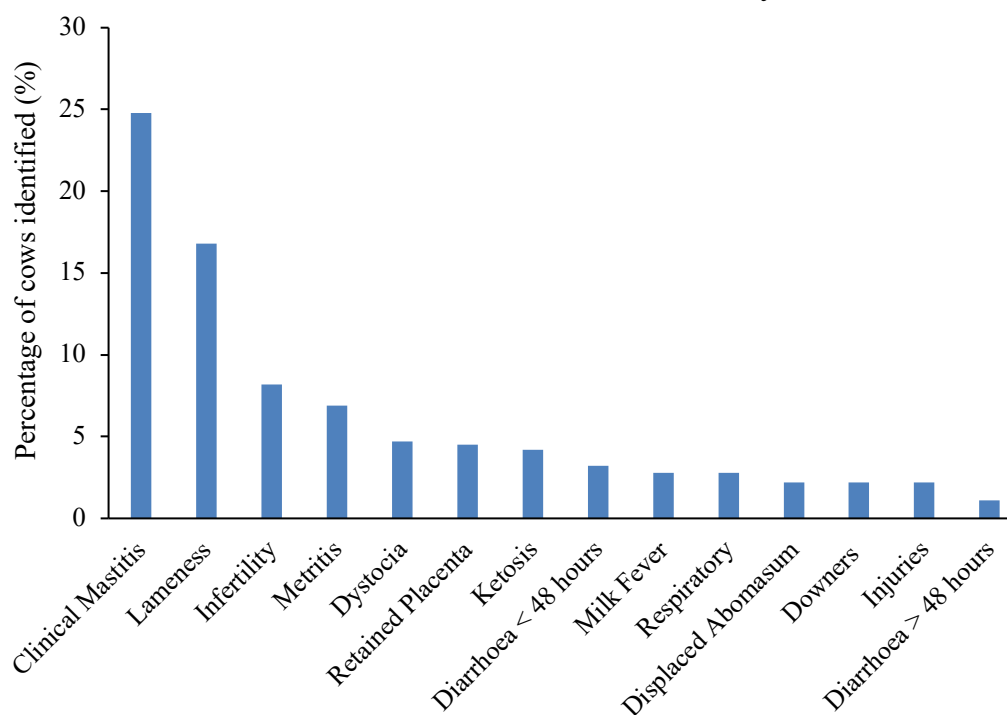
Table 1. Potential impacts of disease on animal welfare (adapted from “Health and disease, in: Animal welfare (3rd ed.)”, by Cockram, M. S. and B. O. Hughes. 2018. Appleby, M. C., Olsson, I. A. S., and Galindo, F. (eds.). C.A.B International, Oxfordshire, UK).

Pain and suffering	Diseases which inflict pain and suffering are of particular concern. Tissue damage and sensitivity to pain can provide an insight to the level of pain experienced in response to disease (Rutherford, 2002). In cattle some painful conditions include dystocia, mastitis, white line disease, digital dermatitis, joint ill and pneumonia (Huxley and Whay, 2006). Additionally, foot rot, flystrike and chronic mastitis have been identified as the most painful diseases affecting sheep (Fitzpatrick et al., 2006).
Feeling ill	Diseased animals are likely to feel ill and may show signs of anorexia, thirst, fever and nausea. In response to fever, compared to healthy calves which often spend time lying with their head and neck raised with an alert appearance and their ears held up, sick calves will often lie down with their neck and head lowered, ears drooped, and their limbs drawn in towards their body.
Fear	Fear may be experienced by diseased animals in response to disorientation or an impaired ability to respond to perceived threats. In response to respiratory disease or fever, calves may show increased fear and decreased exploratory behaviour (Cramer and Stanton, 2015). In cattle, lameness in addition to other factors such as slippery or uneven flooring surfaces or restricted movement, can cause insecurity in response to perceived threats.
Distress	Distress may be experienced as a result of impaired physiological functioning in response to disease. For example, hypoxaemia during respiratory disease can lead to breathlessness (Beausoleil and Mellor, 2015).
Physical discomfort and reduced rest	Disease can result in discomfort, leading to reduced time spent resting (Berriatua et al., 2001) and sleeping. In response to mastitis for example, cows have been found to decrease the amount of time spent lying (Siivonen et al., 2011). Prolonged periods of time spent lying in response to lameness or disease can result in pressure injuries and potentially muscle and skin damage (e.g., lame sows can develop shoulder wounds (Bonde et al., 2004), and broiler chickens can develop hock burn or breast blisters (Broom et al., 2006)).
Weakness	Disease can weaken an animal, thus reducing an animal’s ability to compete for essential resources such as food, which in turn leads to malnutrition. Additionally, as part of the immune response to overcome disease, prolonged energy expenditure (Colditz, 2002; Straub et al., 2010) can result in fatigue. Weakness may also impact an animal’s ability to care for their offspring.
Thermal discomfort	Diseases which trigger a fever response or result in prolonged periods of immobility can lead to increased heat loss. As a consequence, an animal may feel cold, and a reduced ability to move away from environmental conditions such as draughts, precipitation or solar radiation, combined with anorexia, dehydration or nutrient malabsorption can result in thermal discomfort (Balsbaugh et al., 1986).
Hunger, thirst and reduced rest	Lameness, injury and weakness can limit an animal’s mobility, which restricts their ability to obtain essential resources such as food, water or a suitable lying area. Lameness in broiler chickens can prevent the ability to access food and water and consequently they can succumb to dehydration and starvation (Butterworth et al., 2002).
Impaired physical condition limiting function	Disease can lead to emaciation (e.g., Johne’s disease, BVD) and impaired or total loss of function (e.g., blindness, deafness, paralysis and congestive heart failure). These effects can decrease vigour and impact an animal’s ability to perform natural behaviours and experience pleasurable activities (McMillan, 2003; Reynolds et al., 2010). Weakness or impaired perception as a result of disease can reduce the ability of an animal to avoid attack from conspecifics or predators.

Mastitis

Mastitis is a disease relating to inflammation of the mammary gland which typically results from pathogenic infection (e.g., from bacteria such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*)) of the gland cistern following pathogenic invasion of the teat canal (Banos et al., 2017), and is a disease particularly abundant in cattle, sheep, and goats worldwide (Rashid et al., 2017). Mastitis has been identified as the single most prevalent and costly disease within the dairy industry (Banos et al., 2017), with associated costs arising from issues such as reduced milk production, premature culling and veterinary treatment (Bennett and Ijpelaar, 2005; Aghamohammadi et al., 2018). In the UK, at an estimated cost of £120 million per year, mastitis is considered one of the main diseases affecting ewes (AHDB Beef and Lamb, 2016), and at a cost of £179.7 million per year bovine mastitis is identified as the main disease impacting the UK agricultural industry (Bennett and Ijpelaar, 2005; Down et al., 2016). In New Zealand, it has been estimated that on average 12.7% of cows will be affected with mastitis (McDougall et al., 2007). Further, at an average cost of NZ\$11,500 per herd (based on an average herd size of 315 cows), mastitis is estimated to cost the New Zealand dairy industry a total of NZ\$180 million per year (NMAC, 2006; DairyNZ, 2013). In the USA, mastitis is estimated to cost the dairy industry an estimated US\$1.8 billion annually (Viguier et al., 2009), and during 2013 was the most common disease identified in dairy cows, accounting for 24.8% of all health issues identified by producers (USDA, 2014a) (Figure 1).

Health issues identified in dairy cows



Producer identified health issues

Figure 1. Percentage of health issues identified in dairy cows by producers in the US dairy industry during 2013 (generated from data presented by USDA, 2014a).

Lameness

Referring to painful disorders arising from disease or injury of the hoof or leg, which result in impaired locomotion or an abnormal gait or posture (Van Nuffel et al., 2015), lameness is another key health concern for livestock. Lameness has considerable detrimental impacts on an animal's level of welfare and has been linked to effects such as decreased milk production, feed intake, mobility, fertility, growth rates and weight loss (Hernandez et al., 2001; Rushen, 2001; Warnick et al., 2001; Nieuwhof et al., 2008; Christodouloupolos, 2009; Gelasakis et al., 2010; Wassink et al., 2010; Sadiq et al., 2017). Some common causes for lameness include digital dermatitis, foot rot, white line disease, sole ulcers, osteochondrosis and hoof

overgrowth (Murray et al., 1996; Scott et al., 1996; Gelasakis et al., 2010; Sullivan et al., 2014; Duncan and Angell, 2019). In sheep for example, since 1997, foot rot has consistently been identified as the most common cause of lameness in the UK (Groenevelt et al., 2015), with a UK survey conducted in 2008 indicating that foot rot was an issue for 96.0% of flocks (Kaler and Green, 2008). Foot rot is estimated to cost the UK sheep industry £24 million per year, approximately half of which relates to the cost of preventative measures, with the remaining half incurred through production losses and the cost of treatment (Nieuwhof and Bishop, 2005). In New Zealand, a survey of the Merino industry conducted in 2005, reported that foot rot was present in 59.0% of flocks and estimated that foot rot costs the industry NZ\$11.4 million per year (Hickford et al., 2005).

Respiratory diseases

Respiratory diseases are another concern regarding the health and welfare of livestock. In cattle, respiratory diseases are caused by several pathogens which infect the respiratory tract and collectively result in the clinical condition commonly referred to as bovine respiratory disease (Delabougliise et al., 2017). Some of the common bacterial pathogens associated with the incidence of bovine respiratory disease (BRD) for example include *Mycoplasma bovis*, *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* (Fairly, 1996; Fulton, 2009; Delabougliise et al., 2017). Common viral agents associated with BRD include, bovine respiratory syncytial virus (BRSV), bovine herpesvirus type 1 (BHV-1), parainfluenza virus type 3 (PI₃), bovine coronavirus (BCV), BVDV, and bovine adenovirus type 3 (BA_{DV}-3) (Fairly, 1996; Fulton, 2009; Delabougliise et al., 2017).

The incidence of BRD is identified as the main health concern for the beef industry (Edwards, 2010; USDA 2013). In the USA, BRD is the leading cause of both morbidity and mortality in beef feedlots, associated with 70.0-80.0% and 40.0-50.0% of all cases of morbidity and mortality respectively (Edwards, 2010). Whilst several of the pathogens associated with BRD are present in New Zealand, the prevalence of respiratory disease is minimal by comparison to other countries (Fairly, 1996). It is possible that whilst the pathogens associated with respiratory disease may be present in New Zealand, the particular strains may not be the same as those which result in the same degree of respiratory disease experienced overseas (Fairly, 1996). However, it is also possible that the same strains are in fact present in New Zealand, and it is instead differences in our management practices that minimise the risk of animals contracting respiratory disease (Fairly, 1996). For example, *Pasteurella haemolytica* related pneumonia is a rare occurrence in New Zealand cattle and yet is the leading cause of respiratory disease in North America (Fairly, 1996). Beef feedlots are extremely common in North America, compared to New Zealand where only a few beef feedlots exist and instead of being grain-fed, cattle are typically grass-fed (Fairly, 1996). The risk of contracting respiratory disease is considered to be greatest during or following transportation (Taylor et al., 2010; Cirone et al., 2019). In North America, it is common for animals to be transported across long distances, mixed together in feedlots, and subjected to extreme temperatures which can further increase the susceptibility to respiratory disease. By comparison, in New Zealand cattle typically experience a more stable climate and are not subject to transportation across such long distances (Fairly, 1996).

A current concern for the New Zealand livestock industry is the bacterial disease *Mycoplasma bovis*. Until its detection during July 2017, New Zealand was one of only two countries that were free from *Mycoplasma bovis*. Whilst the disease poses no risk of infection to humans and presents no food safety risk, it does pose serious concerns to cattle health and welfare (DairyNZ, 2020; MPI, 2020). The effects of *Mycoplasma bovis* on the health and welfare of cattle include untreatable mastitis, severe pneumonia, late-term abortion and arthritis (DairyNZ, 2020; MPI, 2020). No country with *Mycoplasma bovis* has ever managed to eradicate the disease and as it stands Norway is the only country currently free from the disease. Internationally, infected countries manage the disease through strict biosecurity practices on-farm, careful selection of replacement and breeding stock, and through ensuring the herd is in good health (MPI, 2020). Whilst it has never been achieved by any other country, New Zealand is currently working to eliminate *Mycoplasma bovis* through a phased programme of eradication. The programme involves continued tracing of all potentially affected cattle, and the testing and culling of herds with infected animals (DairyNZ, 2020). Since the disease was detected in 2017, as of September 2nd 2020, a total of 250 properties have been identified as being infected and 158,080 cattle have been culled (MPI, 2020). However, as of September 2nd 2020, 249 of these infected properties have been cleared leaving just a single property still actively infected with *Mycoplasma bovis* (MPI, 2020). The total cost of eradication over 10 years was estimated at \$886 million, \$16 million of which was associated with the loss of production and the remaining \$870 million associated with the cost of the eradication response (GovtNZ, 2018). Throughout the course of the eradication programme as of September 2nd 2020, a total of \$171.2 million has been paid to farmers as compensation for losses incurred associated with the response

(MPI, 2020). Had New Zealand not taken steps to eradicate the disease it was estimated that over 10 years the disease would have cost the country \$1.3 billion (GovtNZ, 2018).

Other species of livestock are also susceptible to respiratory diseases, for example, pigs are susceptible to several respiratory conditions, often collectively referred to as porcine respiratory disease complex (PRDC) (Hansen et al., 2010; Cheong et al., 2017). Common viral and bacterial pathogens associated with PRDC include porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV-2), swine influenza virus (SIV), *Mycoplasma hyopneumoniae* and *Pasteurella multocida* (Opriessnig et al., 2011; Cheong et al., 2017; Qin et al., 2017). Regarded as one of the main health issues affecting pig production, PRDC triggers lung damage which results in significant economic losses to the swine industry due to poor growth rate, reduced feed intake, treatment costs and increased mortality (Sorensen et al., 2006; Cheong et al., 2017; Qin et al., 2017).

Pestiviruses

Of further concern to the livestock industry are pestiviruses such as bovine viral diarrhoea virus (BVDV), classical swine fever virus (CSFV) and border disease virus (BDV) which respectively cause BVD, CSF and BD (Tao et al., 2013; Mirosław and Polak, 2019). These viruses are capable of being transmitted from an infected mother to her foetus and consequently, she will give birth to offspring which are persistently infected and consequently shed the virus throughout their lives (Newcomer and Givens, 2013). Further complicating the control of these viruses is the issue that they can often present with mild clinical signs which can

mean the disease often goes undetected and thus spreads to further animals (Newcomer and Givens, 2013).

Typically, cattle are the main species impacted by the BVDV. However, it is important to note that BVDV is also capable of infecting other species and as such has been reported in over 40 different species including alpacas, goats, sheep, pigs and deer (Ridpath, 2010; Nelson et al., 2016). In cattle, BVD causes mucosal disease, respiratory and gastrointestinal tract infections and reproductive issues (Tao et al., 2013; Nelson et al., 2016) and can result in weight loss, decreased milk production, and immune suppression which leads to an increased susceptibility to further diseases (BVDSC, 2019a). Referred to as a “hidden disease” whilst it may be actively influencing production, BVD can often go undetected (BVDSC, 2019a). In New Zealand, BVD is identified as one of the key diseases affecting cattle (BVDSC, 2019a). The BVDV is considered a worldwide pathogen (Heuer et al., 2007; Richter et al., 2019), and as such BVD is also of significant concern to other countries where for example in the UK, BVD is recognised as one of the main endemic infections impacting the agricultural sector (Bennett and Ijpelaar, 2005). It is believed that 90.0% of UK beef and cattle herds have been exposed to BVDV and the virus is estimated to cost the industry £25-61 million per year (MSD UK, 2020). At least 80.0% of New Zealand beef and dairy cattle have been exposed to BVD and at any one time, at least 65.0% of beef herds and 15.0% of dairy herds have active BVD infections (BVDSC, 2019a, 2019b). The cost of BVD to dairy farmers is estimated at NZ\$127 million per annum, with a loss of NZ\$70,000 per infected herd (based on an average herd size of 368 cows) (BVDSC, 2019a). For beef farmers with infected herds, BVD is estimated to cost around NZ\$3000-\$9000 per 100 cows (BVDSC, 2019b).

In pigs, CSF is a particularly lethal disease which is often characterised by fever, lethargy, skin lesions, ataxia, abortion, huddling, anorexia, weakness, conjunctivitis, diarrhoea and lameness (OIE, 2009; Blome et al., 2017). North America, Australia and New Zealand are currently free of CSF, but the disease is found in Central and South America, Europe, Asia and Africa (OIE, 2019a). During the 1990s several large outbreaks of CSF occurred across Europe in the Netherlands (1997), Germany (1993-2000), Belgium (1990, 1993, 1994) and Italy (1995, 1996, 1997) (OIE, 2019a). The outbreak in the Netherlands led to the destruction of 11 million pigs at a cost US\$2.3 billion (OIE, 2019a).

Typically affecting sheep and goats, BD shows many similarities to BVD, however, its effects on reproduction are much more severe (Mao et al., 2015); where in sheep, for example, the disease causes abortions, stillbirths, barren ewes, and weak lambs (Mao et al., 2015). In addition to being weak and unable to stand, diseased lambs will often present with a tremor which can vary from a fine trembling of the head, ears and tail to severe rhythmic contracting of the muscles of the hind legs and back (OIE, 2019b). Coupled with these tremors, affected animals may also be identified based on having a hairier fleece than normal and hence BD is often referred to as 'hairy-shaker disease' (OIE, 2019b). Compared to sheep, BD is less common in goats, and as abortion is the main clinical symptom persistent infection in goats is rare as abortion prevents the disease being carried on by the offspring (OIE, 2019b).

Neonatal calf diarrhoea

Recognised as the leading cause of morbidity and mortality in beef and dairy calves (Smith, 2012, 2019; Dillane et al., 2020), neonatal calf diarrhoea (NCD) is a disease of significant concern to beef and dairy industries worldwide, not only in relation

to the impact of NCD on animal welfare, but also in regard to the associated economic losses (Bazeley, 2003; Smith, 2012; Bonelli et al., 2018). The disease predominantly affects calves during their first month of life (Cho and Yoon, 2014; Bonelli et al., 2018) and has an immense detrimental impact on the welfare of affected animals. As an enteric disease, infectious pathogens inflict substantial damage on the intestines either through 1) the destruction of epithelial cells, which hinders the digestive and absorptive functioning of the intestines, and can result in inflammation; or 2) through the release of toxins which trigger an increased secretion of fluid into the gut (Bicknell and Noon, 1993). This damage to the intestines causes affected animals to suffer from severe diarrhoea, which consequently results in dehydration, weight loss, anorexia, acidosis, loss of electrolytes and mortality in extreme cases (Bicknell and Noon, 1993; Stoltenow and Vincent, 2003; Dillane et al., 2020).

A number of bacterial, viral and protozoal agents are associated with the incidence of NCD; the main pathogens identified being *Escherichia coli* (*E. coli*) (Holland, 1990; Bonelli et al., 2018), *Salmonella* (Izzo et al., 2011), rotavirus (Torres-Medina et al., 1983; Rai et al., 2011; Bonelli et al., 2018), coronavirus (Torres-Medina et al., 1983; Rai et al., 2011; Bonelli et al., 2018), and *Cryptosporidium* (O'Donoghue, 1995; Bonelli et al., 2018). These pathogens each present very similar clinical signs, with faecal testing required to reach an aetiological diagnosis (Millemann, 2009; Potter, 2015). It is important to note that these pathogens may also be present in animals which are deemed healthy, and that clinical disease will occur in instances when the animal's ability to resist disease is overcome by infectious pressure (Lorenz et al., 2011).

Bacterial agents

E. coli

Following ingestion, *E. coli* bacterium act through invasion of the gut epithelium where they adhere and replicate within the epithelial cells of the intestinal villi (Cho and Yoon, 2014; Umpiérrez et al., 2017). The small intestine provides a suitable environment for *E. coli* to colonise due to the relatively low pH (>6.5), and hence villous atrophy is often observed in the small intestine due to the loss of infected and damaged cells (Francis et al., 1989). *E. coli* have a reduced ability to adhere to epithelial cells following the calf's first few days of life (de Graaf et al., 1999); consequently, calves are most susceptible to *E. coli*-based infection during the first 4 days of age (Nataro and Kaper, 1998; Foster and Smith, 2009). The most prevalent strain of *E. coli* in cases of NCD is recognised as *E. coli* K99+ (Nataro and Kaper, 1998; El-Seedy et al., 2016), which possessing the K99 antigen is enterotoxigenic, and therefore capable of releasing toxins into the intestines (Stoltenow and Vincent, 2003). The release of toxins triggers an increased secretion of chloride into the gut, which consequently pulls water into the intestinal lumen, and in addition to villous atrophy contributes to calves becoming diarrhoeic (Cho and Yoon, 2014). Calves are susceptible to *E. coli* infection from contaminated faecal material from healthy adult carriers or other diseased calves and can become infected as early as the first 24 hours of life (Stoltenow and Vincent, 2003). Additionally, the younger the calf at the time of infection, the greater the risk of death from severe dehydration (Stoltenow and Vincent, 2003).

Salmonella

Once ingested, *Salmonella* bacterium act through invasion of the epithelial cells of the intestines where they survive within the intestinal macrophages, resulting in disease of the intestinal tract (Tsolis et al., 1999). *Salmonella* produce endotoxins, which when released poison the affected animal, resulting in endotoxic shock and severe illness (Stoltenow and Vincent, 2003). *Salmonella typhimurium* is identified as the most prevalent strain of *Salmonella* in cases of NCD, with infections most commonly occurring in calves < 3 weeks of age (Cho and Yoon, 2014). Calves can shed *Salmonella* to the environment for variable periods of time and intermittently depending on whether they are in a state of clinical or sub-clinical infection (Cho and Yoon, 2014). Apart from other cattle, sources of *Salmonella* infection also include birds, cats, rodents, humans and contaminated water (Stoltenow and Vincent, 2003).

Clostridium perfringens

Although not as common as some of the other causative pathogens, *Clostridium perfringens* (*Cl. perfringens*) is another bacterial agent which has been associated with cases of NCD (Cho and Yoon, 2014) and is often linked to changes in weather, feeding and management practices (Stoltenow and Vincent, 2003). There are five types of *Cl. perfringens* (types: A-E) which are individually distinguished based on the toxins they produce (toxins: alpha (α), beta (β), epsilon (ϵ), and iota (ι)) (Petit et al., 1999; Freedman et al., 2015). Associated with NCD, *Cl. perfringens* type C, produces both α and β toxins, which respectively cause cell lysis and mucosal necrosis (Cho and Yoon, 2014). Infection with *Cl. perfringens* is particularly lethal and can result in diarrhoea with the presence of blood, however, in many cases

death can occur in the absence of overt clinical signs (Stoltenow and Vincent, 2003).

Viral agents

Rotavirus

Typically, rotavirus is transmitted through the ingestion of contaminated faecal material (McNulty, 1983; Cho and Yoon, 2014) which, once having successfully invaded the body, replicates within the cytoplasm of the epithelial cells lining the villi of the small intestine (Holland, 1990). The invaded cells are destroyed, leading to villous atrophy (Figure 2) and inflammation of the submucosa. As a result of the damage to the intestines, and the release of enterotoxins, the ability for water and nutrients to be absorbed across the intestinal epithelium is reduced, hence calves become diarrhoeic and suffer from dehydration (Cho and Yoon, 2014).

Rotavirus based NCD is most common in calves during the first 1-2 weeks of age, where following a relatively short incubation period of just 12-24 hours, calves begin shedding large quantities of the virus in their faeces throughout the following 5-7 days (Cho and Yoon, 2014). This shedding contaminates the environment, increasing the likelihood of transmission to other calves (Cho and Yoon, 2014). Due to the complex nature of its structure, rotavirus is highly stable, which is a factor contributing to the virus's ability to remain infectious in the environment for up to 6 months at 25°C (Barrington et al., 2002). Additionally, it is not uncommon around the time of calving for adult cattle, who are often carriers of rotavirus, to begin shedding increased amounts of the virus (Barrington et al., 2002; Naylor et al., 2009). Hence shedding of rotavirus from adult cattle is one of the key factors

accounting for much of the exposure neonatal calves have to the virus (Barrington et al., 2002).

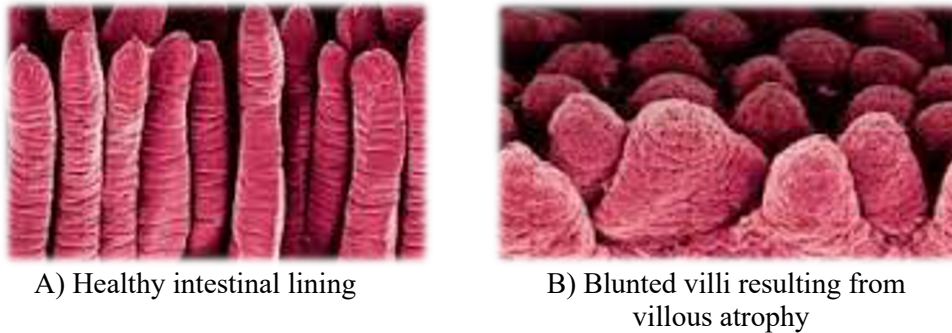


Figure 2. Example of A) a healthy intestinal lining and B) the blunted villi which result from the villous atrophy which occurs during rotaviral infection (diagram obtained from VetEnt, 2019).

Coronavirus

Coronavirus acts very similarly to rotavirus but also damages cells in the intestinal crypts (Stoltenow and Vincent, 2003; Singh et al., 2020). These crypt cells are vital in the production of new intestinal cells and their destruction hinders the healing process of the intestines, and prolongs the recovery period (Stoltenow and Vincent, 2003). Not solely associated with NCD, coronavirus is also linked to diarrhoea in adult cattle and respiratory diseases in both adult and young cattle (Cho and Yoon, 2014; Oma et al., 2016). As with rotavirus, coronavirus typically affects calves during the first 1-2 weeks of age (Cho and Yoon, 2014; Lotfollahzadeh et al., 2020).

Protozoal agents

Cryptosporidium

Four species of *Cryptosporidium* are known to infect cattle (*C. parvum*, *C. bovis*, *C. ryanae* and *C. andersoni*) (Thomson et al., 2017), with *C. parvum* the main

species associated with NCD (Holland, 1990; Chalmers et al., 2011). Calves are prone to *C. parvum* infection following the ingestion of contaminated faecal, water, and food sources (Thomson et al., 2017). Following ingestion, clinical signs of NCD from infection with *C. parvum* typically occur within 2-7 days (Holland, 1990). *C. parvum* oocysts infect the epithelial cells of the small intestine (Cho and Yoon, 2014), leading to villous atrophy which results in loss and blunting of the intestinal villi (Holland, 1990). Further, infection with *C. parvum* leads to reduced mucosal enzymatic activity, which alongside villous atrophy results in impaired digestion and malabsorption of nutrients (Holland, 1990). Through reproduction, *C. parvum* produce further oocysts which result in autoinfection of the host (Cho and Yoon, 2014). These oocysts are later passed from the host into the environment where they act as an immediate source of infection for other animals (Cho and Yoon, 2014; Thomson et al., 2017), commonly infecting calves during their first month of life (Harp et al., 1990).

Pathogen prevalence

Between countries there is considerable variability in the prevalence of pathogens associated with the incidence of NCD (Table 2); however, typically rotavirus and *C. parvum* are the pathogens most commonly associated with NCD (Reynolds et al., 1986; de Graaf et al., 1999; de la Fuente et al., 1999; Langoni et al., 2004; Uhde et al., 2008; Izzo et al., 2011; Içen et al 2012; Al Mawly et al., 2015) (Table 2).

Table 2. Pathogen prevalence reported by country for *Cl. perfringens*, *Salmonella*, *E. coli*, coronavirus, rotavirus and *C. parvum*. To denote those instances where the prevalence of certain pathogens were not reported the abbreviation NR has been used.

Country	<i>Cl. perfringens</i>	<i>Salmonella</i>	<i>E. coli</i>	Coronavirus	Rotavirus	<i>C. parvum</i>
Spain (de la Fuente et al., 1999)	NR	NR	NR	NR	43.0%	52.0%
Belgium (de Graaf et al., 1999)	NR	NR	4.0%	8.0%	20.0%	31.0%
Switzerland (Uhde et al., 2008)	NR	NR	5.5%	7.8%	58.7%	55.0%
Australia (Izzo et al., 2011)	NR	23.8%	17.4%	21.6%	79.9%	58.5%
New Zealand (Al Mawly et al., 2015)	NR	4.0%	11.0%	50.0%	70.0%	50.0%
Great Britain (Reynolds et al., 1986)	NR	12.0%	3.0%	14.0%	42.0%	23.0%
Netherlands (Bartels et al., 2010)	54.0%	NR	2.0%	1.0%	11.2%	15.3%
Turkey (Içen et al., 2012)	NR	NR	26.0%	5.2%	56.9%	47.8%
Brazil Langoni et al., 2004)	NR	11.5%	0.0%	NR	25.1%	21.3%
USA (Cho et al., 2013)	0.0%	9.0%	4.0%	31.7%	27.1%	33.7%

Pathogens have the ability to act both singularly and concurrently (Snodgrass, et al., 1986; de la Fuente et al., 1999; Thomson et al., 2017); in the case of concurrent infections the extent of disease often comes with more severity (Garica et al., 2000; Bazeley, 2003). As with pathogen prevalence, the rate of concurrent infection varies between countries, with reports of the rate of concurrent infection ranging from 5.0-71.0% (de la Fuente et al., 1998; Uhde et al., 2008; Izzo et al, 2011). For example,

in Scotland, a study investigating the occurrence of concurrent infections with two or more pathogens of either rotavirus, coronavirus, *Cryptosporidium*, *E. coli*, and *Salmonella* found concurrent infection to occur in 15.0% of cases of NCD (Snodgrass et al., 1986). Studying the same pathogens, the rate of concurrent infection was much higher in an Australian study which found concurrent infections to occur in 71.0% of cases of NCD (Izzo et al., 2011). The rate of concurrent infection could be a factor that contributes to the overall prevalence of individual pathogens. For example, where concurrent infections occurred at a rate of 71.0% in Australian calves the prevalence of individual pathogens were considerably higher than in Swiss calves (Table 2), where the rate of concurrent infection was only 32.6% (Uhde et al., 2008). Additionally, Izzo et al. (2011) reported that concurrent infections were more common in beef than dairy systems, and that whilst rotavirus and *C. parvum* were the most common pathogens across both production systems, *Salmonella* and rotavirus were more commonly observed in beef systems.

In New Zealand, information regarding the prevalence of pathogens associated with instances of NCD is limited. However, in the first nation-wide study to be conducted, Al Mawly et al. (2015) investigated the prevalence of *E. coli*, coronavirus, rotavirus, *C. parvum* and *Salmonella* across 97 New Zealand dairy farms. Of the 97 farms tested, 96.0% tested positive for at least one pathogen with *E. coli*, coronavirus, rotavirus, *C. parvum* and *Salmonella* identified on approximately 11.0%, 50.0%, 70.0%, 50.0% and 4.0% of the 97 farms respectively (Table 3). Additionally, they identified that concurrent infections with two or more pathogens affected 67.0% of farms, with the most common concurrent infections being rotavirus + *C. parvum*, and rotavirus + coronavirus. They further reported that pathogen prevalence differed with age. In contrast to calves aged between 1-5

days old, in those aged between 9-21 days of age *E. coli* K99+ was not detected. As previously mentioned, calves are most susceptible to *E. coli* infection during the first 4 days of life (Nataro and Kaper, 1998), whilst the bacteria have a greater ability to adhere to the epithelial cells (de Graaf et al., 1999). The nature of *E. coli* infection therefore may reflect why *E. coli* K99+ was not detected in calves aged between 9-21 days of age. However, a greater prevalence of coronavirus, rotavirus, *C. parvum* and *Salmonella* (Table 3) and frequency of concurrent infections was found in calves aged between 9-21 days of age (Al Mawly et al., 2015). This increased prevalence and occurrence of concurrent infections in calves aged between 9-21 days was considered likely to be due to the incubation period of these pathogens during the first few days of life and hence were detected less often in calves aged between 1-5 days old (Al Mawly et al., 2015).

Table 3. The prevalence of pathogens *Salmonella*, *E. coli*, coronavirus, rotavirus and *C. parvum* in diarrhoeic calves aged between 1-5 days old compared to those aged between 9-21 days old, based on findings reported by Al Mawly et al. (2015) in New Zealand dairy calves.

Age	<i>Salmonella</i>	<i>E. coli</i>	Coronavirus	Rotavirus	<i>C. parvum</i>
1-5 days of age	0.7%	11.0%	14.0%	46.0%	18.0%
9-21 days of age	3.1%	0.0%	31.0%	57.0%	52.0%

The same pathogens linked to neonatal diarrhoea in calves have also been associated with neonatal diarrhoea in other species (e.g., lambs, piglets and goat kids), however compared to calves the prevalence levels of the different pathogens show some differences (Table 4). Munoz-Fernandez et al. (1996) reported that similar to calves, for both lambs and goat kids, the leading cause of outbreaks of

neonatal diarrhoea in Spain was *C. parvum* (Table 4). *E.coli* was the next most prevalent pathogen associated with neonatal diarrhoea in both lambs and goat kids, with rotavirus found to be much less prevalent than it often is in calves (Munoz-Fernandez et al., 1996). Coronavirus was not detected in either lambs or goat kids, and *Salmonella* was only detected in outbreaks of diarrhoea in goat kids (Munoz-Fernandez et al., 1996). In piglets, previous studies have reported *Cl. perfringens* and *E. coli* as the most prevalent pathogens associated with incidences of neonatal diarrhoea (Dors et al., 2018; Mesonero-Escuredo et al., 2018). As with calves, lambs, piglets and goat kids are also susceptible to concurrent infections (de Graaf et al., 1999; Dors et al., 2018; Mesonero-Escuredo et al., 2018). Dors et al. (2018), for example reported that across 70 herds of piglets monitored, 60.0% were infected with a single pathogen, and 31.4% were concurrently infected with two or more pathogens.

Table 4. The prevalence of pathogens *Cl. Perfringens*, *Salmonella*, *E. coli*, coronavirus, rotavirus and *C. parvum* in diarrhoeic lambs, piglets and goat kids. To denote those instances where the prevalence of certain pathogens were not reported the abbreviation NR has been used.

Species	<i>Cl. perfringens</i>	<i>Salmonella</i>	<i>E. coli</i>	Coronavirus	Rotavirus	<i>C. parvum</i>
Lambs Munoz-Fernandez et al., 1996 (Spain)	NR	0.0%	60.8%	0.0%	6.5%	65.0%
Goat kids Munoz-Fernandez et al., 1996 (Spain)	NR	7.1%	35.7%	0.0%	14.2%	40.0%
Piglets Mesonero-Escuredo et al., 2018 (Spain)	89.9%	NR	100.0%	NR	NR	NR
Piglets Dors et al., 2018 (Poland)	92.8%	2.9%	30.0%	NR	NR	NR

Risk factors which can influence susceptibility to NCD

In addition to the variety of pathogens associated with NCD, there are a number of management and environmental factors which can influence disease susceptibility. Poor dam nutrition for example can result in calves which show poor performance in terms of both growth and productivity and display a greater susceptibility to disease (Cho and Yoon, 2014). Poor nutrition of the dam also increases the risk of dystocia (difficult calving) (Stoltenow and Vincent, 2003), which further contributes to poor calf performance and susceptibility to disease (Cho and Yoon, 2014). In a previous study, cows that required assistance during calving, due to dystocia, gave birth to calves that were on average 1.44 times more likely to become diarrhoeic (Bendali et al., 1999). Calves which have been assisted during calving will typically take longer to stand following birth and as a consequence may have an increased exposure to pathogens from faecal contamination in the environment during the time they remain lying (Bendali et al., 1999). Dystocia can also result in congestion and swelling of the head and tongue which hinders the calf's ability to feed, preventing adequate colostrum uptake (Cho and Yoon, 2014).

As calves are born agammaglobulinemic (immune deficient), following birth they are reliant upon the consumption of colostrum for the absorption of maternal immunoglobulins (IgGs) (Godden, 2008; Godden et al., 2019). The passive transfer of IgGs from the dam via adequate colostrum uptake is essential for ensuring the calf is protected against infectious diseases immediately following birth (Weaver et al., 2000). To promote successful passive transfer, the adequate and timely feeding of good quality colostrum is recognised as the single most important management factor in reducing morbidity and mortality in preweaned calves (Godden, 2008). In

New Zealand, a recent survey of dairy herds revealed that the pooled colostrum being provided to calves was sub-optimal in quality, and excessive bacterial contamination was detected in a majority of herds (Denholm et al 2017a). Additionally, across 107 New Zealand dairy herds the rate of failure of passive transfer in calves was 33.0% and varied from 5.0-83.0% within herds (Cuttance et al., 2017). Achieving successful passive transfer is beneficial in the long-term for increasing weight gain and feed efficiency, reducing age at first calving, and improving milk production during the postweaning period (Godden, 2008; Godden et al., 2019). Enabling adequate passive transfer, colostrum management is the most important preventive measure for the reduction of neonatal diarrhoea (Arsenopoulos et al., 2017). Insufficient colostrum uptake has been associated with increased risk of diarrhoea and conversely, consuming sufficient amounts of colostrum has been found to reduce mortality in dairy heifer calves in the first 21 days of life by 31.0% (Barrington et al., 2002).

The timing of colostrum feeding is a critical factor in ensuring successful passive transfer (Moran, 2012). In the initial 12 hours of life, the cells of the intestinal wall mature, eventually ceasing their absorptive ability (Moran, 2012). Furthermore, following the first 24 hours of life, the abomasum begins producing acids which improve the functioning of the milk-digestive proteins, however, these same acids degrade the IgGs and thus hinder their effectiveness (Moran, 2012). Additionally, for every half an hour that colostrum feeding is delayed following birth, antibody transfer decreases by approximately 5.0% (Moran, 2012). Colostrum should therefore be fed immediately after birth whilst the intestines still have the ability to absorb IgGs (Jaster, 2005).

In a New Zealand based study the quality of pooled colostrum samples declined by up to 8.5 and 9.5% after being stored for 3 and 7 days respectively (Denholm et al., 2017b). This highlights the importance of feeding colostrum as close as possible to the time at which it is collected to maintain quality. This importance is further demonstrated by the findings that colostrum quality decreases from the time of calving the longer milking is delayed (Moore et al., 2005; Morin et al., 2010; Denholm et al., 2018). From a survey of New Zealand dairy herds, Denholm et al. (2018) reported that 78.0% of the colostrum samples collected from individual cows had a Brix reading <22.0% (an ideal Brix reading being $\geq 22.0\%$ (Bartier et al., 2015; Buczinski and Vanderweerd, 2016)). Additionally, Denholm et al. (2017a) found that 90.0% of pooled colostrum samples had Brix readings <22.0%. To improve the quality of pooled colostrum which is provided to new born calves careful selection of donor colostrum cows would be beneficial (Denholm et al., 2018).

Disease susceptibility has also been found to increase with the length of time calves remain with their dams following birth (Quigley et al., 1994; Trotz-Williams et al., 2007). Compared to calves that were removed from their dams within the first hour following birth, for those which remained longer with their dam, the odds of becoming diarrhoeic increased by 39.0% (Trotz-Williams et al., 2007). The incidence of diarrhoea has also been found to be higher in calves raised with their dam compared to those raised in groups using automated calf feeders (Roth et al., 2009). Additionally, in one week old calves, the prevalence of *C. parvum* was significantly higher in calves which were allowed to nurse from their dams over a three day period compared to calves which were removed before they could nurse and were hand-fed colostrum (Quigley et al., 1994). Dam cleanliness is another

factor associated with disease susceptibility, with an increased incidence of diarrhoea in calves from dams which were considered dirty (Bendali et al., 1999), as these calves have an increased exposure to pathogens on the udder and coat of the dam (Lorenz et al., 2009). The increased prevalence of *C. parvum* reported by Quigley et al. (1994) in calves that had been left to nurse from their dam was attributed to contamination from the dam herself and the surrounding environment (Quigley et al., 1994).

In an attempt to reduce the transfer of pathogens between dam and calf, it has become a common practice on farm to vaccinate cows against pathogens including rotavirus (McNulty, 1983; Saif and Fernandez, 1996), *E. coli*, and coronavirus (Cho and Yoon, 2014) prior to calving. These vaccines increase the antibodies present in the maternal colostrum, which when consumed by the calf can enhance their passive immunity against the pathogen/s for which the dam has been vaccinated (Kohara et al., 1997; Smith et al., 2014). Following whole herd vaccination against rotavirus, New Zealand studies have found increased colostrum quality compared to herds with only partial or no herd vaccination (Denholm et al., 2017a, 2018). As these antibodies are passed on through colostrum, it is vital that calves receive sufficient good quality colostrum to ensure such vaccinations are fully effective, and that the effort and cost of administering vaccinations is worthwhile (Stoltenow and Vincent, 2003). Currently there is no vaccine available for *C. parvum* (Meganck et al., 2014), however calves born to dams which have been treated with preventative calf-scour medications have been found less likely to shed *C. parvum* oocysts (Trotz-Williams et al., 2007).

Other factors which may increase the incidence of diarrhoea include nutrition, herd size, stocking density, housing system, and environmental conditions (Barrington et al., 2002; Klein-Jöbstl et al., 2014). For example, following colostrum feeding, due to the expense of whole milk, dairy calves are often reared on milk replacer, the composition (e.g., fat content, carbohydrates, vitamins, minerals and proteins) of which may vary (Barrington et al., 2002). As a result of their composition, some milk replacers can result in poor growth rates and inadequate nutrition (Fisher, 1976; Bartlett et al., 2014), which can increase disease susceptibility (Barrington et al., 2002).

Increased herd size has also been associated with an increased incidence of diarrhoea due to the greater potential for larger outbreaks of disease and higher stocking densities to promote the spread of disease (Frank and Kaneene, 1993). Furthermore, as herd size increases, the amount of time farmers are able to spend monitoring individual animals decreases, and this lack of individual attention in larger herds has been linked to higher mortality rates in diarrhoeic calves (Lance et al., 1992). In terms of stocking density, Bendali et al. (1999) estimated the risk of diarrhoea to be 1.74 times greater in calves with insufficient space in both tie and free stall systems, where sufficient space allowance was deemed to be 1.6 m² and 1.0 m² per calf respectively. Additionally, Svensson et al. (2006) reported that calves reared in smaller pens (6.2 to <12.6 m²) were 4-10 times more likely to become diarrhoeic than those reared in larger pens (≥ 12.6 m²).

Within the dairy industry, there are a number of different types of housing systems used for rearing calves (e.g., group housed vs individual housing systems) (Marcé et al., 2010). In New Zealand, it is standard practice for calves to be group housed.

Internationally across Europe and the USA the majority of calves are raised in individual housing systems (Marcé et al., 2010; USDA, 2014b), however group housing systems are increasingly being implemented in the USA (Pereira, et al., 2014). At present, approximately 20.0% of dairy operations in the USA are utilizing group housing systems for preweaned calves (Urie et al., 2018). It has been suggested that the type of housing system in which calves are raised may influence their susceptibility to disease, however the impact of housing on disease susceptibility is not always clear or consistent. For example, Linton et al. (1974) reported a higher incidence of *Salmonella* infection in group housed calves compared to those housed individually, further Maatje and Verhoeff (1991) reported a higher incidence of both respiratory and enteric diseases in group housed calves. However, a previous study reported no significant difference in the incidence of respiratory or enteric disease between group or individually housed calves (Waltner-Teows et al., 1986), and additionally other studies have instead reported a lower incidence of diarrhoea in group housed calves (Hänninen et al., 2003). Furthermore, some studies have shown increased mortality rates in group-housed calves (Waltner-Teows et al., 1986; Gulliksen et al., 2009), a finding more evident in calves housed in large groups compared to those housed individually or in smaller groups (Losinger and Heinrichs, 1997). However, it is important to note that this association between housing system and mortality is not always evident (Costa et al., 2016). From these studies, it is evident that the effect of housing on disease susceptibility is unclear, perhaps suggesting its effect is influenced by other animal, management and environmental factors as well.

Weather conditions can also increase the risk of disease (Cho and Yoon, 2014). During the neonatal period, calves are unable to effectively thermoregulate and are

consequently at risk of becoming hyper- or hypo-thermic, especially during extremely cold weather conditions (Cho and Yoon, 2014). Poor weather conditions act as stress factors for calves, negatively impacting their immune system and increasing their susceptibility to disease (Cho and Yoon, 2014). For example, Klein-Jöbstl et al. (2014) reported increased rates of NCD in Austrian dairy calves which were individually housed in outdoor calf igloos compared to calves which were individually barn housed. For the igloo housed calves, the low temperatures and fluctuations in temperature to which they were exposed were considered to have contributed to their increased rates of diarrhoea (Klein-Jöbstl et al., 2014). One way to mitigate the impact of extreme weather conditions is through the provision of adequate clean and dry housing which provides shelter from drafts, precipitation, and solar radiation (Stoltenow and Vincent, 2003; Roland et al., 2016).

Impact of NCD on animal welfare

Although efforts have been made to reduce the incidence and impact of NCD, the disease continues to be recognised as one of the biggest challenges for both beef and dairy industries worldwide (Meganck et al., 2015). The welfare of calves suffering from NCD is initially impacted as a result of the significant intestinal damage inflicted by the causative pathogens which results in symptoms such as nutritional malabsorption and severe diarrhoea (Lorenz et al., 2011). Further, calves suffering from NCD become lethargic, dehydrated, and are subject to significant reductions in appetite and body weight. However, even once individuals have been treated and overcome the disease, NCD continues to have a detrimental impact in the long-term for the once diseased animals later in life. The long-term impacts of NCD include, increased age at first calving, decreased weight gain and decreased

milk production during the first lactation. Instances of diarrhoea during the neonatal period for example, have been associated with reductions of body weight of approximately 10.7 kg at the time of weaning (Wittum et al., 1994). Impacting the age at time of first calving, those animals with a known history of diarrhoea in the first 90 days of life have also been found 2.86 times more likely to reach first calving after 900 days of age (Waltner-Toews, 1986). This finding is in contrast to animals without a known history of diarrhoea who were more likely to reach first calving prior to 900 days of age (Waltner-Toews, 1986). Similarly, Aghakeshmiri et al. (2017) reported a 10 day increase in the time to first calving for animals with a known history of diarrhoea and suggested that this delay may reflect their stunted growth rate during the rearing period. Additionally, it was found that farmers were 2.5 times more likely to sell animals with a history of diarrhoea in the first 90 days of life as opposed to those which had no prior history of diarrhoea (Waltner-Toews, 1986). The sale of animals with a history of diarrhoea was considered to reflect the actual or anticipated under performance of those animals (Waltner-Toews, 1986). Additionally, first lactation milk production has been found to decrease during the first 305 days of lactation by 344 kg in animals with a history of having had diarrhoea during their first three months of life (Svensson and Hultgren, 2008).

In addition to the initial and long-term impacts of NCD, there is also the issue of mortalities which arise in those individuals which are so severely affected by NCD that they succumb to the disease. Highlighting the extent of the impact of diarrhoea on animal welfare in terms of mortalities, between 1991-2013, diarrhoea accounted for more than 50.0% (range: 52.2-62.1%) of all preweaned heifer calf deaths in the US dairy industry (USDA, 2008; USDA, 2014a). Consequently, between 1991-2013, diarrhoea was consistently the leading cause of death in preweaned heifer

calves in the US dairy industry compared to other causes such as respiratory diseases, joint/navel ill and lameness (USDA, 2008; USDA, 2014a) (Figure 3).

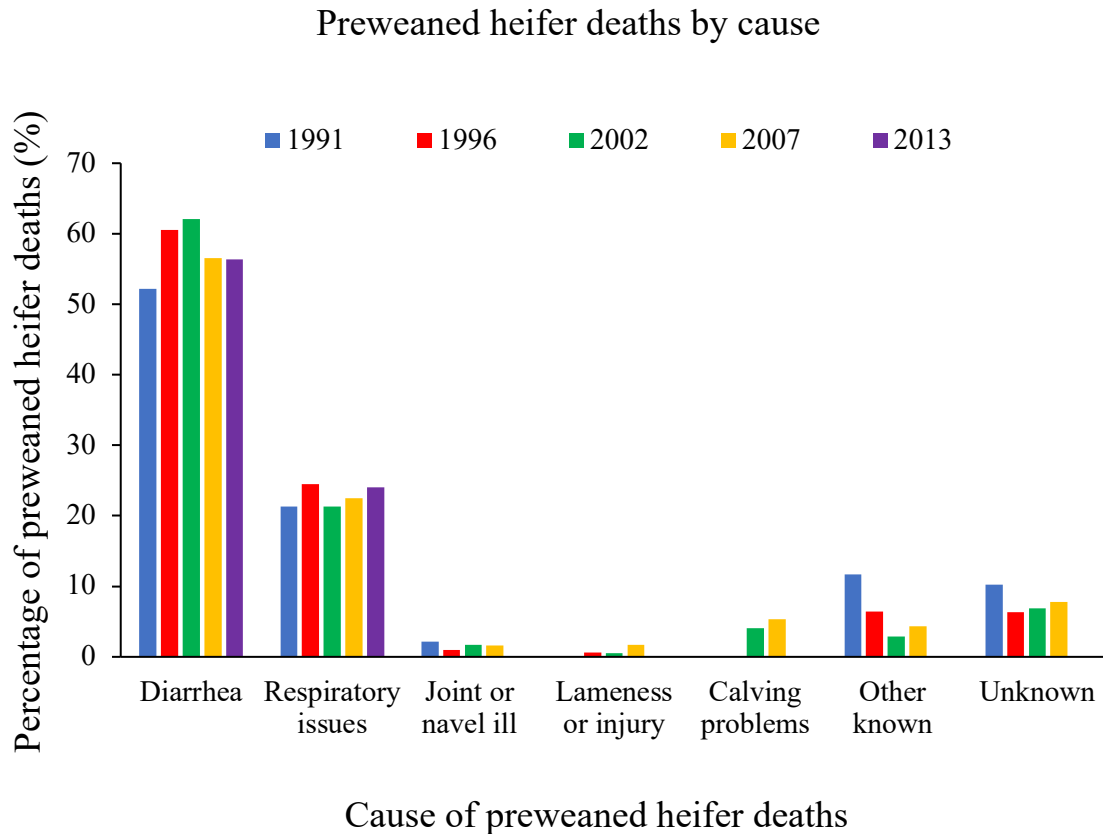


Figure 3. Percentage of preweaned heifer deaths in the US dairy industry by causation from 1991-2013 (adapted from data presented by USDA, 2008 and USDA, 2014a).

Treatment of NCD

Once identified as having NCD calves should be isolated to prevent the spread of disease to other pen mates and treatment should be administered to prevent the amount of time the animal suffers. Calves suffering from NCD lose an average of 10.0% but up to 20.0% of their body weight in fluid (Lorenz, 2013). Therefore, once a calf has been identified as having NCD, oral rehydration with electrolytes is the most critical component of their treatment to help them overcome the disease (Lorenz et al, 2011). Continued milk feeding throughout the treatment period is

generally recommended to provide the energy required for weight gain and growth and additionally to provide the nutrients needed to support the recovery of the intestinal mucosa (Lorenz, 2013). As the consumption of electrolytes can affect the clotting of milk in the abomasum, it is generally advised for electrolyte and milk feeds to be separated by at least 2-3 hours. However, there are types of electrolyte therapy available such as Revive (Virbac, Hamilton, New Zealand) that can be provided alongside a milk feed. In cases of severe dehydration the administration of intravenous (IV) fluids may also be essential for their recovery. The degree of dehydration can be assessed through evaluating the degree of eye recession into the orbit (enophthalmos), performing a tent test to measure skin elasticity and by observing the general appearance of the calf including its ability to stand and suckle (Berchtold, 2009; González-Montaña et al., 2017; Taylor et al., 2017).

The use of antibiotics for treating NCD is controversial with arguments both for and against its use (Smith, 2015). Antibiotics may not always be a suitable option for the treatment of NCD especially in those instances in which viruses (e.g., coronavirus and rotavirus) or protozoal pathogens (e.g., *Cryptosporidium parvum*) are responsible for the infection due to an inability for antibiotics to act on such pathogens (Animart, 2014; Anexa, 2015). Instances in which antibiotic treatment may be suitable during cases of NCD include those attributed to bacterial infection and in which the calf is displaying symptoms such as anorexia, dehydration, depression or fever as these may indicate septicaemia or a high presence of coliform bacteria in the small intestine (Smith, 2015). The overuse of antibiotics is also a concern regarding the risk of antibiotic resistance which could result in the development of pathogen strains that are resistant to treatment and by altering

intestinal flora the administration of antibiotics may actually induce diarrhoea (Anexa, 2015; Smith, 2015).

Given the pain and discomfort calves may experience when suffering from NCD the administration of pain relief can also be beneficial. The use of a nonsteroidal anti-inflammatory drug (NSAID) can help to reduce inflammation of the gastrointestinal tract and reduce the effects of endotoxaemia and septicaemia. The administration of the NSAID meloxicam has previously been shown to improve appetite and performance and is considered to be effective in the treatment of NCD (Todd, 2010).

Economic impact of NCD

Economically the incidence of NCD is a major concern for beef and dairy industries worldwide. This economic concern not only stems from calf loss but also the long-term effects on calf performance (e.g., as a result of reduced weight gain, stunted growth and age to first calving), the cost of treatments (e.g., antibiotics and rehydration fluids) and the cost of additional labour required to treat sick calves (de Graaf et al., 1999; Bazeley, 2003; Smith, 2012; Windeyer et al., 2014). There appears to be little information regarding estimates as to the economic impact of NCD in New Zealand. However, it has been suggested that in the case of a rotavirus outbreak, costs could be up to NZ\$6000 per affected farm (Howe et al., 2011). In the UK, it has previously been estimated that 30.0% of all calves born will be affected by NCD, and that NCD is the cause of approximately 50.0% of all calf deaths, suggesting that 100,000 calves will die annually as a result of NCD (Byron, 1997). The treatment costs of NCD in the UK have been estimated at £26.00 per sick animal. Based on this estimate and taking into account the subsequent long-

term effects of NCD, the average cost of an NCD outbreak per 100-cow herd has been estimated at £3691.00 or the equivalent of £36.91 per calf born (Byron, 1997). Research has further estimated that NCD costs the UK economy approximately £11 million per year (Bennett and Ijpelaar, 2003). In Australia, costs associated with NCD have been estimated at AUD\$73 per calf (Gunn, 2003), whilst the estimated cost to the US industry is approximately US\$250 million per annum (Frank and Kaneene, 1993), with one third of US beef farmers stating that NCD impacts them economically (Lorenz et al., 2011).

Behavioural and physiological responses to disease

The incidence of disease can be thought of as consisting of 3 stages: infection, sickness and recovery (Hart and Hart, 2019) (Figure 4). During the infection stage, in response to viral, bacterial and protozoal infection (Viljoen and Panzer, 2003) activated immune cells (i.e., monocytes, macrophages and lymphocytes) (Vollmer-Conna, 2001) trigger the production of pro-inflammatory cytokines including tumour necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6) (Dantzer 2004). Cytokines increase activation of the immune system by conveying information to the brain, which during the sickness stage triggers behavioural and physiological responses in the form of sickness behaviours, fever and pituitary-adrenal axis activation (Dantzer 2004; Quan and Banks, 2007). Sickness behaviours encompass a series of specific behavioural responses that will often be displayed by animals whilst experiencing disease (Hart, 1988; Millman, 2007). These behavioural responses include lethargy, anorexia, adipsia, anhedonia, hyperalgesia, depression, and decreases in social interaction, grooming, and mental ability (Hart, 1988; Gregory, 1997; Viljoen and Panzer, 2003; Millman, 2007; Adelman and

Martin, 2009; Lopes et al., 2016). These sickness behaviours are often used to identify sick animals and depending on the disease are generally accompanied by a febrile response (Hart, 1988).

Contrary to initial beliefs that sickness behaviours were maladaptive or an effect of debilitation, Hart (1988) proposed that the behaviour of a sick animal is instead a highly organised behavioural strategy which has evolved to facilitate the role of fever to combat infection and consequently increase the chances of survival (Hart, 1988). The development of fever is recognised as a significant component of the immune response (Kluger et al., 1975), whereby achieving a body temperature of 38-40°C potentiates immune cell activity and inhibits pathogenic growth (Aubert, 1999). In addition to causing fever, IL-1 acts to inhibit pathogenic growth by reducing blood iron levels (Hart and Hart, 2019). Serum Fe concentrations have been found to decrease rapidly in response to inflammation (Baydar and Dabak, 2014), and in cattle have been found to decrease in response to diseases such as mastitis, reticuloperitonitis, and BVD (Lohuis et al., 1990; Erskine and Bartlett, 1993; Middleton et al., 2004; Aich et al., 2009; Baydar and Dabak, 2014). During the recovery phase the immune system acts to inhibit pathogen growth and facilitates the elimination of pathogens from the body (Hart and Hart, 2019). Additionally, during the recovery phase the cytokine activity ceases and the animal will begin to recover and resume normal activities (Hart and Hart, 2019).

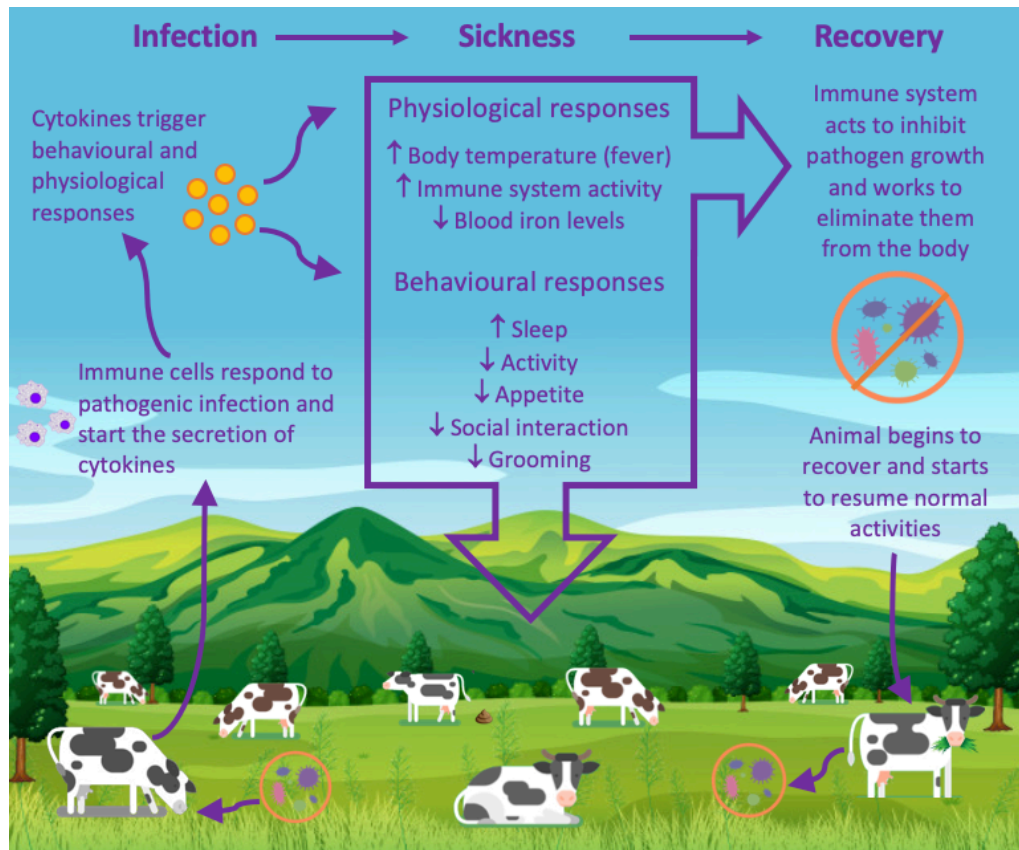


Figure 4. Illustration of the infection, sickness and recovery stages of disease in which pathogenic infection triggers the production of cytokines which results in physiological and behavioural responses during the sickness stage which act to ensure recovery through pathogen inhibition and removal.

Mounting a febrile response is an energetically demanding task; in humans it is estimated that a 1°C increase in body temperature requires a 13.0% increase in metabolic rate (Hart, 1988). The increase in metabolic rate in animals varies among species, and in relation to differences in body size, coat density and habitat (Hart and Hart, 2019). Due to the high energetic demands of fever, in order for a fever to be sustained, behavioural changes are required to reduce the energetic demands for other functions (Millman, 2007). Diseased animals may display increased lying behaviour and reduced activity, both of which are generally considered beneficial for conserving energy and preventing heat loss (Hart, 1988). Whilst lying, animals may alter their typical lying postures for example by curling up or tucking their

limbs in towards their bodies in order to reduce the total surface exposure to the environment to further conserve heat and reduce shivering (Hart, 1988). Increasing the insulative properties of an animal's coat, piloerection can also help to reduce heat loss (Hart and Hart, 2019). Increased sleep has also been associated with less severe clinical symptoms in response to disease. In rabbits for example, following experimental infection with *E. coli*, *S. aureus* or *Candida albicans*, mortality rate was higher in rabbits which spent less time sleeping compared to those which survived the infection (Toth et al., 1993). A reduction in activity is beneficial in conserving energy for use in the immune response and enhancing the ability to overcome infection (Hart, 1988; Medzhitov et al., 2012). This reduction in activity can also reduce, for example, the rate of social encounters (Lopes et al., 2016) and allogrooming (Stockmaier et al., 2018).

During sickness, animals experiencing fever require sufficient calories to support the elevated body temperature and prevent muscle wastage (Hart and Hart, 2019). However, there is a trade-off, particularly for animals in the wild, where the effort of foraging or hunting in order to obtain food can often require a significant expenditure of energy (Hart and Hart, 2019). Therefore, to conserve energy and meet the increased metabolic demands of fever, in response to disease many animals will display signs of anorexia. Additionally, as mentioned earlier a reduction in iron concentration in the blood helps to inhibit pathogen growth, and a reduction in feed intake can be beneficial in reducing the amount of iron being brought into the body (Hart and Hart, 2019). It is also common in response to disease for animals to display a significant reduction in grooming behaviours and consequently they may be identified as having an oily, dirty or scruffy looking appearance (Hart and Hart., 2019). In response to metritis for example, cows have

been found to reduce the amount of time spent grooming (Mandel et al., 2017). As with obtaining food, grooming also requires significant energy expenditure which could otherwise be spent supporting the febrile response. Furthermore, grooming can also lead to increased heat loss through exposure of the body surface and an increased loss of water through the saliva used for grooming (Hart and Hart., 2019). Whilst a reduction in grooming can result in an increased parasite load (e.g., fleas and ticks) (Mooring et al., 1996; Eckstein and Hart, 2000), a return to grooming once the animal has overcome disease can soon see the removal of such parasites (Hart and Hart, 2019).

It is important to note that sickness behaviours are context-dependent (Millman, 2007), where their display can be interrupted if the need to overcome disease is outweighed by the motivation to perform other activities. For example, following a lipopolysaccharide (LPS) challenge, in warm conditions (24°C), female mice still retrieved stray pups but showed reduced nest building behaviours (Aubert, 1999). When the temperature was lowered to 6°C, female mice still retrieved their stray pups, but showed no difference compared to controls in the amount of nest building behaviours (Aubert, 1999). This finding illustrates that the motivation to express sickness behaviours was outweighed by the mother's motivation to perform nest building behaviours in order to ensure the survival of her offspring (Aubert, 1999). Additionally, infected animals might have a tendency to avoid healthy conspecifics and potentially vice versa with healthy animals tending to avoid infected conspecifics (Boillat et al., 2015; Gervasi et al., 2018). This change in behaviour which enables avoidance may be an important factor influencing the degree of pathogen transmission (Silk et al., 2017). However, it is important to note that in species such as vampire bats, rhesus macaque, and banded mongoose the benefits

of social interactions might outweigh the benefits of avoiding sick conspecifics and, in such scenarios, avoidance may not occur to the same extent (Stockmaier et al., 2018).

Automated disease detection

As discussed, in order to identify an animal as being affected by disease, an animal can be monitored for signs of sickness behaviours and dependent on the specific disease the presence of a fever. Traditionally, it is common for behaviours to be monitored by live or video observations. However, these traditional methods of observation are often time consuming, labour intensive and may be subjective (Weary et al., 2009; Daigle and Siegford, 2014). Furthermore, detecting a fever typically relies on the collection of rectal temperatures, which are invasive and require animal restraint and handling (Chung et al., 2010).

When assessing livestock, the reliance on behavioural observations to identify overt clinical signs of disease on-farm can become further problematic given that many farm animals, being prey species, are stoic in nature (Weary et al., 2009). Displaying stoicism, farm animals therefore have a higher tendency to mask signs of illness and vulnerability, which in the wild would potentially serve as a strategy to protect them against predation (Weary et al., 2009). As a result, there is a dependence on those observing animals to be able to reliably detect often subtle changes in behaviour which are indicative of illness. When suffering from mastitis for example, cows can be identified based on the expression of typical sickness behaviours which can be observed through decreases in feed intake, rumination and grooming (Fogsgaard et al., 2012; Dittrich et al., 2019). Furthermore, whilst an increase in lying time is typically observed in response to disease (Broom, 2006),

in response to mastitis, cows have been found to decrease the amount of time spent lying which is considered to be in response to the pain associated with inflammation of the udder (Siivonen et al., 2011; Cyples et al, 2012; Fogsgaard et al., 2015). As previously mentioned, animals suffering from NCD can be identified based on clinical symptoms including depression or apathy, diarrhoea, dehydration, fever and decreased feed intake (de verdier Klingenberg, 2000). For animals suffering from tick-borne disease, detection is reliant on clinical symptoms including pale mucous membranes, increased heart and respiratory rates, fever, weakness, lethargy and anorexia (Kocan et al., 2010).

At present, there is an increasing shift towards automation within the livestock industry (Hamadani and Kahn, 2015; Seiferth, 2020) and this shift is largely driven by a desire to reduce labour costs and pressures on animal management (Gargiulo et al., 2018). In the dairy industry for example, increasing herd sizes contributes to added complexity regarding the ability for farmers to successfully monitor and manage individual cows within the herd and as such requires increased management capabilities (Edwards et al., 2015; Bewley, 2016). The adoption of automated technologies and sensor systems (i.e., precision technologies) on-farm provides farmers with a means to reduce labour and improve animal management (Bewley, 2010; Eastwood et al., 2012, 2015; Rodenburg, 2017). Some of the automated technologies currently available to farmers include, automated cup removers, sorting gates, calf feeders, robotic milking systems and electronic cow identification systems (Edwards et al., 2015; Rodenburg, 2017).

Whilst automation offers a number of benefits, it can reduce traditional “hands on” approaches to farming which when coupled with fewer experienced stockpersons

in the industry and increasing herd sizes, can be to the detriment of animal welfare and production. In the case of disease, for example, this less “hands on” approach coupled with fewer experienced stockpersons capable of detecting the often subtle signs of disease can lead to animals presenting clinical signs going undetected. Furthermore, as discussed earlier increasing herd sizes can also contribute to instances of disease going undetected as this reduces the amount of time which can be spent monitoring individual animals (Lance et al., 1992). Additionally, by the time an animal displays clinical symptoms of disease much of the damage may have already occurred internally. This is evident in calves which are diseased with NCD, as by the time clinical signs (e.g., diarrhoea) are evident significant internal damage to the intestines has already occurred, as it is this intestinal damage which results in calves becoming diarrhoeic. It would therefore be useful if through future developments, automated systems could be developed which incorporate monitoring systems with the capabilities to enable early disease detection in order to minimise the impact of disease on animal welfare and production. The development of automated systems would also help overcome some of the issues which arise when using traditional methods to monitor animals for signs of disease. Previous studies conducted overseas have already demonstrated a number of automated methods for detecting disease based on changes in physiology and behaviour, for example changes in infrared temperatures (Schaefer et al., 2004, 2007, 2012), grooming (Mandel et al., 2017) and feeding behaviours (Svensson and Jensen, 2007; Borderas et al., 2009; Wolfger et al., 2015). However, automated systems have not been thoroughly investigated in New Zealand conditions where livestock are exposed to different diseases, pathogen prevalence and management practices and hence is an area requiring further investigation.

Infrared thermography

Infrared thermography (IRT) is a non-invasive method of detecting the amount of infrared energy being emitted from an object and can be measured from all objects with a temperature above absolute zero (-273°C) (Usamentiaga et al., 2014). As a function of temperature, the more infrared energy an object radiates the greater the temperature of the object (Usamentiaga et al., 2014). Infrared energy is not visible to the human eye, therefore infrared devices process information regarding the amount of infrared energy being emitted and convert this information into a temperature measurement. Based on these temperature measurements, infrared devices are then able to generate visual false-colour images (Figure 5) known as ‘thermograms’ by assigning different colours to represent different temperatures which reflect the different levels of infrared energy being emitted by the object (Redaelli and Caglio, 2013).

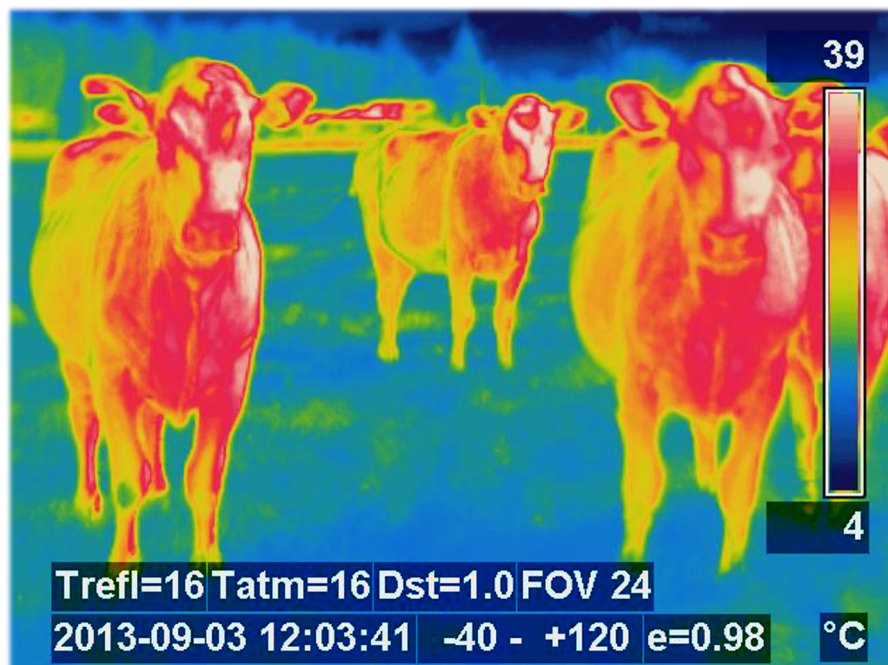


Figure 5. Infrared image illustrating how different temperatures are represented through the use of different colours to produce a visual false-colour image. As indicated by the temperature scale on the right hand side of the image, the warmest areas appear as red whilst the coolest areas appear in blue.

In relation to animal physiology, based on measuring changes in heat transfer and blood flow which occur in response to environmental and physiological conditions (McManus et al., 2016), IRT acts as a remote, non-invasive method of detecting changes in an animal's surface temperature (Cook and Schaefer, 2013). Using IRT, the absolute temperature values detected and their distribution may be associated with underlying physiological, metabolic and behavioural processes and mechanisms (Mitchell, 2013). When an animal experiences stress for example, the hypothalamic-pituitary-adrenocortical axis is activated and heat is produced in response to increased catecholamine and cortisol concentrations, and there are corresponding changes in blood flow (Schaefer et al., 2002). These changes in heat production and blood flow impact the amount of heat being lost from the animal (Schaefer et al., 2002), and hence by enabling changes in surface temperature to be measured, infrared thermography can be a useful tool for indicating stress (McManus et al., 2016). Based on changes in different anatomical regions (e.g., eye, neck, flank, and rump) infrared thermography has been used in previous studies to detect stress (Stewart et al., 2007; Montanholi et al., 2008; Paim et al., 2012; Luzi et al., 2013; Cannas et al., 2018; Redaelli et al., 2019). It is also possible that by measuring changes in surface temperature, IRT can also be applied as a method for detecting diseases in animals based on the detection of fever/inflammation (Rekant et al., 2015). Recent reviews of the use of IRT in livestock and veterinary applications include those by Luzi et al. (2013), Rekant et al. (2015), and McManus et al. (2016). Some examples of the applications for which IRT has been applied include the detection of bluetongue virus in sheep (Pérez de Diego et al., 2013), foot and mouth disease (FMD) in mule deer (Dunbar et al., 2009), rabies in raccoons (Dunbar and MacCarthy, 2006), thoracolumbar vertebral disk disease in dogs

(Grossbard et al., 2014), determining pregnancy in zebras and black rhinoceros (Hilsberg et al., 1997) and for predicting meat quality in pigs (Lawrence et al., 2001; Weschenfelder et al., 2013). In regards to cattle welfare, some of the specific applications for which IRT has been used include diagnosing mastitis (Colak et al., 2008; Polat et al., 2010; Pezeshki et al., 2011; Zaninelli et al., 2018) and lameness (Nikkah et al., 2005; Alsaad et al., 2015; Harris-Bridge et al., 2018) by detecting areas of inflammation and as a method for measuring stress and pain in response to procedures such as disbudding (Stewart et al., 2007), castration (Stewart et al., 2010), transport (Schaefer et al., 1988; Cuthbertson et al., 2020), and handling (Stewart et al., 2008). Studies have also investigated IRT as a method for the early detection of BRD, BVD and FMD, in which the use of IRT demonstrated changes in temperature prior to the presence of clinical symptoms of disease (Schaefer et al., 2004, 2007, 2012; Rainwater-Lovett et al., 2009).

Whilst investigating IRT as a method for detecting BVD, Schaefer et al. (2004) collected infrared images from various anatomical locations and found temperatures increased by 1.5 - 4.0°C prior to clinical signs, with changes of <1°C being clinically significant. Furthermore, the different anatomical areas measured showed varied levels of sensitivity (Schaefer et al., 2004). Whilst clinical symptoms of BVD were not evident until 8-9 days post inoculation, changes in eye temperature occurred as early as 1 day post infection; however, changes in nose, ear, hoof, lateral and dorsal temperatures were not significant until 5-6 days post infection (Schaefer et al., 2004). The increased sensitivity of the eye compared to other anatomical locations likely relates to the presence of vascularisation close to the skin surface providing a more accurate measure of changes in blood flow as a function of thermoregulation, enabling more accurate detection in the eye region

(Ng et al., 2004). During a febrile response, an animal may display an initial increase in core temperature by reducing heat loss through vasoconstriction, which may result in a decrease in surface temperature. As the fever develops, there will be a continued increase in core body temperature and consequently increased heat loss will occur resulting in an increase of the animal's surface temperature (Schaefer and Cook, 2013).

Images of different anatomical locations have also been collected in previous studies investigating the use of IRT for assessing feed efficiency (Montanholi et al., 2009, 2010; Martello et al., 2016). Martello et al. (2016) found that compared to cattle with high residual feed intake (RFI), low RFI cattle, had higher infrared temperatures on the front of the head, whilst other anatomical locations (i.e., eye, ribs, flank, rump, and feet) showed no significant difference regarding an animal's RFI status (Martello et al., 2016). In contrast, Montanholi et al. (2009, 2010) found that infrared temperatures of the snout, cheek, and hoof were more suitable indicators of feed efficiency. However, it is important to note that the findings of these studies go beyond those associated with the suitability of different anatomical locations for indicating feed efficiency. Consistent with the hypothesis that more feed efficient animals waste less energy, converting more energy towards growth than less efficient animals, Montanholi et al. (2009, 2010) found a positive correlation between radiated temperature and RFI. In contrast, Martello et al. (2016) found a negative correlation between radiated temperature and RFI with low RFI animals displaying higher radiated temperatures than high RFI animals. Although inconsistencies were reported between these studies (Montanholi et al., 2009, 2010; Martello et al., 2016), a consistent finding was that infrared temperatures collected from the extremities were the most useful indicators for determining feed

efficiency. The differences observed in studies focussed on BVD and feed efficiency suggest that the suitability of different anatomical areas is dependent on the specific application for which IRT is being applied and therefore needs to be considered when determining which anatomical areas will act as the best indicators.

Similar to the application of IRT for detecting diseases such as BVD, BRD and FMD there is the potential that IRT could also be applied for the early detection of NCD, where different anatomical locations may also display varying degrees of sensitivity to disease. In response to the onset of NCD, an animal may reach a state of fever, by restricting blood flow to the skin and extremities in an attempt to reduce heat loss to the environment. As discussed earlier, sickness behaviours are often accompanied by the development of a fever which helps enable the animal to overcome infection. Through the use of IRT, changes in surface temperature which occur in response to fever may be observed as the animal attempts to maintain homeostasis. In addition to fever, IRT may also be able to detect a change in surface temperature in response to the localised inflammation of the intestines which results from the pathogenic damage which occurs during infection. Furthermore, IRT can be used to continuously monitor animals over extended periods of time which enables a history of baseline data to be collected where deviations from what is considered 'normal' can be used as an indicator for early disease detection (Cook and Schaefer, 2013). Further, with the use of radio frequency identification (RFID) ear tags, in addition to monitoring whole groups of animals, IRT can be used to monitor animals on an individual basis (Cook and Schaefer, 2013). This is another capability which could enable animals displaying early signs of disease (e.g., NCD) to be identified and treated on an individual basis.

During the collection of infrared images, there are a number of image collection, environmental and animal factors which should be taken into consideration to ensure accurate results (Rekant et al., 2015). The distance and angle of the camera to the animal, for example, have a significant impact on the accuracy and precision of the recorded temperature (Church et al., 2014; Talukder et al., 2014; Faye et al., 2015), and as such these factors should be kept as stable as possible throughout the observation period (Cook and Schaefer, 2013). One way to ensure accurate and precise results are obtained is through capturing images whilst animals are stationed in front of a fixed-position camera, for example when animals are in a milking bail (Kunc et al., 2007), a cattle crush (Schaefer et al., 2004, 2007) or at a water station (Schaefer et al., 2012). In the case of NCD, automated calf feeders offer a potential platform to which IRT could be integrated to support the collection of infrared images. Automated calf feeders consist of a narrow chute within which the animals stand as they feed. This chute limits the movement of the animal and would help keep animals stable during image collection to ensure distance and angle are kept constant. As not all farms have access to automated calf feeders, a water station such as that used by Schaefer et al. (2012) could be an alternative area for image collection, where images are collected whilst calves visit a water trough.

In addition to distance and camera angle, surface temperature is also influenced by environmental conditions especially in outdoor environments when animals are exposed to sunlight, wind chill and precipitation. Precipitation for example can result in lower temperatures as a result of the body surface becoming wet (Cook and Schaefer, 2013); in contrast, direct sunlight causes solar loading which can warm the body surface and result in a higher temperature reading (Cook and Schaefer, 2013). To account for changes in ambient temperature and humidity over

the observation periods, the accuracy of the infrared camera can be improved through calibration by entering information pertaining to these variables directly into the camera at the time of image collection or into analysis software during image analysis. If IRT was integrated into an automated system such as an automated calf feeder or a robotic milking system, systems which are generally housed indoors, the impact of environmental conditions could be reduced. Furthermore, as part of the integration of IRT into an automated system, data pertaining to ambient temperature and humidity could be recorded and updated automatically at specified intervals directly into the system.

It is also possible that animal surface temperatures can be impacted by circadian and infradian rhythms which influence the amount of blood flow and thermoregulation occurring at different times within or between days (Alsaod et al., 2015; Rekant et al., 2015). For example, whilst investigating the use of infrared thermography as a potential tool for indicating mastitis, such an impact on surface temperature was reported by Berry et al. (2003), where within-day monitoring at 2h intervals revealed that the surface temperature of the udder has a circadian rhythm. Hence the impact of such rhythms and their impact on blood flow and thermoregulation should be considered during the interpretation of thermographic images.

Another animal-based factor which can significantly influence the surface temperature of an animal measured using infrared thermography is the presence and thickness of pelage or plumage (e.g., fur, hair, wool and feathers) (Cilulko et al., 2012; Mitchell, 2013). For animals or anatomical regions which are densely covered (e.g., sheep), infrared temperatures will reflect the surface temperature of

the pelage or plumage, which will be influenced considerably by air temperature (Mitchell, 2013). In contrast, animals or anatomical regions with minimal coverage (e.g., pigs, elephants, and rhinoceros and facial or limb regions) will more closely reflect skin temperature and will be less impacted by air temperature (Hilsberg-Merz, 2008; Mitchell, 2013). The presence of moisture or dirt on the animal can also impact the infrared surface temperature readings, interfering with the emissivity value of the surface (Alsaad et al., 2015; Rekant et al., 2015). In addition, the colour of the pelage or plumage may also influence infrared temperature measurements (Rekant et al., 2015). In zebra for example, during the day black stripes appear warmer than the contrasting white stripes which likely reflects a greater amount of solar loading occurring at the black stripes (Benesch and Hilsberg, 2003). The black stripes of Zebra have previously been shown to be at least 10°C warmer than their white stripes when exposed to full sunlight (McCafferty, 2007). At night however, the opposite occurs with the black stripes instead appearing cooler than the white stripes which might be related to the ability for thermal energy to be released by the different stripes (Benesch and Hilsberg, 2003). Similar observations have been noted in cattle, with black patches appearing warmer than white patches (Hellebrand et al., 2003). The eye is one specific location which can be measured without being impacted by the presence or pelage or plumage (Stewart et al., 2007), and compared to other anatomical locations (e.g., nose, ear, body and hooves) has previously been shown to provide more consistent temperature changes in relation to early disease detection in cattle (Schaefer et al., 2004).

Physical activity prior to image collection can also increase infrared temperature readings by increasing the amount of heat being released by the skeletal muscles to

the body surface (Hilsberg-Merz, 2008). Reducing the amount of physical activity prior to image collection would help reduce impacts on the results. As stress is also known to impact the thermal profile of an animal, to prevent stress confounding the results it is recommended that images be collected following a period of acclimatisation, allowing the animal to adjust to its environment and the presence of the camera operator (Hilsberg-Merz, 2008; Cilulko et al., 2012; Alsaad et al., 2015).

Automated feeding systems

Automated calf feeders are a form of automated feeding system increasingly being used on-farm as they are beneficial in reducing labour costs and have the capability to promote more natural feeding behaviour, reducing future health problems and associated costs (Janzekovic et al., 2011). Compared to traditional manual feeding methods, automated calf feeders also allow calves to be fed in smaller portions more frequently which helps enable proper digestion, promotes growth and limits the amount of milk wasted (Janzekovic et al., 2011). Automated feeders use electronic identification (EID) technology built into the calf's ear tag to individually identify animals as they approach the feeder and have the capability to record information pertaining to feeding behaviour including milk consumption, drinking speed, visit duration and number of visits both rewarded (calf receives milk) and unrewarded (calf does not receive milk) calves make to the feeder.

Using automated calf feeders, previous studies have investigated the use of changes in feeding behaviours as early indicators of disease (Svensson and Jensen, 2007; Borderas et al., 2009; Johnston et al., 2016; Knauer et al., 2017; Swartz et al., 2017; Sutherland et al., 2018). Svensson and Jensen (2007) found that in response to

disease calves showed a decrease in the number of unrewarded visits made to the feeder, however, showed no change in the number of rewarded visits, drinking speed, or milk consumption. Similarly, Sutherland et al. (2018) and Knauer et al. (2017) reported a decrease in the number of unrewarded visits, however in contrast to Svensson and Jensen (2007) both also reported a decrease in milk consumption in response to disease. It is evident based on the discrepancies between these studies that the effect of disease on milk consumption is conflicting. However, it has been suggested that the effect of disease on feeding behaviour can be influenced by the amount of milk provided (Borderas et al., 2009). Borderas et al. (2009) reported that when provided a high milk allowance (12 L/day or ad libitum), in response to disease calves showed a decrease in both milk consumption and total number of visits to the feeder and increased visit duration. In contrast, when calves were provided a low milk allowance (4 L/day) diseased calves showed no change in milk consumption or in the total number of visits, and visit durations were instead found to decrease. These findings by Borderas et al. (2009) highlight the importance of considering the milk allowance provided when attempting to identify diseased animals based on changes in feeding behaviour.

The specific disease being monitored may also be a potential factor contributing towards the discrepancies amongst the different studies. In a previous study by Knauer et al. (2017) calves were deemed to be suffering from disease when they presented signs of either pneumonia, diarrhoea or general ill-thrift. When these causes of illness were considered collectively, calves were found to decrease milk consumption, the number of unrewarded visits and drinking speed prior to clinical signs of disease. However, when the causes of illness were considered separately to one another, there were some considerable differences of the effect of disease on

feeding behaviours. Prior to overt clinical signs of disease, drinking speed and unrewarded visits were both lower for diarrhoeic calves and those showing general signs of ill-thrift. For calves diagnosed with respiratory illness, drinking speed and unrewarded visits were only lower on the day of clinical detection. Milk consumption was only found to change prior to clinical signs for diarrhoeic calves. Overall, Knauer et al. (2017) reported that calves with diarrhoea displayed the earliest and most consistent changes in feeding behaviours prior to clinical signs of disease followed by calves with general ill-thrift and finally those with respiratory illness.

Aside from automated calf feeders, electronic feed bins have also been used to monitor changes in dry feed intake and feeding behaviours for disease detection. Through the use of electronic feed bins, Oliveira Júnior et al. (2018), reported that in response to tick-borne disease, weaned dairy calves showed a decrease in daily feed intake during days -1 to +1 relative to clinical detection. Additionally, calves also decreased the daily frequency and total duration of visits to the feed bins across days -3 to +4. Through the use of electronic feed bins, a decrease in feed intake and feeding duration has also been observed in cows with metritis, where these differences could be detected as early as 2 weeks prior to clinical signs (Huzzey et al., 2007). Currently, one of the most common automated feed monitoring systems available is the GrowSafe System (Airdrie, Alberta, Canada) (Richeson et al., 2018). The GrowSafe System has been used in previous studies to determine, for example, the effects of vaccination (Arthington et al., 2013), trace mineral source in low- or high- sulfur diets (Hartman et al., 2017) and the early detection of BRD (Wolfger et al., 2015; Jackson et al., 2016; Kayser et al., 2019). Using the GrowSafe System, Wolfger et al. (2015) demonstrated the ability to predict BRD in cattle 7

days prior to clinical signs based on the mean intake per meal, mean meal duration and meal frequency.

Accelerometers

Accelerometers are another example of automated monitoring devices which are increasingly being used on farm. These devices are non-invasive and are commonly attached to an animal's leg, neck or ear tag in order to monitor lying behaviour (Mattachini et al., 2013; Sepúlveda-Varas et al., 2014), activity (Medrano-Galarza et al., 2012), feeding behaviour (Mattachini et al., 2016) and rumination (Hamilton et al., 2019) and have been used for detecting estrus (Valenza et al., 2012) and disease (Sepúlveda-Varas et al., 2014). Time spent lying is a particularly sensitive indicator of disease and has been investigated previously for example in response to BRD, where dairy calves were found to increase the amount of time spent lying (Olivett et al., 2014; Hixson et al., 2018). Similar to this, in a study by Borderas et al. (2008) in which calves were injected with LPS to stimulate a fever response, calves increased the amount of time they spent lying. It has also been suggested that in addition to appearing lethargic and having a reduced appetite, diarrhoeic calves will lie down for increased periods of time (Mainau et al., 2013); as was found in a previous study by Sutherland et al. (2018), where prior to presenting overt clinical signs of NCD calves increased the amount of time spent lying. An increase in time spent lying has also been observed in dairy cattle in response to lameness (Blackie et al., 2011). In addition to lying time, previous studies have also reported a decrease in the number of lying bouts in response to the onset of diseases such as NCD (Sutherland et al., 2018) and BRD (Swartz et al., 2017). Additionally, in response to BRD, previous studies have reported that diseased calves are less active,

performing fewer steps than healthy calves (Swartz et al., 2017; Hanzlicek et al., 2010) and demonstrate increased lying laterality (i.e., a preference for lying on a particular side) (Hixson et al., 2018).

When suffering from mastitis, cows have been found to decrease the amount of time spent lying (Siivonen et al., 2011; Cyples et al., 2012; Fogsgaard et al., 2012; Medrano-Galarza et al., 2012), and additionally show an increased preference for lying laterality (Medrano-Galarza et al., 2012). The decrease in time spent lying may be indicative of the pain being experienced due to inflammation of the udder (Medrano-Galarza et al., 2012), whilst increased lying laterality may be representative of the animal's level of discomfort (Ledgerwood et al., 2010). Additionally, in the first 3 days following mastitis detection, mastitic cows have previously been found to exhibit increased stepping, lifting and kicking behaviour during milking which being indicative of restlessness likely reflects the degree of pain and stress experienced whilst suffering from mastitis (Medrano-Galarza et al., 2012).

Rumination is also a key indicator of health and welfare in cattle, whereby cattle that are diseased will typically consume less feed, and consequently spend less time ruminating (Hamilton et al., 2019). Compared to dairy cattle, the use of accelerometers in beef cattle is relatively limited (Marchesini et al., 2018). However, accelerometers have been used successfully to measure rumination and activity in beef cattle for the purpose of detecting BRD and lameness (Marchesini et al., 2018). Based on decreased rumination and activity, accelerometers were capable of detecting BRD and lameness in beef cattle 3-6 days prior to the presence of overt clinical signs (Marchesini et al., 2018). This finding is similar to those

reported in dairy cattle, in which decreased rumination and/or activity measured using accelerometers have also shown the capability of detecting lameness (Van Hertem et al., 2013), and other illnesses such as ketosis (Kaufman et al., 2016; Stangaferro et al., 2016a; Gáspárdy et al., 2014), metritis (Gáspárdy et al., 2014), and mastitis (Stangaferro et al., 2016b) prior to the presence of overt clinical signs.

The use of accelerometers in livestock is not limited to cattle, as shown for example where accelerometers have been used previously for detecting lameness in sheep (Barwick et al., 2018) and disease in pigs (Martinez-Avilés et al., 2015). In relation to pigs, accelerometers have previously been used following experimental infection with African swine fever as a means of early disease detection (Martinez-Avilés et al., 2015). As part of a monitoring system which was developed, prior to infection biosensors and accelerometers were mounted to the animal's ear tag in order to monitor body temperature and movement. When data pertaining to these two variables were considered simultaneously, based on an increased body temperature and a decrease in activity, infection could be detected 1-3 days prior to identification based on overt clinical signs (Martinez-Avilés et al., 2015).

Automated water systems

Water is an essential nutrient which animals require (Drackley, 2008). However, although the importance of water is acknowledged, and a freedom from thirst is expressed as one of the ideals of animal welfare in the concept of five freedoms (FAWC, 2013), the provision and quality of water on farm are often neglected (Beede, 2005). Previous studies researching water intake in adult cattle have shown that even partial water deprivation can result in reduced feed intake (Little et al., 1978) and milk production (Little et al., 1980), behavioural problems (e.g.,

increased aggression) (Little et al., 1980), physiological changes (Hogan et al., 2007), and altered urine and faecal concentrations (Hogan et al., 2007). Similar responses are also observed in sheep, where water deprivation results in reduced feed intake, milk production, and reproduction and further leads to immune suppression which increases the animal's susceptibility to disease (Chedid et al., 2014).

In previous studies, drinking behaviour and water intake of adult cattle for example, have typically been monitored manually from live or video observations (Huzzey et al., 2005, Mitlohner et al., 2001), or through measuring back residual amounts of water left after being provided to animals for a given period of time (Morris et al., 2010). The problem with these manual methods is that they are often labour intensive and are not always suitable for monitoring individual animals. However, as an alternative to these manual methods, in a study by Chapinal et al. (2007), an automated water system (Insentec, Marknesse, Netherlands) was validated as a method for measuring drinking behaviour and water intake in adult cattle. Automated water systems have also been developed for use in pigs to record drinking behaviours at both the group (Madsen and Kristensen, 2005) and individual level (Maselyne et al., 2016). Compared to manual methods, automated water systems are beneficial in that they are less labour intensive and provide the capability to monitor animals on an individual basis whilst still being group housed. Through the use of automated systems the drinking behaviour of pigs has been suggested as a potential indicator of disease (Madsen and Kristensen, 2005; Kruse et al., 2011). Madsen and Kristensen (2005) for example reported the drinking behaviour of pigs was relatively stable as long as the animals remained healthy. However, when affected by disease pigs were often found to change their drinking

behaviour as demonstrated by an increase in water consumption, which in the case of diarrhoea, indicated an outbreak of disease a day prior to the presence of overt clinical signs (Madsen and Kristensen, 2005).

Few studies have investigated the water intake and drinking behaviour of calves, and it is therefore an area requiring research in order to determine for instance, the age at which calves begin to consume water, how much they consume and the frequency with which they visit the water trough. Research into the drinking behaviour of calves could then provide an insight into their water requirements and the effects of water intake on their health, welfare and productivity. Similar to the automated water systems which have been developed for pigs and adult cattle, the development of an automated water system suitable for calves, could enable remote, continuous monitoring of calf health and welfare and could potentially be used for early disease detection based on changes in drinking behaviour and water intake. There is also the potential that an automated system developed for calves could be integrated into other automated data collection systems on farm, for example, as demonstrated in a previous study by Schaefer et al. (2012) whereby infrared images were collected automatically as calves visited a water trough for the early detection of BRD. The integration of water intake and drinking behaviours alongside other measures could be used collectively to monitor calf health and welfare and may potentially strengthen the ability to successfully predict disease onset prior to the presence of clinical signs.

Development of machine learning algorithms to support automation

Once reliable indicators have been identified, machine learning algorithms are needed to enable the technology to be used in a manner as to enable animals to be

monitored and information provided in real-time. Due to the importance of alerting farmers when a cow is due to calve for example, a number of studies have focused on the development of real-time predictive models to achieve this using animal mounted sensors (Titler et al., 2015; Borchers et al., 2017; Krieger et al., 2018). For example, Miller et al. (2020) investigated whether integrating data-streams from accelerometers mounted in two positions on the dam could be used to develop machine learning algorithms to predict calving. The accelerometers used by Miller et al. (2020) consisted of neck mounted Silent Herdsman collars (Afimilk Ltd., Israel) which recorded the time spent ruminating and eating as well as relative activity, and tail mounted tri-axial accelerometers (Ax3 3-Axis logging accelerometer; Axivity, Newcastle upon Tyne, UK) to detect tail-raise events. Machine learning forest algorithms were developed to predict calving using single-sensor variables and integrating multiple sensor data streams (Miller et al., 2020). Using machine learning techniques, tail-raise events were determined to be the single best predictor of calving for both beef and dairy cattle, giving optimum indication 2 hours immediately preceding calving (Miller et al., 2020). For predicting calving, the tail mounted sensor achieved a sensitivity of 78.6% and a specificity of 83.5% for dairy cattle (Miller et al., 2020). In beef cattle these values dropped marginally to a sensitivity of 76.1% and a specificity of 83.3% (Miller et al., 2020). Similar results have also been reported by Borchers et al. (2017) using accelerometers placed on the neck and leg which recorded rumination and time spent standing or lying and step count respectively which were able to predict calving by 8 hours with a sensitivity of 82.8% and a specificity of 80.4%. Borchers et al. (2017) were able to generate these alerts to this degree of sensitivity and specificity using neural network machine learning techniques. The benefits of

predicting calving include the provision of calcium to high risk cows to reduce the risk of hypocalcaemia (Oetzel and Miller, 2012), the ability to administer NSAIDs to reduce labour-induced pain (Newby et al., 2013) and the ability to prevent neonatal losses (Jensen, 2012).

Algorithms have also been developed to support real time, automated monitoring for the detection of lameness (Hertem et al., 2014; Van Nuffel et al., 2015; Norton and Berckmans, 2017; Wu et al., 2020). Lameness is a leading concern for cattle welfare and significantly impacts milk production and fertility (Wu et al., 2020). Conducting manual locomotion scoring to detect lameness is often considered time consuming and subjective (Van Hertem et al., 2014). As an alternative, automated method for detecting lameness, Van Hertem et al. (2014) have validated the use of a 3D-video based algorithm to detect lameness based on arching of a cow's back. As is common in manual locomotion scoring, Van Hertem et al. (2014) suggested that other indicators such as head bob and gait asymmetry are parameters which could be included in future algorithm development. Similarly, algorithms have also been developed using real time object detection using a cow's legs as targets in order to determine lameness based on relative step size (Wu et al., 2020). Through the use of a YOLOv3 deep learning algorithm Wu et al. (2020) demonstrated an ability to detect the legs of cows to determine relative step size with an accuracy of 99.2% which enabled lame and non-lame cows to be classified with 98.6% accuracy.

The detection of estrus in cattle is another purpose for which algorithms have been developed (e.g., Talukdar et al., 2014). Estrus detection helps to achieve optimal herd conception rates by ensuring that a high proportion of the herd are inseminated

prior to ovulation (Hockey et al., 2010). Using IRT, Talukder et al. (2014) developed an algorithm capable of detecting estrus and providing an indication of ovulation based on changes in muzzle and vulval temperature. Although the sensitivity of the algorithm (73.0%) was greater than by visual observation (67.0%), the specificity and predictive value of the algorithm was lower than that measured by visual observation (Talukdar et al., 2014). This reduced specificity and predictive value resulted in the algorithm over predicting the incidence of ovulation (Talukdar et al., 2014). To improve the specificity and positive predictive value, Talukder et al. (2014) suggested that monitoring other anatomical areas such as the eye and ears be investigated in future work as potentially more suitable indicators. Algorithms have also been developed to support the automated use of IRT for the purpose of detecting mastitis (Zaninelli et al., 2018). The algorithm developed by Zaninelli et al. (2018) enables the automatic detection of the udder surface and subsequent calculation of the udder surface temperature (USST) based on the maximum number of pixels in the infrared image. Based on a somatic cell count (SCC) of 200,000 cells/ml to classify subclinical mastitis the algorithm demonstrated a sensitivity of 78.6% and a specificity of 77.9%. An increase in SCC to 400,000 cells/ml resulted in a sensitivity of 71.4% and a specificity of 71.6%. Zaninelli et al. (2018) acknowledged that these levels of sensitivity and specificity were lower than studies involving the non-automated analysis of infrared analysis for detecting mastitis (e.g., Polat et al., 2010). However, Zaninelli et al. (2018) suggested that future improvements could be made through further image processing and enhancement to support its use as an automated method for mastitis detection.

The potential for early disease detection to mitigate human pandemics

The development of automated monitoring systems which enable early disease detection provide benefits not only for the health and welfare of domestic animals, but also for the benefit of human health. In particular, in the case of zoonoses, there is significant concern surrounding the threat of such diseases to human health (Bennett, 2003; McElwain and Thumbi, 2017). As a disease which passes from wild or domestic animals to humans, zoonotic diseases have often proven to be some of the deadliest known (Rodriguez-Morales et al., 2018, 2020) and account for 60.0% of all human infectious diseases (Taylor et al., 2001). The number of zoonotic diseases have been rising since the introduction of agriculture and domesticated animals (Pearce-Duvel, 2006) and include diseases such as H1N1 influenza, Middle East Respiratory Coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). A key concern with the transmission of zoonotic diseases is their potential to escalate and result in the development of human pandemics such as the current COVID-19 pandemic. COVID-19 is a severe respiratory disease caused by the pathogen coronavirus 2 (SARS-CoV-2) (Zheng et al., 2020). Having emerged in late 2019, the virus has spread rapidly across the world and as of 8th September 2020, the World Health Organisation (WHO) had reported a total 216 countries, areas or territories with cases of COVID-19 from which have been a combined total of 27,032,617 confirmed cases and 881,464 deaths (WHO, 2020). As yet, the exact source of SARS-CoV-2 outbreak is unknown; however sequence-based analysis suggests bats as a primary source (Shereen et al., 2020) with the bat genome Bat-CoV-RaTG13 being 96.0% identical at the whole genome level to SARS-CoV-2 (Zhou et al., 2020). However, as direct contact between bats and humans is rare, it is possible that the transmission of SARS-CoV-2 to humans

occurred through an intermediate host (e.g., pangolins) as was observed in the SARS-CoV and MERS-CoV outbreaks (Liu et al., 2020).

The earlier an emerging zoonotic disease can be detected, the sooner actions can be taken to reduce the extent of transmission and ultimately minimise morbidity, mortality and economic losses to both domestic animal and human populations (NRC (US), 2009). The development of automated monitoring systems with the capabilities of enabling early disease detection have the potential to minimise the extent of disease that animals experience. Minimising the extent and spread of disease in animals could consequently act to prevent the risk of transmissions to humans, ultimately helping to prevent human pandemics.

Conclusions and implications

Within the livestock industries, it is clear the incidence of disease is of worldwide concern from both economic and animal health and welfare perspectives; it is therefore imperative that going forward, steps are taken to mitigate the impact of disease. An ability to detect disease sooner than is currently possible based on overt clinical symptoms is essential for enabling earlier treatment and isolation of affected animals. Early disease detection is also necessary to reduce the severity and extent of disease and overall to minimise the detrimental impacts of disease on animal health and welfare. Early disease detection could also be beneficial for mitigating the emergence of zoonotic diseases which pose concern to human health and have the potential to trigger human pandemics. Therefore, methods which could reliably enable early disease detection need to be developed. Furthermore, with an increasing shift towards automation occurring across the livestock industries, it would be useful if these methods could be developed with the intention of being

integrated into automated systems. This integration is necessary in order to ensure that a reliable means of monitoring animal health and welfare on-farm is maintained in response to a less 'hands on' approach to farming, fewer experienced stockpersons in the livestock industry and increasing herd sizes.

In calves, NCD is recognised as the leading cause of ill-health and mortality in both beef and dairy industries worldwide, and as with other diseases it is acknowledged that the incidence of NCD has a substantial impact from both animal welfare and economic perspectives. Currently, the major issue with NCD is that its diagnosis relies on the presence of overt clinical symptoms which do not arise until significant damage has already been inflicted internally on the intestines. A number of automated systems such as IRT, automated calf feeders, accelerometers and water systems offer themselves as potential systems which could be utilised for the purpose of early NCD detection. Additionally, it is possible that instead of being used independently, these systems could be combined in order to develop highly accurate composite measures for disease detection. Furthermore, these measures may not be limited to detecting NCD, and may have further application for the detection of other diseases including those occurring in other species.

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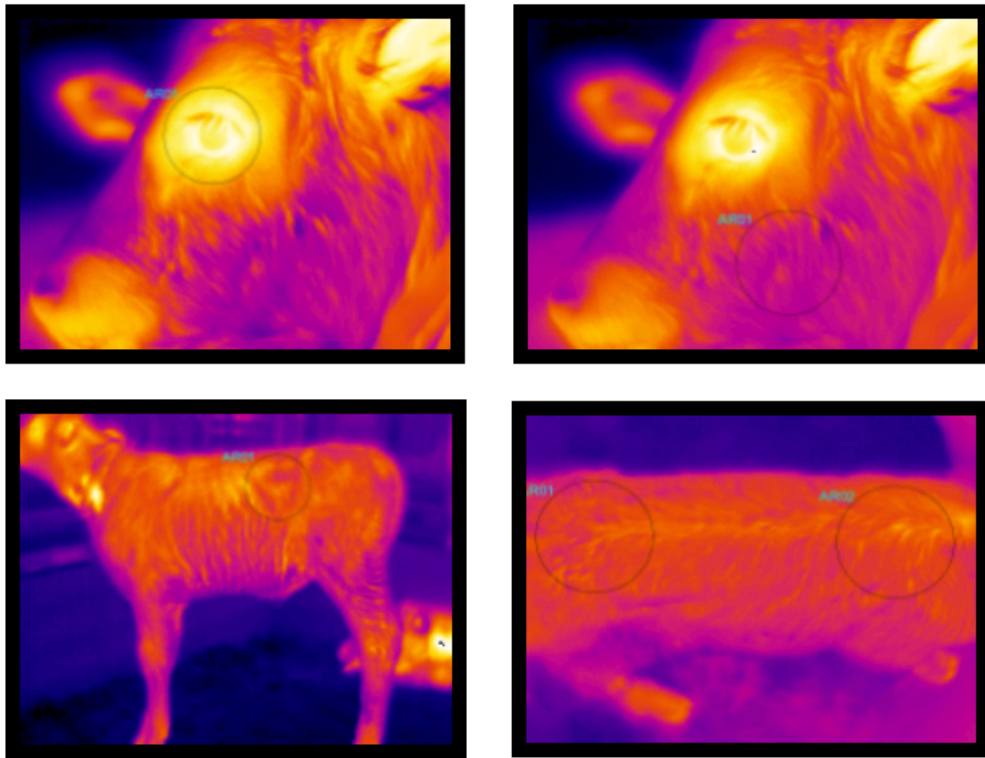
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Chapter Three

*Physiological and behavioural responses as indicators for early
disease detection in dairy calves*



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Abstract

This study investigated physiological and behavioral responses associated with the onset of neonatal calf diarrhea (NCD) in calves experimentally infected with rotavirus and assessed the suitability of these responses as early disease indicators. The suitability of infrared thermography (IRT) as a noninvasive, automated method for early disease detection was also assessed. Forty-three calves either (1) were experimentally infected with rotavirus (n = 20) or (2) acted as uninfected controls (n = 23). Health checks were conducted on a daily basis to identify when calves presented overt clinical signs of disease. In addition, fecal samples were collected to verify NCD as the cause of illness. Feeding behavior was recorded continuously as calves fed from an automated calf feeder, and IRT temperatures were recorded once per day across 5 anatomical locations using a hand-held IRT camera. Lying behavior was recorded continuously using accelerometers. Drinking behavior at the water trough was filmed continuously to determine the number and duration of visits. Respiration rate was recorded once per day by observing flank movements. The effectiveness of inoculating calves with rotavirus was limited because not all calves in the infected group contracted the virus; further, an unexpected outbreak of *Salmonella* during the trial led to all calves developing NCD, including those in the healthy control group. Therefore, treatment was ignored and instead each calf was analyzed as its own control, with data analyzed with respect to when each calf displayed clinical signs of disease regardless of the causative pathogen. Milk consumption decreased before clinical signs of disease appeared. The IRT temperatures were also found to change before clinical signs of disease appeared, with a decrease in shoulder temperature and an increase in side temperature. There were no changes in respiration rate or lying time before clinical signs of disease

appeared. However, the number of lying bouts decreased and lying bout duration increased before and following clinical signs of disease. There was no change in the number of visits to the water trough, but visit duration increased before clinical signs of disease appeared. Results indicate that milk consumption, IRT temperatures of the side and shoulder, number and duration of lying bouts, and duration of time spent at the water trough show potential as suitable early indicators of disease.

Keywords: dairy calf; early disease detection; infrared thermography; feeding

Introduction

Neonatal calf diarrhea (NCD), an enteric disease affecting beef and dairy industries worldwide, is a significant concern from both economic and animal welfare perspectives. Typically affecting calves during the first 28 d of life (Cho and Yoon, 2014), NCD causes severe diarrhea, resulting in dehydration, weight loss, and potentially death (Schroeder et al., 2012). Pathogens associated with NCD include rotavirus, coronavirus, *Cryptosporidium*, and *Salmonella*, which may act exclusively or in combination. The 2 most prevalent pathogens associated with NCD in New Zealand are rotavirus and *Cryptosporidium* (Al Mawly et al., 2015). Neonatal calf diarrhea inflicts a substantial amount of damage on the intestines as a result of the pathogens causing villous atrophy and inflammation of the submucosa (Todd et al., 2010; Cho and Yoon, 2014). As a consequence of this damage, there is a reduction in the surface area over which fluid and nutrients can be absorbed across the intestinal epithelium; this results in a greater amount of fluid being lost, leading to severe diarrhea (Cho and Yoon, 2014). Neonatal calf diarrhea generates major economic losses, and reducing the prevalence of NCD is

considered one of the biggest challenges affecting both beef and dairy industries worldwide (Meganck et al., 2015). In addition to the cost of mortalities, other economic losses associated with NCD include treatment costs (e.g., antibiotics and electrolytes) and time spent caring for the affected animals. In the longer term, significant losses are associated with growth and production as a result of reduced weight gain and stunted growth in affected animals (de Graaf et al., 1999). In addition, the extensive use of antibiotics to treat infected calves raises concerns regarding antibiotic resistance.

When experiencing illness, it is common for animals to display sickness behaviors (Millman, 2007); these are associated with physiological and behavioral changes, the purpose of which are to increase the animal's ability to build up an immune response and thus enable the animal to focus its energy on fighting off the disease. At present, to identify an animal as being affected by disease, farmers will observe animals for clinical signs of illness (e.g., depression or apathy, diarrhea, dehydration, and decreased feed intake; de Verdier Klingenberg, 2000). The issue with relying on clinical signs to identify a diseased animal is that many farm animals are prey species and as a result are typically stoic in nature. Being stoic, these animals have a tendency to mask signs of illness and vulnerability, which is a strategy that in the wild would potentially protect them against predation (Weary et al., 2009). As a result, those responsible for observing animals need to be able to reliably detect subtle changes in behavior that are indicative of illness. The challenge with NCD is that by the time a calf exhibits clinical signs (e.g., diarrhea and dehydration), much of the internal damage to the intestines has already occurred. Therefore, systems need to be developed that would enable disease to be

detected earlier than is currently possible based on when an animal presents overt clinical signs of disease.

Over time, due to a desire to reduce labor costs, reliance on automated systems has increased, leading to a less hands-on approach to farming as illustrated, for example, by the shift from cows being milked by hand to cows being milked using robotic milking systems. Additionally, increasing herd sizes leads to less individual animal contact and, with fewer experienced stock people in the industry, can lead to an increase in the number of cases of NCD going undiagnosed; this results in reduced welfare and production and increased mortalities. Therefore, there is a need to develop measures that could be incorporated within automated systems on-farm to enable remote and reliable monitoring of animal health and welfare. Automated monitoring systems have been found to be capable of detecting disease based on changes in infrared temperatures (Schaefer et al., 2004, 2007, 2012) and feeding behavior (Svensson and Jensen, 2007; Borderas et al., 2009). In contrast to the situation overseas, these automated systems have not been investigated in New Zealand conditions, where calves have different susceptibilities to certain diseases (e.g., prevalence of NCD vs. respiratory disease).

Infrared thermography (**IRT**) is a noninvasive method of detecting radiated heat. Previous studies have investigated the use of IRT for detecting early signs of bovine respiratory disease (**BRD**) and bovine viral diarrhea (**BVD**; Schaefer et al., 2004, 2007, 2012). Infrared thermography has also been used to investigate how temperature changes at different anatomical areas relate to the onset of BVD (Schaefer et al., 2004) and differences in feed efficiency (Montanholi et al., 2009, 2010; Martello et al., 2016). Moreover, changes in feeding behaviors, measured

using automated calf feeders, have been used to assess disease in calves (Svensson and Jensen, 2007; Borderas et al., 2009; Swartz et al., 2017; Sutherland et al., 2018). Automated calf feeders are able to record information, including milk consumption, visit duration, and the number of rewarded (calf receives an allocation of milk) and unrewarded (calf does not receive an allocation of milk) visits calves make to the feeder. The accelerometer is another automated device that has been used in previous studies to monitor lying (Mattachini et al., 2013; Sepúlveda-Varas et al., 2014) and feeding (Mattachini et al., 2016) behavior and for detecting estrus (Valenza et al., 2012) and disease (Sepúlveda-Varas et al., 2014). Previous studies have found that lying behaviors change in diseased calves (Hart, 1988; Johnson, 2002; Sutherland et al., 2018); therefore, there is potential for accelerometers to be used to measure lying behavior to detect the onset of NCD.

Water intake, drinking behavior, and respiration rate (**RR**) also have the potential to be used as early indicators of disease. However, current methods for measuring these responses are often labor intensive and are not always suitable for monitoring individual animals. However, an automated water system (Insentec, Marknesse, the Netherlands) has been validated as a method of measuring drinking behavior and water intake in adult cattle (Chapinal et al., 2007). The development of a similar system for calves would provide insights into the drinking behavior of calves and how water intake influences their health, welfare, and productivity. In addition to the above, RR can provide valuable information regarding stress, pain, and overall cow welfare (Pastell et al., 2006).

The purpose of our study was to identify physiological and behavioral responses associated with the early onset of NCD in calves infected with rotavirus and to

assess the suitability of those responses as indicators for early disease detection. In addition, by assessing the thermal responses of different anatomical regions in response to disease, we investigated the suitability of IRT as a noninvasive method for early disease detection.

Materials and methods

All procedures involving animals in this study were approved by the University of Waikato Animal Ethics Committee (protocol no. 955) under the New Zealand Animal Welfare Act 1999.

Animals and experimental design

The study was undertaken at a farm in the Waikato region of New Zealand (37°48'29.6"S, 175°04'48.7"E) between August and October 2015. Forty-three mixed-breed calves (20 Friesian males and 23 Hereford females) were sourced from commercial farms and transported to the facility for enrollment in the study at 4 d of age. Unfortunately, BW could not be reliably recorded upon enrollment due to an equipment fault. Within a single barn, calves were housed in 1 of 2 equal-sized (6.6 × 8.1 m) pens constructed on a solid concrete base with solid walls on all 4 sides. Within each pen, an area (30 m²) of wood chip (20 cm deep) was provided as bedding. Each pen contained a water trough (PT10; Stallion Limited, Palmerston North, New Zealand), hay feeder (purpose built, measuring 75 × 65 × 22 cm), meal feeder (purpose built using one half of a 210-L closed-head drum; ES Plastics, Hamilton, New Zealand), and automated calf feeder (RFID Calf Feeder; A. and D. Reid, Temuka, New Zealand) as used by Sutherland et al. (2018). Calves were fed as described below using the automated calf feeders in addition to being provided

ad libitum access to meal that consisted of 18.0% CP, 10.0% crude fiber, and 5.0% crude fat (Moozlee; NRM, Auckland, New Zealand). Additionally, calves were given ad libitum access to water and meadow hay. On arrival to the facility, calves were individually identified using numbered, colored collars (Calf Neck Bands; Shoof International Ltd., Cambridge, New Zealand).

The trial consisted of 2 replicates of 2 treatments into which calves were randomly assigned upon arrival. The treatment groups were referred to as treatment group 1, in which calves were experimentally infected with rotavirus at 6 d of age (n = 20; replicate 1, n = 10 and replicate 2, n = 10), and treatment group 2, in which calves acted as uninfected controls (n = 23; replicate 1, n = 10 and replicate 2, n = 13). Calves in treatment group 1 were infected with rotavirus through an oral drench containing a mixture of 40 mL of water and 6 mL of feces collected from 2 calves known to be positive for rotavirus. Control and infected animals were housed separately using the 2 pens but were otherwise handled in the same manner. Health checks, IRT, and feeding, drinking, and lying behaviors were recorded daily as described below.

Clinical observations

Health checks were carried out each morning to assess the calves' general well-being and to identify when each calf began to display clinical signs of illness. Health checks assessed calves on the basis of their general appearance, coat condition, gut fill, and fecal consistency, with definitions presented in Figure 1. Dehydration levels were assessed by monitoring calves for sunken eyes and by performing a tent test to measure skin elasticity in which the skin of the neck was pinched and the time for the skin to return to its normal position was recorded (Ghanem et al., 2012).

Health checks were also used to monitor signs of nasal and ocular discharge and navel infection (a disease resulting from bacterial infection via the umbilical cord soon after birth). Rectal temperatures were taken once per animal during each health check using a digital thermometer (MC-343; Omron, Kyoto, Japan). Temperatures were split into 3 categories: low ($\leq 37.9^{\circ}\text{C}$), normal ($38\text{--}39.5^{\circ}\text{C}$), and high ($\geq 39.6^{\circ}\text{C}$). As part of the health checks, RR was measured by observing flank movements to record the time taken for each calf to complete 10 breaths; this was then used to calculate RR (breaths/min). Calves were defined as being clinically ill when they were observed as being diarrheic. To be considered diarrheic, a calf had to be witnessed passing malodorous feces with a loose to watery consistency, with the possibility of blood present in severe cases (a score of 2 or 3 for fecal consistency; Figure 1). For calves that had not been observed passing feces but were suspected of being diarrheic due to loose fecal matter present on the top of the tail or hind legs, a fecal sample was taken to confirm whether the calf was diarrheic. From all diarrheic calves a fecal sample was collected for analysis to confirm NCD as the cause of illness and to identify the specific pathogen responsible. Once deemed clinically ill, calves were treated accordingly with electrolytes (Dexolyte; Bayer New Zealand Ltd., Auckland, New Zealand) and antibiotics (Amphoprim; Virbac New Zealand Ltd., Hamilton, New Zealand) as needed to help them overcome the disease.









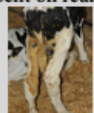
	0	1	2	3
Appearance	Normal, alert & active, head and ears held up 	Slightly unresponsive, droopy ears 	Depressed, lethargic, head held down, droopy ears, unsteady balance. 	Severe depression, reluctance to stand. 
Coat	Bright/Sleek	Rough/Dull	N/A	N/A
Sunken Eyes	Normal, bright, no recession into the orbit 	Dull, slightly sunken, recessed 2-4mm into the orbit 	Sunken, recessed 5-7mm into the orbit 	Deeply sunken, ≥8mm of recession into the orbit 
Ocular Discharge	No discharge 	Minimal discharge 	Moderate discharge 	Heavy discharge 
Nasal Discharge	No discharge 	Minimal discharge 	Moderate discharge 	Heavy discharge 
Tent Test	0 seconds (<6% dehydration)	1-3 seconds (6-8% dehydration)	4-5 seconds (9-12% dehydration)	≥6 seconds (>12% dehydration)
Navel Ill	Normal	Navel is slightly swollen, not warm to touch	Navel is swollen, warm with slight discharge	Navel is very swollen, warm with excessive discharge
Joint Ill	Normal	Joints are slightly swollen, not warm to touch	Joints are swollen, warm, animal displays mild signs of lameness	Joints are very swollen, warm and animal displays severe signs of lameness
Gut Fill	Full	Hollow	N/A	N/A
Rear End	Clean 	Loose faecal matter present on rear end 	N/A	N/A
Faecal Consistency	Firm consistency	Soft to loose consistency	Scours, loose to watery consistency with a strong odour	Severe scours, strong odour, consistency of water, blood may be present.

Figure 1. Daily health check definitions used to assess calves for the onset of neonatal calf diarrhea based on a scoring system of 0 to 3 for general appearance, sunken eyes, ocular discharge, nasal discharge, tent test, navel ill, joint ill, and fecal consistency. A scoring system of 0 or 1 was used to assess coat condition, rear end cleanliness, and gut fill. Measures for which scores were not applicable are denoted as N/A.

Fecal sample analysis

Fecal samples were analyzed (New Zealand Veterinary Pathology, Hamilton, New Zealand) to determine the presence of rotavirus, coronavirus, *Cryptosporidium*, and *Salmonella* to verify NCD as the cause of illness. The presence of *Cryptosporidium* was assessed by performing an acid-fast stain analysis, which caused oocysts to stain from light pink to red. Broth enrichment and selective plating were used to assess fecal samples for the presence of *Salmonella*. Samples that tested positive for *Salmonella* were then sent to the Institute of Environmental Science and Research (Auckland, New Zealand) for serotyping and phage typing to identify the individual strain of *Salmonella* present. A commercially available ELISA kit (Pourquier ELISA Calves Diarrhea; Institut Pourquier, Montpellier, France) was used to determine the presence of rotavirus and coronavirus.

Feeding behavior

Each pen was fitted with an automated calf feeder that individually identified calves as they approached the feeder based on the electronic identification in their ear tags. Upon arrival to the facility, calves were trained to use the automated feeder. This was done by handlers encouraging calves into the feeder and guiding them toward the teat, where they were trained to press down on a lever with their nose as they suckled in order for milk replacer (Brown Bag CMR; Fonterra Ltd., Auckland, New Zealand) to be dispensed. The milk replacer consisted of 21.0% protein, 21.0% fat, and 48.5% lactose and was provided at a mixing rate of 150 g/L. Initially, when calves were 4 to 7 d of age, they were given a total allowance of 4 L of milk replacer/d, which was increased at 8 d of age to 6 L/d. Each daily allowance was split into 2-L allocations. Between the full consumption of each 2-L allocation a

stand-down period of 6 h (when no milk was delivered) had to pass before the calf could receive the next allocation of milk (per standard farm practice in New Zealand). The automated calf feeders recorded milk intake, the number of both rewarded and unrewarded visits, and the total number of visits (sum of both unrewarded and rewarded visits) calves made to the feeder. A rewarded visit was defined as one in which the calf visited the automated calf feeder and received an allocation of milk. There was no set minimum amount that had to be consumed; any visit in which the calf consumed milk, regardless of the amount, was regarded as a rewarded visit. An unrewarded visit was one in which the calf visited the feeder but did not receive an allocation of milk and hence no amount of milk was consumed. The automated feeders did not record visit duration or drinking speed. Milk was heated to approximately 26°C before being dispensed to the calf. Milk temperature was monitored using Thermochron iButton temperature data loggers (DS1922L-F5#; Maxim Integrated, San Jose, CA).

Infrared thermography

Infrared thermography images of the animals' side (lateral), shoulder (proximal dorsal area over the trapezius muscle), back (dorsal), eye (orbital), and cheek (mandible area over the maxillary muscle) areas were collected every morning following health checks using a hand-held IRT camera (ThermaCAM S60; FLIR Systems AB, Danderyd, Sweden). The camera was calibrated daily for ambient temperature, humidity, and emissivity (0.98). Temperature and relative humidity were measured continuously using data loggers (EL-USB-2-LCD+; Lascar Electronics Ltd., Salisbury, UK) located next to each automated calf feeder. Images were collected by an observer as they walked around the pen capturing images at a

set distance from each anatomical area (i.e., 1 m from the eye, 2 m from the back and shoulder, and 3 m from the side) at an angle of 90° to the animal. With the exception of the dorsal view, all other images were collected from the animals' left side. Images were analyzed using ThermoCAM Researcher Software (version 2.10; FLIR Systems AB) to calculate the maximum, minimum, and average temperatures for each area. This analysis involved tracing a circle over the area of interest as shown in Figure 2. For the eye, this circle was placed so it included the eyeball and area surrounding the eyelid. The cheek area was defined by placing the circle over the cheek muscle. The side was defined by placing the circle over the rumen fossa. Dorsal images were divided into 2 areas of interest, the back and shoulders, which were defined by placing one circle over the lower (distal) back across the hips, including the ilium, and another over the shoulders, including the scapula. Image analysis was carried out by a single observer, and intraobserver reliability was calculated based on the reanalysis of 10% of the images from each area. Initial observations were compared with secondary observations, revealing intraobserver reliability ranging from 96 to 99% for all areas measured, with a combined overall average of 98%. The level of intraobserver reliability was calculated in Excel (version 16.10; Microsoft Corp., Redmond, WA) using the correlation function.

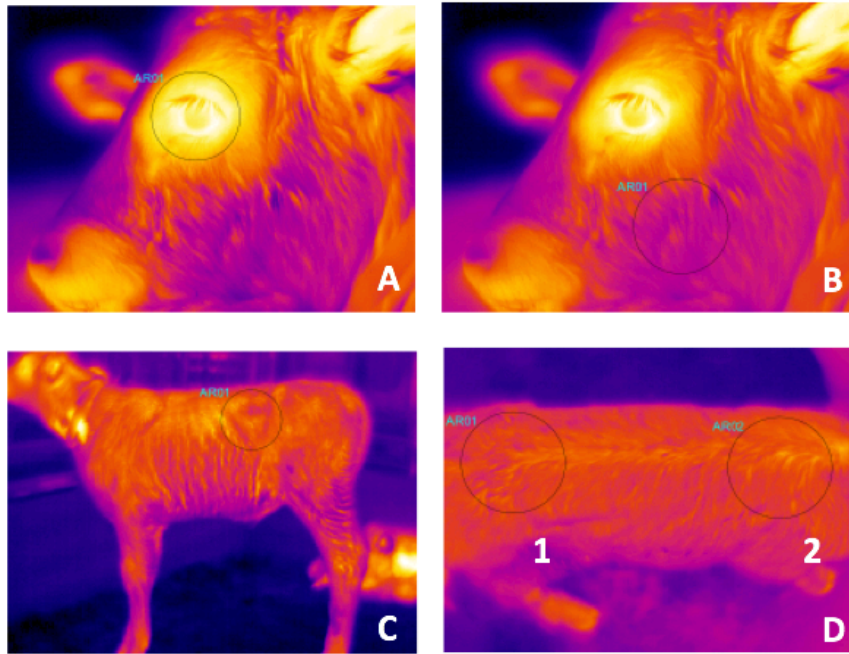


Figure 2. Infrared images of the (A) eye, (B) cheek, (C) side, and (D) dorsal surface (1 = back, 2 = shoulder) showing the circle traced to define each area during analysis of the images.

Lying behavior

Lying and standing behavior were recorded continuously using Hobo pendant G data loggers (64k, Onset Computer Corp., Bourne, MA) set at 1-min intervals using the y- and z-axes as recommended in previous validation studies (Ledgerwood et al., 2010; Bonk et al., 2013). The Hobo loggers were fitted into purpose-made fabric pouches that were attached on the lateral side of the right hind leg above the metatarsophalangeal joint using Velcro and Kamar glue (Livestock Improvement Corp., Hamilton, New Zealand). Data loggers were placed horizontally on the leg such that the x- and z-axes ran parallel to the ground, with the x-axis pointing in the cranial direction and the z-axis pointing toward the mid plane of the calf. The y-axis ran perpendicular to the ground, pointing in the dorsal direction. Data loggers were initialized and downloaded using Onset HOBOWare Pro software (version 3.7.2; Onset Computer Corp.). The output was converted into daily summaries of

standing and lying behavior using SAS software (version 9.3; SAS Institute Inc., Cary, NC).

Drinking behavior

For the second replicate only (n = 23: n = 10 infected calves and 13 uninfected controls), drinking behavior at the water trough was recorded continuously using Panasonic video cameras (HC-V270, Panasonic, Osaka, Japan). Cameras were secured to camera stands at a height of 2 m from the ground, which were attached to the side of the pen. Three red lights (PAR38 80W, Red Globe; Philips, Shanghai, China) were placed in each pen 1.7 m above the ground to enable behavioral observations to be conducted at night with minimal disturbance to the calves' behavior. A drinking behavior was defined as "calf's head is over the water trough." Video footage was analyzed continuously for each animal to record the frequency and duration of visits to the trough. Video footage was analyzed using Adobe Premiere Pro CC (version 12.0 Haberdasher; Adobe Systems, San Jose, CA). Video analysis was carried out by a single observer, and intraobserver reliability was calculated based on the reanalysis of the video footage at 3 stages of the trial (beginning, middle, and end). Initial observations were compared with secondary observations, revealing that intraobserver reliability ranged from 95 to 99% with a combined overall average of 98%. The level of intraobserver reliability was calculated in Excel (version 16.10, Microsoft Corp.) using the correlation function.

Statistical analysis

The effectiveness of the rotavirus infection treatment was limited because not all calves infected with rotavirus contracted the virus (only 8 of 19 treated animals

became infected). In addition, some calves in the control group also contracted rotavirus (8 of 24 control animals contracted rotavirus). Furthermore, an unexpected outbreak of *Salmonella* (an NCD-causing pathogen) during the trial led to all calves, including those in the healthy control group, developing NCD. Although all calves developed NCD, the clinical dates on which they were identified as being clinically ill varied across individuals. Therefore, it was decided that treatment would be ignored and instead each calf would be analyzed as its own control with data analyzed with respect to when each calf displayed clinical signs, comparing measures both pre- and post-clinical signs regardless of the causative pathogen. During analysis, 3 animals from the first replicate were excluded due to insufficient data. Data for feeding behavior (milk consumption, total visits, and unrewarded visits), IRT temperatures (eye, cheek, back, shoulder, and side), lying behavior (lying time, number of lying bouts, and lying bout duration), drinking behavior (number of visits and visit duration), and RR were modeled with REML using Genstat (version 19; VSN International Ltd., Hemel Hempstead, UK). Data for each of these variables were fitted with splines for each animal to (1) account for some of the noise in the data, (2) account for some animals not having data on some of the days, and (3) model the correlations between the repeated observations on the animals. Restricted maximum likelihood with smoothing splines was fitted to the data to enable the general trends for all variables to be observed across d -7 to 7 (relative to the clinical identification of disease, d 0). We then summarized and examined in more detail particular time frames pre- and post-onset of disease based on trends found using the REML models. For all variables, comparisons of the mean values were made between d -7 to -4 and d -3 to 0 and between d -7 to -1 and d 0 to 6 using the 1-sample sign test (exact binomial probability; Dixon and Mood,

1946; Conover, 1980). The 1-sample sign test was used to measure the significance of change of each animal between the average daily value between periods being compared; standard error of the means was used to measure the level of variability. Table 1 presents the number of positive changes detected from the total number of pairs tested using the sign test for each variable measured. These time periods were chosen to enable a comparison to be made between 2 periods of time before the clinical identification of disease (d -7 to -4 and d -3 to 0) while also allowing for a comparison between the period of time before the clinical identification of disease (d -7 to -1) and the period of time post-clinical identification (d 0 to 6). The time periods before clinical identification of disease (d -7 to -4 and d -3 to 0) were chosen based on the earliest time at which a change in response to the onset of NCD could be detected because with any disease, the sooner it can be detected the sooner animals can be isolated to prevent the spread of disease and enable treatments to be administered. The sign test was chosen over other analyses (e.g., ANOVA) because the data were not normally distributed. Age relative to clinical diagnosis was included as a covariate in the analysis for all variables and showed a significant influence only on lying time ($P < 0.001$). For all IRT data, ambient temperature ($^{\circ}\text{C}$) and relative humidity (%) were included as additional covariates in the REML model to adjust for changes in environmental conditions that were highly significant for all anatomical locations ($P < 0.001$) with the exception of the eye, which is less sensitive to changes in environmental conditions ($P = 0.288$). The inclusion of temperature and humidity as covariates improved the fit of the REML model for all anatomical locations, including the eye. For the REML model, data on drinking behavior were square root transformed before analysis to meet the assumptions of homogeneous variance and normal distribution of the data.

Results

Feeding behavior

Figure 3 shows the changes in milk consumption, total number of visits, and percentage of unrewarded visits made to the automated calf feeder over the days before and after (d -7 to 7) clinical signs of disease were identified (d 0). Milk consumption was higher during d -7 to -4 compared with the 4 d (d -3 to 0) immediately before the clinical identification of disease (d 0; Table 1). No other significant changes in feeding behavior were found during d -7 to -4 compared with the 4 d (d -3 to 0) immediately before the clinical identification of disease (d 0). However, milk consumption, total number of visits, and percentage of unrewarded visits to the automated calf feeder were all lower in the 7 d after clinical signs occurred compared with the 7 d before (Table 1).

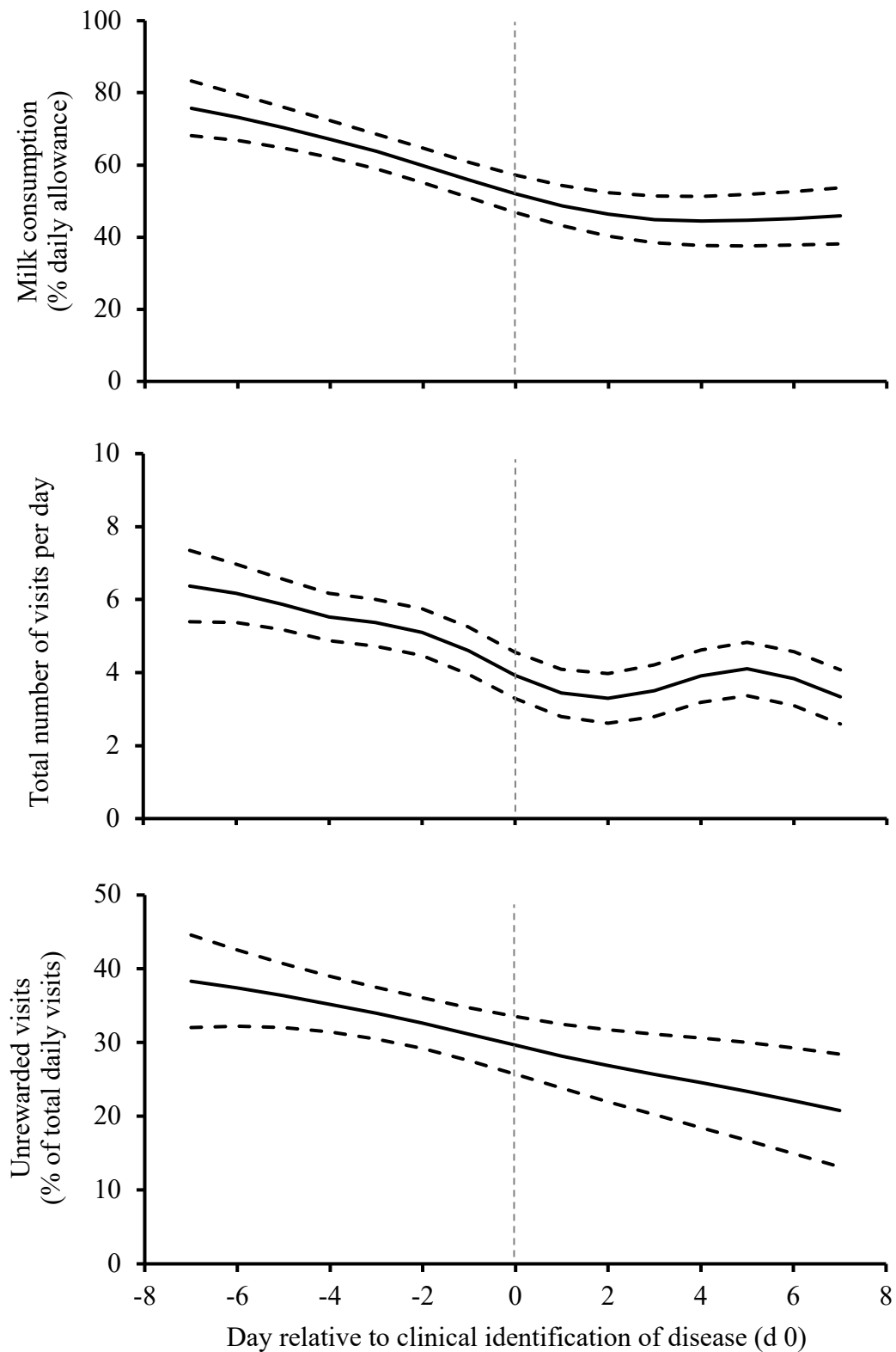


Figure 3. Mean (\pm SEM, dashed lines) milk consumption, total number of visits, and percentage of unrewarded visits made to the automated calf feeder per day during d -7 to 7 relative to the clinical identification of disease (d 0) based on outcomes of the REML model.

Infrared thermography

Figure 4 shows the changes in IRT temperature in response to the onset of disease for the different anatomical areas measured (eye, cheek, back, shoulder, and side) over the days before and after (d -7 to 7) clinical signs were identified (d 0). When comparing the 4 d immediately before the clinical identification of disease (d -3 to 0) and the previous 4 d (d -7 to -4), IRT temperatures changed significantly, with an increase in side temperature (Table 1) and a decrease in shoulder temperature (Table 1). No other significant changes in IRT temperature were found during d -7 to -4 compared with the 4 d (d -3 to 0) immediately before the clinical identification of disease (d 0). However, when comparing the 7 d after clinical signs occurred and the 7 d before, IRT temperatures changed significantly, with a decrease in eye, back, and shoulder temperatures and an increase in cheek and side temperatures (Table 1).

Lying behavior

Figure 5 shows the changes in lying behavior in terms of lying time, number of lying bouts, and bout duration over the days before and after (d -7 to 7) clinical signs of disease were identified (d 0). When comparing the 4 d immediately before the clinical identification of disease (d -3 to 0) and the previous 4 d (d -7 to -4), there was a significant decrease in the number of lying bouts and an increase in bout duration (Table 1). When comparing the 7 d after clinical signs occurred and the 7 d before, significant changes were seen, with decreases in both lying time and the number of lying bouts and an increase in bout duration (Table 1).

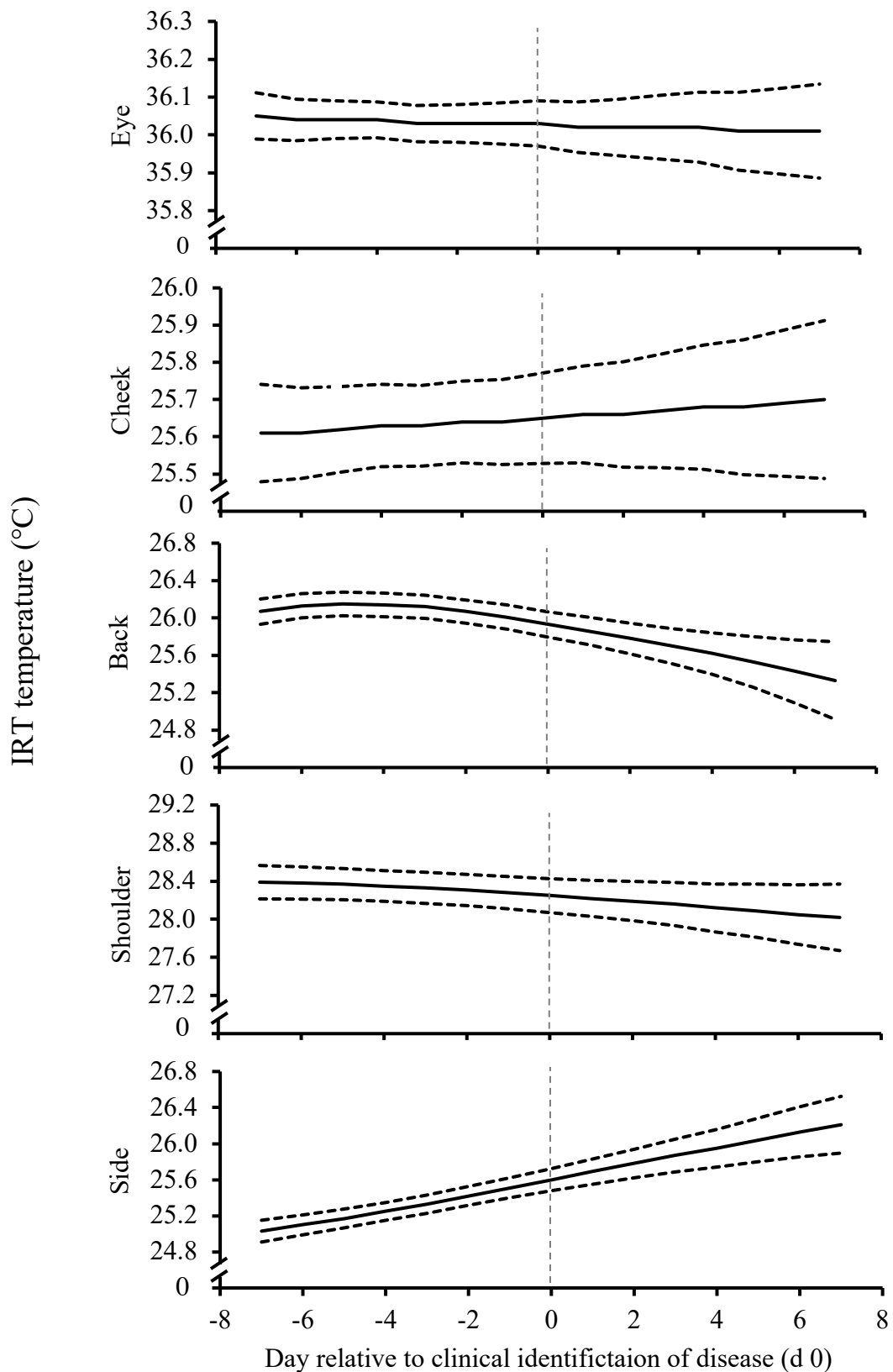


Figure 4. Mean (\pm SEM, dashed lines) maximum infrared thermography (IRT) temperature for the different anatomical areas (eye, cheek, back, shoulder, and side) measured during d -7 to 7 relative to the clinical identification of disease (d 0) based on outcomes of the REML model.

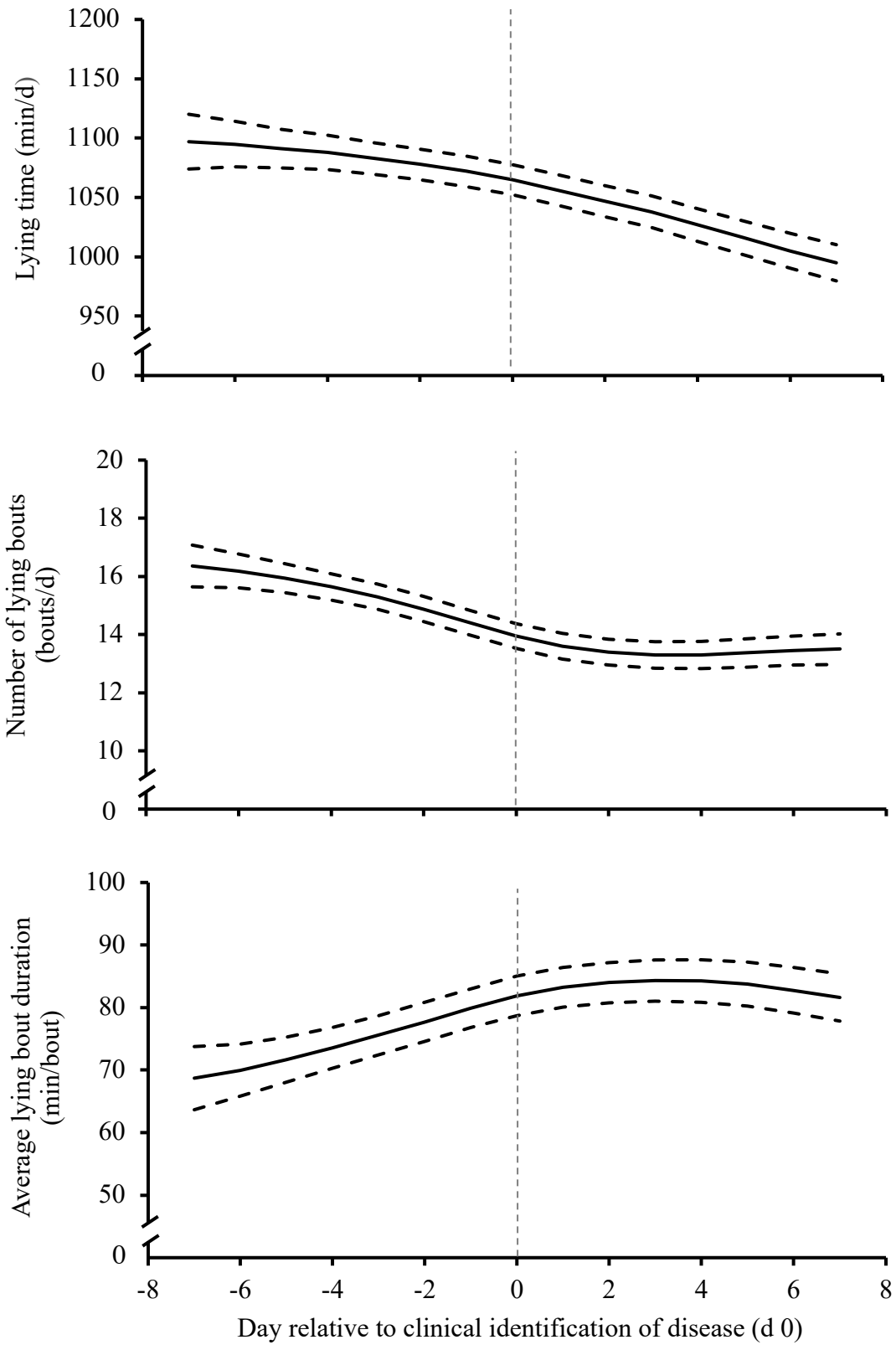


Figure 5. Mean (\pm SEM, dashed lines) lying time, number of lying bouts, and average bout duration during d -7 to 7 relative to the clinical identification of disease (d 0) based on outcomes of the REML model.

Drinking behavior

Figure 6 shows changes in drinking behavior in terms of the number of visits and visit duration over the days before and after (d -7 to 7) clinical signs of disease were identified (d 0). There was no change in the number of visits to the water trough before or following clinical identification of disease. However, when comparing the 4 d immediately before the clinical identification of disease (d -3 to 0) with the previous 4 d (d -7 to -4), there was a significant increase in visit duration (Table 1).

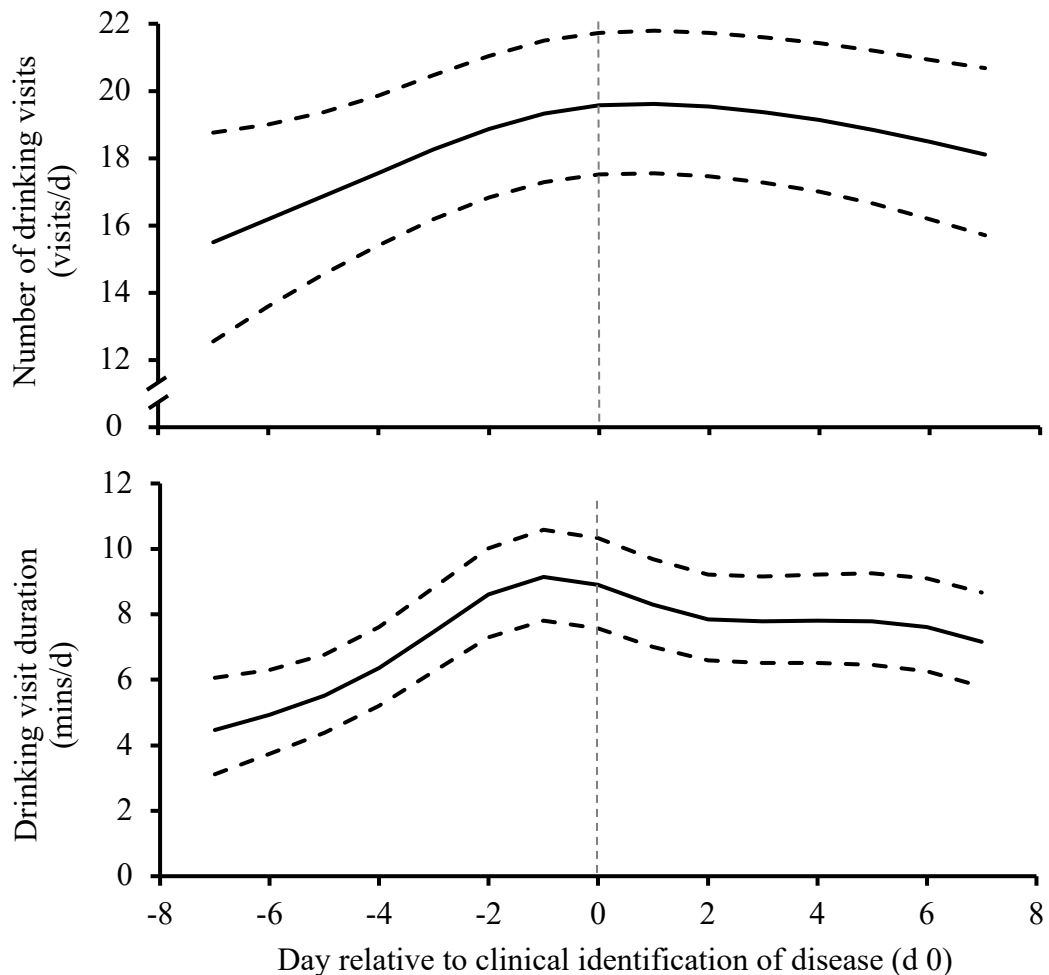


Figure 6. Mean (\pm SEM, dashed lines) number of drinking visits and drinking visit duration during d -7 to 7 relative to the clinical identification of disease (d 0) based on outcomes of the REML model.

Respiration rate

Figure 7 shows the changes in RR over the days before and after (d -7 to 7) clinical signs of disease were identified (d 0). When comparing the 4 d immediately before the clinical identification of disease (d -3 to 0) and the previous 4 d (d -7 to -4), there was no significant change in RR. However, when comparing the 7 d after clinical signs with the 7 d before, RR was found to decrease (Table 1).

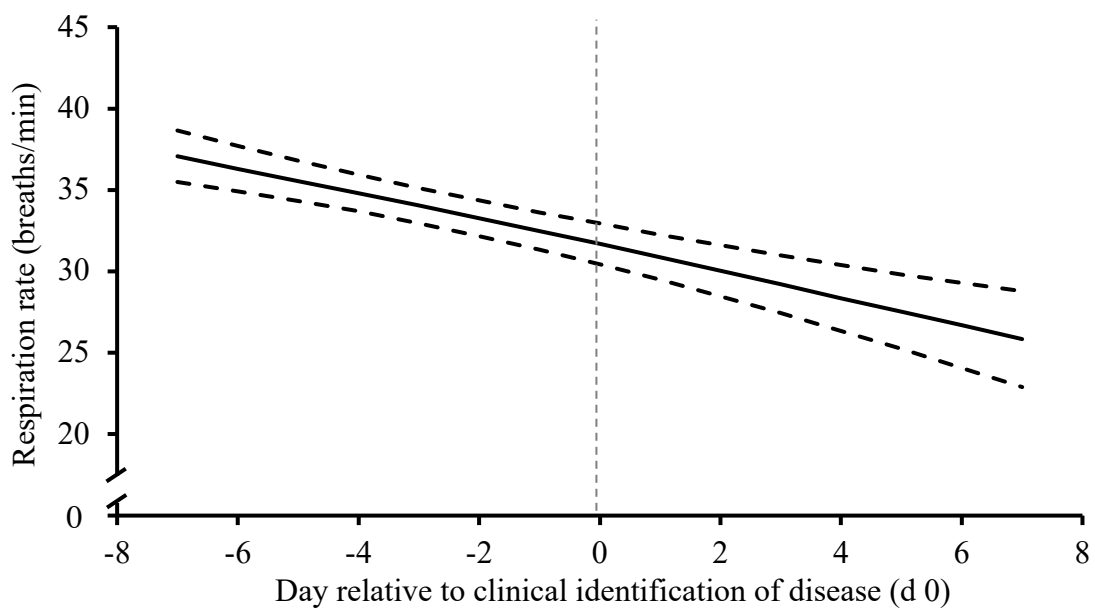


Figure 7. Mean (\pm SEM, dashed lines) respiration rate during d -7 to 7 relative to the clinical identification of disease (d 0) based on outcomes of the REML model.

Table 1. Average (\pm SEM) and difference for all variables consisting of feeding behavior, infrared thermography temperatures, lying behavior, drinking behavior, and respiration rate comparing d -7 to -4 with d -3 to 0 and comparing d -7 to -1 with d 0 to 6 relative to the clinical identification of disease (d 0) based on results of the sign test; the number of positive changes detected from the total number of pairs tested during each comparison for the different variables using the sign test is also presented.

Variable	Average		Difference	No. positive changes ¹	P-value	Average		Difference	No. positive changes ¹	P-value
	d -7 to -4	d -3 to 0				d -7 to -1	d 0 to 6			
Feeding behaviour										
Milk consumption (% of daily allowance)	71.34 \pm 3.09	58.85 \pm 3.15	-12.49	8 (40)	<0.001	67.25 \pm 2.56	41.07 \pm 3.24	-26.18	2 (40)	<0.001
Total visits (no./d)	5.74 \pm 0.65	5.36 \pm 0.27	-0.38	21 (40)	0.511	5.87 \pm 0.44	3.78 \pm 0.23	-2.09	4 (40)	<0.001
Unrewarded visits (% of total visits/d)	33.72 \pm 2.58	32.01 \pm 1.92	-1.71	23 (40)	0.132	34.45 \pm 2.06	26.02 \pm 1.58	-8.43	5 (40)	<0.001
Infrared thermography										
Eye ($^{\circ}$ C)	35.99 \pm 0.04	36.01 \pm 0.03	0.02	18 (40)	0.749	35.99 \pm 0.03	35.92 \pm 0.04	-0.07	8 (40)	0.003
Cheek ($^{\circ}$ C)	25.60 \pm 0.10	25.56 \pm 0.09	-0.04	23 (40)	0.337	25.57 \pm 0.09	25.63 \pm 0.12	0.06	26 (40)	0.003
Back ($^{\circ}$ C)	26.23 \pm 0.10	26.11 \pm 0.11	-0.12	13 (40)	0.053	26.19 \pm 0.10	26.00 \pm 0.16	-0.19	2 (40)	<0.001
Shoulder ($^{\circ}$ C)	28.49 \pm 0.18	28.39 \pm 0.19	-0.10	8 (40)	<0.001	28.44 \pm 0.19	28.06 \pm 0.20	-0.38	8 (40)	0.024
Side ($^{\circ}$ C)	25.09 \pm 0.09	25.36 \pm 0.09	0.27	34 (40)	<0.001	25.17 \pm 0.08	25.93 \pm 0.11	0.76	30 (40)	<0.001
Lying behavior										
Lying time (min/d)	1075.17 \pm 17.41	1073.66 \pm 11.40	-1.51	15 (40)	0.200	1084.48 \pm 9.90	1040.06 \pm 14.54	-44.42	10 (40)	0.002
Lying bouts (no./d)	16.13 \pm 0.46	14.91 \pm 0.53	-1.22	11 (40)	0.014	15.66 \pm 0.45	13.50 \pm 0.49	-2.16	6 (40)	<0.001
Bout duration (min/bout)	70.06 \pm 2.51	77.45 \pm 2.89	7.39	39 (40)	<0.001	73.57 \pm 2.40	83.52 \pm 3.08	9.95	31 (40)	<0.001
Drinking behaviour										
Number of visits (no./d)	17.51 \pm 3.48	20.91 \pm 2.13	3.40	15 (23)	0.134	19.81 \pm 2.42	17.96 \pm 1.73	-1.85	8 (23)	0.286
Visit duration (min/d)	6:12 \pm 1:28	9:32 \pm 1:17	3:20	17 (23)	0.017	8:02 \pm 1:19	7:34 \pm 0:52	-0:28	11 (23)	1.000
Respiration rate (breaths/min)	35.54 \pm 1.40	34.00 \pm 1.40	-1.54	15 (40)	0.337	34.90 \pm 1.59	29.88 \pm 1.43	-5.02	5 (40)	<0.001

¹ Total number of pairs tested in parentheses.

Discussion

Little change in feeding behavior occurred in response to the onset of disease until after calves had displayed clinical signs of disease. Milk consumption was the only feeding behavior to show a significant change, with a decrease before clinical signs of disease were evident. This decrease in milk consumption is likely a result of the calves having a reduced appetite before the onset of disease. Following clinical signs, milk consumption, number of visits, and percentage of unrewarded visits to the automated calf feeder all decreased significantly in response to disease. In contrast to these findings, previous studies using automated calf feeding systems for detecting disease found no effect on milk consumption (Svensson and Jensen, 2007). However, as in the current study, Borderas et al. (2009) found milk consumption to be influenced by the onset of disease: when fed high milk allowances (12 L/d), sick calves decreased milk consumption and visits to the feeder and increased visit duration (changes that were also most apparent following clinical diagnosis). In contrast, when sick calves were fed low milk allowances (4 L/d) following clinical diagnosis, Borderas et al. (2009) found no change in milk consumption, whereas visit duration was found to decrease. These changes in feeding behavior are indicative of disease: compared with healthy calves, diseased calves visit the feeder less often and the visits are of a longer duration (it takes a greater amount of time for the calf to consume their allowance). Borderas et al. (2009) highlighted the importance of considering an animal's milk allowance when using changes in feeding behavior to identify diseased animals. Although the current study provided calves with a daily milk allowance of 4 to 6 L/d (which follows standard farm practice in New Zealand), according to Borderas et al. (2009), this would be considered a low allowance. It is possible in the current study that changes in feeding behavior may have been greater had calves been provided with a greater milk allowance. Also, similar to our findings, previous studies (Svensson and Jensen, 2007; Sutherland et al., 2018) have reported that sick calves decrease the number of unrewarded visits

in response to disease; Sutherland et al. (2018) also detected this decrease in the days before clinical diagnosis. Age and breed could be factors contributing to the differences in feeding behaviors across the studies. Compared with drinking rate and milk consumption, Svensson and Jensen (2007) suggested that the number of unrewarded visits was the most useful indicator of disease. Unrewarded visits represent calves testing the feeder to see whether milk is available, so a decrease in the number of unrewarded visits is suggestive of a reduction in appetite.

The IRT temperatures of all anatomical areas measured were found to change in response to disease. This is consistent with known chronology for the onset of disease with thermal biometric values (Cook and Schaefer, 2013). However, before clinical signs of disease appeared, significant changes in IRT temperatures were found only for the shoulder and side. The decrease in shoulder temperature may be due to the animal generating a state of fever to fight against the infection by restricting blood flow to the skin and extremities. This reduces heat loss to the environment, which helps the animal maintain homeostasis. The decrease in temperature could also be a result of a reduction in feed and metabolic activity in response to the onset of disease. The contrasting increase in side temperature is likely a result of the area being situated over the rumen fossa, resulting in the side area being in close proximity to the site of infection and localized inflammation of the intestines. Previous studies investigating the use of IRT as a method for the early detection of BRD and BVD in beef cattle found IRT temperatures to increase before clinical signs of disease were evident (Schaefer et al., 2004, 2007). Inconsistencies across studies could be due to differences in pathogenesis associated with the modes of action corresponding to the different diseases as well as the specific anatomical area being measured and differences in environmental

temperature and relative humidity. Similar to the current study, Schaefer et al. (2004) collected images from various anatomical locations (side, back, eye, ear, and nose) and found that IRT temperatures of these areas increased by 1.5 to 4.0°C before clinical signs of BVD and that changes of <1°C were clinically significant. In the current study, changes in IRT of <0.3°C before clinical signs appeared were found to be significant ($P < 0.001$). Additionally, before clinical signs of disease, a significant ($P < 0.001$) change in IRT temperature was found for the side and shoulder but not for the eye, cheek, or back. This finding is similar to Schaefer et al. (2004) reporting that different anatomical areas presented differing levels of sensitivity; for example, significant changes in eye temperature occurred as early as 1 d post-infection compared with changes in nose, ear, side, or back temperatures, which were not found to be significant until 5 to 6 d post-infection (Schaefer et al., 2004).

Through measuring temperature changes at different anatomical locations, IRT has also been investigated in previous studies (Montanholi et al., 2009, 2010; Martello et al., 2016) for assessing feed efficiency. When comparing cattle with high residual feed intake (**RFI**) and those with low RFI, Martello et al. (2016) found that cattle with a low RFI had a higher IRT temperature on the front of the head, whereas other anatomical locations (i.e., eye, ribs, flank, rump, and feet) showed no significant difference in relation to RFI. However, other studies (Montanholi et al., 2009, 2010) found that temperatures of the snout, cheek, and hoof were more suitable indicators of feeding efficiency. Compared with core body areas, a consistent finding across all 3 studies (Montanholi et al., 2009, 2010; Martello et al., 2016) was that temperature differences measured at the extremities were the most useful indicators of feeding efficiency. In contrast, the current study found core body areas, rather

than extremities, to be the most useful indicators of disease. This possibly suggests that the suitability of different areas is dependent on the specific application for which they are being measured and needs to be considered when determining which indicators will be the most suitable.

Lying time, number of lying bouts, and bout duration all changed significantly following clinical signs of disease, with decreases in both lying time and the number of lying bouts and an increase in bout duration. In addition, before clinical signs appeared, there was a decrease in the number of lying bouts and an increase in bout duration. Ollivett et al. (2014) investigated the effect of BRD on the lying behavior of dairy calves and found that lying time increased in response to the onset of disease. Similarly, in a study by Borderas et al. (2008), calves that were given an injection of bacterial LPS to stimulate a fever response also increased the amount of time they spent lying. Sutherland et al. (2018) reported that before clinical signs of NCD became evident, calves increased the amount of time spent lying and typically performed fewer lying bouts. In the current study, before clinical signs appeared, there was no change in lying time; however, similar to Sutherland et al. (2018), calves performed fewer lying bouts. Additionally, the current study found that the duration of lying bouts increased in response to the onset of disease. This change in the number of lying bouts and bout duration suggests that calves have fewer periods of activity. This is perhaps due to a reduced appetite and calves becoming lethargic and attempting to conserve energy in response to the onset of disease.

In terms of drinking behavior, the only significant change observed in the current study was an increase in visit duration before clinical signs of disease appeared.

Although water intake was not measured in the current study, this increase in visit duration may suggest an increase in the amount of water being consumed during each visit. Previous studies (Jenny et al., 1978; Wenge et al., 2014) found water intake to increase in diarrheic calves. Similar results have also been found in pigs, whereby water intake increased the day before overt clinical signs of an enteric disease were seen (Madsen and Kristensen, 2005). Neonatal calf diarrhea subjects the intestines to substantial damage, which results in severe diarrhea. Therefore, in the current study, increasing visit duration and potentially water intake could be a mechanism that restores hydration levels as calves attempt to overcome the disease.

Respiration rate decreased only following clinical signs of disease, suggesting that it may not be a suitable early indicator for disease detection. The lack of change in RR before clinical signs were evident could be due to NCD being a gastrointestinal disease as opposed to a respiratory disease such as BRD, where RR would be expected to change more rapidly.

Milk consumption, IRT temperatures of the side and shoulder, number and duration of lying bouts, and duration of drinking visits all showed changes before the clinical identification of disease, therefore demonstrating potential suitability as early indicators of NCD. The integration of these measures into an automated on-farm system could enable earlier treatment and isolation of diseased animals to prevent further spread of disease by alerting farmers to diseased animals earlier than is currently possible based on overt clinical signs. In addition to facilitating decision-making abilities for the farmer, the development of such a system would reduce costs on-farm and to the industry as a whole and ultimately improve calf health and welfare.

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Chapter Four

*Automated collection and analysis of infrared thermograms for
measuring eye and cheek temperatures in calves*



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Abstract

As the reliance upon automated systems in the livestock industry increases, technologies need to be developed which can be incorporated into these systems to monitor animal health and welfare. Infrared thermography (IRT) is one such technology that has been used for monitoring animal health and welfare and, through automation, has the potential to be integrated into automated systems on-farm. This study reports on an automated system for collecting thermal infrared images of calves and on the development and validation of an algorithm capable of automatically detecting and analysing the eye and cheek regions from those images. Thermal infrared images were collected using an infrared camera integrated into an automated calf feeder. Images were analysed automatically using an algorithm developed to determine the maximum eye and cheek (3×3 -pixel and 9×9 -pixel areas) temperatures in a given image. Additionally, the algorithm determined the maximum temperature of the entire image (image maximum temperature). In order to validate the algorithm, a subset of 350 images analysed using the algorithm were also analysed manually. Images analysed using the algorithm were highly correlated with manually analysed images for maximum image ($R^2 = 1.00$), eye ($R^2 = 0.99$), cheek 3×3 -pixel ($R^2 = 0.85$) and cheek 9×9 -pixel ($R^2 = 0.90$) temperatures. These findings demonstrate the algorithm to be a suitable method of analysing the eye and cheek regions from thermal infrared images. Validated as a suitable method for automatically detecting and analysing the eye and cheek regions from thermal infrared images, the integration of IRT into automated on-farm systems has the potential to be implemented as an automated method of monitoring calf health and welfare.

Keywords: Infrared thermography; algorithm validation; automated systems; early disease detection; calves

Introduction

Over time, the livestock industry has seen a significant change in response to an increasing reliance on automated systems. This reliance has largely been driven by a need to reduce labour costs and has resulted in the development of automated systems such as the robotic milking and automated calf feeder systems seen in modern dairy farming systems [1]. This increasing level of automation in the livestock industry [2] has resulted in a less “hands-on” approach to farming, and additionally, there are fewer experienced stock people in the industry [3]. These effects of automation, along with increasing herd sizes and less individual animal contact, can result in a reduced ability to monitor and identify animals displaying signs of compromised health and welfare. It is therefore important as part of the future development of automated systems that they are designed to incorporate methods which provide the capability to reliably monitor animal health and welfare on-farm.

Infrared thermography (IRT) is a technology which has the potential to be integrated into automated systems for monitoring animal health and welfare [3]. IRT detects the amount of infrared energy an object radiates; the more infrared energy, the greater the temperature of the object [4]. IRT is a noninvasive, remote method of measuring an animal’s surface temperature [5], where the temperatures detected and the distributions may be associated with underlying physiological, metabolic and behavioural processes and mechanisms [6]. There is the potential that, by measuring changes in surface temperature, IRT can be applied as a method

for detecting disease based on the detection of fever/inflammation [7]. The use of IRT in livestock and veterinary applications was most recently reviewed by Luzi et al. [8]. Applications for which IRT has been applied include the detection of bluetongue virus in sheep [9], of foot and mouth disease in mule deer [10], of rabies in raccoons [11], of thoracolumbar vertebral disk disease in dogs [12], of pregnancy in zebras and black rhinoceros [13] and of impaired meat quality in pigs [14]. With specific relevance to cattle welfare, some of the applications that IRT has been used for include diagnosing mastitis [15] and lameness [16,17] by detecting areas of inflammation and as a method for measuring stress and pain responses to procedures such as disbudding [18], castration [19], handling [20] and transport [21]. IRT has also been used in previous studies as a method for the early detection of diseases such as bovine viral diarrhoea (BVD), bovine respiratory disease (BRD) [22–24] and neonatal calf diarrhoea (NCD) [1,25] and, additionally, as a method for detecting differences in feed efficiency [26–28]. Previous studies have collected infrared images from various anatomical regions. Whilst investigating IRT as a method for detecting BVD for example, Schaefer et al. [22] found changes in eye temperature as early as 1 d postinfection; however, changes in nose, ear, hoof, lateral and dorsal temperatures were not significant until 5–6 days postinfection. The increased sensitivity of the eye was considered to be attributed to the blood flow being closer to the surface and thus providing a more accurate reflection of core body temperature [5].

Generally, thermal infrared images are collected and analysed manually, where an observer collects images using a handheld infrared camera (e.g., ThermaCAM S60; FLIR systems AB, Danderyd, Sweden) [1,26]. These images are then analysed using specific analysis software (e.g., ThermaCAM Researcher; FLIR systems AB,

Danderyd, Sweden). This software requires observers to select the region of interest (ROI) in each image to obtain the minimum, median and maximum temperatures of the area. These manual methods are often time-consuming, especially when dealing with large groups of animals and large data sets and requires the skill of trained observers during image collection and analysis [29]. Additionally, handheld infrared cameras are often cumbersome and impractical to use and can, in some situations, cause disturbances for the animals, which can affect the results [29]. The development of automated methods for the collection and analysis of thermal infrared images would provide an alternative to current manual methods [29]. Furthermore, the development of algorithms is necessary for the successful integration of IRT into automated systems where it could then be utilized for noninvasive monitoring of animal health and welfare. The integration of IRT into automated systems could potentially enable diseased animals to be identified sooner than is currently possible based on overt clinical signs [1]. This would enable sick animals to be identified and isolated from their pen mates to prevent the spread of disease and would enable treatments to be administered sooner [1]. A system providing the ability to detect early disease onset would also facilitate decision-making abilities for farmers, reducing costs on-farm and for the industry as a whole through reduced labour, mortalities and veterinary costs, and, overall, would lead to improvements in both production and animal welfare [1].

This study was part of a larger project investigating a wide range of automated methods for early disease detection in calves. The purpose of the current project was 1) to develop an algorithm capable of detecting and analysing temperatures of the eye and cheek regions from thermal infrared images (collected automatically

while calves visited an automated calf feeder) and 2) to validate the algorithm through comparison with manual methods of analysis.

Materials and methods

All procedures involving animals in this study were approved by the University of Waikato Animal Ethics Committee (Protocols #955 and 985) under the New Zealand Animal Welfare Act 1999.

Development of eye and cheek algorithm

An algorithm was developed with the capability of automatically detecting and analysing the eye and cheek from thermal infrared images collected from calves. Within each captured image, individual pixels acted as floating-point numbers, indicating temperature in degrees Kelvin. Images were converted from floating-point raw data into 8-bit grey-scale portable network graphics (PNG) images using the following conversion formula:

$$x' = 255 \frac{(x - 260)}{315 - 260}$$

where x is the original temperature, x' is the scaled pixel value, 260 is the minimum temperature and 315 is the maximum temperature. All temperatures which fell outside the minimum and maximum temperatures were clamped at those values to maximise the temperature resolution when converting the raw data into a PNG image. The original temperature (x) was calculated from the PNG images using the following formula:

$$x = \frac{x'(315 - 260)}{255} + 260$$

The calculation of x was subject to a conversion accuracy of approximately ± 0.2 °C as a result of the level of error in converting from float to byte values.

The eye detection component of the algorithm used a cascade of AdaBoost classifiers using decision stumps and Haar wavelets [30] and was generated using the OpenCV (version 3.4.3) [31] utility `opencv_traincascade` with the following parameters: `stageType = BOOST`; `featureType = HAAR`; `width = 24`; `height = 24`; `boostType = GAB`; `minHitRate = 0.99`; `maxFalseAlarm = 0.4`; `maxDepth = 1`; `maxWeakCount = 100`; `featSize = 1`; and `mode = BASIC`.

For further development of the eye detection component, the OpenCV utility required a set of training images. Training images were collected during a previous study by our group [32] using an infrared camera (Thermovision A300 (accuracy: ± 2.0 °C; sensitivity: < 0.05 °C; resolution: 320×240 ; temperature range: -20 °C to 120 °C; spectral range: $7.5\text{--}13$ μm); FLIR Systems AB, Danderyd, Sweden) set up at an automated calf feeder (RFID Calf Feeder, A&D Reid, Temuka, New Zealand (as used previously by Lowe et al. [1])) in the same manner as the current study (as described below). Training images were collected from 23 animals, with the number of images captured for each individual varying from 162 to 252 images, yielding a total of 5250 images. For training purposes, images were split into both positive and negative examples.

Positive examples were images that, with the exception of including some surrounding area, contained only the ROI. Including some surrounding area to the ROI has been found to improve the robustness of the detector in face detection applications [33] and hence was included in the positive training images. From the

eye, maximum temperature has been found to be the most relevant diagnostic [24]; therefore, for training purposes, the location of the maximum temperature was identified manually in 1364 images. To create the positive training images, a 72×72 -pixel sub-image was extracted from the original image based on the thermal maximum with a top-left position of $(x - 12, y - 36)$ and a bottom right position of $(x + 60, y + 36)$. Once extracted, these sub-images were resized for efficiency purposes to 24×24 pixels for the training process. The process of creating the positive training sub-images is shown in Figure 1.

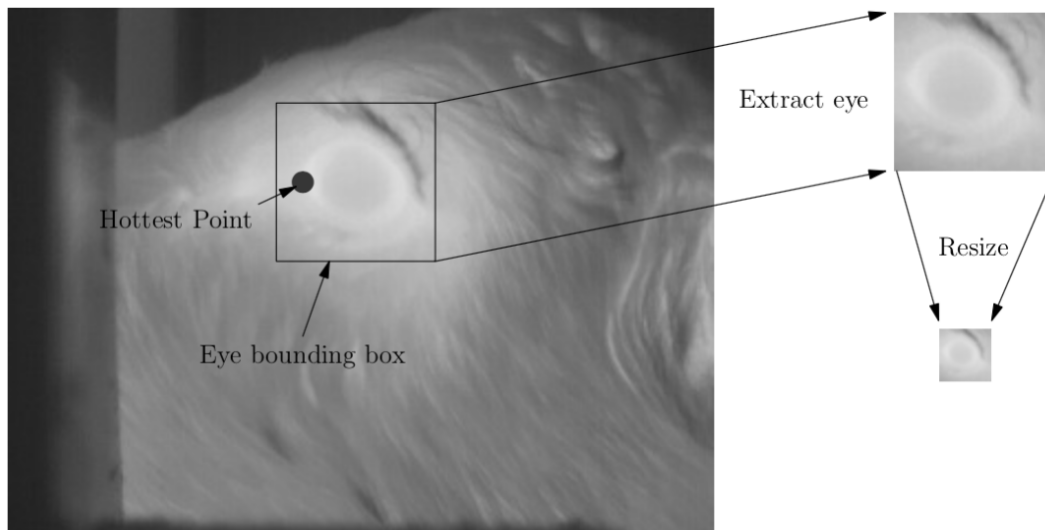


Figure 1. Automated thermal infrared image collected as a calf fed from the automated calf feeder showing the square the algorithm traced around the region of interest (ROI) in order to create the sub-image which was then resized for efficiency purposes to a 24×24 -pixel image.

Negative training images did not include the whole eye region but consisted of parts of an eye. In addition to images which only partially included the eye, negative training examples included those in which the eye was fully or partially closed, as these types of images reduce the ability to acquire an accurate temperature measurement. Examples of negative training images are shown in Figure 2.

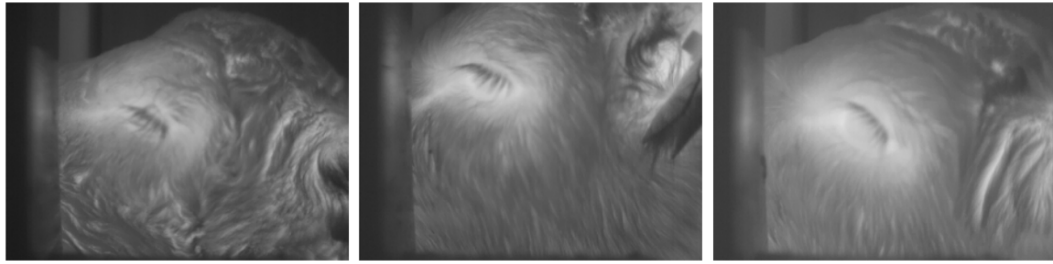


Figure 2. Examples of negative training images, where the eye being fully or partially closed prevented an accurate temperature measurement of the eye.

The training process resulted in an 8-stage cascade, with each stage consisting of 3, 3, 3, 5, 4, 6, 8 and 9 weak classifiers respectively. Each sub-image was passed through the cascade detector in order to determine whether an eye was present within the sub-image. Images which were considered to potentially consist of an eye were passed onto subsequent stages of the cascade until a definite determination on whether the image contained an eye could be made. If a sub-image was considered not to contain an eye, the image was eliminated from the cascade. An illustration of how the cascade worked is shown in Figure 3.

Each cascade stage is an AdaBoost classifier using Haar wavelets as features and decision stumps as weak classifiers within AdaBoost. A decision stump is a simple threshold rule computed over a single feature (a Haar wavelet). A Haar wavelet feature is a weighted sum of pixel values lying within two, three or four connected rectangles. Figure 4 shows the five possible feature shapes. Each shape has 4 trainable shape parameters: x and y offset from the origin of the window and the width and height of the rectangles.

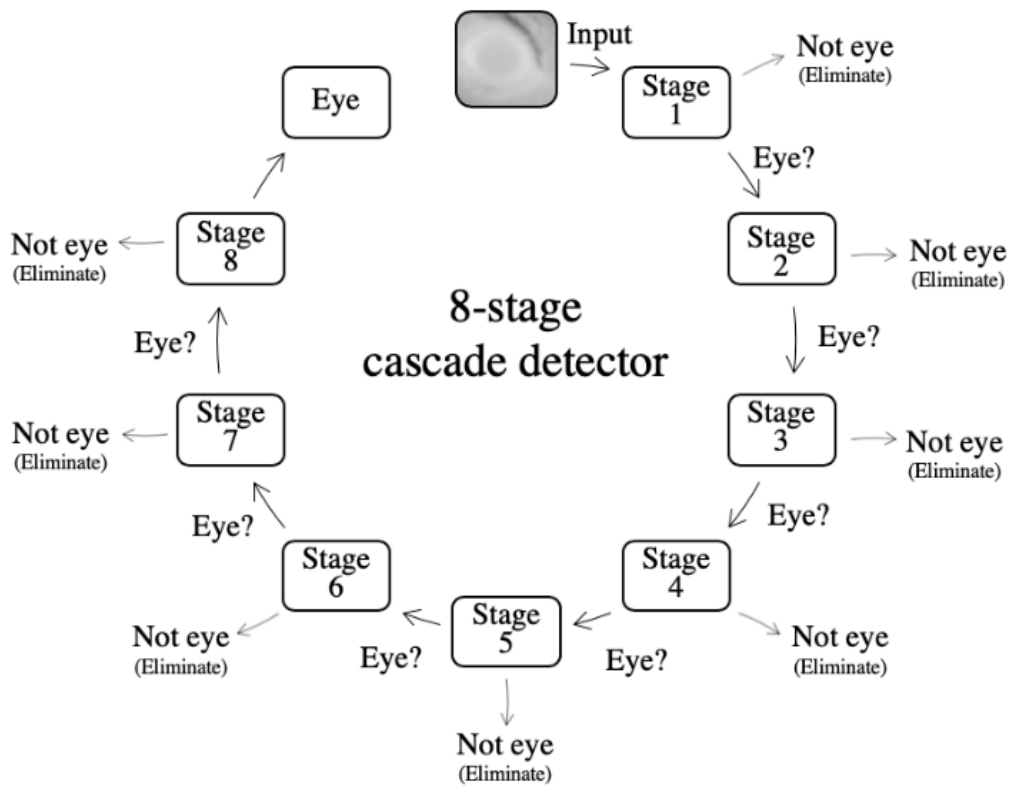


Figure 3. Illustration of the 8-stage cascade detector: At each stage, if the sub-image potentially contained an eye, the sub-image was passed onto the next stage of the cascade. If determined not to contain an eye, the sub-image was eliminated from the cascade.

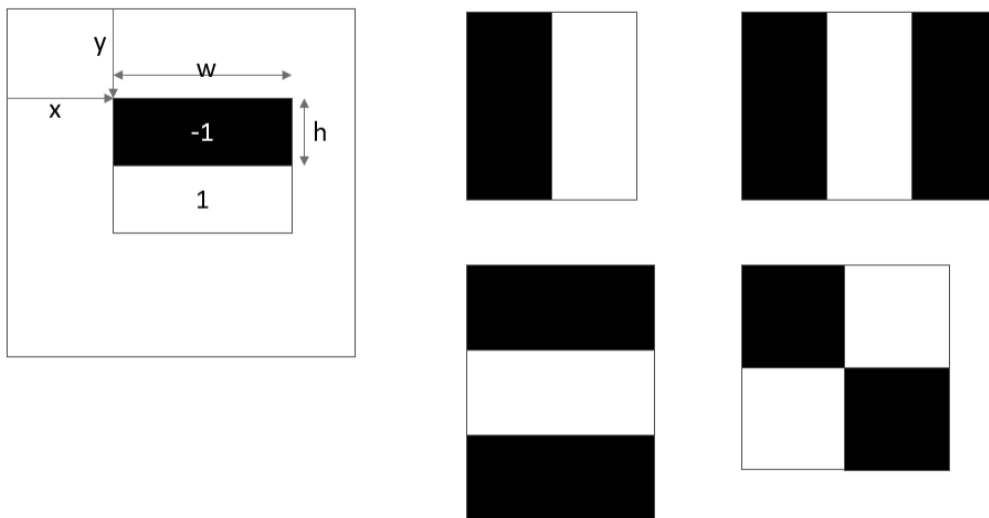


Figure 4. Illustration of the Haar wavelet features showing the five possible feature shapes.

The features are evaluated by summing up the pixel values under the white rectangles and by subtracting the sum of the pixel values under the black rectangles (suitably weighted to normalize area). There are over 150,000 possible features which are evaluated exhaustively on the training set so that the best features are chosen at each cascade stage. A threshold is automatically determined to maximally separate positive and negative training examples, both within each weak classifier and within a single cascade stage. A decision stump, D , is evaluated on a window, w , according to the following equation:

$$D(w) = \text{If } F(w) < t \text{ then } v1 \text{ else } v2$$

where $F(w)$ evaluates the Haar feature on the window w (a weighted sum of the pixel values in each rectangle), t is the learned threshold, and $v1$ and $v2$ are the values returned depending on if the feature is above or below the threshold. The cascade stage is evaluated as the sum of all weak classifiers in the stage as follows:

$$S(w) = D_1(w) + D_2(w) + D_3(w) + \dots$$

If $S(w)$ is greater than the stage threshold, then the window passes on to the next stage, and if not, it is rejected as outlined in Figure 3.

For example, the first cascade stage has 3 weak classifiers and eliminates approximately 60% of sub-windows. In this case, the three weak classifiers are as follows:

$$D_1(w) = \text{If } F_1(w) < 0.081 \text{ then } -1.0 \text{ else } 0.6$$

$$D_2(w) = \text{If } F_2(w) < 0.068 \text{ then } -0.95 \text{ else } 0.59$$

$$D_3(w) = \text{If } F_3(w) < 0.056 \text{ then } -0.97 \text{ else } 0.42$$

The features F_1 , F_2 and F_3 are shown in Figure 5. As can be seen from the figure, the features tend to find areas where there is high contrast in the target class, and this is typical of all the features used by such classifiers.

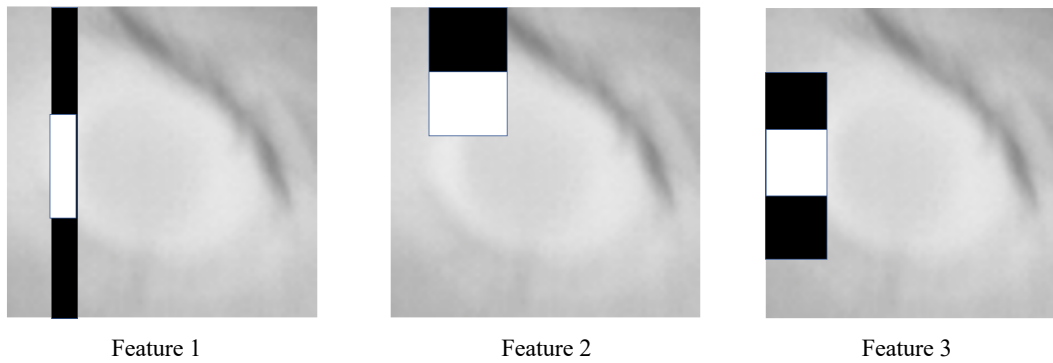


Figure 5. Features used in the first cascade stage.

Each cascade layer was trained to achieve a true positive rate of 0.99 and a false positive rate of 0.4. Therefore, the theoretical accuracy of the entire 8-stage cascade was a true positive rate of $0.99^8 = 0.92$ with a false positive rate of $0.4^8 = 0.00066$. Example images from cascade stage-8 indicating the location of the maximum eye temperature are shown in Figure 6.

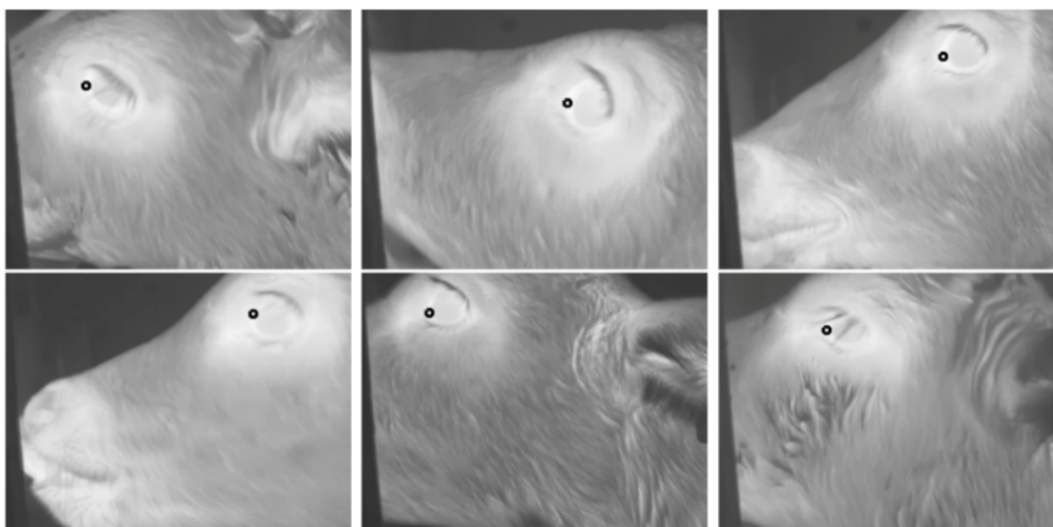


Figure 6. Examples of eye images from cascade stage-8: The marked positions indicate the location of the algorithm maximum eye temperature.

In addition to the eye detection component of the algorithm, we further developed the algorithm in order to detect the cheek region. The development of the cheek component of the algorithm required a set of training images. Training images were collected manually using a handheld infrared camera (ThermaCAM S60, FLIR Systems AB, Danderyd, Sweden) during a previous trial [1]. A total of 465 training images were collected from 43 calves, within which the cheek region was manually selected within each image by tracing a circle over the cheek muscle using ThermaCAM Researcher software (version 2.10; FLIR Systems AB, Danderyd, Sweden) (as described by Lowe et al. [1]). Based on the location of the cheek region as specified in the manually analysed images, the eye was used as a reference point in order to train the algorithm to determine the location of the cheek. The cheek region was identified by the algorithm tracing a rectangle down from the eye as shown in Figure 7. At the base of this rectangle, 3×3 and 9×9 -pixel areas were automatically traced by the algorithm, from which the maximum temperatures of those areas were generated (Figure 8). The location of the cheek being determined using the eye as a reference point allowed the eye and cheek temperatures to be collected from the same images.



Figure 7. Example images which, based on the location of the eye, show the rectangle the cheek component of the algorithm traced for determining the location of the cheek.

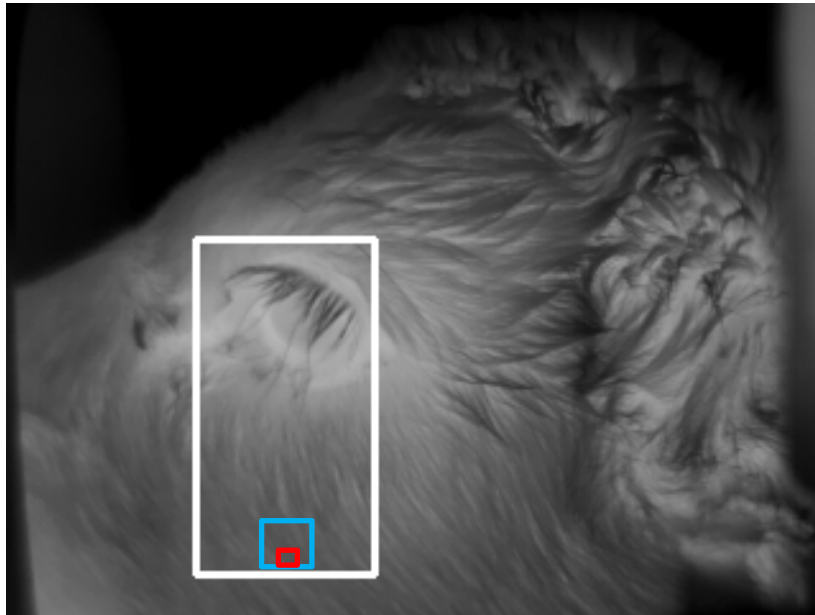


Figure 8. Example image showing the 3×3 (red square) or 9×9 (blue square) pixel area traced by the algorithm within the cheek ROI from which the maximum temperatures of those areas were determined.

The algorithm developed consists of two modes: 1) single-image mode and 2) multiple-image mode. In single-image mode, each image is treated independently and, hence, temperatures from all detected eyes or cheeks are reported; this mode is most useful when only a few images of each animal are recorded. In multiple-image mode, the algorithm assesses all images on an individual animal basis, reporting the median of the maximum eye or cheek temperatures across all images as the temperature for that animal. Multiple-image mode is most advantageous when numerous images of the same animal are being recorded.

For both modes of image analysis, the eye component of the algorithm records the maximum temperature in degrees Celsius in two ways: 1) as the maximum temperature measured from the hottest pixel located within the eye region (algorithm: eye maximum temperature) and 2) as the maximum temperature measured from the hottest pixel within the entire image (not necessarily the eye)

(algorithm: image maximum temperature). Similarly, the cheek component of the algorithm also records the maximum temperature in degrees Celsius in two ways: 1) as the maximum temperature within a 3×3 -pixel area (algorithm: cheek 3×3 pixel maximum temperature) and as the maximum temperature within a 9×9 -pixel area (algorithm: cheek 9×9 pixel maximum temperature).

Validation of the eye and cheek algorithm as an automated method for thermal infrared image analysis

In order to validate the eye and cheek algorithm, thermal infrared images were collected and analysed automatically and compared to manual methods of analysis as described below.

Animals

This validation component of the study was undertaken at a farm in the Waikato region of New Zealand ($38^{\circ}04'15.6''S$ $175^{\circ}19'42.5''E$) from July to October 2016. One hundred and twenty mix breed heifer calves (66 dairy calves (Friesian, Jersey and cross breeds) and 54 Hereford calves) (36.4 ± 4.33 kg, mean \pm SD) were enrolled into the study at two days of age and were observed until 24 ± 14.4 (mean \pm SD) days of age.

Automated thermal infrared image collection and analysis

Throughout the course of the study, calves were fed whole milk using two automated calf feeders (RFID Calf Feeder, A&D Reid, Temuka, New Zealand (as used previously by Lowe et al. [15])). Thermal infrared images were collected automatically using an infrared camera (Thermovision A300 (accuracy: ± 2.0 °C;

sensitivity: <0.05 °C; resolution: 320×240 ; temperature range: -20 °C to 120 °C; spectral range: $7.5\text{--}13$ μm); FLIR Systems AB, Danderyd, Sweden) integrated into each calf feeder. The left side panel of the calf feeder was modified by cutting out a square viewing hole so that the infrared camera could be placed in such a position that, as the calf fed, it could continuously collect images of the facial region during each visit (Figure 9). Thermal infrared images were captured at 60 frames per second with a resolution of 320×240 pixels. Calves were individually identified using an automatic electronic identification (EID) reader (G03113 EID tag reader controller R; Gallagher, Hamilton, New Zealand) and antenna system (G03121 EID tag reader antenna panel 600; Gallagher, Hamilton, New Zealand) as they entered the feeder based on the EID in their ear tags. The infrared camera was programmed to begin capturing images once the EID of the calf visiting the feeder had been detected. The infrared cameras were connected to a laptop which, through interface software, enabled the individual tag information and thermal infrared images to be collected and stored. For consistency, all images were collected at a set distance of 0.5 m and at an angle of 90° to the animal. Each infrared camera was set at an emissivity of $\varepsilon = 0.98$, which is accepted as a suitable emissivity for measuring an animal's surface temperature [34] and has been used previously in cattle studies [1,3,29]. During the present study, thermal infrared images were collected during a total of 29,637 visits to the calf feeder. A subset of 350 randomly selected images were analysed using the eye and cheek detection algorithm as described above to determine the image, eye, cheek 3×3 -pixel and cheek 9×9 -pixel maximum temperatures.



Figure 9. Infrared thermography (IRT) camera installed on the left side of the automated calf feeder collecting thermal infrared images of the facial region through the square viewing hole as a calf feeds from the feeder.

Manual image analysis

The 350 images analysed using the algorithm were also analysed manually in order to validate the algorithm. Images were analysed manually to determine the maximum image, eye and cheek (3×3 and 9×9 -pixel areas) temperatures using MATLAB analysis software (R2019b; MATLAB, MathWorks Inc., Natick, MA, USA). As shown in Figure 10, the “manual: image maximum temperature” was determined by tracing the black square around the entire image. The “manual: eye maximum temperature” was determined by tracing the green square around the eye to include the eyeball and area surrounding the eyelid. The “manual: cheek 3×3 -pixel maximum temperature” and “manual: cheek 9×9 -pixel maximum temperatures” were determined respectively by tracing the red and blue squares over the cheek muscle using the eye as a reference point.

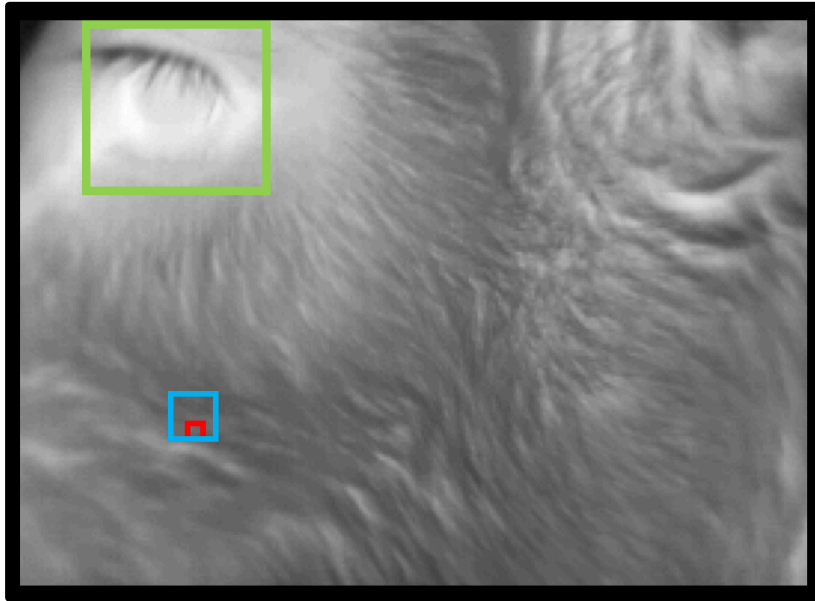


Figure 10. Example image showing the areas traced during the manual analysis to determine the “manual: image maximum temperature” (black square), “manual: eye maximum temperature” (green square), “manual: cheek 3 × 3-pixel maximum temperature” (red square) and “manual: cheek 9 × 9-pixel maximum temperature” (blue square).

Statistical analysis

Using Microsoft Excel (version 16.26; Microsoft Corporation, Redmond, WA, USA), data recorded automatically using the eye and cheek detection algorithm from infrared images collected in the current study were regressed against the data gathered from manual analysis of the same images. This enabled the level of agreement between the two different methods of analysis to be assessed. Bias was assessed using Bland Altman analyses. In addition, a Lin’s concordance analysis was carried out to assess the level of equality between the different types of temperature measurement.

Results

Images analysed using the algorithm were highly correlated with those analysed manually for maximum image ($R^2 = 1.00, p < 0.001$), eye ($R^2 = 0.99, p < 0.001$), cheek 3×3 -pixel ($R^2 = 0.85, p < 0.001$) and cheek 9×9 -pixel ($R^2 = 0.90, p < 0.001$) temperatures (Figure 11). Bland Altman analysis of the differences between the algorithm and manual analysis plotted against the average showed no evidence of any change in bias across the range of values, and the average bias was not significant for maximum image (0.00 ± 0.000), eye (0.00 ± 0.001), cheek 3×3 -pixel (0.07 ± 0.027) and cheek 9×9 -pixel (0.08 ± 0.031) (mean difference \pm standard error of the mean (SEM)) temperatures. In addition, Lin's concordance analysis showed strong levels of equality between the two methods of analysis for maximum image ($Qc = 1.00, p < 0.001$), eye ($Qc = 0.99, p < 0.001$), cheek 3×3 -pixel ($Qc = 0.92, p < 0.001$) and cheek 9×9 -pixel ($Qc = 0.95, p < 0.001$) temperatures.

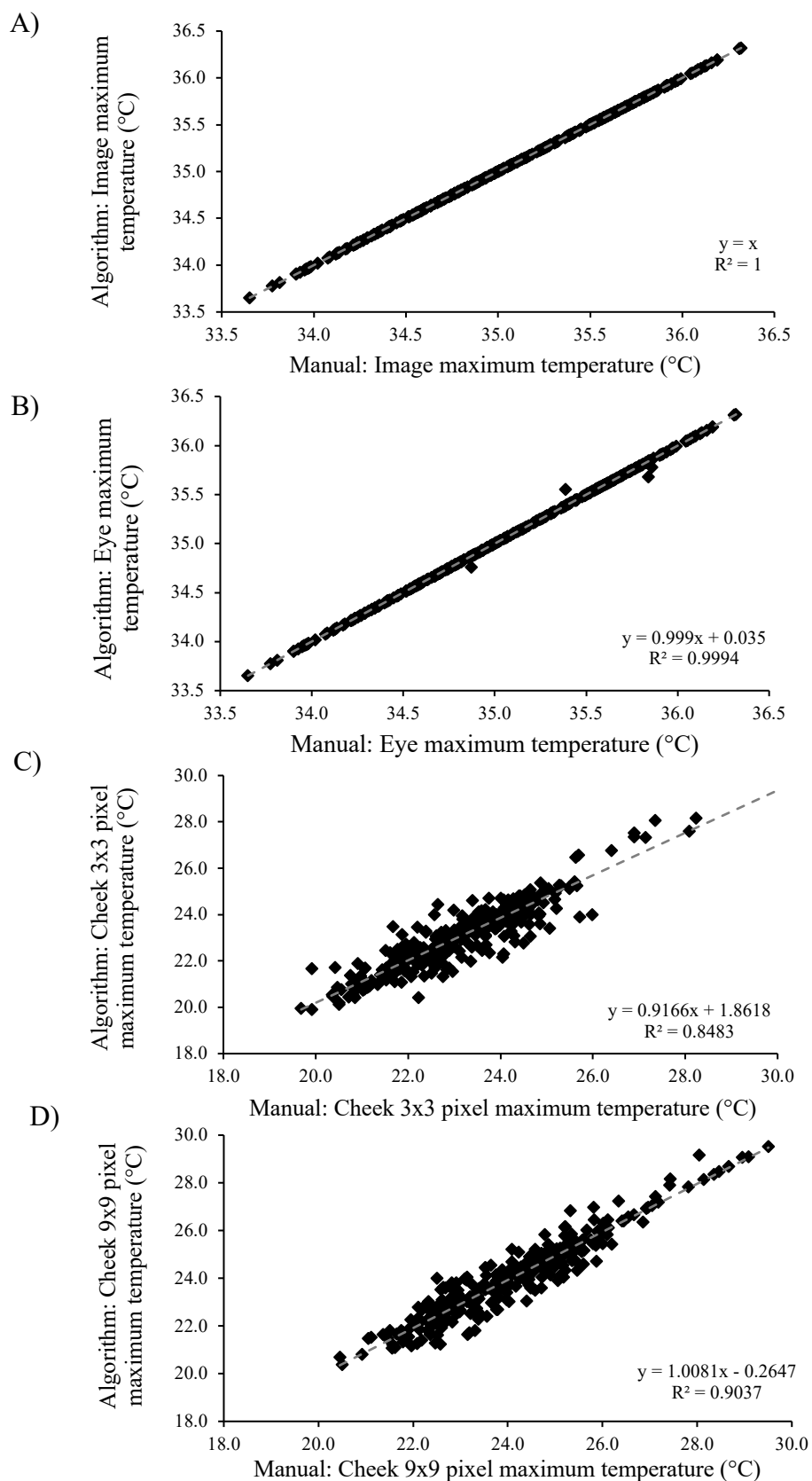


Figure 11. Correlations between images analysed using the algorithm and manually for the maximum (A) image, (B) eye, (C) cheek 3 × 3-pixel and (D) cheek 9 × 9-pixel temperatures.

Discussion

The current study provides a demonstration of an automated IRT system where infrared cameras were programmed to collect images automatically as calves fed from automated calf feeders. The system used for automatically collecting thermal infrared images in the current study is similar to that used by Schaefer et al. [24], who provided the first example of an automated IRT system for the early detection of BRD in beef calves. Schaefer et al. [24] demonstrated that IRT cameras could be used to noninvasively collect thermal infrared images as calves visited a water station. Further, the current study validated an algorithm developed as an automated method of detecting and analysing the eye and cheek regions from thermal infrared images collected from young calves.

The strongest level of agreement between the algorithm and manual method of analysis occurred when determining the maximum image temperature. This finding reflects both methods of analysis, assessing the entire image in order to determine the temperature of the single hottest pixel within the image. The maximum eye temperature also showed a strong level of agreement between both methods of analysis. This reflects the distinct appearance of the eye, making it possible for this region to be easily distinguished from the rest of the image and therefore enabling the eye region to be selected to determine the maximum eye temperature. In comparison, whilst the cheek 3×3 and cheek 9×9 -pixel maximum temperatures also showed strong agreement between both methods of analysis, the level of agreement was lower than for the maximum eye temperature. In contrast to the eye region, the cheek has a less distinguishable appearance and the region of selection is instead based on using the eye as a reference point to determine the location of

the cheek, which can result in some variability in the exact location selected. This level of variability consequently leads to some reduction in the level of agreement between methods when determining the maximum temperature of the cheek region. As demonstrated in the current study, the cheek 9×9 -pixel area showed a stronger level of agreement between methods than the cheek 3×3 -pixel area. This finding is due to the increase in pixel area, reducing the variability in the area being selected between the two methods of analysis. Validated as an automated method of analysing the eye and cheek regions from thermal infrared images, this algorithm would support the integration of IRT into automated systems for noninvasive monitoring of animal health and welfare.

Previous studies [22–24] investigating the use of IRT for early detection of BRD and BVD have found IRT capable of detecting the onset of the diseases based on changes in eye temperature. Schaefer et al. [23,24] found that eye temperature increased significantly in response to the onset of BRD, and these changes were found to occur several days to a week before clinical signs of disease were apparent. Similarly, Schaefer et al. [22] found a significant increase in eye temperature in response to the onset of BVD, and this increase was found to occur as early as 1-day postinfection. The integration of IRT into automated systems on-farm would enable continuous monitoring of individual animals. For the purpose of detecting diseases such as BRD and BVD, for example, this would enable a history of baseline data to be established per animal. Baseline data is essential in order for parameters to be set in such a way that deviations from what is considered “normal” for each animal can be detected. Deviations could then be used to generate alerts to notify farmers of animals displaying early signs of disease. Early disease detection would enable farmers to identify and isolate sick animals to prevent the spread of

disease and would enable treatments to be administered sooner to reduce the degree of suffering [1]. Early disease detection would facilitate decision-making abilities for farmers, would reduce costs on-farm and for the industry as a whole through reduced labour, mortalities and veterinary costs and, overall, would lead to improvements in both production and animal welfare [1]. Robotic milking systems, automated feeders and automated water stations are potential systems that could support the integration of IRT. Integration of IRT into such systems would support the simultaneous collection of IRT alongside other behavioural and physiological measures. For example, Lowe et al. [1] found that milk consumption, number and duration of lying bouts, and duration of drinking visits all changed prior to the onset of NCD and suggested that they could be useful early indicators for detecting NCD. Additionally, Lowe et al. [1] manually collected and analysed thermal infrared images from a number of anatomical locations and found thermal changes of the side and shoulder of the calf to have the best potential as early indicators of NCD; therefore, these may be other anatomical areas which are worth developing further automated detection algorithms. Additionally, it may be useful to combine thermal changes, measured using IRT, with behavioural changes in feeding and drinking behaviours to provide stronger predictive composite indicators of disease. In addition to disease, the automation of IRT could also be beneficial for other applications including genetic selection and breeding and as a method for measuring stress and pain responses. Additionally, although the algorithm discussed in the current study was developed for use in young calves, with future developments, it may have further applications for use in adult cattle and other species.

Conclusions

In conclusion, this study reports on the development and validation of an algorithm with the ability to automatically detect and analyse the eye and cheek regions from thermal infrared images collected from calves, which is a significant step towards the integration of IRT into automated systems. It is possible that further algorithms could also be developed to automatically detect and analyse other anatomical locations. With the support of algorithms, IRT could be integrated into automated systems, where, alongside other behavioural and physiological measures, IRT could be implemented as a noninvasive method of monitoring animal health and welfare.

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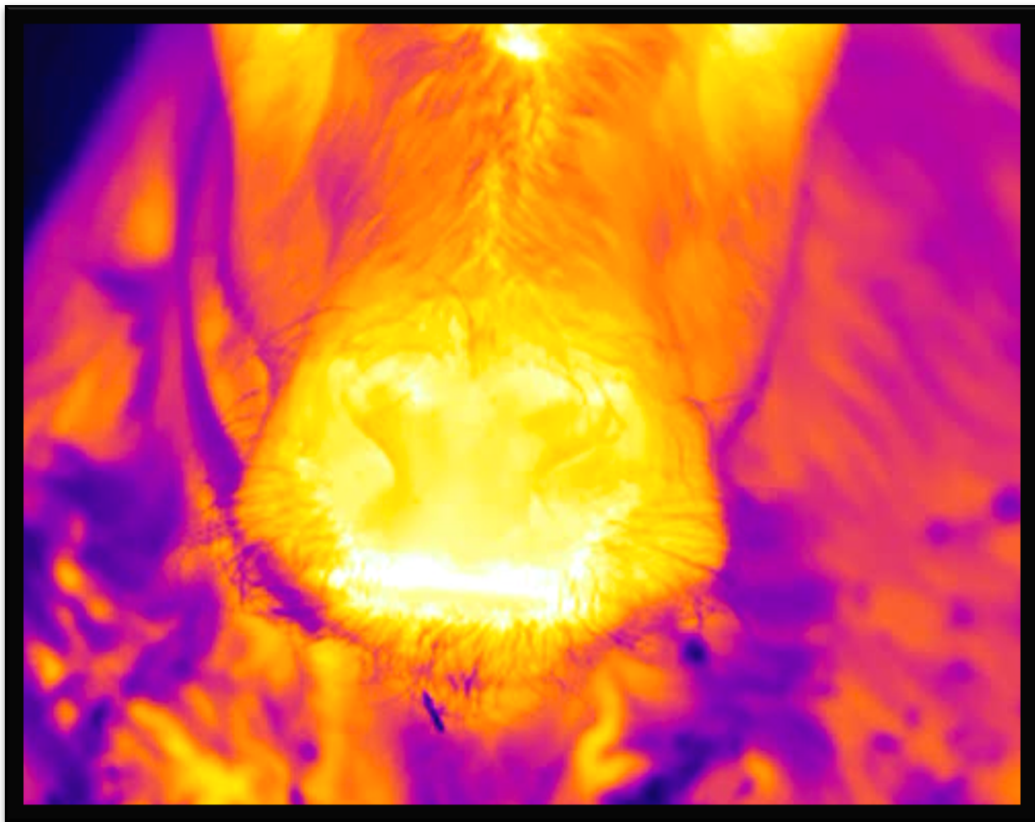
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Chapter Five

*Infrared thermography - a non-invasive method of measuring
respiration rate in calves*



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Abstract

Respiration rate (RR) is a common measure of cattle health and welfare. Traditionally, measuring RR involves counting flank movements as the animal inhales and exhales with each breath. This method is often considered difficult, labour-intensive and impractical. We validated the use of infrared thermography (IRT) as an alternative method of non-invasively measuring RR in young calves. RR was simultaneously recorded in two ways: (1) by observing flank movements from video recordings; and (2) by observing thermal fluctuations around the nostrils during inhalations and exhalations from infrared recordings. For each method, the time taken to complete five consecutive breaths (a breath being a complete inhalation/exhalation cycle) was recorded and used to calculate RR (breaths/min). From a group of five calves, a total of 12 video recordings and 12 infrared recordings were collected. For each procedure, 47 sets of five consecutive breaths were assessed. The RRs measured from video recordings of flank movements and thermal fluctuations around the nostrils from infrared recordings were highly correlated ($R^2 = 0.93$). Validated as a suitable method for recording RR, future research can now focus on the development of algorithms to automate the use of IRT to support its integration into existing automated systems to remotely monitor calf health and welfare.

Keywords: respiration rate; infrared thermography; flank movements; thermal fluctuations; calves; cattle health and welfare

Introduction

Respiration rate (RR) is a vital sign that can provide valuable information relating to disease [1], stress [2], pain [3] and overall cattle health and welfare [4]. Traditionally, RR is measured manually by recording the time taken for an animal to complete a specified number of breaths (e.g., the time taken to complete 10 [5] or 20 breaths [2]) or by recording the number of breaths completed in a fixed period of time (e.g., the number of breaths completed within 30 [6] or 60 s [7]); this is accomplished by observing flank movements as the animal inhales and exhales with each breath [2,7]. However, the method is often considered to be time-consuming, labour-intensive and impractical, especially in the case of long-term studies [8]. Furthermore, compared to summer conditions when rapid and heavy breathing is often observed, in cold environments flank movements are typically slower and less pronounced and, therefore, can be difficult to observe [9]. In order to accurately record flank movements, observers are also required to stand in close proximity to the animal, which may influence behavior, thereby altering RR and consequently the reliability of the results [8].

Alternative methods for recording RR have been validated, including the use of thermistors [7], infrared lasers [4], thoracic belts [10], differential pressure sensors [8] and spirometry masks [11]. However, as with flank movements, these methods have their limitations. For example, thoracic belts, spirometry masks and thermistors have to be fitted to individual morphologies and can influence natural behavior. When battery powered, these methods are also limited by battery life. Further, thoracic belts can slip from the desired position or become dislodged by other animals during the recording period, compromising the accuracy of results

[8]. Similarly, thermistors (which measure thermal changes of the nostrils) can display reduced accuracy when environmental and external body surface temperatures are similar [8]. Infrared lasers offer a non-invasive means of measuring RR but, as noted by [4], they were found to be unsuitable on black cows due to their black hair absorbing the light emitted by the laser.

Infrared thermography (IRT) is another non-invasive method that has been validated for the measurement of RR in adult dairy cattle [9]. Infrared thermography detects the amount of infrared energy an object radiates; the more infrared energy, the greater the temperature of the object [12]. Infrared energy is not visible to the human eye, so infrared devices assign different colors to different levels of infrared energy to produce a false-color image visible to the human eye, known as a thermogram [13]. Stewart et al. [9] used IRT to collect recordings of the nose in order to detect thermal fluctuations associated with air movement from the nostrils during inhalation/exhalation cycles. During inhalation, cool air is drawn in from the environment, resulting in a cooling of the nostrils and a subsequent darker appearance of the nostrils in infrared recordings. In contrast, during exhalation, warm air is expelled into the environment, resulting in a warming of the nostrils and a subsequent warmer reading (and brighter appearance) of the nostrils in infrared recordings. Stewart et al. [9] recorded the time taken to complete 10 breaths (converting this into breaths per min (i.e., RR)), and found that RR measured using IRT was highly correlated with RR measured by observing flank movements in both real time and from video recordings of adult dairy cattle.

Although IRT has been validated for measuring RR in adult cattle, IRT has not been validated for measuring RR in young calves, and it should not be assumed that it

would necessarily be a valid method in calves. Compared to adult cattle, both the nostrils and the amount of air displaced during inhalation and exhalation by young calves are smaller, potentially influencing the ability to accurately observe thermal fluctuations. Furthermore, as calves are more active than adult cattle, manual measures of RR become more challenging; hence, alternative methods of recording RR need to be developed. Therefore, the present study investigated the suitability of using IRT as an alternative method of non-invasively measuring RR in calves, and discusses its future potential as a technology that, through further research and development, could be integrated into automated recording systems to track the RR of calves for extended periods of time.

Materials and methods

The current study was part of a larger project undertaken at the AgResearch Ruakura Research farm (40°44'30.822" S, 73°59'21.508" E) located in Hamilton, New Zealand from April to June 2017. All procedures involving animals were approved jointly by the University of Waikato Animal Ethics Committee (Protocol #1017) and the Ruakura Animal Ethics Committee (Protocol #14089). For the purposes of the present study, both video and infrared recordings were collected from a group of five Hereford calves of mixed sex (two females and three males); the animals were 27 ± 3.7 days old (range 22–32 days old) and recordings were collected on a single day within a 3.0 x 6.0 m pen (within an indoor barn).

Video recordings

Video recordings were collected from calves during this period only at times whilst they were standing still, by using a hand-held video camera (HC-V270; Panasonic,

Osaka, Japan). For consistency, recordings were collected by a single operator standing within the pen, 1m in front of the calf. The field of view of the video camera was focussed on the flank area in order to observe the inward and outward flank movements that occurred as the animal exhaled and inhaled, respectively. To calculate RR, video recordings were analysed by a single observer using Adobe Premiere Pro CC (version 12.0 Haberdasher; Adobe Systems, CA, USA) to play back the recordings. The observer counted the flank movements from the video and recorded the time taken for each calf to complete five breaths (a single breath being a completed inhalation/exhalation cycle).

Infrared recordings

Infrared recordings were collected using a hand-held IRT camera (T650sc (accuracy: ± 1.0 °C, sensitivity: < 0.02 °C; resolution: 640 x 480; temperature range: -40 °C to 2000 °C; spectral range: 7.5–14 μm); FLIR systems AB, Danderyd, Sweden). Individual infrared recordings were collected at the same time as the corresponding video recordings by a second observer. For the purpose of validation, it was essential that the infrared and video recordings were collected simultaneously on the same individual to allow for a direct comparison to be made to assess the level of correlation between the two methods. Recordings were collected within an indoor barn to minimise the impact of sunlight, a factor that can influence the accuracy of infrared measurements. The IRT camera was calibrated prior to recordings being collected by entering the ambient temperature (16.5 °C), relative humidity (RH, 77%) and emissivity ($\epsilon = 0.98$ (in accordance with the general known emissivity of an animal's body [14] and as used previously for cattle [9,15])) into the camera settings. Ambient temperature and humidity were recorded using a

Kestrel meter (Kestrel 3000 PocketWeather Meter (temperature-accuracy: ± 1.0 °C; resolution: ± 0.1 °C; range: -29.0 °C to 70.0 °C; humidity accuracy: $\pm 3.0\%$ RH; resolution: 0.1% RH; range: 5.0 – 95.0% RH); Nielsen-Kellerman, PA, USA). As with the video recordings, infrared recordings were collected by a second operator standing within the pen, at a distance of 1 m in front of the calf while the calf was standing. The field of view for the infrared recordings was focussed on the nose in order to observe the thermal fluctuations that occurred as air moved in and out of the nostrils as the animal inhaled and exhaled with each breath (Figure 1). For consistency and accuracy of the results, in addition to (1) minimising sunlight; (2) adjusting for environmental conditions; and (3) keeping a consistent distance from the calf, infrared recordings were collected at an angle of 90° in relation to the front of the nose. To calculate RR, infrared recordings were analysed by a single observer using Adobe Premiere Pro CC (version 12.0 Haberdasher; Adobe Systems, CA, USA) to play back the recordings. From the infrared recordings, the observer monitored the color change that occurred as a result of the thermal fluctuations around the nostrils during inhalation and exhalation in order to record the time taken to complete five breaths.

Calculating RR

For both video and infrared recordings, the time taken to complete five breaths was converted into the number of breaths per minute (i.e., RR) using the following equation:

$$\text{RR (breaths/minute)} = \left(\frac{60}{x}\right) \times y$$

where 60 is the number of seconds in a minute, x is the time taken to complete five breaths, and y is the number of breaths.

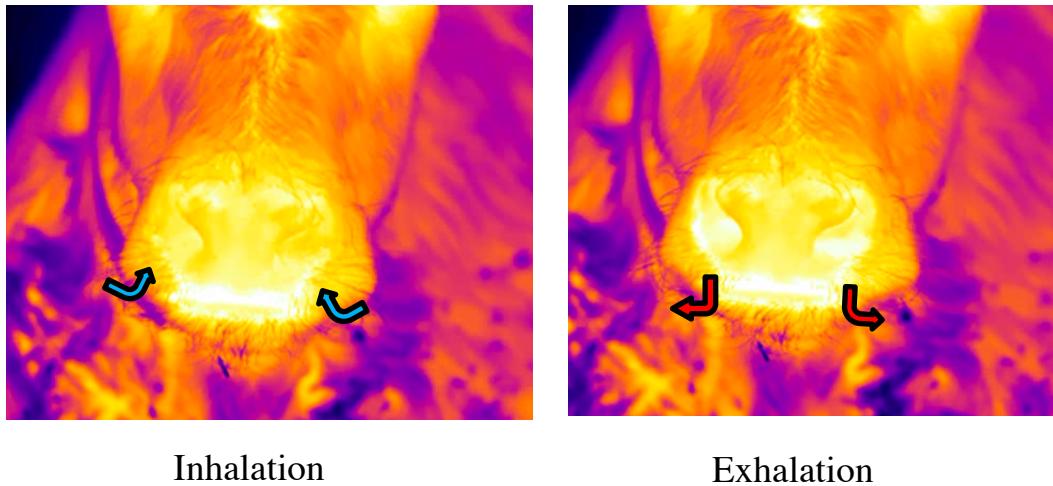


Figure 1. Example infrared images showing the thermal changes which occur at the nostrils during inhalation, when cold air (illustrated as blue arrows) is drawn in through the nostrils from the environment, and exhalation, when warm air (illustrated as red arrows) is expelled through the nostrils back into the environment.

Additional recording information

From the five animals observed, a collective total of 12 video recordings and 12 IRT recordings were obtained (the number of recordings collected ranged from 1 to 4 recordings/animal with an average recording length of 58 s (range: 16–152 s per recording)). Multiple sets of five consecutive breaths were collected from each recording giving a total of 47 sets of five consecutive breaths, from which RR was calculated. Each breath was only included in a single set of breaths to prevent any individual breath being counted more than once. For the purpose of this study, series of five breaths were counted to reduce the likelihood of calves moving during recordings (as occurred frequently when 10 consecutive breaths were recorded). Using Adobe Premiere Pro CC (version 12.0 Haberdasher; Adobe Systems, CA, USA) enabled the timestamps of the recordings to be displayed. These timestamps were needed for both infrared and video recordings to ensure the same period of time was being observed across the two methods.

Statistical analysis

Once the RR for each set of breaths (from both the video and infrared recordings) had been determined, analysis was carried out using Genstat (version 19; VSN International Ltd., Hemel Hempstead, UK). A regression analysis was performed in order to assess the level of agreement between the two types of recording. Bias was assessed using a Bland Altman analysis and Lin's concordance analysis was carried out to assess the equality between the two methods. Further regression analyses were carried out to test for any slope or intercept differences across animals.

Results

Measured from video recordings, RRs were found to be highly correlated with those measured from infrared recordings ($R^2=0.93$, $p < 0.001$, Figure 2). A Bland Altman analysis of the differences between infrared and video RR recordings plotted against the average infrared and video RR recordings showed no evidence of any change in bias across the range of values and the average bias was not significant (0.02 ± 0.255 (mean difference \pm standard error of the mean (SEM))) (Figure 3). In addition, Lin's concordance analysis showed a strong level of equality between the two methods ($Q_c = 0.9621$, $p < 0.001$). Testing for any animal differences in the relationship, further regressions indicated neither slope ($p = 0.281$) nor intercept ($p = 0.999$) showed any significant differences between animals, suggesting no animal dependence in the relationship between infrared and video RR recordings.

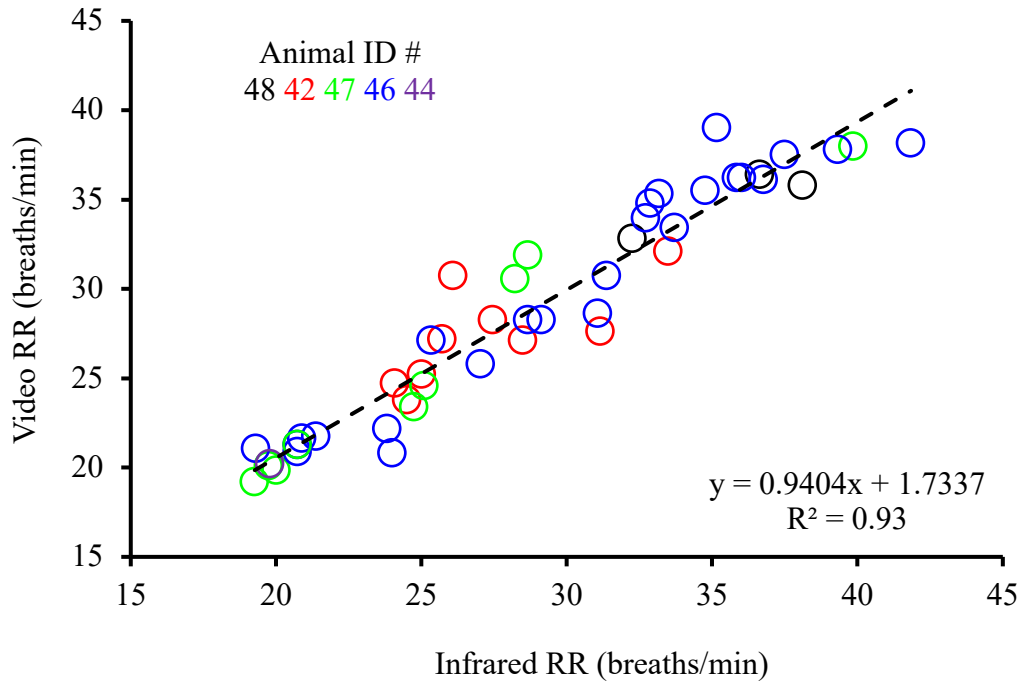


Figure 2. Correlation between respiration rates (RR) measured from infrared recordings and respiration rates measured from video recordings for 47 recordings.

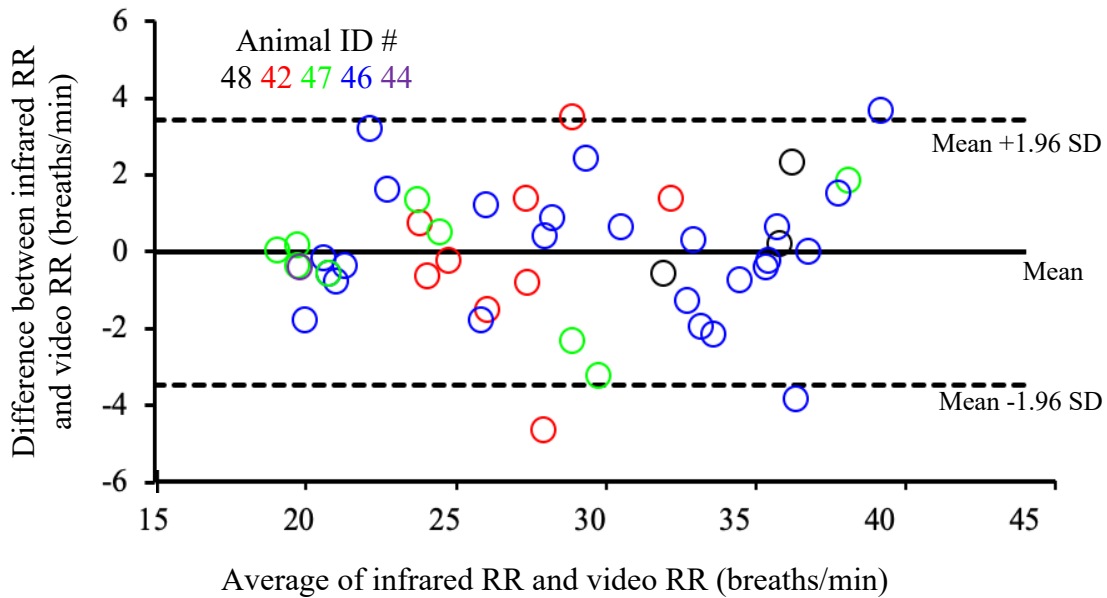


Figure 3. Bland Altman analysis of the average respiration rates (RR (breaths/min)) from both infrared and video recordings plotted against the differences between infrared and video recordings of RR (breaths/min).

Discussion

The results suggest that IRT is a suitable method for recording the RR of young calves based on thermal fluctuations occurring as air passes through the nostrils during inhalation and exhalation. Our results were consistent with those reported by Stewart et al. [9] for adult cattle. In contrast to other techniques which have been developed to estimate RR, IRT offers a non-invasive means of recording RR. Having been successfully validated, IRT could be used to record RR in young calves to provide valuable information relating to calf health and welfare during a vulnerable stage of life. Additionally, through future research, the development of algorithms which are necessary for the automation of IRT and integration into on-farm systems to enable RR to be recorded and analysed automatically, are within reach [9].

For calves, automated milk feeders and drinking systems provide potential platforms for integration. Such integration would enable long-term monitoring of RR and, if collected alongside other physiological and behavioral measures (e.g., feeding, drinking and lying behavior, and thermal changes), could be combined to provide highly accurate composite indicators of calf health and welfare. For example, with respect to disease onset, Lowe et al. [15] found milk consumption, duration of drinking visits, number and duration of lying bouts, and thermal fluctuations (of the side and shoulder) to show changes prior to the onset of neonatal calf diarrhea (NCD), suggesting that the measures were suitable as early indicators for detecting NCD. These indicators could be combined with RR, measured using IRT, to improve the ability to monitor calf health and welfare.

Furthermore, in relation to disease, previous studies [16,17] investigating the use of IRT for early detection of bovine respiratory disease (BRD) found IRT capable of indicating the onset of disease based on changes in eye temperature. Schaefer et al. [16,17] found that eye temperature increased significantly in response to the onset of BRD, and these changes were found to occur several days to a week before clinical signs of disease were apparent. As with other behavioral and physiological indicators, it is possible that thermal fluctuations of the eye could be recorded alongside RR in an automated system. Recording RR alongside other behavioral and physiological measures has potential for the development of highly accurate composite measures of health and welfare.

Typically, flank movements are fairly distinct and easy to see on adult cattle, except when animals are in cold environments. Measuring RR in calves based on flank movements comes with added difficulty due to calves being more active, which makes the capture of long stationary periods difficult, compromising the accuracy of the results. Focusing instead on thermal fluctuations around the nostrils, IRT provides an alternative method for measuring RR that would help overcome the difficulty of relying on flank movements to record RR in calves. If IRT was integrated into automated calf feeder or water systems, for example, these systems typically require calves to stand individually within a narrow chute, allowing RR to be measured whilst activity is restricted. Additionally, a number of automated calf feeders are fitted with a shutter that has to open before a calf is provided access to the teat. If integrated into an automated calf feeder, RR could be recorded before the calf is given access to the teat so that the action of suckling and the presence of warm milk does not influence the accuracy of the results.

As were considered in the current study, there are aspects of using IRT to record RR that future studies and operators need to consider to ensure accurate results are obtained. For example, for calibration purposes, changes in environmental conditions over recording periods need to be entered into the IRT camera at the time of recording or during data analysis. As part of the integration of IRT into an automated system, data pertaining to environmental variables could be obtained (e.g., using a weather station or temperature and humidity loggers) and updated automatically at specified intervals directly into the system. Infrared cameras are also sensitive to sunlight, so this needs to be considered in the environments in which IRT is to be used.

Furthermore, similar to thermistors, it is possible that in hot conditions the environmental temperatures may be similar to those of the nostrils during inhalation and exhalation, making it potentially difficult to observe the thermal fluctuations in order to determine RR. This requires future investigation. However, again, if integrated into an automated system such as an automated milk feeder, these systems are generally housed indoors where exposure to sunlight and heat is minimised. The automation of IRT would also enable stationary IRT cameras to be used, as opposed to the need for observers to operate hand-held cameras, which can cause disturbances to the animal. The distance of the camera to the animal also needs to be considered and kept constant to ensure reliable results. This can be managed during the collection of both manual and automated IRT recordings by having a set distance that operators stand or IRT cameras have to be installed away from the animal. Although changes in environmental variables, distance from the animal and exposure to sunlight are all factors that could influence the results being generated whilst using IRT as a method for recording RR, with consideration,

operators can act to ensure the effect of these factors can be overcome or at least minimised.

Conclusions

In conclusion, IRT was found to be a suitable method for recording RR in calves. The validation of IRT as a method for recording RR is a necessary step to be taken before this method can be automated. Having been validated, the automation of IRT is now reliant upon the future development of algorithms to enable IRT to be used as a method to automatically record and analyse RR. Furthermore, as mentioned there are a number of factors that can influence the results that are obtained when using IRT. Therefore, to further support the automation of this method it would be worthwhile for future research to investigate the influence of such factors (e.g., sunlight and environmental conditions) on the use of IRT as an automated method of recording RR. Automation would support the integration of IRT into existing automated systems where, alongside other measures, it could be implemented as a tool for monitoring calf health and welfare.

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Chapter Six

Effect of milk allowance on the suitability of automated behavioural and physiological measures as early disease indicators in calves



Authors note: Chapter six is presented in the style of the international journal with which it is currently under review as:

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Abstract

This study investigated the effect of milk allowance on the suitability of behavioural and physiological responses, individually and in combination, as early disease indicators. A total of 120 heifer calves were assigned into a 5 L/d or 10 L/d milk allowance treatment group. Daily health checks were conducted to determine when calves presented clinical signs of neonatal calf diarrhoea (NCD). Automated calf feeders recorded milk feeding behaviour, and infrared cameras automatically recorded eye and cheek temperatures. Accelerometers recorded lying behaviour, and water drinking behaviour was recorded through the use of an automated water system and video observations. Respiration rate was recorded once per day as part of the health check as the time taken to complete 10 breaths by counting flank movements. Calves were used as their own controls, with data analysed relative to the day of clinical identification (d 0). Based on changes which occurred over the 6 days prior to clinical signs of disease (across d -6 to 0), typically, feeding behaviours only changed significantly for calves on the 10 L/d milk allowance with an increase in total ($P<0.001$) and rewarded visits to the feeder ($P=0.013$), and a decrease in milk consumption ($P=0.011$). Infrared temperatures only changed significantly prior to clinical signs for calves on the 5 L/d milk allowance, with a decrease in eye ($P=0.013$) and cheek ($P=0.006$) temperatures. Regardless of milk allowance, lying time ($P=0.028$, 5 L/d; $P=0.011$, 10 L/d), number of lying bouts ($P<0.001$, 5 L/d; $P=0.007$, 10 L/d), and average bout duration ($P<0.001$, 5 L/d; $P=0.002$, 10 L/d), all changed significantly prior to clinical signs. The only significant change in water drinking behaviour prior to clinical signs was an increase in total trough visit duration for calves on the 5 L/d milk allowance ($P=0.029$). Respiration rate showed no significant change prior to clinical signs

regardless of milk allowance. For calves on the 10 L/d and 5 L/d milk allowances, the most suitable indicator of disease was the total number of visits to the feeder ($P<0.001$) and the number of lying bouts ($P<0.001$), respectively. Regardless of milk allowance, combinations of feeding and lying behaviours, and additionally infrared temperatures in the case of calves on the 5 L/d milk allowance, provided the strongest composite indicators of disease. Overall, results suggest milk allowance can influence the suitability of behavioural and physiological responses as early disease indicators. Milk allowance should be considered when determining which measures will act as the optimum indicator/s when developing and implementing algorithms into automated on-farm systems for disease detection.

Keywords: calves; early disease detection; automated; infrared thermography; feeding behaviour; milk allowance

Introduction

The incidence of disease is a significant concern for livestock industries worldwide. These concerns stem from the detrimental impacts of disease on animal health and welfare (Tomley and Shirley, 2009), the economic impacts associated with disease, and in the case of zoonoses, the risks such diseases pose to human health (Morris, 1988; Bennett, 2003). Neonatal calf diarrhoea (NCD) is a disease of significant concern to beef and dairy industries worldwide (Smith, 2012). Recognised as one of the leading causes of mortality and morbidity in young calves (Todd et al., 2010), NCD is an enteric disease which typically affects calves during their first month of life, and is caused by infectious bacterial, viral, and protozoal pathogens (Cho and Yoon, 2014). Common pathogens associated with NCD include *Escherichia coli*, *Salmonella*, rotavirus, coronavirus, and *Cryptosporidium* (Gunn et al., 2009). These

pathogens inflict substantial damage on the intestines and depending on the specific causative pathogen/s can result in intestinal inflammation, villous atrophy and severe secretory or malabsorptive/maldigestive diarrhoea (Gunn et al., 2009; Cho and Yoon, 2014). Whilst efforts have been made to reduce its incidence, NCD is still recognised as one of the leading challenges for beef and dairy industries worldwide (Meganck et al., 2015).

In response to pathogenic infection (Viljoen and Panzer, 2003), activated immune cells (i.e., monocytes, macrophages and lymphocytes) trigger the production of pro-inflammatory cytokines (Vollmer-Conna, 2001). Cytokines, including tumour necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6) act to increase activation of the immune system, triggering behavioural and physiological responses in the form of sickness behaviours, fever and pituitary-adrenal axis activation (Dantzer, 2004). Sickness behaviours are thought to be part of a behavioural strategy which has evolved to support the fever response in order to combat infection and thus increase an animal's chances of survival (Hart, 1988). These sickness behaviours include responses such as lethargy, anorexia, adipsia, hyperalgesia, depression and decreased social interaction, grooming and mental ability (Hart, 1988; Gregory, 1997; Viljoen and Panzer, 2003; Millman, 2007; Adelman and Martin, 2009) and are often used to identify animals experiencing disease (Hart, 1988). Relying on sickness behaviours to identify diseased animals can become problematic on-farm as being stoic, many farm animals have a tendency to mask signs which may indicate sickness or vulnerability (Weary et al., 2009). The reliance on stockpersons identifying diseased animals based on sickness behaviours is further impacted by the current shift towards automation occurring within the livestock industry. This shift is largely driven by a desire to reduce labour

costs (Hamadani and Kahn, 2015) and whilst offering a number of benefits, automation can result in a less “hands on” approach to farming which can be detrimental to animal health and welfare. When coupled with fewer experienced stockpersons who are capable of accurately identifying sickness behaviours, and increasing herd sizes, this shift towards automation can result in less time available to spend monitoring individual animals and can contribute to cases of disease going undiagnosed (Lance et al., 1992). Furthermore, by the time clinical symptoms of disease are evident, significant damage may have already been inflicted on the internal organs. A major challenge with NCD for example, is that by the time clinical symptoms are evident much of the internal damage to the intestines has already occurred. It would therefore be useful if methods could be developed to enable disease to be detected before clinical symptoms are evident, to help minimise the impact of disease by promoting earlier treatment and isolation of diseased animals. One approach is to integrate algorithms capable of monitoring health and welfare responses into on-farm automated systems.

Using automated calf feeders, previous studies (Svensson and Jensen, 2007; Borderas et al., 2009; Knauer et al., 2017; Swartz et al., 2017; Sutherland et al., 2018) have investigated changes in feeding behaviours as early indicators of disease, however, the results across studies are often conflicting. As highlighted by Knauer et al. (2017), one potential factor influencing the impact of disease on feeding behaviour is the specific disease being monitored (e.g., respiratory vs enteric diseases). However, it has also been suggested that the effect of disease on feeding behaviour may be influenced by milk allowance, and should be considered when using changes in feeding behaviour to identify diseased animals (Borderas et al., 2009). Automated water systems, accelerometers and infrared thermography are

other potential systems which could be integrated for the purpose of disease detection, for example by monitoring changes in drinking and lying behaviours, and body surface temperatures as have been investigated in previous studies (Schaefer et al., 2004; Hanzlicek et al., 2010; Swartz et al., 2017; Sutherland et al., 2018; Lowe et al., 2019a). Furthermore, as opposed to using a single measure, the potential for combining automated behavioural and physiological information as a more sensitive measure for early disease detection needs to be investigated.

The purpose of this study was to investigate the effect of milk allowance on the behavioural and physiological responses which occur in response to the onset of NCD, and further, to assess how milk allowance influences the suitability of these responses both individually and in combination as indicators for early disease detection.

Materials and methods

All procedures involving animals in this study were approved by the University of Waikato Animal Ethics Committee (protocol no. 985) under the New Zealand Animal Welfare Act 1999.

Animals, housing and experimental design

The study was carried out on a farm in the Waikato region of New Zealand (38°04'15.6"S 175°19'42.5"E) between July and October 2016. A total of 120 mix breed heifer calves (66 dairy calves (Friesian, Jersey and cross breeds) and 54 Hereford calves) (36.4 ± 4.33 kg, mean \pm SD) were monitored during the study from 2 days of age. Upon enrolment to the study, to enable individual identification, calves were assigned coloured collars (Calf neck bands; Shoof International Ltd.,

Cambridge, New Zealand), and fitted with numbered (Allflex; TX, USA) and electronic identification (EID) ear tags (Allflex; TX, USA) placed in the left and right ears respectively.

Within an indoor barn, calves were housed in a single pen (7.5 x 14.0 m) constructed on a solid dirt floor with walls on all four sides. The pen contained two water troughs (PT10; Stallion Limited, Palmerston North, New Zealand (34 cm (width) x 40 cm (length) x 31 cm (depth)), a hay feeder (Meal bar; Milkbar, McInnes Manufacturing Ltd., Whangarei, New Zealand (30 cm (width) x 100 cm (length)) and two purpose built meal feeders (31 cm (width) x 77 cm (length) x 26 cm (depth)). The pen was also fitted with two automated calf feeders (RFID Calf Feeder; A&D Reid, Temuka, New Zealand) (as used by Lowe et al., 2019a)), which provided calves with whole milk. Calves were individually identified as they entered the automated feeders using an automatic tag reader (G03113 EID tag reader controller R; Gallagher, Hamilton, New Zealand) and antenna system (G03121 EID tag reader antenna panel 600; Gallagher, Hamilton, New Zealand). Calves were given *ad libitum* access to water, straw and meal (consisting of 18.0% crude protein, 20.0% starch and 30.0% neutral detergent fibre (Superior; OSP Stock Feeds, Auckland, New Zealand)). Within the pen, an area (100 m²) of woodchip was provided as bedding, and an area of stones (2.3 m²) provided as drainage around both calf feeders.

The trial consisted of two treatments into which calves were randomly assigned upon enrolment into the study. Calves assigned to treatment Group 1 (n=60), were provided a milk allowance of 5 L/d (as per standard practice in New Zealand) whilst calves in treatment Group 2 (n=60), were provided a milk allowance of 10 L/d. The

lower milk allowance of 5 L/d is similar to the conventional feeding practice of providing calves with a supply of milk equivalent to 10% of body weight (BW) at birth (Kahn et al., 2011) and is comparable to the low milk allowances of previous studies (Borderas et al., 2009; Haisan et al., 2019). The higher milk allowance of 10 L/d is similar to the increasing trend towards providing calves with a supply of milk equivalent to 20% of BW at birth (Haisan et al., 2019) and is again comparable to the high milk allowances used in previous studies (Borderas et al., 2009; Haisan et al., 2019). Calves were enrolled into the trial at 2 days of age and were observed up until 24 ± 14.4 (mean \pm SD) days of age. Due to the calving spread, calves were gradually enrolled into the study as they became available. At the end of each calf's observation period, they were returned to the farmer and a new calf was enrolled into the study. At any one time, 33 ± 10.0 (mean \pm SD) calves were housed within the study pen. Health checks, infrared temperatures, respiration rate, milk feeding, lying, and water drinking behaviours were recorded daily as described below.

Clinical observations

To monitor calves for the incidence of NCD, health checks were conducted each morning to assess the calves' general health and identify when each calf began to display overt clinical signs of illness. The study used a simplified version of the scoring system used by Lowe et al. (2019a) to assess calves for signs of NCD. Calves' were assessed based on their general appearance, coat condition, eye appearance, gut fill, rear end cleanliness and faecal consistency, based on the definitions outlined in the scoring system presented in Fig.1. As a component of the daily health checks, respiration rate (RR) was measured by observing flank movements in order to record the time taken for each calf to complete 10 breaths;

this was then used to calculate RR (breaths/min). Calves were defined as being clinically ill when they were observed as being diarrhoeic. Based on Lowe et al. (2019a), to be deemed diarrhoeic, a calf had to be witnessed passing malodorous faeces with a loose to watery consistency, with the possibility of blood present in severe cases (a score of 2 or 3 for faecal consistency; Fig.1). If a calf was suspected of being diarrhoeic due to the presence of loose faecal matter on the top of the tail/hind legs, but had not been observed passing faeces, a faecal sample was taken to confirm whether the calf was diarrhoeic. Faecal samples were collected from all diarrhoeic calves for analysis to identify the causative pathogen/s. If deemed clinical, dehydration levels were assessed by performing a ‘tent test’ to measure skin elasticity whereby the skin of the neck was tented and the time for the skin to return to its normal position recorded and assigned a score of 0-3 based on the definitions presented in Fig. 1. Dehydration levels were further assessed by monitoring whether the extremities of the animal (e.g., ears, legs and feet) were warm or cold to touch and assigned a score of 0 or 1 respectively. Rectal temperatures were taken using a digital thermometer (MC-343; Omron, Kyoto, Japan) and classified as being: low ($\leq 37.9^{\circ}\text{C}$), normal ($38-39.5^{\circ}\text{C}$) or high ($\geq 39.6^{\circ}\text{C}$). Once identified as being clinical, calves were treated with electrolytes (NutriCare® Calf Electrolyte; Nutritech, Auckland, New Zealand) and anti-inflammatories (Metacam; Boehringer Ingelheim Limited, Auckland, New Zealand) and antibiotics (Bivatop; Boehringer Ingelheim Limited, Auckland, New Zealand) as required.









	0	1	2	3	
Appearance	Normal, alert and active, head and ears held up 	Slightly unresponsive, droopy ears 	Depressed, lethargic, head held down, droopy ears, unsteady balance. 	Severe depression, no interest in getting up. 	
Coat	Bright/Sleek	Rough/Dull	N/A	N/A	
Sunken Eyes	Normal, bright, no recession into the orbit 	Dull, sunken, recessed into the orbit 	N/A	N/A	
Gut Fill	Full	Hollow	N/A	N/A	
Rear End	Clean 	Loose faecal matter present on rear end 	N/A	N/A	
Faecal Consistency	Firm to soft consistency	Soft to loose consistency	Scours, loose to watery consistency with a strong odour	Severe scours, strong odour, consistency of water, blood may be present	
Respiration Rate	Record respiration rate: Time taken for calf to complete 10 breaths.				
If Sick	Tent Test	0 seconds (<5 dehydration)	1-3 seconds (6-8 dehydration)	4-5 seconds (9-12 dehydration)	≥6 seconds (>12 dehydration)
	Extremities	Warm	Cold	N/A	N/A
	Rectal Temperature	Record rectal temperature: low (≥37.9°C), normal (38-39.5 °C) and high (≤39.6 °C).			

Figure 1. Daily health check definitions used to assess calves for the onset of NCD, based on a scoring system of 0-3 for appearance and faecal consistency. A scoring system of 0-1 was used to assess coat condition, sunken eyes, gut fill, and rear end cleanliness. Respiration rate was recorded by measuring the time taken for a calf to complete 10 breaths. If an animal appeared sick a tent test was carried out and the level of dehydration recorded using a scoring system of 0-3 and extremities were recorded as being warm or cold using a 0-1 scoring system. Rectal temperatures were recorded and categorised as being low, normal or high. Measures for which scores were not applicable are denoted as N/A. This scoring system was modified from Lowe et al., 2019a and simplified for the purposes of the current study.

Faecal sample analysis

To identify the causative pathogen, faecal samples were sent for analysis (New Zealand Veterinary Pathology (NZVP), Hamilton, New Zealand) to test for the presence of rotavirus, coronavirus, *Cryptosporidium*, and/or *Salmonella*. An acid fast stain analysis was used to determine the presence of *Cryptosporidium*. The presence of *Salmonella* was determined using broth enrichment and selective plating. Samples which tested positive for *Salmonella* were additionally sent to the Institute of Environmental Science and Research (ESR, Auckland, New Zealand) for serotyping and phage typing in order to identify the individual strain of *Salmonella* present. The presence of rotavirus and coronavirus was determined using an ELISA kit (Pourquier® ELISA Calves Diarrhoea; Institut Pourquier, Montpellier, France).

Milk feeding behaviour

Upon enrolment to the study pen, calves were trained to use the automated feeders. This involved observers training calves to press on a lever with their nose as they suckled in order for whole milk to be dispensed. Regardless of treatment group, calves were started on an allowance of whole milk of 5 L/d. For calves assigned to treatment Group 1, this 5 L/d milk allowance was maintained for the duration of the study. Calves assigned to treatment Group 2, were gradually adjusted to a higher milk allowance by increasing this amount (by 1 L/d) until they reached an allowance of 10 L/d; this allowance was then maintained for the remainder of the study period. Regardless of treatment group, each daily milk allowance was split into 3 equal allocations. Between the full consumption of each allocation a stand-down period of 6 h (when no milk was provided) had to pass before a calf could

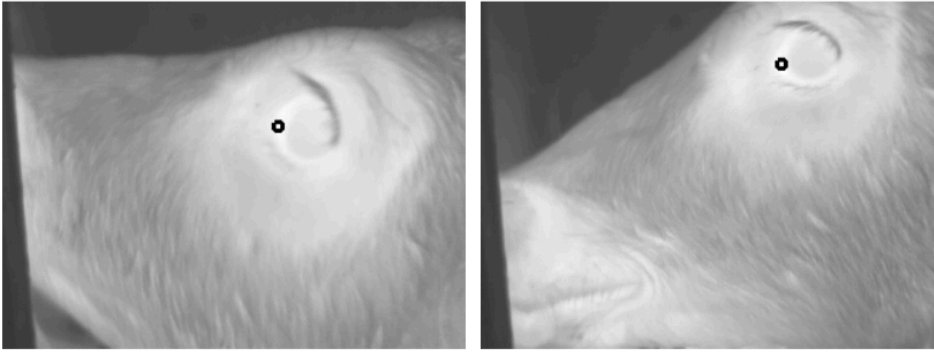
receive their next allocation of milk. The automated feeders recorded milk consumption, total number of visits, and the number of both rewarded and unrewarded visits calves made to the feeder. Any visit in which milk was consumed (regardless of the amount) was regarded as a rewarded visit. In contrast, any visit in which the calf visited the feeder but did not receive an allocation of milk was considered an unrewarded visit. The automated feeders did not have the capability to record visit duration or drinking speed. Milk was heated to approximately 38-40°C before being dispensed to the calf. Milk temperature was controlled using an in-built thermostat which was monitored using Thermochron iButton temperature data loggers (DS1922L-F5#; Maxim Integrated, San Jose, CA, USA) and digital thermometers (ST-3 Electronic thermometer; Aqua One, Southampton, UK).

Infrared thermography

Infrared images were collected automatically from an infrared camera (Thermovision A300 (accuracy: $\pm 2.0^{\circ}\text{C}$; sensitivity: $<0.05^{\circ}\text{C}$; resolution: 320 x 240; temperature range: -20°C to 120°C ; spectral range: 7.5-13 μm); FLIR Systems AB, Danderyd, Sweden) integrated into each automated feeder. The left side panel of each feeder was modified by cutting out a viewing window so that an infrared camera could be installed in a position that enabled images of the facial region to be collected as calves fed. The cameras were programmed to begin continuously capturing images at a rate of 60 frames/s for the duration of each feeding bout. For consistency, images were collected at a set distance of 0.5 m and angle of 90° to the animal. Each infrared camera was set to an emissivity of $\varepsilon=0.98$, as accepted for use in animals (Redaelli et al., 2014), and as used previously in cattle (Stewart et al., 2017; Lowe et al., 2019a, 2019b). Temperature and relative humidity were

measured continuously using data loggers (EL-USB-2-LCD+; Lascar Electronics Ltd., Salisbury, UK) located next to each calf feeder, data from which were used during analysis to adjust for environmental changes. Infrared images were analysed to determine the maximum temperature using an algorithm developed in a previous study by Lowe et al. (2019c) to automatically detect and analyse the eye and cheek regions as shown in Fig. 2. Across the entire study period, the algorithm obtained eye temperatures from 408,950 images, with readings obtained from 152 ± 79.42 (mean \pm SD) images per animal/d, and 26 ± 21.38 (mean \pm SD) readings obtained per feeding bout. The algorithm obtained cheek temperatures from a total of 945,187 images, with readings obtained from 310 ± 139.98 (mean \pm SD) images per animal/d; 29 ± 24.66 (mean \pm SD) readings were obtained per feeding bout. Readings were summarised for each animal to provide a daily maximum value for each anatomical area.

A)



B)

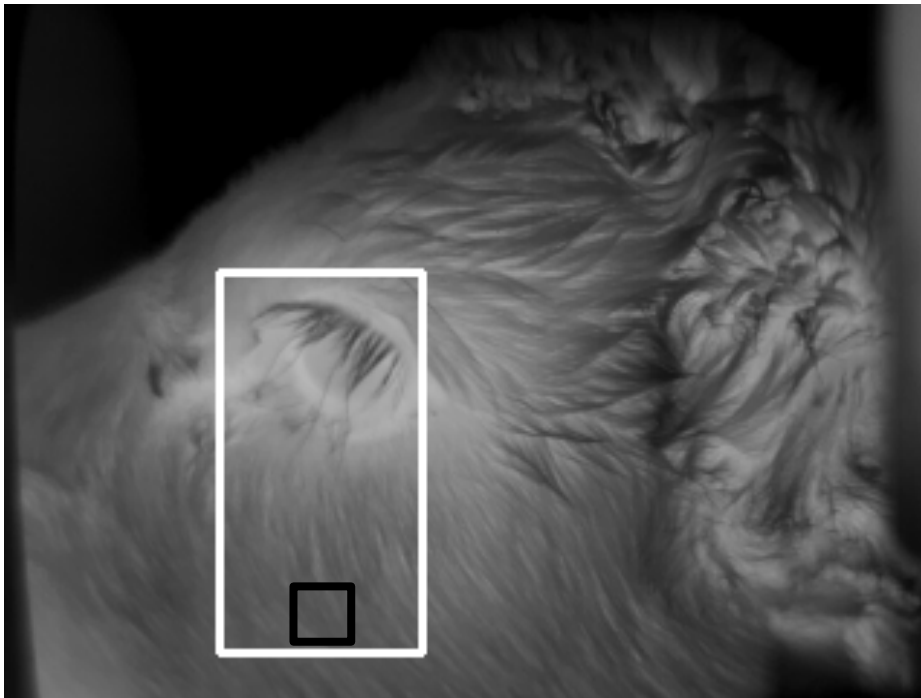


Figure 2. Example infrared images collected of the facial region with the marked position indicating the location of the maximum eye temperature (A) as determined by the eye detection algorithm. The location of the cheek region (indicated by the black square) (B) is determined by the cheek detection algorithm based on the positioning of the white rectangle which is traced using the eye as a reference point.

Lying behaviour

Tri-axial accelerometers (HOBO pendant G data loggers 64k; Onset Computer Corporation, MA, USA) set at 1-min intervals using the y- and z-axes (as recommended in previous validation studies (Ledgerwood et al., 2010; Bonk et al., 2013)), were used to continuously record lying behaviour. Following Lowe et al. (2019a), accelerometers were attached to the lateral side of the right hind leg above the metatarsophalangeal joint in purpose made fabric pouches using Velcro and KAMAR glue (KAMAR®; Livestock Improvement Corporation, Hamilton, New Zealand). The accelerometers were orientated horizontally on the leg such that the x and z axes ran parallel to the ground with the x-axis pointing in the cranial direction and the z-axis toward the mid-plane of the calf. The y-axis ran perpendicular to the ground, pointing in the dorsal direction. Accelerometers were initialised and downloaded using Onset HOBOWare Pro software (version 3.7.2; Onset Computer Corporation, MA, USA); the output was converted into daily summaries of lying behaviour using SAS software (version 9.3; SAS Institute Inc., NC, USA).

Water drinking behaviour

The duration and number of visits to the water trough were determined using two automated water systems, validated by Lowe et al. (2017). Briefly, the automated systems each consisted of a water trough positioned inside a narrow chute (0.4 x 1.2 m). As with the automated calf feeders, calves were individually identified as they entered the water system using an automatic EID tag reader (G03113 EID tag reader controller R; Gallagher, Hamilton, New Zealand) and antenna system (G03121 EID tag reader antenna panel 600; Gallagher, Hamilton, New Zealand).

The system determined the number and duration of visits to the trough based on the beam from an overhead photoelectric sensor (WTB27-3S1511; SICK, Germany) being broken and then reconnected as the calf entered and backed out of the chute. Unfortunately, whilst the troughs were fitted with flow meters (SPX-075; Seametrics, USA), due to a distributor error, water intake could not be recorded. Although water intake could not be recorded, calves drinking behaviour at the water trough was recorded continuously at a rate of 30 frames/s using overhead security cameras (DS-2CD2332-I; Hikvision, Hangzhou, China) to determine the number of rewarded (drinking) and unrewarded (non-drinking) visits to the water trough. A rewarded visit to the water trough was defined as “the calf’s head is situated over the water trough and with the muzzle lowered towards the water; the water is seen to move”. An unrewarded visit was defined as “the calf’s head is not situated over the water trough, or in the case that the head is situated over the trough, the muzzle is not lowered towards the water and the water is not seen to move”. Cameras were secured to the ceiling of the calf barn at a height of 2 m from ground level and built-in infrared lights enabled behavioural observations to be conducted at night without influencing the calves’ behaviour. Video footage was analysed continuously by a single observer for each animal throughout the trial using Adobe Premiere Pro CC (version 12.0.1 Haberdasher; Adobe systems, CA, USA). During video analysis, intra-observer reliability was completed at three stages of the trial (start, middle and end). During each session of intra-observer reliability, a 24 hour period of footage was reanalysed. Initial observations were compared against those made during the reliability sessions to calculate the level of reliability based on the percent agreement using Excel (version 16.26; Microsoft Corporation, WA, USA). Intra-observer reliability ranged from 88.9-100% with a combined overall average of

95.7%.

Statistical analysis

During the study, 112 calves developed NCD: the 8 calves that did not develop NCD were excluded from all analyses. Each calf which developed NCD was analysed as its own control, with data analysed relative to the date it was deemed clinical. Data for feeding behaviour (total and percentage of rewarded visits to the feeder and milk consumption), infrared temperature (eye and cheek regions), lying behavior (lying time, number of lying bouts and lying bout duration), water drinking behaviour (total trough visits, percentage of rewarded trough visits, average trough visit duration, and total trough visit duration) and RR were modeled with REML using Genstat (version 19; VSN International Ltd., Hemel Hempstead, UK). For each variable measured, a linear mixed model with random smoothing splines for each animal was fitted to the data in order to observe the general trends which occurred over d -6 to 6 (relative to clinical identification of disease (d 0)). Smoothing splines helped to (1) account for some of the animals not having data on all days, (2) account for some of the noise in the data, and (3) model the correlation between repeated observations on the animals. Then using the trend across d -6 to 0 we looked to see how well each variable and combinations of variables were at indicating the onset of disease. To achieve this, we used a robust slope estimator, the Theil-Sen method (Theil, 1950) which uses, for each animal, the median of the slopes between all possible pairs of data points. The significance of the change over this period (d-6 to 0) was then tested using the t-test. Based on the t-ratio the variables and combinations of variables were then ranked based on their suitability for indicating disease onset whereby the higher the t-ratio the more

suitable the given variable/s for indicating disease onset.

Results

Pathogen prevalence

The 112 calves that developed clinical signs of disease were subsequently faecal sampled to identify the causative pathogen/s, the prevalence of which are presented in Table 1. Regardless of milk allowance, *Cryptosporidium* and rotavirus were identified as the most prevalent pathogens, identified in 42.8% and 14.3% of cases of NCD respectively. Co-infection with two or more pathogens, occurred in 7.2% of cases with the most common co-infection being *Cryptosporidium* + rotavirus (5.4%), followed equally by *Cryptosporidium* + coronavirus (0.9%) and *Cryptosporidium* + coronavirus + rotavirus (0.9%). Approximately 33.0% of samples came back as negative for the four pathogens tested, but based on the clinical symptoms presented, these calves were still diagnosed with NCD. In 83.0% of cases, calves were ≤ 10 d of age at the time of clinical identification, compared to 17.0% of calves which were identified as being clinical at >10 d of age. Of the 112 calves which were diagnosed as having NCD, 48.2% were calves on the 5 L/d milk allowance and 51.8% were calves on the 10 L/d milk allowance.

Table 1. Pathogen prevalence (*Cryptosporidium*, rotavirus, coronavirus and *Salmonella*) in faecal samples collected from calves identified as being clinically ill. Pathogen prevalence is presented separately based on age at diagnosis ≤ 10 or > 10 days of age for both 5 L/d and 10 L/d milk allowances and collectively as a combined total regardless of milk allowance.

Test result	Pathogen prevalence (%)						
	5 L/d milk allowance (n=54)			10 L/d milk allowance (n=58)			Combined (n=112)
	≤ 10 days of age ¹	> 10 days of age ¹	Total ¹	≤ 10 days of age ¹	> 10 days of age ¹	Total ¹	Total ¹
<i>Cryptosporidium</i>	42.6 (23)	3.7 (2)	46.3 (25)	36.2 (21)	3.4 (2)	39.7 (23)	42.8 (48)
Rotavirus	13.0 (7)	1.9 (1)	14.8 (8)	8.6 (5)	5.2 (3)	13.8 (8)	14.3 (16)
Coronavirus	0 (0)	0 (0)	0 (0)	1.7 (1)	0 (0)	1.7 (1)	0.9 (1)
<i>Salmonella</i>	1.9 (1)	0 (0)	1.8 (1)	0 (0)	0 (0)	0 (0)	0.9 (1)
<i>Cryptosporidium</i> + Rotavirus	1.9 (1)	1.9 (1)	3.8 (2)	4 (6.9)	0 (0)	6.9 (4)	5.4 (6)
<i>Cryptosporidium</i> + Coronavirus	0 (0)	0 (0)	0 (0)	1.7 (1)	0 (0)	1.7 (1)	0.9 (1)
<i>Cryptosporidium</i> + Coronavirus + Rotavirus	0 (0)	0 (0)	0 (0)	1.7 (1)	0 (0)	1.7 (1)	0.9 (1)
Negative for tested pathogens	24.1 (13)	9.3 (5)	33.3 (18)	25.9 (15)	8.6 (5)	34.5 (20)	33.9 (38)

¹ Number of calves presented in parentheses.

Milk feeding behaviour

Changes in feeding behaviour (total visits, rewarded visits, and milk consumption) across d -6 to 6 are presented in Fig. 3. Prior to clinical identification (d -6 to 0), only calves in the 5 L/d milk allowance group showed a significant increase in the total number of visits to the feeder (Table 2). In contrast, calves on the 10 L/d milk allowance were found to significantly increase both the total number of visits and percentage of rewarded visits to the feeder and decrease milk consumption (Table 2). Therefore, with the exception of total visits, changes in feeding behaviour prior to clinical identification (d -6 to 0) were typically only significant for calves on the 10 L/d milk allowance.

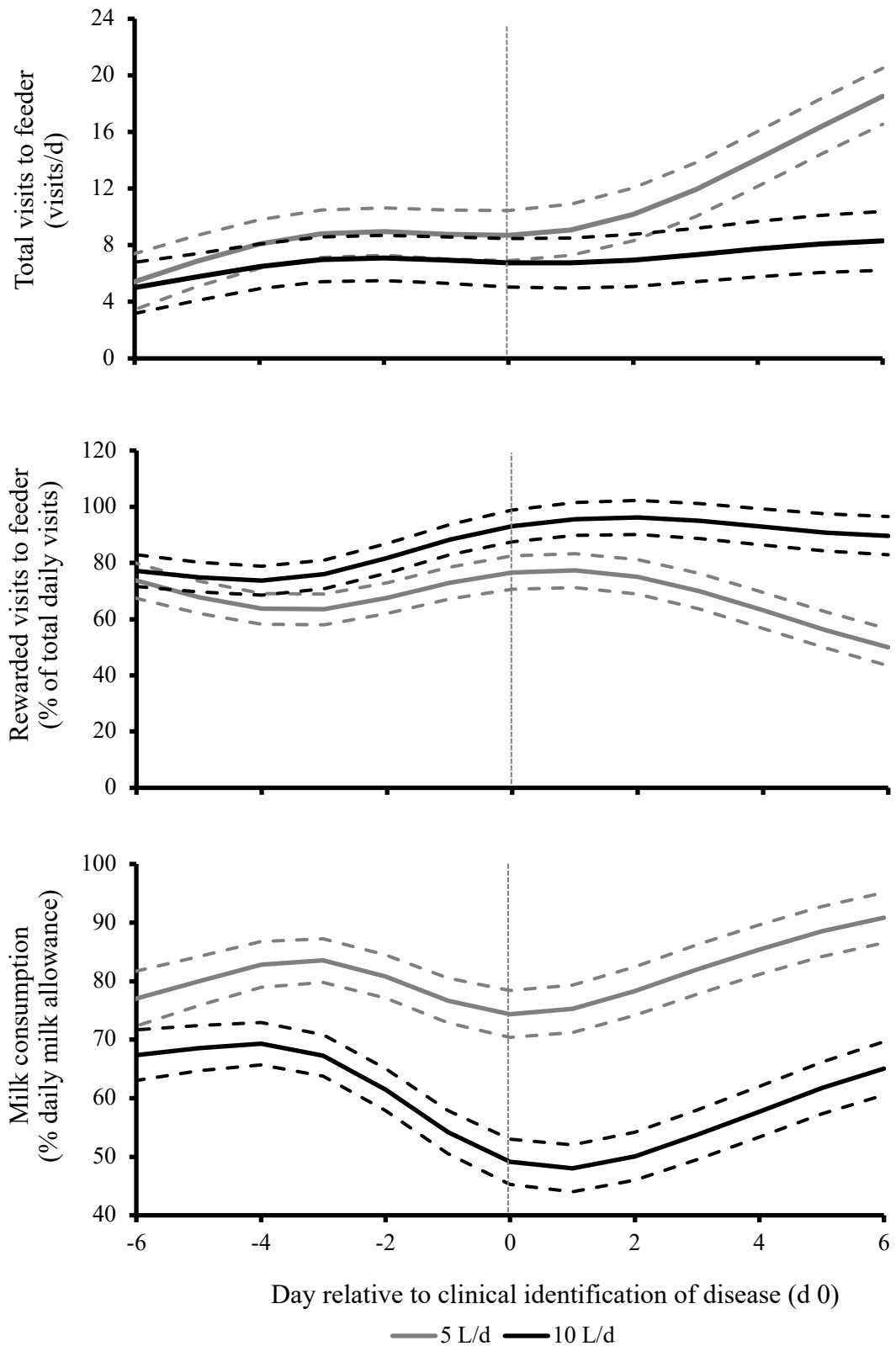


Figure 3. Mean (\pm SEM, dashed lines) total and rewarded visits to the feeder and milk consumption per day during d -6 to 6 relative to the clinical identification of disease (d 0) for calves on both 5 L/d and 10 L/d milk allowances based on results of the REML model.

Infrared thermography

Changes in infrared temperatures of the eye and cheek across d -6 to 6 are presented in Fig. 4. Prior to clinical identification (d -6 to 0), calves on the 5 L/d milk allowance showed significant decreases for both eye and cheek temperature prior to clinical identification (Table 2). However, calves on the 10 L/d milk allowance showed no significant change in infrared temperature for either the eye or cheek regions (Table 2).

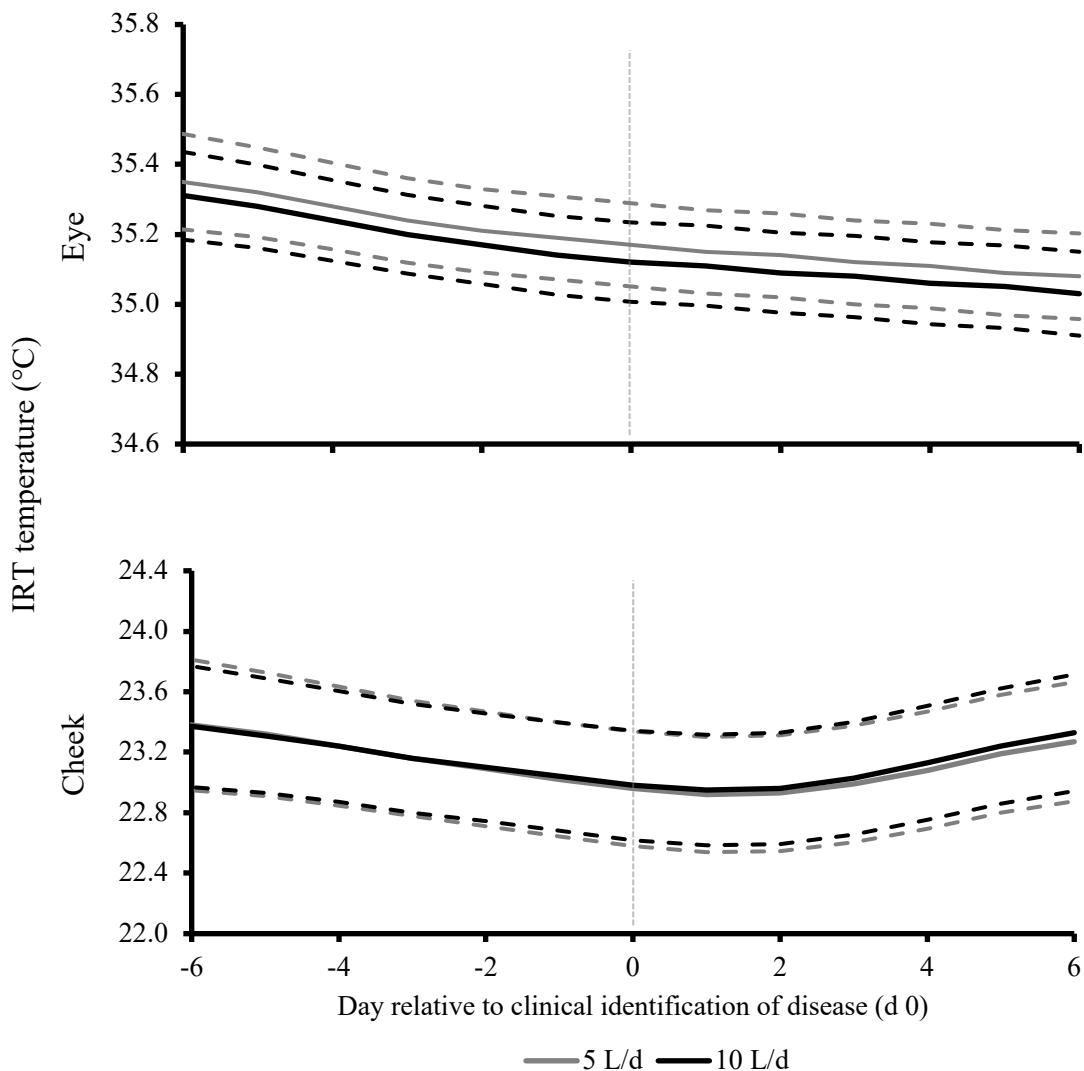


Figure 4. Mean (\pm SEM, dashed lines) maximum infrared thermography (IRT) temperature for the eye and cheek regions measured during d -6 to 6 relative to the clinical identification of disease (d 0) for calves on both 5 L/d and 10 L/d milk allowances based on results of the REML model.

Lying behaviour

Changes in lying behaviour (lying time, number of lying bouts, and average lying bout duration) across d -6 to 6 are presented in Fig. 5. Prior to clinical identification (d -6 to 0), regardless of milk allowance, calves showed a significant decrease in the number of lying bouts and an increase in average lying bout duration (Table 2). Lying time significantly decreased for calves on the 5 L/d milk allowance, but significantly increased for calves on the 10 L/d milk allowance (Table 2).

Water drinking behaviour

Changes in water drinking behaviour (total trough visits, percentage of rewarded trough visits, average trough visit duration, and total trough visit duration) across d -6 to 6 are presented in Fig. 6. Prior to clinical identification (d -6 to 0), the only variable that was found to change significantly was total trough visit duration, with an increase for calves on the 5 L/d milk allowance (Table 2).

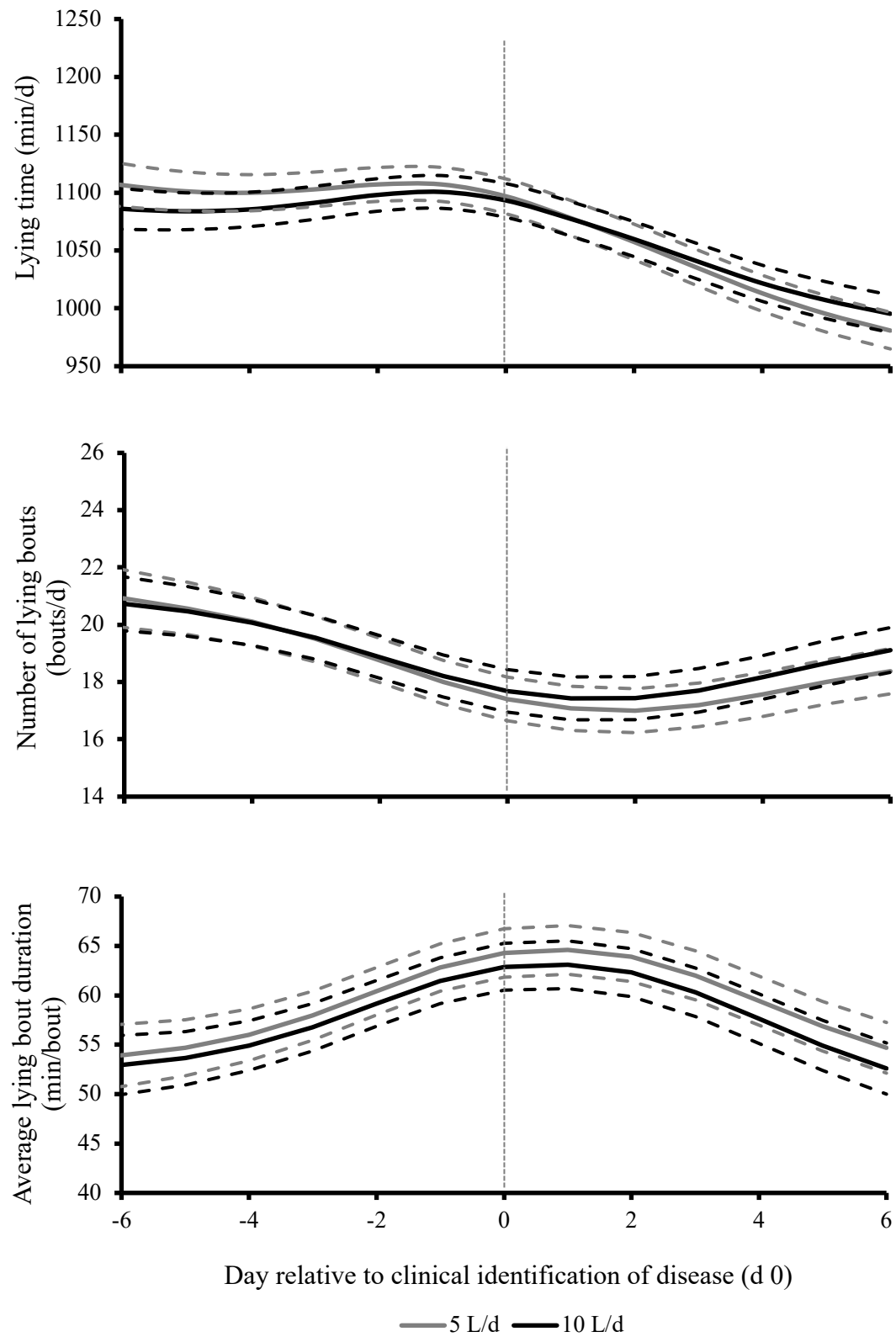


Figure 5. Mean (\pm SEM, dashed lines) lying time, number of lying bouts, and average lying bout duration during d -6 to 6 relative to the clinical identification of disease (d 0) for calves on both 5 L/d and 10 L/d milk allowances based on results of the REML model.

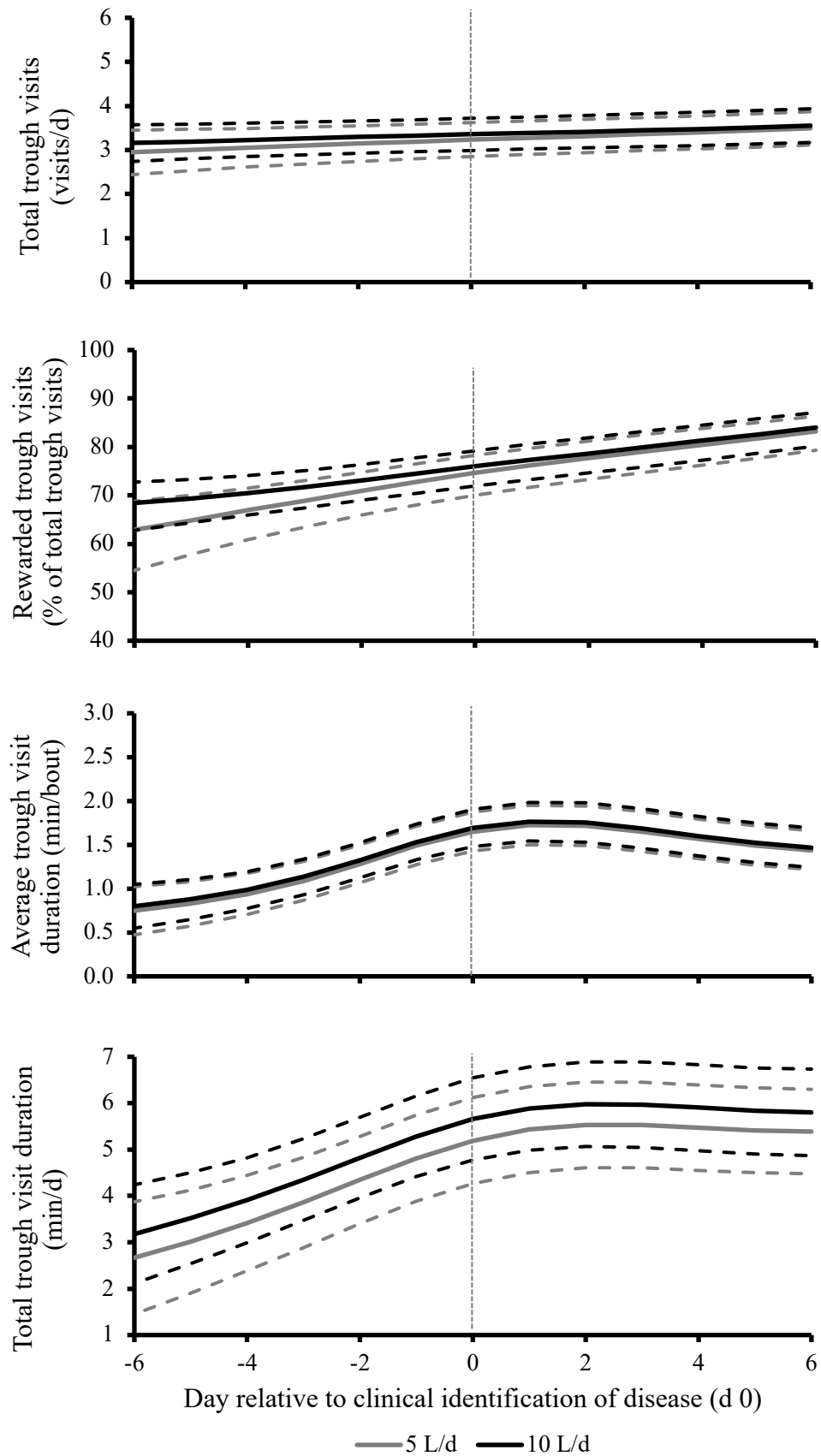


Figure 6. Mean (\pm SEM, dashed lines) total trough visits, rewarded trough visits, average trough visit duration, and total trough visit duration during d -6 to 6 relative to the clinical identification of disease (d 0) for calves on both 5 L/d and 10 L/d milk allowances based on results of the REML model.

Respiration rate

Changes in RR during d -6 to 6 are presented in Fig. 7. Prior to clinical identification (d -6 to 0) no significant change in RR was detected for calves on either milk allowance.

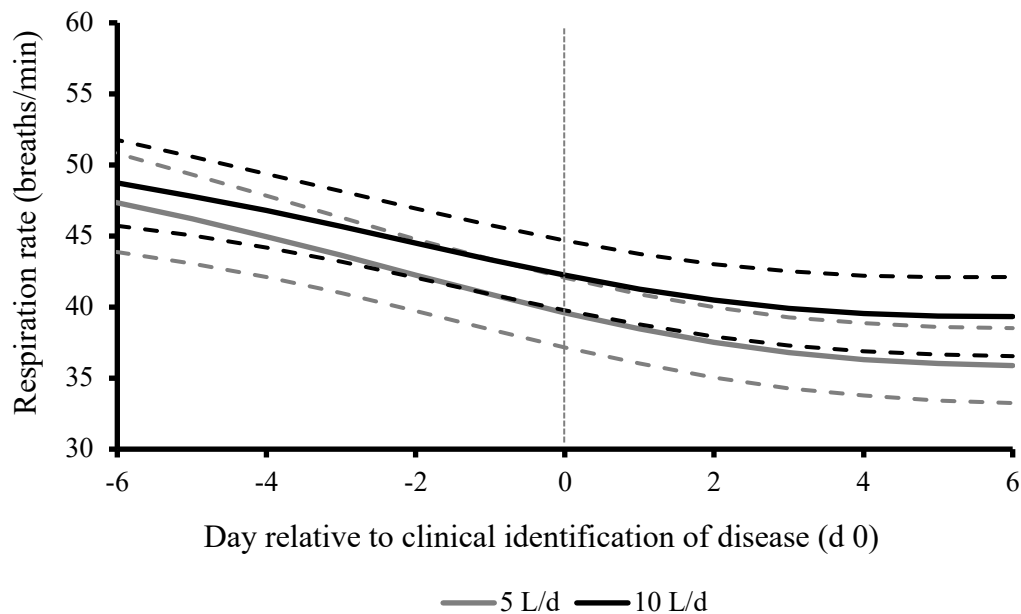


Figure 7. Mean (\pm SEM, dashed lines) respiration rate during d -6 to 6 relative to the clinical identification of disease (d 0) for calves on both 5 L/d and 10 L/d milk allowances based on results of the REML model.

Suitability of individual and combinations of responses as early disease indicators

Based on the changes which occurred prior to clinical identification (d -6 to 0), the suitability of each individual variable as an indicator for early disease detection was ranked from 1-13 (highest-lowest) (Table 2). For calves on the 10 L/d milk allowance, the variable which acted as the best indicator for early disease detection was total visits to the feeder, followed by lying behaviours (in order of average lying bout duration, number of lying bouts and lying time); the remaining milk feeding behaviours (milk consumption and rewarded visits) were the next most

suitable indicators for disease detection. Compared to lying and feeding behaviours, infrared temperatures, RR and drinking behaviours were less useful as indicators for early disease detection; overall RR was the lowest ranked indicator for disease detection for calves on a 10 L/d milk allowance (Table 2).

In contrast, the most suitable variable for early disease detection for calves on the 5 L/d milk allowance was found to be the number of lying bouts followed by average lying bout duration (Table 2). Total visits to the feeder was the next most useful indicator, but compared to calves on a 10 L/d milk allowance, the remaining feeding behaviours (milk consumption and rewarded visits) were less suitable as indicators for early disease detection. Instead, for calves on the 5 L/d milk allowance, infrared temperatures of the eye and cheek proved to be more suitable. The suitability of drinking behaviours as indicators of disease detection for calves on the 5 L/d milk allowance was similar to that of calves on the 10 L/d milk allowance (Table 2).

When assessing the best two combinations of 2-4 variables as indicators for early disease detection there were some distinct differences between the two milk allowances. Typically, the best combinations of 2-4 variables for calves on the 10 L/d milk allowance included variables relating to feeding behaviour (i.e., total visits to the feeder, percentage of rewarded visits to the feeder and milk consumption) followed by lying behaviours (i.e., number of lying bouts, lying time and average lying bout duration) (Table 3). Variables relating to infrared temperature, drinking behaviour and respiration rate were not included in any of the top combinations of variables for early disease detection for calves on the 10 L/d milk allowance. In contrast, for calves on the 5 L/d milk allowance, the best combinations typically

included the number of lying bouts followed by the total and rewarded visits to the feeder. As with calves on the 10 L/d milk allowance, variables relating to water drinking behaviour and respiration rate were not included in any of the top combinations of variables for early disease detection for calves on the 5 L/d milk allowance. However, in contrast to calves on the 10 L/d milk allowance, infrared temperatures of the cheek or eye were included in several of the top combinations of variables for early disease detection for calves on the 5 L/d milk allowance. Typically, based on the t-ratio, the suitability of the different combinations of variables as indicators for early disease detection were greater for calves on the 10 L/d milk allowance than those on the 5 L/d milk allowance.

Table 2. The ranking of each individual variable measured as an indicator of early disease detection for calves on both 5L/d and 10L/d milk allowances based on the trend over d -6 to 0 relative to the clinical identification of disease (d 0). The higher the t ratio the greater the ranking of each variable as an indicator for early disease detection.

Variable		5 L/d milk allowance					10 L/d milk allowance				
		n	Difference	t ratio	<i>P</i> -value	Indicator ranking	n	Difference	t ratio	<i>P</i> -value	Indicator ranking
Feeding behaviour	Total visits to feeder (visits/day)	53	3.27	2.99	0.002	3	57	1.77	4.04	<0.001	1
	Rewarded visits to feeder (% of total daily visits)	53	2.99	0.48	0.316	12	57	15.88	2.27	0.013	6
	Milk consumption (% of daily milk allowance)	53	-2.63	0.91	0.184	10	57	-18.21	2.35	0.011	5
Infrared temperature	Eye temperature (°C)	53	-0.18	2.30	0.013	5	57	-0.19	0.81	0.211	11
	Cheek temperature (°C)	53	-0.42	2.60	0.006	4	57	-0.39	1.19	0.120	8
Lying behaviour	Lying time (mins/day)	51	-9.79	1.95	0.028	6	52	7.20	2.36	0.011	4
	Number of lying bouts (bouts/day)	51	-3.50	5.13	<0.001	1	52	-3.04	2.55	0.007	3
	Average lying bout duration (min/bout)	51	10.38	3.91	<0.001	2	52	9.96	3.11	0.002	2
Drinking behaviour	Total trough visits (visits/d)	48	0.29	1.10	0.139	9	54	0.20	0.95	0.174	10
	Rewarded trough visits (% total trough visits)	48	11.77	0.04	0.484	13	54	7.55	0.61	0.273	12
	Average trough visit duration (min/bout)	48	0.90	1.27	0.105	8	54	0.89	1.13	0.132	9
	Total trough visit duration (min/d)	48	2.53	1.95	0.029	7	54	2.48	1.42	0.081	7
Respiration rate	Breaths/min	41	-7.74	0.50	0.310	11	46	-6.5	0.43	0.336	13

Table 3. The top two ranking single variables or combinations of 2-4 variables as indicators for early disease detection for calves on both 5L/d and 10L/d milk allowances based on the trend over d -6 to 0 relative to the clinical identification of disease (d 0). The higher the t ratio the greater the ranking of each variable or combination of variables as indicators for early disease detection.

Milk allowance	Number of variables	Indicator ranking	Variable/s	n	t ratio	P-value
5 L/day	1	1	Number of lying bouts	51	5.13	<0.001
		2	Average lying bout duration	51	3.91	<0.001
	2	1	Number of lying bouts, total visits to feeder	51	6.22	<0.001
		2	Number of lying bouts, cheek temperature	51	5.48	<0.001
	3	1	Number of lying bouts, total visits to feeder, rewarded visits to feeder	51	6.54	<0.001
		2	Number of lying bouts, total visits to feeder, milk consumption	51	6.53	<0.001
	4	1	Number of lying bouts, total visits to feeder, rewarded visits to feeder, cheek temperature	51	6.82	<0.001
		2	Number of lying bouts, total visits to feeder, rewarded visits to feeder, eye temperature	51	6.8	<0.001
10 L/day	1	1	Total visits to feeder	57	4.04	<0.001
		2	Average lying bout duration	51	3.11	0.002
	2	1	Total visits to feeder, milk consumption	57	6.06	<0.001
		2	Total visits to feeder, rewarded visits to feeder	57	5.85	<0.001
	3	1	Total visits to feeder, rewarded visits to feeder, average lying bout duration	57	8.37	<0.001
		2	Total visits to feeder, rewarded visits to feeder, number of lying bouts	53	8.01	<0.001
	4	1	Total visits to feeder, rewarded visits to feeder, number of lying bouts, milk consumption	53	9.22	<0.001
		2	Total visits to feeder, number of lying bouts, milk consumption, lying time	53	8.71	<0.001

Discussion

Using automated measures, we monitored the behavioural and physiological responses of calves on-farm in response to the onset of NCD. During the study, 112 calves were identified as having NCD, from which *Cryptosporidium* and rotavirus were the most prevalent pathogens associated with the incidence of NCD, a finding consistent with previous studies (de la Fuente et al., 1999; Langoni et al., 2004; Al Mawly et al., 2015). The rate of co-infection in the current study (7.2%) is similar to that previously reported in Spain (5.0%) (de la Fuente et al., 1998) but considerably lower than previously reported in Australia (71.0%) (Izzo et al., 2011). It is possible that the rate of co-infection may be influenced by the prevalence of individual pathogens. For example, the rate of co-infection in Australian calves and the prevalence of individual pathogens (Izzo et al., 2011) were considerably higher than in Swiss calves where the prevalence of individual pathogens were lower and the rate of co-infection was only 32.6% (Uhde et al., 2008). Individual pathogen prevalence may be a factor contributing to the low rate of co-infection observed in the current study.

Regardless of milk allowance, most calves were diagnosed with NCD at ≤ 10 d of age. Furthermore, milk allowance did not seem to influence the incidence of NCD. There is a perception among some producers that higher milk allowances result in a higher incidence of diarrhoea (Jasper and Weary, 2002). However, previous studies investigating the influence of milk allowance on the incidence of diarrhoea present conflicting results. For example, previous studies have reported that, compared to calves which were restrictively fed, the incidence of diarrhoea was higher in calves which were provided a greater milk allowance (Khouri and

Pickering, 1968; Quigley et al., 2006). Contrastingly, other studies have found no effect of milk allowance on the incidence of diarrhoea (Huber et al., 1984; Jasper and Weary, 2002). In the study by Khouri and Pickering (2012) it is important to note that the incidence of diarrhoea was only higher in calves on a greater milk allowance after 8 weeks of age, during the first 4 weeks of age, milk allowance was not found to affect the incidence of diarrhoea. It is possible inconsistencies observed across studies could be due to other factors, such as differences in management and housing conditions (e.g., sanitation, ventilation and colostrum feeding practices and the quality of the milk or milk replacer provided (Jasper and Weary, 2002; Kahn et al., 2011)).

Milk allowance was found to affect behavioural and physiological responses that occur during the onset of disease. Furthermore, based on the changes which occurred prior to clinical signs of disease, milk allowance was found to influence the suitability of these responses, both individually and in combination, as indicators for early disease detection. Prior to clinical signs of disease, changes in feeding behaviours were found to be influenced by milk allowance. Previous studies using automated calf feeders have also reported changes in feeding behaviour in response to disease, however, across studies findings are inconsistent (Svensson and Jensen, 2007; Borderas et al., 2009; Knauer et al., 2017; Swartz et al., 2017; Sutherland et al., 2018). Svensson and Jensen (2007) for example, reported a decrease in the number of unrewarded visits, but no change in drinking speed, the number of rewarded visits or milk consumption. Similarly, Sutherland et al. (2018) and Knauer et al. (2017) reported a decrease in the number of unrewarded visits. However, in contrast to Svensson and Jensen (2007) both studies also reported a decrease in milk consumption in response to disease.

It has previously been suggested that the effect of disease on feeding behaviours may be influenced by milk allowance (Borderas et al., 2009). When provided a high milk allowance (12 L/d or ad libitum), Borderas et al. (2009) reported that diseased calves showed a decrease in both milk consumption and total number of visits to the feeder and increased visit duration. In contrast, when calves were provided a low milk allowance (4 L/d) diseased calves instead showed no change in milk consumption or the total number of visits, and visit durations were instead found to decrease. The increase in total visits, regardless of milk allowance, observed in the current study is in contrast to the findings of Borderas et al. (2009). In the current study, as a majority of calves were diagnosed with NCD at ≤ 10 days of age, it is possible the increase in total visits prior to disease could be due to calves still learning to use the automated calf feeder, especially during the initial days of observation. For calves on the 10 L/d milk allowance, a significant increase in the percentage of rewarded visits and decrease in milk consumption was also observed in response to the onset of disease. As the percentage of rewarded and unrewarded visits collectively comprise the total number of visits, an increase in the percentage of rewarded visits will occur simultaneously with a decrease in unrewarded visits. Unrewarded visits to the feeder represent calves testing the feeder to determine whether or not milk is available; a decrease in unrewarded visits is therefore suggestive of a reduced appetite in response to disease. An increase in the percentage of rewarded visits also suggests calves are restricting their visits to those in which they consume milk, which may be a means of limiting their activity in order to conserve energy. The changes in milk consumption observed in the current study are similar to those of Borderas et al. (2009) where in response to disease, calves provided a high milk allowance showed a decrease in milk consumption

whilst those on a low milk allowance showed no change. In the current study, it is possible that calves on the lower milk allowance showed fewer significant changes in feeding behaviour prior to clinical signs as they could not afford to decrease their milk consumption or alter the percentage of rewarded visits to the same extent as those on the higher milk allowance whilst still ensuring they consumed enough milk in order to function. The differences in feeding behaviour between milk allowances in the current study support the suggestion by Borderas et al. (2009) that milk allowance should be considered when relying on changes in feeding behaviour to identify diseased animals. Furthermore, the suggestion that the increase in total visits to the feeder prior to clinical signs of disease observed in the current study could be due to calves still learning to use the automated calf feeder also highlights the importance of considering the age of the animals. For example, if the age at which the animals learn to use the automated system is close to the onset of disease this has the potential to influence the results, and should be given consideration when using such changes to predict the onset of disease.

The specific disease being monitored is another factor which may also influence changes in feeding behaviours (Knauer et al., 2017). When considered collectively, calves showing signs of pneumonia, diarrhoea or general illness were found to decrease milk consumption, unrewarded visits and drinking speed prior to clinical identification (Knauer et al., 2017). When the causes of illness were considered individually, milk consumption was only found to decrease for diarrhoeic calves in their study. They also reported that drinking speed and unrewarded visits decreased for diarrhoeic calves and those showing general illness. In calves with pneumonia, drinking speed and unrewarded visits were only lower on the day of clinical identification. Diarrhoeic calves displayed the earliest and most consistent changes

in feeding behaviours prior to clinical identification followed by those showing signs of general illness and finally those with pneumonia (Knauer et al., 2017).

Infrared temperatures of the eye and cheek were found to decrease prior to the clinical identification of disease, however, these changes were only significant for calves on the lower 5 L/d milk allowance. In a previous study, with a similar milk allowance of 6 L/d, eye and cheek temperatures were only found to change following clinical signs of NCD (Lowe et al., 2019a). Compared to the study of Lowe et al. (2019a) in which a single image of each anatomical location was collected manually on a daily basis, in the current study numerous images were collected automatically during each visit to the calf feeder. These differences in methodology may contribute to differences observed between studies. Prior to clinical signs of disease, Lowe et al. (2019a) reported an increase in side temperature and a decrease in shoulder temperature. The increase in side temperature was thought to be due to the close proximity of the side area to the site of intestinal inflammation (Lowe et al., 2019a). The decrease in shoulder temperature prior to clinical signs was suggested to be due to the animal generating a state of fever (Lowe et al., 2019a). Reaching a state of fever helps the animal fight infection by restricting blood flow to the skin and extremities; enabling the animal to reduce heat loss to the environment and maintain homeostasis (Lowe et al., 2019a). Also investigating NCD, it is possible the decrease in infrared temperatures observed in the current study, occurred due to similar physiological responses during the onset of disease. Additionally, Lowe et al. (2019a) suggested that a decrease in infrared temperature could be a result of a reduction in feed and metabolic activity prior to clinical identification. The decrease in infrared temperatures observed in the current study may have only been significant for

calves on the lower milk allowance of 5 L/d, as they had less milk to consume and potentially had a lower metabolic rate than those on the higher milk allowance. Further, as demonstrated by Lowe et al. (2019a), compared to the eye and cheek regions, other anatomical sites (i.e., the side and shoulder) showed more suitability for detecting the onset of NCD. Had it been possible for other anatomical sites to be measured in the current study, it is possible they may have shown greater suitability for detecting NCD, to the extent that they may have shown significant changes in response to the onset of NCD regardless of milk allowance.

Lying behaviours were all found to change significantly prior to clinical signs. Regardless of milk allowance, calves were found to decrease the number of lying bouts and increase the average lying bout duration. These findings indicate fewer lying to standing transitions, and suggest that whilst fewer lying bouts are performed, those that are made, are of a longer duration. Previous studies investigating changes in lying behaviour in relation to NCD and bovine respiratory disease (BRD) have similarly reported a decrease in the number of lying bouts, and an increase in average bout duration in response to the onset of disease (Swartz et al., 2017; Sutherland et al., 2018; Lowe et al., 2019a). As suggested by Lowe et al. (2019a), the change in the number of lying bouts and average bout duration in response to the onset of disease indicates a decreased level of activity, likely due to a lack of appetite, and calves becoming lethargic and consequently attempting to conserve energy. Time spent lying is another lying behaviour which is considered a particularly sensitive indicator of disease. In addition to appearing lethargic and having a reduced appetite, it has been suggested that diarrhoeic calves will increase the amount of time spent lying (Mainau et al., 2013), as reported by Sutherland et al., (2018). Similarly, an increase in lying time has also been observed in calves in

response to BRD (Ollivett et al., 2014; Hixson et al., 2018) and a bacterial endotoxin challenge (Borderas et al., 2008). In the current study, milk allowance appeared to influence lying time, as whilst calves on the 10 L/d milk allowance were found to increase lying time, those on the 5 L/d allowance were instead found to decrease lying time. We are uncertain why calves on a lower allowance showed a decrease in the amount of time spent lying. However, being severely restricted by their milk allowance (compared to what they would drink ad libitum), calves on the low milk allowance were presumably still hungry and their increased activity may reflect a search for food. As unrewarded visits to the milk feeder were not found to change in response to disease onset for calves on the low milk allowance, the decrease in lying time may instead reflect an increased search for other alternative food sources which were provided such as meal and hay. However, as hay and meal intake were not measured in the present study this can not be confirmed.

The lack of changes in water drinking behaviour in the current study could be due to the young age of the calves. In a previous study, for milk fed calves, additional water intake (as opposed to milk-based water intake) was of little importance until the calves were at least eight weeks of age, after which point water intake increased relative to age (Atkeson et al., 1934). It has also been reported that water intake is minimal for milk fed calves until the time of weaning, during which time water intake increases considerably (Hepola et al., 2008). The only significant change in water drinking behaviour in the current study prior to clinical signs was an increase in daily trough visit duration for calves on the 5 L/d milk allowance. This finding is similar to that of Lowe et al. (2019a) who also reported a significant increase in visit duration for calves on a 6 L/d milk allowance in response to NCD. This increase in visit duration may be indicative of increased water consumption,

however, as water intake could not be recorded in the current study it is not possible for this to be confirmed. It has previously been reported that calves that were restrictively fed 6 L/d had a higher water intake than those on a higher milk allowance of 12 L/d (de Passillé et al., 2011). Previous studies have reported an increased consumption of water in diarrhoeic calves (Jenny et al., 1973; Wenge et al., 2014). As NCD results in severe diarrhoea it is not uncommon for calves to experience dehydration in response to the amount of water being lost (Wickramasinghe et al., 2019). Therefore, an increase in water consumption in response to disease onset could be a strategy to aid rehydration.

Regardless of milk allowance, there was no change in RR prior to clinical identification. This result is similar to that of Lowe et al. (2019a) and again may be due to NCD being an enteric disease as opposed to a respiratory disease. For example, in relation to BRD, an increased respiratory rate is often a clinical symptom used to identify diseased animals (Hodgins et al., 2002). Alternatively, the frequency of only recording RR once per day may have been insufficient to enable a significant change to be detected.

The changes which occurred prior to clinical signs were found to reflect the individual suitability of the different behavioural and physiological responses as indicators for early disease detection. For calves on the 10 L/d milk allowance total visits to the calf feeder was found to be the best indicator of disease, followed by lying behaviours (in order of average lying bout duration, number of lying bouts and lying time) and then the remaining milk feeding behaviours (milk consumption and rewarded visits). Compared to feeding and lying behaviours, infrared temperatures, were not found to change significantly for calves on the 10 L/d milk

allowance prior to clinical signs, consequently infrared temperatures were found to be less useful as indicators for early disease detection. In contrast, for calves on the 5 L/d milk allowance the number of lying bouts followed by the average lying bout duration were the most suitable indicators of disease. Total visits to the feeder was the next most suitable indicator, but compared to calves on a 10 L/d milk allowance the remaining feeding behaviours (milk consumption and rewarded visits) showed much less suitability as indicators for early disease detection, due to the lack of significant changes in these variables compared to those on a higher milk allowance. Instead, for calves on the 5 L/d milk allowance, infrared temperatures of the eye and cheek proved to be more suitable than the remaining feeding behaviours. Regardless of milk allowance, the general lack of significant changes in RR and drinking behaviours prior to clinical signs, meant that these variables were found less suitable than others for indicating disease.

Combining responses provided more measures to simultaneously detect changes occurring in response to the onset of disease. Consequently, combinations of certain measures were found to provide stronger indicators for detecting disease, the strength of which increased relative to the number of variables being combined. For calves on the 10 L/d milk allowance, the best combinations of responses were found to consist only of variables relating to feeding and lying behaviour. For calves on the 5 L/d milk allowance, in addition to feeding and lying behaviours, the best combinations included eye and cheek temperature. Typically, combined responses provided greater strength to the indicator for calves on the 10 L/d milk allowance. This reflects the significance of the individual measures comprising the combined responses typically being greater for calves on the 10 L/d milk allowance than the significance for those included in the combinations of responses for calves on the 5

L/d milk allowance.

To enable different behavioural and physiological responses to be monitored simultaneously, individual automated systems could be combined to form a collective monitoring system. For example, as demonstrated in the current study, the integration of infrared cameras with automated calf feeders enabled the collective recording of both infrared temperatures and feeding behaviour as calves visited the feeder. There is also the potential that other automated systems such as accelerometers, which can be downloaded remotely (e.g., via Bluetooth), could be programmed to upload data to a collective database as calves visit the automated feeder to enable lying behaviours to be monitored alongside infrared and feeding behaviour. As discussed, milk allowance, age and the specific disease being monitored are all factors which may influence the suitability of behavioural and physiological responses as early disease indicators. Therefore, such factors need to be considered when determining which indicators are the most suitable for detecting disease onset based on the specific application for which they are being measured. For example, in the current study infrared temperatures of the eye and cheek were found to be suitable for detecting NCD in calves on the lower milk allowance but not for calves on the higher milk allowance. This finding suggests that whilst infrared temperatures may be a suitable indicator of NCD in New Zealand where it is standard practice to provide calves a milk allowance of 5 L/d, in countries in which calves are provided a higher milk allowance, infrared thermography would be a less suitable indicator of NCD. Similarly, in the current study changes in feeding behaviours were typically only suitable for indicating NCD for calves on the higher milk allowance. Therefore, compared to detecting NCD in calves in New Zealand, this finding suggests that feeding behaviours would

be a more suitable measure for detecting NCD in countries which provide calves a higher milk allowance. Through further research, the development and validation of algorithms are needed to support the integration of behavioural and physiological measures into automated systems on-farm to enable remote, real-time, automated monitoring of calf health for the purpose of disease detection. Once validated, algorithms could be developed further by being trained to send out alerts to farmers to notify them of at risk animals that require attention. Alerts would facilitate decision making abilities for farmers regarding the treatment and isolation of sick animals and would lead to improvements in calf health and welfare.

Conclusions

In conclusion, milk allowance was found to affect the behavioural and physiological responses that occur during the onset of disease. Furthermore, based on the changes which occurred prior to clinical signs of disease, milk allowance was found to influence the suitability of these responses, both individually and in combination, as indicators for early disease detection. As opposed to being used individually, in combination, responses were able to provide a stronger composite indicator of disease, especially for animals provided a higher milk allowance. Overall, results suggest that milk allowance and age should be considered when determining which responses will act as the optimum indicator/s for detecting disease. Integrated into automated on-farm systems these indicator/s, particularly combinations of feeding and lying behaviours for calves on a higher milk allowance and combinations of feeding and lying behaviours, and infrared thermography for calves on a lower milk allowance, could be used to develop algorithms that provide farmers with an automated alert system to identify at risk calves. Such systems

would enable animals to be treated and isolated sooner to prevent the spread of disease, and would lead to improved calf health and welfare.

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Chapter Seven

Water intake and drinking behaviour in preweaned group housed calves provided a high or low milk allowance and the suitability of an infrared system at a water station for monitoring calf health and welfare



Authors note: Chapter seven is presented in the style of the international journal for which the majority of this chapter is being prepared for submission as:

Lowe, G. L., M. A. Sutherland, M. Stewart, J. R., Waas, N. R. Cox, and K. E. Schütz. 2019. Water intake and drinking behavior in preweaned group housed calves provided a high or low milk allowance.

Abstract

Knowledge regarding water intake and drinking behavior of calves is limited. The aims of this study were to: 1) determine the suitability of setting up an infrared camera at a water system for monitoring health and welfare, 2) investigate water intake and drinking behavior of calves and how these are influenced by the provision of high or low milk allowances and 3) assess the influence of water provision on body weight and milk, hay and meal feeding, and lying behaviors. Fifty mix-bred calves were used during the study and were individually assigned to one of four treatments: 1) water + high milk allowance (10 L/d) (n=13), 2) no water + high milk allowance (10 L/d) (n=12), 3) water + low milk allowance (5 L/d) (n=12) and 4) no water + low milk allowance (5 L/d) (n=13). Water intake and drinking behavior were determined through the use of a water system and video observations. Milk feeding behaviors were recorded with automated calf feeders and lying behaviors were recorded using accelerometers. Hay and meal feeding behaviors were recorded from video observations. Calves were weighed weekly. Water intake and the total visits to the water trough, and the number of visits where water was consumed (rewarded), increased with age; the increase was greatest for calves on a high milk allowance. A decrease in the number of unrewarded visits (visits in which water was not consumed) to the water trough occurred with age, and was greatest for calves on a low milk allowance. Water intake was positively related to ambient temperature. With age, the difference in the total and unrewarded number of visits to the automated milk feeder between water and no-water treatment groups increased; however, there was no difference in the number of rewarded visits to the feeder. Drinking speed, which increased with age, was slower for calves on a low milk allowance, and at younger ages was slower for the no-

water treatment groups. With age, the increase in the number of visits to the hay feeder was not influenced by milk allowance, however, was greater for calves in the water treatment groups. Lying time, number of lying bouts and average bout duration decreased with age, with calves on a high milk allowance exhibiting greater lying times. Calves on a low milk allowance performed fewer lying bouts and had greater average bout durations than those on a high milk allowance. Lying behaviors were not influenced by the provision of water. The increase in body weight which occurred with age was greatest for calves on a high milk allowance. Body weight increased quicker for calves in the no-water treatment groups. The findings that calves started to consume water within the first week of life, and that water intake was positively related to ambient temperature, suggests that calves should be provided water from birth and highlights the importance of providing water particularly during warm weather. In addition, an infrared system at a water station could provide a suitable and more affordable alternative option than an automated calf feeder for farmers to monitor calf health and welfare.

Keywords: Water intake, drinking behavior, milk feeding behavior; lying behavior; infrared thermography; calves

Introduction

Water is acknowledged as an essential nutrient required by animals (Drackley, 2008). The importance of water for supporting life and ensuring performance is considered second only to oxygen (Beede, 2005). Compared to other essential nutrients (e.g., fats, proteins, carbohydrates, minerals and vitamins), the provision and quality of water on farm are often overlooked, hindering optimal nutrition, performance (Beede, 2005) and welfare in cattle (Schütz et al., 2010, 2019).

Water is required for the digestion and metabolism of energy and nutrients, the transportation of nutrients and metabolites to and from the blood, the excretion of waste products, and for maintaining ion, fluid and heat balances (Houpt, 1984; Murphy, 1992). In adult cattle, partial water deprivation has been shown to result in reduced feed intake (Little et al., 1978; Senn et al., 1996), milk production (Little et al., 1980) and body weight (Little et al., 1980), and causes behavioral (e.g., increased aggression and reduced lying) (Little et al., 1980) and physiological changes (e.g., altered urine and faecal concentrations) (Hogan et al., 2007). The provision of adequate clean water is particularly important in warm weather for enabling animals to reduce heat load and body temperature; cattle increase water intake and time around the water trough in warm weather to cool down, especially if they have no access to shade (Mader et al., 1997; Widowski, 2001; Schütz et al., 2010).

Compared to adult cattle, few studies have investigated water intake and drinking behavior in calves (Atkeson et al., 1934; Jenny et al., 1978; Kertz et al., 1984; Hepola et al., 2008; Wickramasinghe et al., 2019). Consequently, there is a lack of information regarding, for example, the age at which calves begin to consume water, how much they consume and the frequency with which they visit the water trough. Additionally, it is not uncommon on farm for preweaned calves to be denied water beyond that obtained through the consumption of milk (Kertz et al., 1984). The lack of water provision for young calves could have negative effects on welfare and productivity, particularly during warm weather conditions. Whether providing water to preweaned calves is beneficial, is controversial (Kertz et al., 1984). However, previous studies have suggested that water intake is closely associated with increased calf starter intake and body weight gain (Thickett et al., 1981; Kertz

et al., 1984; Khalili et al., 1992). Additionally, milk allowance may influence water intake (Kertz et al., 1984; Richard et al., 1988). Calves provided milk ad libitum were found to consume 0.45 kg water/d until weaning at 5 weeks of age (Richard et al., 1988). Restrictively fed calves however, were reported to consume >1.0 kg water/d during the first 3 weeks of life (Kertz et al., 1984). Further research into water intake and drinking behavior of calves would provide additional insight into the water requirements of preweaned calves and the effects of water intake on their health, welfare and productivity.

By gaining greater insight into calf water intake and drinking behavior, this study aimed to determine the suitability of an infrared system at a water station. An infrared camera system at a water station may provide a more affordable option for monitoring calf health and welfare for farmers that do not have an automated calf feeder. Therefore, the main purpose of this study was to describe water intake and drinking behavior of preweaned, group housed, autumn-born calves provided high or low milk allowances, and further, to assess how water provision effects body weight and milk, hay and meal feeding, as well as lying behaviors.

Materials and methods

All procedures involving animals in this study were approved jointly by the University of Waikato Animal Ethics Committee (Protocol #1017) and the Ruakura Animal Ethics Committee (Protocol #14089).

Animals, housing and experimental design

The study was conducted in the Waikato region of New Zealand at the AgResearch Ruakura Research farm (40°44'30.822"S 73°59'21.508"E), from April to June (late

Autumn to early Winter) 2017. Fifty mix-breed calves, sourced from a commercial farm were used during this trial. Six dairy calves (Friesian, Jersey and cross breeds) and 44 Hereford calves (18 females and 32 males) were transported to the facility and monitored from 5.2 ± 2.1 days of age (mean \pm SD). Upon enrolment to the study, all calves were weighed using a weigh crate (FastWeigh sheep crate; Tru-Test, Auckland, New Zealand) and weigh head (XR3000; Tru-Test, Auckland, New Zealand) (42.2 ± 5.0 kg; mean \pm SD), and fitted with accelerometers (as described below). Calves were individually identified using colored collars (Calf neck bands; Shoof International Ltd., Cambridge, New Zealand), and numbered (Allflex; TX, USA) and electronic identification (EID) ear tags (Allflex; TX, USA). To assist with individual identification during video analysis, calves were further identified through the application of animal marking paint (Tell-tail paint; GEA FIL, New Zealand) in unique patterns across each calf's back.

Calves were housed within a barn, in one of four equal sized pens (3.0 x 6.0 m) constructed on a concrete floor with solid plywood walls on all four sides. Each pen contained two meal feeders (Snack bar; Milk Bar, McInnes Manufacturing Ltd, Waipu, New Zealand (20 cm x 65 cm x 13 cm) and three purpose-built hay feeders (46 cm x 60 cm x 23 cm). Calves were fed milk replacer (Ancalf; NZAgbiz, Hamilton, New Zealand) (consisting of 26.0% protein, 10.0% fat, 43.5% lactose, 3.5% moisture and 7.0% minerals) using automated calf feeders and provided ad libitum access to meal (Moozlee; NRM, Christchurch, New Zealand) (consisting of 18.0% crude protein, 10.0% crude fibre and 5.0% crude fat) and hay. Within each pen, wood shavings (Pressed wood shavings; Supa shavings, Otorohanga, New Zealand) were provided as bedding (30 cm deep, area 17.5 m²), and an area of concrete flooring (0.5 m²) enabled drainage around each automated calf feeder.

The trial consisted of four treatments: 1) water + high milk allowance (10 L/d) (n=13), 2) no water + high milk allowance (10 L/d) (n=12), 3) water + low milk allowance (5 L/d) (n=12), and 4) no water + low milk allowance (5 L/d) (n=13). Treatments were replicated four times across two replicates (reps). In rep 1, a total of 32 calves were assigned equally (n=8) to each of the four treatment groups. For rep 2, only 18 calves were available due to a shortage of calves towards the end of the calving spread, which resulted in a total of five calves being assigned to each of treatment groups 1 and 4, and four calves being assigned to each of treatment groups 2 and 3. Calves were assigned into treatment groups balanced for body weight upon arrival to the facility. Due to calf availability, calves were gradually enrolled into the study as they became available. From the time of enrollment, calves were monitored to 26.5 ± 2.2 days of age (mean \pm SD). Treatment groups 1 and 3 were managed as one group across two pens, and treatment groups 2 and 4 were managed as another group across the remaining two pens so that milk allowance and water provision could be controlled on an individual or group basis respectively. Calves were weighed weekly. Water, milk, hay and meal feeding, and lying behaviors were recorded daily as described below.

Water intake and drinking behavior

In the two pens in which water was provided (treatment groups 1 and 3), a water station was set up whereby a drinking trough (5 L Farmhand Nylon; Shoof International, Cambridge, New Zealand) was positioned inside a narrow plywood chute (1.2 x 0.3m), designed to allow one animal at a time to enter. Water intake was measured in 20g increments, using a weigh scale platform (WS207PMS; Wedderburn, Auckland, New Zealand) connected to a data logger (Space logger

S10; Richard Paul Russel Ltd., Milton on Sea, England). The amount of water consumed during each visit was determined by recording changes in the weight of the water barrel that sat upon the weigh scale and supplied the water trough, between the start and end times of each visit (when the calf entered and left the chute respectively). Water was refreshed twice daily.

The number of visits to the water trough, and the start and end times of those visits were determined by analysing video footage (recorded continuously in real time; 30 frames/s) collected using overhead security cameras (DS-2CD2332-I; Hikvision, Hangzhou, China). The cameras built-in infrared lights enabled night recordings to be carried out without disrupting the calves' behavior. Video footage was analysed by two trained observers using Adobe Premiere Pro CC (version 12.0.1 Haberdasher; Adobe systems, CA, USA). The start time of a visit to the water trough was defined as being when "the poll of the calf's head is situated in front of the marked halfway point into the chute". The end of a visit to the water trough was defined as being when "the calf backs up such that the calf's entire body is situated behind the marked halfway point of the chute". A rewarded visit to the water trough was defined as being when "the calf's head is situated over the water trough, with the muzzle lowered towards the water, and based on the weigh scale readings, there is a consumption of water". An unrewarded visit to the water trough was defined as "the calf's head is not situated over the water trough, or in the case that the head is situated over the water trough, the muzzle is not lowered towards the water and based on the weigh scale readings there is no consumption of water". During video analysis, intra-observer reliability was completed at four equally interspersed stages during the trial. During each session of intra-observer reliability, a 24 hour period of footage was reanalysed. The level of reliability between observers was calculated

based on the percent agreement using Excel (version 16.26; Microsoft Corporation, WA, USA). The start and end times of visits to the water trough were considered to be in agreement when the recorded times were within 1 s of each other. Intra-observer reliability ranged from 82.4-100%, with a combined overall average of 95.7%.

Milk feeding behavior

Each pen was fitted with a Lely automated calf feeder (Lely calm; Lely, Hamilton, New Zealand) which individually identified calves as they entered the feeder based on their EID ear tags. Upon arrival to the facility, calves were individually trained to use the automated feeder. This was accomplished by handlers gently guiding calves into the feeder and teaching them to wait for a hydraulic slide to open which then allowed them access to the teat. Calves were then guided to follow the observers hands towards the teat, and taught to suckle in order for milk to be dispensed. Regardless of treatment group, calves were started on a total milk allowance of 5 L/d. For calves assigned to the low milk allowance treatment groups this was their allowance for the entire trial. However, for those assigned to the high milk allowance treatment groups this amount was gradually increased (by 1 L/d) until an allowance of 10 L/d was reached; this allowance was then maintained for the remainder of the study. For each treatment group, each daily milk allowance was split into three equal allocations. Between the full consumption of each allocation a 'stand down' period of at least 4 h passed before the calf could receive the next allocation of milk. When a calf entered the feeder, if they were entitled to feed, the automated feeder prepared the allocated amount of milk by mixing the milk replacer with warm water (150g milk replacer/L of water) which was then

delivered fresh to the calf at a flow rate of 0.7 L/min. Milk was heated to approximately 39°C before being dispensed to the calf. The automated feeders recorded milk consumption, drinking speed and the total, rewarded and unrewarded number of visits calves made to the feeder.

Hay and meal feeding behavior

Hay and meal were provided ad libitum and were split evenly into the relevant feeders to provide enough space for all calves to have access simultaneously. The number of visits to the hay and meal feeders were determined by analysing video footage (recorded continuously in real time; 30 frames/s) collected using overhead security cameras (DS-2CD2332-I; Hikvision, Hangzhou, China). The cameras' built-in infrared lights enabled night recordings to be carried out without disturbing the calves' behavior. Using 5 minute scan sampling, video footage was analysed for the entire duration of the trial by four trained observers using Adobe Premiere Pro CC (version 12.0.1, Haberdasher; Adobe systems, CA, USA) to determine the number of visits to the hay and meal feeders. During video analysis, intra-observer reliability was completed at six equally interspersed periods during the trial. During each session of intra-observer reliability, a 24 hour period of footage was reanalysed. The level of intra-observer reliability was calculated using the Pearson product-moment correlation function in Excel (version 16.26; Microsoft Corporation, WA, USA). The level of intra-observer reliability ranged from $r=0.92$ to $r=1.00$, with a combined overall average of $r=0.96$.

Lying behavior

Lying behavior was recorded continuously using tri-axial accelerometers (Hobo

pendant G data loggers 64k; Onset Computer Corp., Bourne, MA) set at a 1-min recording interval using the y- and z-axes (as validated by Ledgerwood et al. (2010) and Bonk et al. (2013)). As described by Lowe et al. (2019), accelerometers were fitted into purpose-made fabric pouches that were affixed to the lateral side of the right hind leg above the metatarsophalangeal joint using Velcro and KAMAR glue (KAMAR®; Livestock Improvement Corp., Hamilton, New Zealand). The accelerometers were orientated horizontally on the leg so that the x- and z-axes ran parallel to the ground, the x-axis pointed in the cranial direction and the z-axis pointed toward the mid plane of the calf. The y-axis ran perpendicularly to the ground, pointing in the dorsal direction. Accelerometers were initialized and downloaded using Onset HOBOWare Pro software (version 3.7.2; Onset Computer Corp.). The outputs were converted into daily summaries of lying behavior using SAS software (version 9.3; SAS Institute Inc., Cary, NC).

Environmental conditions

Ambient temperature and relative humidity were recorded at 10 minute intervals within the barn using data loggers located within each pen (EL-USB-2-LCD+; Lascar Electronics Ltd., Salisbury, UK).

Statistical analysis

The data consist of repeated observations over time of each calf. The calves were grouped into 2 reps; rep 1 consisting of 8 calves per treatment, while rep 2 consisted of 11 calves in total, split unequally between treatments (1) water + high milk allowance (10 L/d) (n=1), 2) no water + high milk allowance (10 L/d) (n=3), 3) water + low milk allowance (5 L/d) (n=3), and 4) no water + low milk allowance

(5 L/d) (n=4)). The lack of calves in rep 2 was caused by a shortage of calves at the end of the calving spread. Additionally, 7 calves were excluded from rep 2 due to being clinically identified with neonatal calf diarrhea during the observation period. Data for water drinking behavior (water intake, and total, rewarded and unrewarded visits), milk feeding behavior (total, rewarded and unrewarded visits, and drinking speed), hay and meal feeding behavior (visits to the hay and meal feeders), lying behavior (lying time, number of lying bouts and lying bout duration) and body weight were modelled with REML using Genstat (version 19; VSN International Ltd., Hemel Hempstead, UK). Data for each of these variables were fitted with splines for each animal to (1) model the correlations between the repeated observations on the animals, (2) account for noise in the data, and (3) account for some animals not having data at some of the ages. The REML consisted of fixed effects for (1) the water and milk treatments and their interaction, (2) calf age (ranging from 4-28 days of age) fitted as a linear term, and (3) linear terms for calf age varying by each treatment and their interaction. The random spline (non-linear) terms in the REML consisted of (1) calf age, (2) calf age varying by each treatment and their interaction, and (3) calf age varying by rep and by calf. Additionally, the REML included random terms for rep and calf. For analysis purposes, data for water drinking behavior (water intake, and total, rewarded and unrewarded visits) were log transformed, with the back-transformed predictions presented. Due to an accelerometer becoming corrupt during the observation period, the lying data from one calf in rep 1, treatment 3 (water + low milk allowance (5 L/d)), was excluded from the analysis. Ambient temperature and relative humidity were included as covariates in the analysis of water intake.

Results

Water intake and drinking behavior

Figure 1 shows the average daily water intake for calves provided a low (5 L/d) or high (10 L/d) daily milk allowance from 4 to 28 days of age. Regardless of milk allowance, water intake increased with age ($P<0.001$), however this increase was greatest for calves on a high milk allowance ($P=0.017$). Water intake was positively influenced by ambient temperature ($P<0.001$) but not relative humidity ($P=0.115$).

Table 1 presents the average water intake, and the total, rewarded and unrewarded number of visits to the water trough across 1-4 weeks of age. Regardless of milk allowance, overall, with age the total ($P=0.004$) and rewarded ($P<0.001$) number of visits to the water trough increased, and the number of unrewarded visits decreased ($P=0.003$). The increase in total visits ($P<0.001$) and number of rewarded visits ($P=0.008$) was greatest for calves on the high milk allowance, whilst the decrease in unrewarded visits was greatest for calves on the low milk allowance ($P=0.023$).

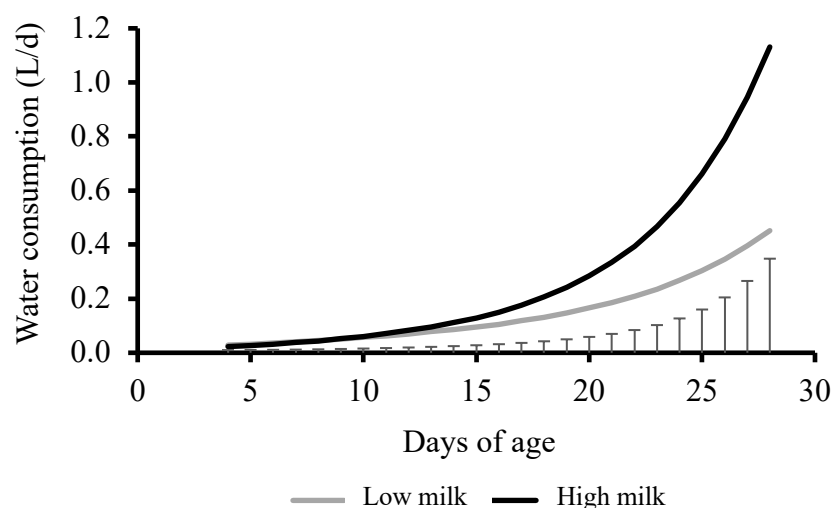


Figure 1. Mean daily water intake (L/d) for calves provided a low (5 L/d) or high (10 L/d) daily milk allowance from 4 to 28 days of age. Error bars show the average standard error of the difference (SED) between treatments at each day of age.

Table 1. Mean (\pm SEM) water intake, total, rewarded and unrewarded number of visits made to the water trough for calves provided a low (5 L/d) or high (10 L/d) daily milk allowance from 1- 4 weeks of age.

Variable	Milk allowance	Week 1	Week 2	Week 3	Week 4
Water intake (ml/d)	Low (5 L/d)	38.2 \pm 8.15	68.0 \pm 10.88	131.4 \pm 20.61	282.8 \pm 59.38
	High (10 L/d)	35.3 \pm 8.58	81.2 \pm 15.33	214.8 \pm 59.39	632.1 \pm 165.48
Total visits (no. visits/d)	Low (5 L/d)	3.6 \pm 0.47	5.6 \pm 0.53	4.4 \pm 0.43	4.2 \pm 0.56
	High (10 L/d)	2.6 \pm 0.40	5.5 \pm 0.64	5.7 \pm 0.64	6.5 \pm 1.02
Rewarded visits (no. visits/d)	Low (5 L/d)	1.6 \pm 0.19	2.5 \pm 0.21	2.6 \pm 0.21	3.2 \pm 0.39
	High (10 L/d)	1.5 \pm 0.20	2.7 \pm 0.27	3.4 \pm 0.31	5.1 \pm 0.71
Unrewarded visits (no. visits/d)	Low (5 L/d)	3.1 \pm 0.39	3.6 \pm 0.30	2.6 \pm 0.22	1.7 \pm 0.21
	High (10 L/d)	2.4 \pm 0.35	3.5 \pm 0.37	3.0 \pm 0.30	2.2 \pm 0.30

In addition, of the average of 22 days that calves were monitored (range: 21-24 days), calves were found to visit the water trough on 92.0% (range 76.0-100%) of those days, visiting the trough on an average of 19 of the monitoring days (range 18-24 days).

Milk feeding behavior

Calves in the low milk allowance treatment groups (5 L/d) consistently consumed their total daily milk allowance whilst calves on the high milk allowance consistently consumed at least 9.5 L/d of their 10 L/d allowance. Figure 2 shows the change in the total, rewarded and unrewarded number of visits made to the automated calf feeder and drinking speed for calves on all treatments from 4 to 28 days of age. Regardless of treatment group, for all variables there was no significant change with age. There was also no significant difference between milk allowances for the total and unrewarded number of visits to the feeder. However, calves in the high milk allowance treatment groups made more rewarded visits to the feeder at

younger ages ($P < 0.001$). The difference in the total ($P = 0.017$) and unrewarded number of visits ($P = 0.016$) to the feeder between water and no-water treatment groups increased with age. There was no difference between water and no-water treatment groups for the number of rewarded visits.

Drinking speed increased with age regardless of treatment group ($P < 0.001$), but was slower for calves in the low milk allowance treatment groups ($P < 0.001$). The difference in drinking speed increased between water and no-water treatment groups ($P < 0.001$), with calves in the no-water treatment groups drinking slower than calves in the water treatment groups at younger ages.

Hay and meal feeding behavior

Figure 3 shows the change in the number of visits to the hay and meal feeders for calves on all treatments from 4 to 28 days of age. Regardless of treatment group, the number of visits to the hay feeder increased with age ($P < 0.001$); however, this increase was greater for calves in the water treatment groups than those in the no-water treatment groups ($P = 0.004$). There was no significant difference between high and low milk allowance treatment groups for the number of visits to the hay feeder. Regardless of treatment group, number of visits to the meal feeder increased with age ($P < 0.001$), and this increase was greater for calves on a low milk allowance ($P = 0.035$). Additionally, calves on a low milk allowance visited the meal feeder more often ($P < 0.001$) than those on a high milk allowance. Furthermore, the number of visits to the meal feeder was greater for calves in the no-water treatment groups ($P = 0.035$) than those in the water treatment groups.

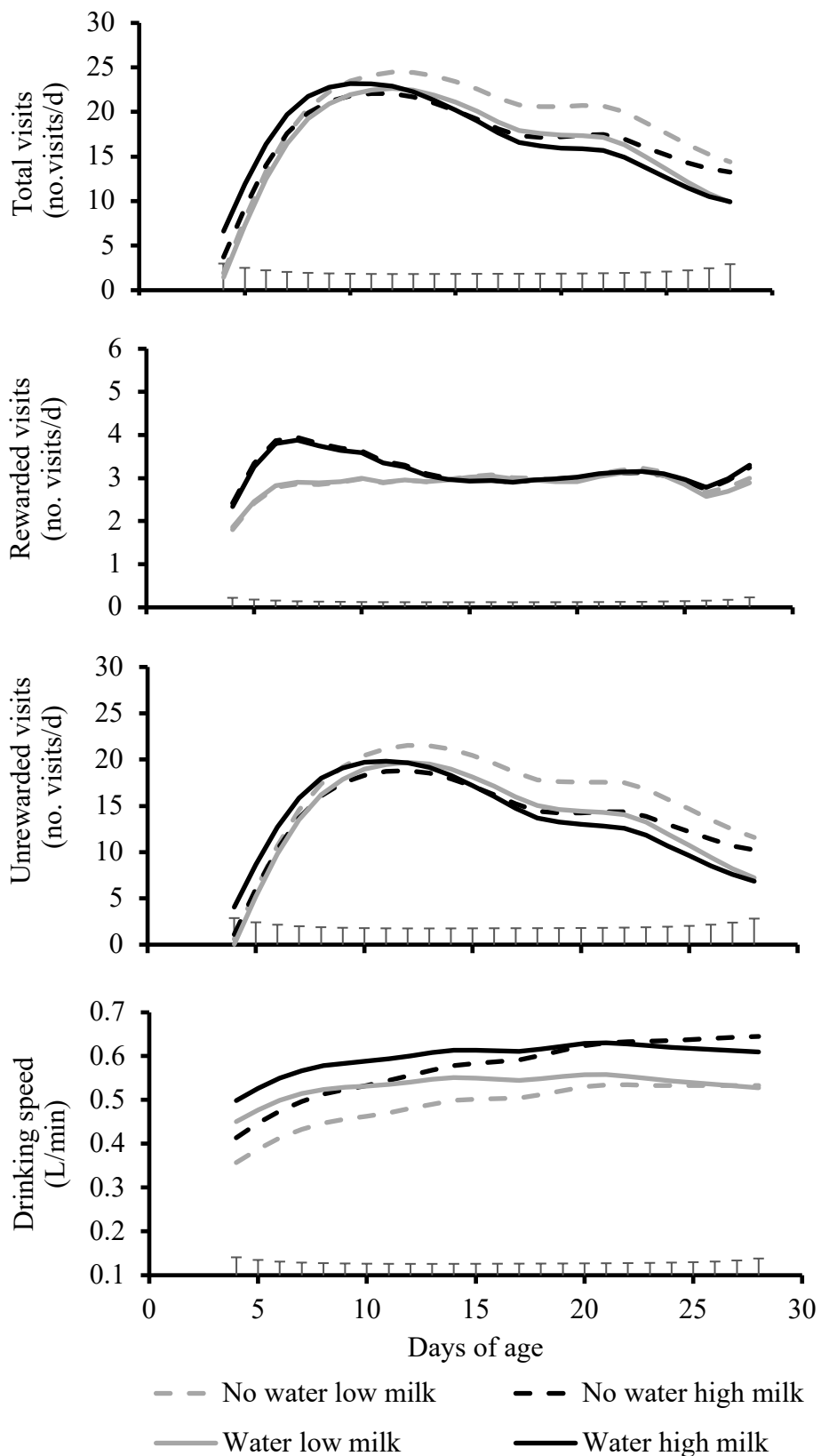


Figure 2. Mean total, rewarded and unrewarded number of visits made to the automated calf feeder and drinking speed for calves allocated to treatments 1) water + high milk allowance (10 L/d), 2) no water + high milk allowance (10 L/d), 3) water + low milk allowance (5 L/d) and 4) no water + low milk allowance (5 L/d) from 4 to 28 days of age. Error bars show the average standard error of the difference (SED) between treatments at each day of age.

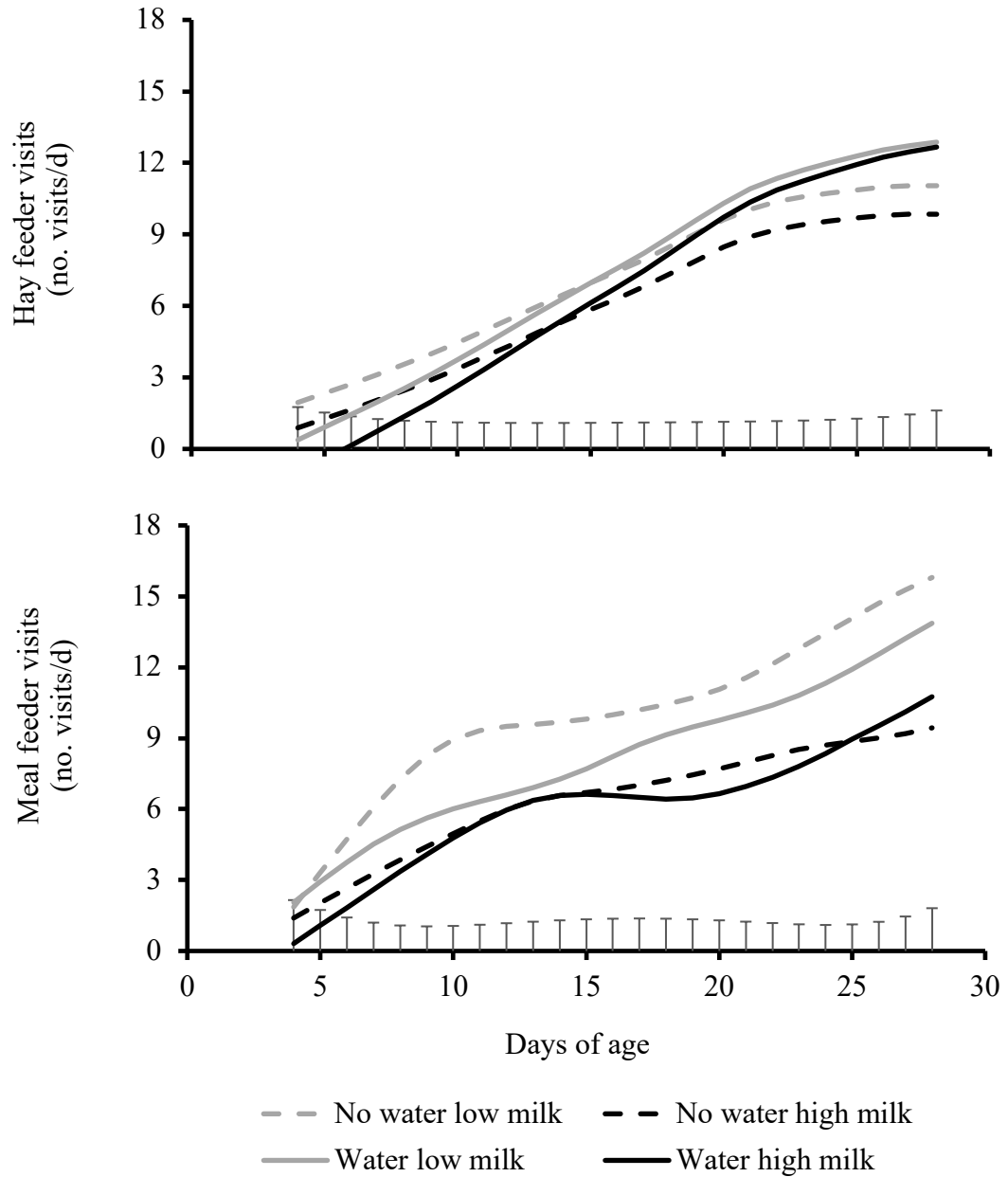


Figure 3. Mean number of visits to the hay and meal feeders for calves allocated to treatments 1) water + high milk allowance (10 L/d), 2) no water + high milk allowance (10 L/d), 3) water + low milk allowance (5 L/d) and 4) no water + low milk allowance (5 L/d) from 4 to 28 days of age. Error bars show the average standard error of the difference (SED) between treatments at each day of age.

Lying behavior

Figure 4 shows the changes in lying behavior in terms of lying time, number of lying bouts and average lying bout duration for calves on all treatments from 4 to 28 days of age. Regardless of treatment group, lying time decreased with age ($P < 0.001$), with calves on a high milk allowance exhibiting greater total lying times than those on a low milk allowance ($P = 0.009$). Regardless of treatment group, the number of lying bouts ($P = 0.014$) and average lying bout duration ($P = 0.046$) decreased with age. Calves on a low milk allowance performed fewer lying bouts ($P = 0.010$) than those on a high milk allowance. Additionally, average lying bout duration was greater for calves on a low milk allowance ($P = 0.006$). There was no significant difference between water and no-water treatment groups for either total lying time, number of lying bouts or average lying bout duration.

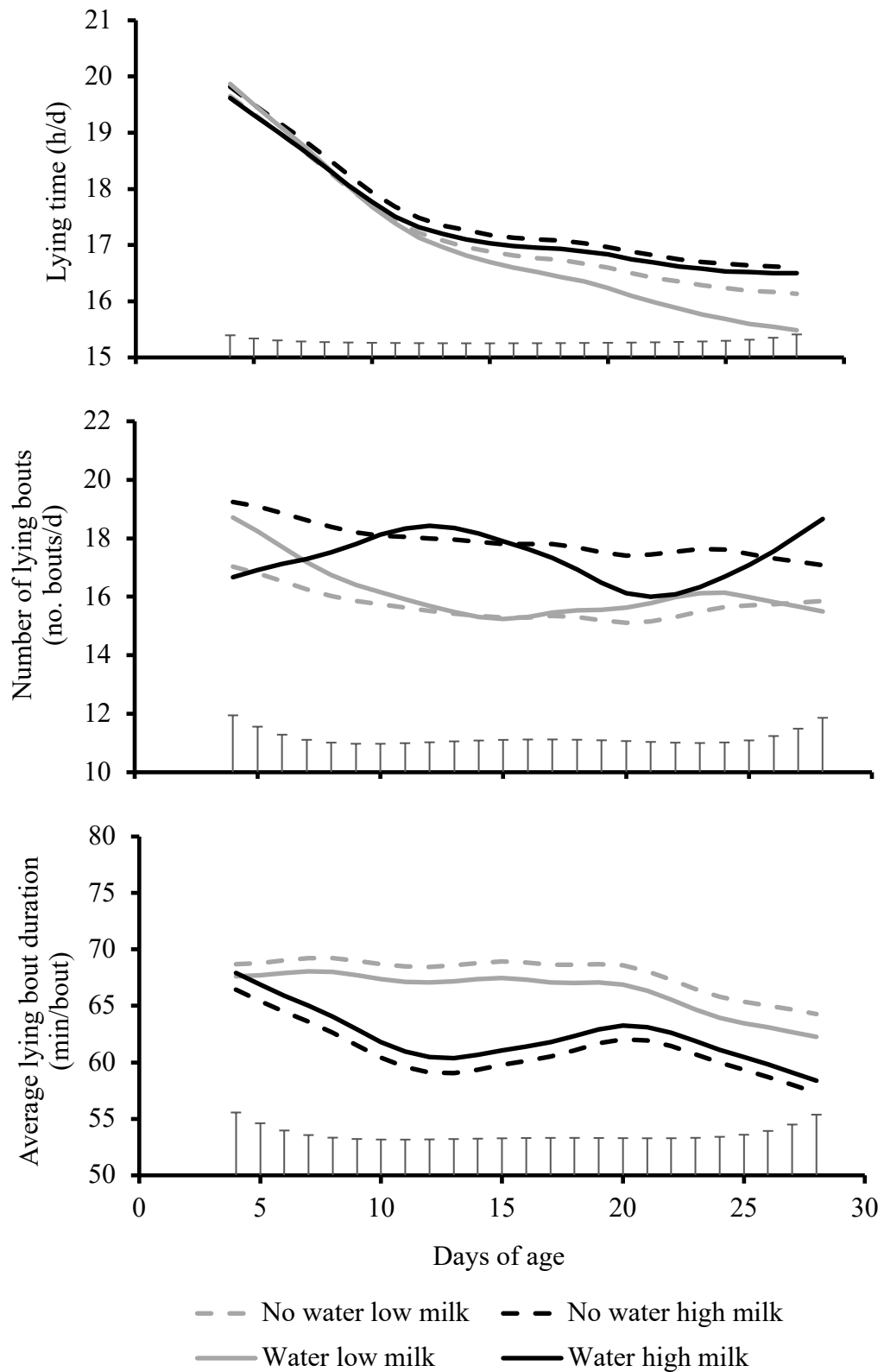


Figure 4. Mean lying time, number of lying bouts and lying bout duration for calves allocated to treatments 1) water + high milk allowance (10 L/d), 2) no water + high milk allowance (10 L/d), 3) water + low milk allowance (5 L/d) and 4) no water + low milk allowance (5 L/d) from 4 to 28 days of age. Error bars show the average standard error of the difference (SED) between treatments at each day of age.

Body weight

Figure 5 shows the mean body weight for calves on all treatments from 4 to 28 days of age. Regardless of treatment group, body weights increased with age ($P < 0.001$), with a greater increase for those in the high milk allowance treatment groups ($P < 0.001$). Compared to calves in the water treatment groups, body weight increased faster for calves in the no-water treatment groups ($P = 0.044$).

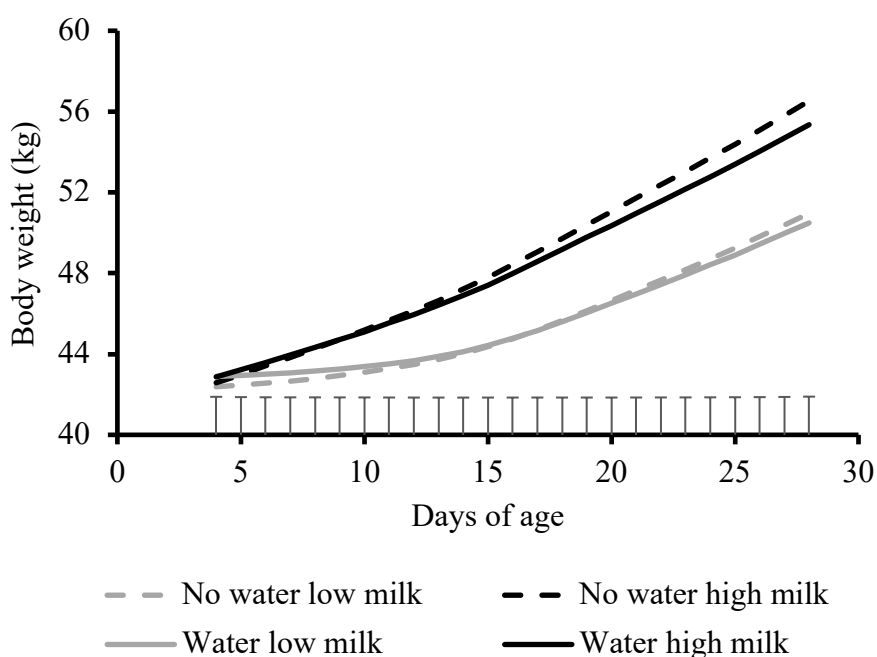


Figure 5. Mean body weight for calves allocated to treatments 1) water + high milk allowance (10 L/d), 2) no water + high milk allowance (10 L/d), 3) water + low milk allowance (5 L/d) and 4) no water + low milk allowance (5 L/d) from 4 to 28 days of age. Error bars show the average standard error of the difference (SED) between treatments at each day of age.

Environmental conditions

During the study period, the ambient temperature in the research facility was on average 14.2°C (range: 6.3 to 19.2°C) and relative humidity was on average 77.6% (range: 63.7 to 89.9%).

Discussion

Consistent with previous studies (Atkeson et al., 1934; Wickramasinghe et al., 2019), water intake increased relative to age, an increase which in the present study became more pronounced after 2 weeks of age. Whilst water intake increased with age, in the current study the average daily water intake was still considerably lower than that reported by Wickramasinghe et al. (2019). They reported average daily water intake as being 750 g/d during days 0-16 of age, increasing to 820 g/d during days 17-42 of age. The contrasting findings may be due to ambient temperature. The study by Wickramasinghe et al. (2019) occurred during late Summer and throughout Autumn, while the current study took place during late Autumn to early Winter (i.e., temperatures were considerably lower). However, in the current study there was a positive relationship between ambient temperature and water intake; an observation consistent with previous studies in both calves (Wenge et al., 2014; Wickramasinghe et al., 2019) and adult cattle (Winchester and Morris, 1956; Arias and Mader, 2011). This relationship between water intake and ambient temperature reinforces the importance of providing animals with adequate drinking water, particularly during warm weather when water intake may increase in an attempt to reduce thermal load (Beede and Collier, 1986). Low water intake in the current study may also reflect differences in breed across studies. The current study used predominantly Hereford calves while Wickramasinghe, et al. (2019) used Holstein calves; however, little is known about the effect of breed on water intake.

In addition to increasing with age and ambient temperature, water intake and drinking behaviors were also influenced by milk allowance. Calves provided a high milk allowance visited the water trough (total and rewarded visits) more and this

corresponded with a greater water intake by these animals. The greater water intake and visits to the water trough, observed in calves on a high milk allowance, could be attributed to these animals satisfying their level of satiety by drinking more water. In the present study, calves on the high milk allowance were only fed 10 L/d while previous studies have shown that given ad libitum access to milk, Holstein calves will drink approximately 10-12 L/d (equivalent to 20% body weight/d) (Jasper and Weary, 2002; Kahn et al., 2007; Sweeny et al., 2010). In contrast, calves on the low milk allowance consumed less water and visited the water trough less but consumed more meal, which could be attributed to these calves satisfying their hunger and obtaining more nutrients by consuming more meal rather than water.

In the current study, calves in the water treatment groups had more visits to the hay feeder but fewer visits to the meal feeder than calves in the no-water treatment groups. These results support our prior suggestion that calves in the no-water treatment groups likely performed more visits to the meal feeder in search of nutrients. Correspondingly, with a greater importance on obtaining nutrients, calves in the no-water treatment groups likely performed fewer visits to the hay feeder due to its comparatively lower nutritional value. However, hay intake was not measured in the present study- it would be of interest in future studies to determine if there is a relationship between hay and water intake.

With increasing age, calves on the no-water treatment had a greater total and unrewarded number of visits to the automated calf feeder than those provided with water. This may suggest that these calves were visiting the feeder more frequently in an attempt to compensate for the lack of free water by attempting to obtain more milk-based water. The total and unrewarded number of visits, increasing relative to

age, suggests that the desire to compensate for the lack of free water became more important with age, reflecting the increasing nutritional requirements needed for growth and development. This is also supported by the increase in water intake observed in calves provided with water in the present study, especially after 2 wk of age.

The increase in drinking speed, which occurred relative to age, was slower for calves not provided water and those on a lower milk allowance. Whilst drinking speed has been investigated relative to sickness (Mattje et al., 1993; Svensson and Jensen, 2007; Knauer et al., 2017), there is a general lack of knowledge surrounding drinking speed in healthy calves. To our knowledge the influence of milk allowance or the provision of water on drinking speed has not previously been assessed. The lower drinking speed of calves provided a low milk allowance and denied access to water may be due to these calves having a lower energy intake which potentially reduces how vigorously calves are able to drink.

In the current study, body weights were found to increase faster for calves in the no-water treatment group. It is unclear why calves not provided water would gain more weight, however these animals visited the meal feeder more frequently which may suggest they were consuming more meal and hence why they gained weight more quickly. As with previous studies (Jasper and Weary, 2002; Brown et al., 2005; Quigley et al., 2006; de Passille et al., 2011; Rosenberger et al., 2017), the increase in body weight was greater for calves on a high milk allowance likely due to a greater calorie intake enabling them to put on more weight than low milk fed calves.

Overall, a provision of water in the present study increased visits to the hay feeder

and milk drinking speed, and resulted in fewer total and unrewarded visits to the automated calf feeder, as well as lower body weight gain. It has previously been reported that water intake is minimal for milk fed calves until the time of weaning, at which point water intake increases considerably (Hepola et al., 2008; Wickramasinghe et al., 2019). Hepola et al. (2008) found that, prior to weaning (< 7 weeks of age), calves consumed 360 g of water per day. Following weaning, water intake was found to increase considerably to 8 to 9 kg of water per day. A similar response was observed by Wickramasinghe et al. (2019) whereby water intake was found to increase from 2 kg/d during partial weaning (43 to 49 days of age) to over 5 kg/d once calves were fully weaned. It is possible, had the current study monitored calves over a longer period of time, greater levels of water intake may have been observed.

Milk allowance, irrespective of the provision of water, was not found to influence the number of visits to the hay feeder. This finding is consistent with that of Rosenberger et al. (2017) who also reported that prior to weaning the number of visits to the hay feeder was not influenced by milk allowance. Milk allowance did however effect the number of visits to the meal feeder; calves on the low milk allowance visited the meal trough more often. Previous studies (Kertz et al., 1984; Hill et al., 2010; Kiezebrink et al., 2015; Rosenberger et al., 2017) have reported higher meal intake for calves fed lower milk allowances. As suggested by Rosenberger et al. (2017) this increase in meal by calves on a lower milk allowance could be attributed to calves attempting to compensate for a lack of nutrients due to the lack of milk. Although meal consumption was not measured in the current study, it is possible the increase in number of visits to the meal feeder may reflect a greater level of consumption.

In the present study, milk allowance influenced the number of rewarded visits to the automated calf feeder. The greater number of rewarded visits observed in younger calves on a high milk allowance could be attributed to the greater volume of milk which calves initially consumed in smaller amounts until they were capable of consuming the entire 10 L/d allowance across fewer rewarded visits.

Lying behaviors were not influenced by the provision of water; however, calves on a higher milk allowance spent a greater amount of time lying and performed more lying bouts than calves on a low milk allowance. The greater time spent lying by calves on the high milk allowance may suggest they were more comfortable or content due to the greater amount of milk being received in their diets. Longer lying times have also been associated with cow and calf comfort in previous studies in relation to more comfortable lying surfaces (Tucker et al., 2009; Sutherland et al., 2014; Worth et al., 2015). The finding that calves on a high milk allowance performed more lying bouts, and that these bouts were of a shorter average duration, reflects an increase in the number of lying to standing transitions and overall suggests an increased level of activity. Providing a higher milk allowance has previously been found to increase active behaviors such as play (Krachun et al., 2010; Duve et al., 2012; Jensen et al., 2015). Increases in play behavior likely reflect the greater energy intake for calves on a higher milk allowance. Krachun et al. (2010) reported that play running decreased in calves due to a decrease in energy intake due to low milk allowances and weaning. Further, Jensen et al. (2015) suggested calves on a lower milk allowance may have a lower motivation to perform play behavior due to a greater level of hunger, and it is likely that they spend more time conserving energy and searching for milk. It is possible high milk allowance calves in the current study were more active than calves feed a low milk

allowance, and may have been exhibiting greater levels of play behavior; however, it was beyond the scope of this study to record play and active behaviors in the home pen.

For the purpose of monitoring calf health and welfare, an infrared camera system at a water station could offer farmers a more affordable alternative than at an automated calf feeder. Chapter 6 reported the water drinking behavior (e.g., number and duration of visits to the water trough) of calves in relation to the onset of disease. However, to be able to evaluate the suitability of setting up an infrared camera system at a water station (as an alternative to an automated feeder) for monitoring NCD we needed to gain a better understanding of the drinking behavior of healthy calves. In particular, we needed to determine at what age calves start drinking water, and the frequency with which they visit the water trough. In the current study we found that calves started consuming water within the first week of life and visited the water trough an average of 3 times per day during this period. This was similar to the finding in Chapter 6, where calves visited the water trough on average 3 times per day in response to the onset of disease. In the present study, the average number of rewarded visits to the automated feeder also averaged 3 visits per day. Findings in Chapter 6 also showed that in response to disease, calves did not reduce the total, rewarded or duration of visits to the water trough; calves on a low milk allowance actually increasing the duration of visits to the water trough. Given that (1) the incidence of disease does not appear to influence water drinking behaviors, (2) healthy calves make use of a water source during the first week of age and (3) calves visit the water trough as often as they make rewarded visits to an automated calf feeder, collection of infrared images at a water trough may be frequent enough to make this a feasible option. However, of the total visits made to

the water trough, only about half of these during the first week were rewarded visits. Therefore, if the infrared camera system was programmed to collect images during total visits rather than only rewarded visits, this would help to increase the number of images collected. Additionally, the findings that calves increased the total and rewarded number of visits to the water trough suggests that with age, the opportunity to collect images would increase. The number of days that calves visit the feeder is one factor that may influence the suitability of setting up infrared monitoring systems at water troughs. If a farmer is going to rely upon an automated system to identify most cases of disease, in an ideal situation each calf would need to be identified at least once daily by the system. In the current study, each calf visited the water trough for an average of 92% (range 76-100%) of the days monitored. As not all calves were recorded at least once daily by the system, this may impact disease detection when using an automated infrared system. The impact of days where calves were not recorded by the system on the ability to reliably detect disease is something which could be investigated in future studies.

In conclusion, water intake and the total and rewarded number of visits to the water trough were found to increase relative to age and these behaviors were greater for calves on a high milk allowance. Results suggested that providing a higher milk allowance may potentially improve calf comfort and contentment, consequently, providing a high milk allowance was considered to be beneficial for calf welfare. For the purpose of monitoring calf health and welfare from a very young age, a water station was found to be a potential platform for an infrared system that would be more affordable for some dairy farmers compared to an automated calf feeder. The positive relationship between water intake and ambient temperature highlights the importance of providing calves with an adequate water supply, especially during

warm weather conditions. Further, although the volume of water intake may be relatively low, the finding that calves do make use of a water source at such young ages suggests that water should be provided to calves as early as possible.

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Chapter Eight

General Discussion



This thesis investigated the behavioural and physiological changes occurring during the onset of neonatal calf diarrhoea (NCD) and assessed their suitability as early disease indicators. To support the future integration of these indicators into automated systems for non-invasive monitoring of animal health and welfare, the thesis further developed and validated several automated methods of recording behavioural and physiological responses. Finally, the thesis investigated water intake and drinking behaviour of calves and assessed how these variables were influenced by disease, milk allowance and age. In this chapter, the main findings and overall conclusions of the research comprising this thesis are reviewed and synthesized to provide an overview of its academic accomplishments. Further, research limitations and areas for future research are identified. Lastly, the subsequent animal welfare and economic implications of this research are discussed.

Main findings and overall conclusions

This section first outlines the results of the studies which investigated the behavioural and physiological changes which occurred during the onset of NCD and their suitability as early disease indicators (Chapters 3 and 6). Second, this section discusses the results of the validation studies (Chapters 4, 5, and Appendix 1) which were run in parallel with Chapters 3 and 6. Lastly, the results of the study investigating the water intake and drinking behaviour of healthy calves, and the suitability of setting up an infrared camera at a water station (Chapter 7) are discussed.

Behavioural and physiological responses as early disease indicators (Chapter 3 and 6)

The first research chapter (Chapter 3) provided initial insights into the behavioural and physiological changes occurring during the onset of disease, based on observations of calves experimentally infected with NCD. Through assessing the thermal changes which occurred in response to disease, the optimal anatomical regions for infrared image collection were determined. The only variables which changed prior to clinical signs of disease were a decrease in milk consumption, the number of lying bouts and shoulder temperature, and an increase in lying bout duration, side temperature and the duration of visits to the water trough.

Changes in milk feeding and lying behaviours prior to clinical signs were thought to occur due to calves experiencing a reduction in appetite, and becoming lethargic and attempting to conserve energy in response to the onset of disease. The changes in infrared temperatures were consistent with the known chronology of the onset of disease with thermal biometric values (Cook and Schaefer, 2013). A decrease in shoulder temperature prior to clinical signs is thought to be due to restricted blood flow to the skin and extremities associated with animals generating a fever in order to overcome the infection, thus preventing heat loss and helping the animal to maintain homeostasis. The temperature decrease was also thought to relate to a reduction in feed intake and metabolic activity. An increase in side temperature prior to clinical signs was probably associated with the location of the side area, over the rumen fossa, being in close proximity to the underlying localised infection of the intestines. Exhibiting the greatest degree of change prior to clinical signs, the side was determined as the optimal anatomical location for infrared image

collection. The increasing durations spent at the water trough at each visit over time may correspond with an increase in water intake. As NCD results in severe diarrhoea and consequent water loss, it was believed increasing water intake may act to restore hydration, helping the animal to overcome the disease as has been suggested in previous studies (Jenny et al., 1973; Wenge et al., 2014).

The study presented in Chapter 6 further focussed on the behavioural and physiological changes which occur prior to clinical signs. Additionally, Chapter 6 investigated the effect of milk allowance on the suitability of the different behavioural and physiological responses (individually and in combination) as early disease indicators. To more closely reflect the occurrence of disease on-farm, Chapter 6 monitored calves for natural incidences of NCD.

Milk allowance influenced the suitability of behavioural and physiological responses, individually and in combination, as early disease indicators. Prior to clinical signs, milk feeding behaviours typically only changed for calves on a high milk allowance (10 L/d). In response to disease, calves on a high milk allowance likely experience a reduced appetite, so reduce their milk consumption to a level required for maintaining basic function. However, calves on the low allowance are already so severely restricted by their milk allowance, that in response to disease onset they attempt to maintain their level of milk consumption in order to maintain basic function. As in Chapter 3, the decrease in milk consumption in response to disease onset is similar to previous studies (Borderas et al., 2009; Knauer et al., 2017; Sutherland et al., 2018). Interestingly, although the milk allowances of 5 L/d and 6 L/d were similar between studies (Chapter 3 and 6), a decrease in milk consumption was only observed for calves on a 6 L/d milk allowance in Chapter 3.

This finding may suggest that the additional 1 L/d that these calves were receiving was enough for them to exhibit a decrease in milk consumption in response to disease whilst still maintaining the ability to function. However, it is important to note that differences in study protocols between Chapters 3 and 6 may have also contributed to inconsistency across these studies. In Chapter 3 for example, the calves were Friesian or Hereford whilst the calves in Chapter 6 were Friesian, Jersey, Hereford or cross breeds. Furthermore, calves in Chapter 3 were provided milk replacer whilst calves in Chapter 6 were provided whole milk. Therefore, it is possible that the inconsistency across studies may relate to differences in breed and milk quality. As unrewarded visits represent calves testing the feeder to determine whether more milk is available, a decrease in the percentage of unrewarded visits prior to clinical signs, for calves on the high milk allowance, further suggested a reduction in appetite. A decrease in unrewarded visits prior to the identification of clinical disease has also been reported in earlier studies (Svensson and Jensen, 2007; Knauer et al., 2017; Sutherland et al., 2018). A simultaneous increase in the percentage of rewarded visits indicated that calves on a high milk allowance were restricting their visits to those in which milk was consumed, potentially representing a strategy to limit their activity as they attempted to conserve energy.

Compared to Chapter 3 in which eye and cheek temperatures changed only following clinical signs, in Chapter 6, eye and cheek temperatures were found to decrease prior to clinical signs. The same logic for the decrease in shoulder temperature in Chapter 3 is applicable to the decrease in eye and cheek temperatures in Chapter 6. The inconsistencies in the response of the eye and cheek regions between Chapters 3 and 6 may be attributed to the different methodologies used for image collection and analysis. In Chapter 3, a single infrared image was manually

collected from each anatomical location on a daily basis using a handheld infrared camera and were then analysed manually. In Chapter 6, images of the eye and cheek regions were collected non-invasively using an automated infrared camera as calves fed from an automated calf feeder. This automated method of image collection enabled numerous images to be collected throughout the course of each feeding bout which were then analysed automatically using the eye and cheek detection algorithm developed and validated in Chapter 4. Based on a greater number of images, the infrared temperatures observed in Chapter 6 may have more accurately represented the changes which occurred during the onset of disease as demonstrated previously by Scoley et al. (2019). Interestingly, in Chapter 6, the changes in eye and cheek temperatures prior to clinical signs were only found to occur for calves on a low milk allowance (5 L/d). As mentioned in Chapter 3, which had a similar milk allowance of 6 L/d, these decreases in infrared temperature may have occurred due to the lower milk consumption meaning that they were consuming fewer calories which potentially reduced their metabolic activity, lowering radiated temperatures.

Regardless of milk allowance, a decrease in the number of lying bouts and an increase in average lying bout duration were observed prior to clinical signs (Chapter 6). These changes in lying behaviour suggested fewer standing to lying transitions and suggest an overall decrease in activity as calves attempted to conserve energy. Interestingly, in contrast to Chapter 3 in which lying time did not change prior to clinical signs, in Chapter 6, lying time was found to decrease and increase respectively for calves on a low or high milk allowance. In response to disease, calves have previously been reported to increase the amount of time spent lying (Borderas et al., 2008; Ollivett et al., 2014; Hixson et al., 2018; Sutherland et

al., 2018). Whilst an increase in lying time was consistent with the response observed for calves on the higher milk allowance in Chapter 6, the decrease in lying time for calves on a low milk allowance was not expected. The reason calves on a lower milk allowance spent less time lying in response to the onset of disease is unclear. However, it is interesting to note in the study presented in Chapter 7, in which lying times were monitored in healthy calves on high and low milk allowances, again calves on a high milk allowance spent a greater amount of time lying. In the case of healthy calves, greater lying times for calves on a high milk allowance may suggest these animals were more content and comfortable, due to the greater milk intake. With milk consumption being so severely restricted by their milk allowance (compared to what they would drink *ad libitum*), calves on the low milk allowance in Chapter 6 were presumably still hungry and their increased activity may reflect a search for food. As unrewarded visits to the milk feeder were not found to change in response to disease onset for calves on the low milk allowance in Chapter 6, the decrease in lying time may instead reflect an increased search for other alternative food sources which were provided such as meal and hay.

Water drinking behaviours did not change prior to clinical signs for calves on a high milk allowance (Chapter 6). In contrast, calves on a low milk allowance increased the number of visits to the water trough prior to clinical signs. Additionally, in Chapter 3 in which calves were provided a milk allowance of 6 L/d, calves also increased the number of visits to the water trough. It has previously been suggested that calves will increase water consumption in response to the onset of NCD (Jenny et al., 1973; Wenge et al., 2014). The increase in the number of visits to the water trough observed in Chapters 3 and 6 may be suggestive of increased water

consumption. As NCD results in severe diarrhoea it is not uncommon for calves to become dehydrated and therefore increasing water consumption may serve as a strategy to maintain hydration. Additionally, as changes in drinking behaviour were only observed prior to clinical signs for calves on a low milk allowance (5-6 L/d), it is possible that these calves increase water consumption to account for receiving less milk-based water.

Chapter 6 further assessed the suitability of behavioural and physiological responses individually and in combination as early disease indicators. The most suitable indicator for calves on the high and low milk allowances were the total number of visits to the automated calf feeder and the number of lying bouts respectively. In this study we found that providing more measures to simultaneously detect changes occurring in response to the onset of disease, combined responses proved more suitable as early disease indicators. Combinations of milk feeding and lying behaviours, and additionally infrared temperatures (eye and cheek) in the case of calves on the low milk allowance, provided the strongest composite indicators of disease. Combining individual automated systems could form a collective monitoring system which would enable these combinations of responses to be monitored simultaneously.

Integrated into automated on-farm systems, behavioural and physiological responses particularly combinations of milk feeding and lying behaviours, and infrared temperatures, could be used to develop algorithms that provide farmers with an automated alert system to identify at-risk calves. Such systems would enable early disease detection, allowing animals to be treated and isolated sooner to prevent the spread of disease, and would lead to improved calf health and welfare

and reductions in the associated economic costs of disease.

Developments towards automated monitoring of behavioural and physiological responses for early disease detection (Chapters 4, 5, and Appendix 1)

The increasing shift towards automation within the livestock industries is generating an increasing need to develop and integrate early disease indicators into automated on-farm systems for the purpose of non-invasively monitoring animal health and welfare. Therefore, in addition to investigating the behavioural and physiological responses which occur during the onset of NCD and assessing the suitability of these responses as early disease indicators, this thesis additionally focussed on the development and validation of automated methods of recording these responses.

The first of these studies (Chapter 4) demonstrated an automated system for non-invasively collecting infrared images automatically as calves fed from automated calf feeders. Further, the study developed and validated an algorithm capable of detecting the eye and cheek regions from infrared images. This automated method of image collection and analysis was utilised successfully in Chapter 6 for the purpose of detecting NCD, where both eye and cheek temperatures were found to decrease prior to overt clinical signs for calves on a low milk allowance (5 L/d). The ability to collect images automatically at an automated calf feeder provides an alternative to the automated infrared thermography (IRT) system described by Schaefer et al. (2007) who showed that infrared images could be collected non-invasively as calves visited a water station (for the purpose of detecting bovine respiratory disease (BRD)). Further, automated methods of image collection and analysis provide an alternative to traditional manual methods which are not always

practical, require trained observers, and are often time consuming when dealing with larger groups of animals. To detect BRD, Schaefer et al. (2012) developed an algorithm for automatically detecting the eye as calves visited a water station. However, the eye and cheek detection algorithm developed in this study (Chapter 4) is the first example of an algorithm with the capability to monitor the eye and cheek simultaneously at an automated calf feeder. The successful validation of this algorithm is a significant step necessary for the integration of IRT into automated systems for monitoring animal health and welfare on-farm.

As an additional component of this thesis, IRT was validated as a method of recording respiration rate (RR) for use in young calves (Chapter 5). The validation of IRT for recording RR provides an alternative method to the often time consuming and impractical traditional method of recording RR (eg., manual observations of flank movements) (Stewart et al., 2017). Having been successfully validated, future research can now focus on the development of algorithms to automate the use of IRT to support its integration into existing automated systems where it could potentially be used for remotely monitoring calf health and welfare. In adult cattle and pigs for example, Jorquera-Chavez et al. (2019, 2020) have developed and validated algorithms capable of recording RR using IRT to detect changes in pixel intensity occurring around the nose during inhalations and exhalations. However, Jorquera-Chavez et al. (2019) noted that further research is needed to investigate the feasibility of implementing this method on a larger scale and to decrease the impacts of potential environmental and animal factors. It is also possible that as an alternative to measuring pixel intensity, algorithms could be developed to determine RR on the basis of numerical values representing the temperature of each pixel during peaks and troughs occurring as the animal inhales and exhales.

Finally, as information regarding the water intake and drinking behaviour of young calves is limited, this thesis also aimed to develop and validate an automated water system for recording water intake and drinking behaviour. The use of an automated water system would support research into water intake and drinking behaviour of calves to determine, for example, the age they begin to consume water, how much water they consume, and the frequency and duration with which they visit the water trough. A greater knowledge of the water intake and drinking behaviour of calves could then be used to determine water requirements and the effects of water intake on calf health, welfare and productivity. As presented in Appendix 1, this thesis developed and validated an automated water system as a reliable method for recording drinking behaviour in terms of the number and duration of visits to the water trough. Unfortunately, a distributor error prevented the flow meters from working accurately and consequently the automated system was not successfully validated as a method of recording water intake. This automated system was used successfully in Chapter 6 as a means of recording drinking behaviour to assess how drinking behaviour is influenced by disease and milk allowance. In Chapter 7, the flow meters were replaced with weigh scales to enable water intake to be recorded.

Water intake and drinking behaviour of preweaned group housed calves and the suitability of an infrared system at a water station for monitoring calf health and welfare (Chapter 7)

Due to the limited amount of information available regarding water intake and drinking behaviour of young calves, the final study (Chapter 7) conducted as part of this thesis investigated water intake and drinking behaviour of healthy, preweaned, group housed, autumn-born calves and assessed how these behaviours

are influenced by milk allowance. The study further investigated the influence of water provision on body weight and milk, hay and meal feeding, and lying behaviours. Finally, for monitoring calf health and welfare, the suitability of setting up an IRT system at a water station was assessed based on the age at which calves began to make use of the water station and how frequently they visited. The age and frequency with which calves make use of a water station are important for determining whether an IRT system set up at a water station would be able to collect sufficient data to support early disease detection. This study did not collect infrared images at the water station. However, the ability to collect infrared images of calves at a water station has previously been demonstrated by Schaefer et al. (2007, 2012) for the purpose of detecting BRD.

Similar to previous studies (Atkeson et al., 1934; Wickramasinghe et al., 2019), calves consumed water as early as the first week of life, with water intake and the total and rewarded number of visits to the water trough increasing with age. Visits and water intake were more pronounced after 2 weeks of age and were greatest for calves on a high milk allowance. It was suggested that the lower water intake for calves on a low milk allowance may relate to these calves satisfying their hunger by consuming more meal rather than water in an effort to compensate for the lack of nutrients obtained due to being on a lower milk allowance. Compared to Chapters 3 and 6, when water intake could not be recorded, the ability to record water intake in Chapter 7 confirmed that an increase in the number of visits to the water trough was associated with an increase in water intake. In addition, water intake was positively related to ambient temperature as has previously been observed in studies of both adult cattle and calves (Winchester and Morris, 1956; Arias and Mader, 2011; Wenge et al., 2014; Wickramasinghe et al., 2019). The finding that calves

made use of a water source at such young ages suggests that calves should be provided with water from birth. Additionally, the positive relationship between water intake and ambient temperature suggests the provision of water is increasingly important during warm weather conditions as discussed in detail in Chapter 7.

A lack of water provision was found to decrease visits to the hay feeder and milk drinking speed, and resulted in greater total and unrewarded visits to the automated calf feeder and increased body weight gain. Fewer visits to the hay feeder were attributed to calves making more visits to the meal instead, as calves try to satisfy their hunger by consuming a food with a greater nutritive value than hay. Additionally, the lower drinking speed for calves provided a low milk allowance and denied access to water was considered to be due to these calves having a lower energy intake, which potentially reduced how vigorously these calves were able to drink. Further, the finding that calves without a provision of water performed a greater total and unrewarded number of visits to the automated calf feeder could be attributed to these calves visiting the feeder more frequently in an attempt to overcome their thirst by compensating for the lack of free water, by attempting to obtain more milk-based water. The total and unrewarded number of visits were also found to increase with age, which suggests that the desire to compensate for the lack of free water became considerably more important with age; reflecting the increasing nutritional requirements needed for growth and development. Finally, the higher body weight gain for calves denied access to water was considered to relate to these animals visiting the meal feeder more frequently which potentially suggests they were consuming more meal.

As not all farms have access to automated calf feeders, gaining a greater insight into the water intake and drinking behaviour of healthy and diseased calves through the research contained in this thesis was beneficial in assessing whether a water station would be a suitable alternative for an IRT system. The studies presented in Chapters 3 and 6, showed that prior to clinical signs, water drinking behaviours (number and duration of visits to the water trough) were not negatively impacted by the onset of disease. Further, in the studies presented in Chapters 6 and 7, which used similar water station set-ups, healthy and diseased calves were found to perform the same number of visits to the water trough per day. Additionally, healthy calves were found to start consuming water within the first week of life and performed the same number of visits to the automated calf feeder as to the water trough. Collectively, these observations regarding the water intake and drinking behaviour of healthy and diseased calves suggest that an IRT system set up at a water station may be a suitable alternative to an automated calf feeder for the purpose of monitoring calf health and welfare. However, as mentioned in Chapter 7, the issue of calves failing to visit the water trough everyday may influence the ability of the system to reliably detect disease. The impact of having days where calves are absent from the system, on the ability to reliably detect disease is a concern which could be assessed through future research. The ability to detect that a calf has been absent from the system itself could alert farmers to animals worthy of paying closer attention to. Similar alerts are already provided by automated calf feeders such as the Lely Calm feeder (Lely, Hamilton, New Zealand) which can detect that calves have not visited the feeder for extended periods of time. However, ultimately, to support early disease detection an automated system needs to alert farmers of a diseased animal before it is indicated based on an absence from the system. This is because, by the point

animals are absent from the system, they are likely already displaying clinical symptoms. Through the use of data algorithms, the issue of having data missing on days an animal is absent from the system could potentially be overcome or at least minimised and again this is something which could be investigated in future research.

Practical considerations and limitations

Milk allowance was found to influence the milk feeding behaviours of calves in response to disease. This effect of milk allowance on milk feeding behaviours in sick calves (e.g., a decrease in unrewarded visits) is consistent with those reported by Borderas et al., (2009) and further supports the suggestion that milk allowance needs to be considered when relying on changes in milk feeding behaviours to identify disease in calves. However, in addition to the above suggestion by Borderas et al. (2009), the results from this research further suggest that milk allowance also influences the ability to identify diseased animals based on changes in infrared temperatures and lying and drinking behaviours. Therefore, it is suggested that milk allowance should also be considered when determining the suitability of infrared temperatures, and lying and drinking behaviours as early disease indicators.

The time taken for an animal to be fully trained to use an automated system is another factor which could impact the ability to accurately detect disease. The results from Chapters 3 and 6 for example, demonstrated an increase in total number of visits to the automated feeder prior to clinical signs. However, this change in behaviour was thought to relate to calves still learning to use the feeder during the initial days of observation. Therefore, the sooner an animal can be trained to use an automated system the sooner their behaviour can be reliably monitored. This is

especially important with diseases such as NCD where calves are susceptible to infection immediately following birth, meaning changes in behaviour can potentially occur in the very initial days of life. The impact of the initial days over which animals become accustomed to using an automated system on predicting disease onset is something which needs to be taken into consideration when monitoring animals using automated systems.

Calves develop rapidly and changes in behavioural and physiological responses overtime may simply reflect this development process. To ensure that changes in behavioural and physiological responses can be attributed to the onset of disease, and not the process of development, age needs to be considered when identifying diseased animals. In Chapters 3 and 6, age was included as a covariate in the statistical analysis and of the different behavioural and physiological variables measured was only found to have a significant effect on lying time in Chapter 3. It is generally accepted that lying time decreases relative to age with previous studies showing calves decrease their lying time from 70.0-74.0% of the day during the first week of life to 35.0-39.0% of the day at one month of age (Walczak, 2005; Wojciech, 2013). Chapter 7 demonstrated a similar decrease in lying time relative to age in healthy calves. Animals will typically increase the amount of time spent lying in response to the onset of disease, as demonstrated in calves in response to diseases such as BRD and NCD (Olivett et al., 2014; Swartz et al., 2017; Hixson et al., 2018; Sutherland 2018). As the decrease in lying time relative to age opposes the increase in lying time which occurs in response to disease, potentially, the influence of age could reduce the degree of change in lying time observed in response to the onset of disease. However, the impact of age should not be considered solely on its influence on lying behaviour. Instead, the impact of age

should be considered across any potential measures which could be influenced such as milk feeding, water intake and drinking behaviours. In healthy calves, milk feeding, water intake and drinking behaviours for example, were demonstrated to change relative age in Chapter 7, and the impact of age was considered in regards to the responses observed in Chapters 3 and 6.

Finding that milk allowance influenced not only milk feeding behaviour, but other behavioural and physiological responses, may impact the suitability for these measures as early disease indicators depending on different management systems. In New Zealand, for example, where it is standard practice to provide calves a milk allowance of approximately 5 L/d, based on the findings of this research, infrared temperatures may be a more suitable indicator of NCD than in other countries where calves are typically provided greater milk allowances. Additionally, the research in this thesis typically found milk feeding behaviours to change for calves on a high milk allowance. Therefore, in contrast to infrared temperatures, milk feeding behaviours may be better suited for use as early NCD indicators in calf rearing systems where the provision of a higher milk allowance is common practice.

To enable early disease detection, it is advantageous to collect as much baseline data as possible in order for deviations from what are considered “normal” to be detected. During the course of this thesis, the lack of baseline data collected prior to clinical signs was identified as a limitation. This lack of baseline was partly due to calves presenting with clinical signs of NCD as young as ≤ 10 days of age. The early age at which calves became infected in Chapter 6 is likely due to calves being more susceptible to pathogenic infection during the first few weeks of life (Cho and Yoon, 2014). In addition, the incubation periods of the different pathogenic agents

associated with NCD also impacts upon the ability to obtain baseline data. For example, calves are most susceptible to *E. coli* based infection during the first four days of life and similar to rotavirus can become infected as early as within the first day of life (Stoltenow and Vincent, 2003). Further, following infection with *Cryptosporidium*, clinical signs can typically occur anywhere within the first 2-7 days of age (Holland, 1990). This varied incubation period could result in a significant level of variation in the amount of baseline data which is obtained, particularly in those calves with lower incubation periods. Due to these pathogens generally infecting calves at such a young age, the amount of baseline data collected is also limited due to the time calves are manually fed colostrum and the subsequent period of time taken to train calves how to use the automated calf feeders. Being susceptible to pathogenic infection from birth means that by the time calves are trained to use automated feeders they may already be infected and potentially showing clinical signs. Therefore, the integration of early disease indicators into automated calf feeders may be better applied for use in older calves and potentially for the detection of other diseases, such as BRD. Calves are typically affected by BRD during the first 2-6 months of age (Murray et al., 2018), which compared to NCD, would enable a greater amount of baseline data to be collected before calves start presenting with clinical signs of disease. Compared to other countries, the incidence of BRD is relatively uncommon in New Zealand (Fairly, 1996), consequently, the application of early disease indicators for BRD detection could be better utilised in overseas systems. However, it is important to note that the integration of early disease indicators into an automated calf feeder means that they can only be useful prior to weaning off a milk-based diet.

In addition to the ages at which calves were identified as being clinical, a further factor which limited the amount of baseline data obtained in this thesis for Chapters 3 and 6 is the high number of calves who became ill. The study presented in Chapter 3 was originally designed to have separate control and infected treatment groups. However, an unexpected outbreak of *Salmonella* meant all calves including those in the control group for both replicates of this study contracted the disease. Consequently, the data from the control animals could not be used to establish a baseline for comparison against calves in the “infected” treatment groups. Similarly, in Chapter 6 there was a high prevalence of disease, so again there was an insufficient baseline for comparison against known sick animals. Instead each calf had to be treated as its own control with data analysed relevant to when they each displayed clinical signs of NCD. Although this enabled an assessment to be made as to the potential suitability of different behavioural and physiological variables for early disease detection, it prevented the ability to determine their diagnostic efficacy to establish how well they would perform in real-time. The diagnostic efficacy of the different variables is something which could be established in future studies now that potential suitable indicators for detecting NCD have been identified.

The cost of equipment would be another limitation for some farmers. Hawkins et al. (2019) for example provide a detailed economic analysis regarding the initial set-up and ongoing costs associated with using automated feeders with direct comparisons to the costs associated with other management strategies. In New Zealand, an automated calf feeder system, comprised of four feeding stations that could collectively support feeding 100-120 calves, costs approximately NZ\$60,000, with each additional feeding station estimated to cost a further

NZ\$10,000. Although in the long-term, the reduced labour costs are a financial benefit, the initial outlay to purchase an automated calf feeder is a large investment that many farmers may struggle to afford. In addition, infrared cameras vary considerably in price, for example the cameras used in this thesis (Thermovision A300; FLIR Systems AB, Danderyd, Sweden) cost approximately NZ\$15,000. However, the price of infrared cameras has reduced significantly over the past few years and it would be worth testing the accuracy of lower end cameras for this purpose. Another way to try and reduce the costs of implementing IRT equipment would be to investigate whether it is necessary to set up infrared cameras at each feeding station or whether sufficient data could be collected by only integrating IRT into some of the feeding stations. This necessity may be influenced by factors such as calves preference for one feeding station over another and the layout of the calf shed, for example, whether calves are housed in groups that only have access to a single calf feeder or are housed with access to multiple calf feeders simultaneously. As not all farmers can afford to invest in automated calf feeders, this thesis determined that a water station may also provide a suitable alternative platform for setting up an infrared system, for monitoring calf health and welfare. The automated water system developed for the purpose of this research cost approximately \$2500, with \$2000 of that associated with the electronic identification reader (G03113 EID tag reader controller R; Gallagher, Hamilton, New Zealand) and antenna system (G03121 EID tag reader antenna panel 600; Gallagher, Hamilton, New Zealand) for enabling individual identification. Moreover, accelerometers for recording lying behaviour, are a comparatively low cost investment, with the accelerometers used in this research (Hobo pendant G data loggers; Onset Computer Corp., Bourne, MA) costing NZ\$130 per unit. However, in an on-farm situation the cost of

providing each calf with an individual accelerometer can soon become costly, for example, on a farm with 100-120 calves it would cost NZ\$13,000-15,600 to invest in accelerometers for every animal. It is likely, with increasing technological advancement and demand, many of these technologies (e.g., IRT technology, accelerometers, automated calf feeders and electronic identification systems) will become more affordable for farmers.

Whilst a water station may be an alternative platform for setting up an IRT system, the added benefit of an automated feeder is the capability to simultaneously record milk feeding behaviours and infrared temperatures, which combined with lying behaviours for calves on a low milk allowance were found to be the most suitable for detecting the onset of NCD. However, as mentioned above, this is dependent on the different calf management systems and normal practices regarding milk allowances for calves, which can vary between countries. Whilst it would provide a more affordable alternative for farmers than an automated calf feeder, the results of this thesis suggest that setting up an infrared camera at a water station may reduce the ability for the early detection of NCD for calves on lower milk allowances. An infrared camera set up at a water station may however prove beneficial for monitoring other diseases or other aspects of calf health and welfare, which could be determined through future research. For example, an infrared camera system at a water station has previously been used successfully to monitor calves for detecting BRD (Schaefer et al., 2012). As opposed to an automated calf feeder which can only enable calves to be monitored prior to weaning, a benefit of integrating an IRT system into a water station is the ability to monitor older calves post-weaning off milk.

Respiration rate did not change prior to clinical signs, likely due to NCD being an enteric disease compared to a respiratory disease. Alternatively, this lack of response may be due to the frequency of only recording RR once per day which may have been insufficient to enable a change to be detected. Although the research in this thesis considered RR was not a suitable measure for NCD detection, IRT was validated as an alternative method for measuring respiration rate; this method could still provide other valuable information relating to other aspects of calf health and welfare and other diseases such as BRD. In the case of BRD, RR would be expected to change more rapidly and is a clinical sign which is commonly used to identify bovine respiratory disease (BRD) (Hodgins et al., 2002). It may also be useful for other species in different management systems, for example, monitoring respiratory disease in pigs, goats and sheep.

Limitations associated with the use of IRT

As considered in this thesis, there are a number of factors (as reviewed by Rekant et al., (2015)) when using IRT that need to be considered in order to ensure accurate results are obtained during future research. The angle and distance of the camera from the animal need to be kept as consistent as possible throughout the observation period. The impact of these factors can be minimised by specifying a set distance and angle at which images are collected, which is kept constant throughout the observation period. In the case of automated image collection, the effect of distance and angle can be minimised by stationing the animal in front of a fixed-position camera as has been previously demonstrated whilst animals were restrained in a milking bail (Kunc et al., 2007), cattle crush (Schaefer et al., 2004, 2007) or as they visited a water station (Schaefer et al., 2012). As demonstrated in this thesis, for the

purpose of detecting NCD, infrared cameras integrated into automated calf feeders provided a suitable platform for image collection. Automated calf feeders consist of a narrow chute in which animals have to stand as they feed, this chute is beneficial for infrared image collection as it helps to limit the degree of movement which consequently acts to ensure distance and angle are kept constant. An automated water system (like the one developed in this thesis) also consists of a narrow chute, which again would help ensure distance and angle are kept constant during image collection. To further reduce the effect of distance, some cameras enable operators to adjust for this by entering this information directly into the camera (Rekant et al., 2015).

Environmental factors such as ambient temperature, sunlight, humidity, wind chill and precipitation can significantly impact the accuracy of surface temperature measurements obtained when using IRT (Rekant et al., 2015). To account for changes in ambient temperature and relative humidity some cameras enable this information to be entered into the camera or use inbuilt calibration systems in order to adjust for these variables to improve the accuracy of the camera (Nguyen et al., 2010). Alternatively, ambient temperature and relative humidity can be accounted for at the time of image analysis by entering information relating to ambient temperature and relative humidity, recorded at the time of image collection, directly into the analysis software. In support of the integration of IRT into automated systems, environmental data could easily be recorded using weather stations or temperature and humidity loggers and uploaded automatically at specified intervals directly into the system. Infrared cameras are also sensitive to sunlight which can result in solar loading that increases the body surface temperature (Cook and Schaefer, 2013). Additionally, the presence of dirt or moisture on the body surface

can also influence the temperatures obtained by affecting the animals' emissivity (Campbell and Norman, 1998). The effects of environmental factors can be minimised by collecting images indoors, in dry and sheltered environments. The integration of IRT into automated systems such as automated calf feeders and robotic milking systems would reduce the impact of environmental conditions on infrared recordings as these systems are typically housed indoors. Additionally, as part of integrating IRT into automated systems, environmental data could be recorded and updated automatically at specified intervals directly into the system to ensure camera accuracy in real-time.

In addition to environmental conditions, animal surface temperatures can also be impacted by circadian and infradian rhythms which influence the amount of blood flow and thermoregulation occurring at different times within or between days (Alsaad et al., 2015; Rekant et al., 2015). Such an impact on surface temperature has previously been reported by Berry et al. (2003), where whilst investigating the use of infrared thermography as a potential tool for indicating mastitis, within-day monitoring at 2 h intervals revealed that temperature changes of the udder occur with a circadian rhythm. The impact of such rhythms and their consequent impact on blood flow and thermoregulation should be considered during the interpretation of thermographic images.

A further consideration is the pelage or plumage of the animal (fur, hair, coat, wool or feathers) as this can greatly influence the heat exchange that occurs between the animal and its environment, which consequently impacts skin temperature (Mitchell, 2013). For animals or anatomical regions which are densely covered (e.g., sheep), infrared temperatures will reflect the surface temperature of the pelage

or plumage, which will be influenced considerably by air temperature (Mitchell, 2013). In contrast, animals or anatomical regions with minimal coverage (e.g., pigs and facial or limb regions) will more closely reflect skin temperature and will be less impacted by air temperature (Mitchell, 2013). The presence of moisture or dirt on the animal can also impact infrared surface temperature readings, interfering with the emissivity value of the surface (Alsaad et al., 2015; Rekant et al., 2015). In addition, the colour of the pelage or plumage may also influence infrared temperature measurements (Rekant et al., 2015). The eye is one specific location which can be measured without being impacted by the presence of pelage or plumage (Stewart et al., 2007), and compared to other anatomical locations (e.g., nose, ear, body and hooves) has previously been shown to provide more consistent temperature changes in relation to early disease detection in cattle (Schaefer et al., 2004).

The number of images collected and the temperature variable used (e.g., minimum, median or maximum temperature) are other factors which could influence the results. From images collected of the eye, Schaefer et al. (2012) reported the maximum temperature to be the most relevant diagnostic. Similarly, Scoley et al. (2019) also demonstrated increased precision was achieved when the maximum temperature value was used and this was a consistent finding across all anatomical areas measured (left eye, right eye and rectal area). Additionally, Scoley et al. (2019) reported that precision was improved with increasing repetition whereby the standard error of a single measurement was reduced by half when the average of five replicate images were taken and by half again when 30 replicate images were taken. As suggested by Scoley et al. (2019), for IRT to be a practical tool for use on-farm, it is important to achieve sufficient precision to ensure an accurate

diagnosis of ill-health is made. In agreement with Byrne et al. (2017), Scoley et al. (2019) suggest that a total of three replicate images are needed to achieve a sufficient level of precision. The ability to collect numerous images is a considerable challenge when images are collected manually due to labour constraints and potential disruption to the animal. Additionally, the need to handle the animals in order to collect the images could potentially cause the animals stress and as the stress response is known to influence infrared temperatures this could potentially confound the results. However, through integration into an automated system, such as a calf feeder, IRT could be used to automatically and remotely collect numerous images increasing the level of precision achieved. Collecting infrared images using an automated system would also remove the need to handle animals, which would prevent the results being confounded due to a stress response.

As outlined, there are numerous factors that need to be considered when using IRT that can influence the precision and accuracy of the results. As discussed, with consideration, the impact of these factors can be overcome or at least minimised for the successful use of IRT in future research and on-farm applications.

Further developments and future research

This research has seen significant progress of a prototype, automated system, for early disease detection in calves. Algorithms for detecting temperatures in certain areas of the body have been developed and a number of potential automated measures have been identified. The next step for development of a commercial system is to conduct real-time testing on-farm of a collective automated system that has the capability to monitor different behavioural and physiological measures concurrently. The findings of this research suggest that milk feeding and lying

behaviours, and additionally infrared temperatures (eye and cheek) for calves on a low milk allowance provided the strongest composite measure for early disease detection. On-farm testing is required to confirm the suitability and accuracy of these combinations of indicators to successfully predict NCD in a commercial farming situation. During on-farm testing, a number of combinations, in addition to those identified, could be triggered to alert farmers to potentially sick animals, in order to assess the accuracy of different combinations for detecting the onset of disease in real-time. As part of this assessment it would be worthwhile testing systems set up at automated calf feeders and water stations simultaneously to determine which is most accurate and practical on-farm. Testing of an IRT system set up at a water station would also enable combinations that do not include milk feeding behaviours to be assessed as to their suitability for predicting disease.

As demonstrated throughout this research, milk feeding behaviours and infrared temperatures of the eye and cheek can be recorded automatically through the integration of an infrared camera into an automated calf feeder. Further, lying behaviours can be recorded automatically using accelerometers. During this thesis, the type of accelerometers used had to be downloaded manually, however for integration into an automated system, accelerometers that can download data remotely (e.g., via infrared, Bluetooth or wi-fi) would be useful. These are already available (e.g., Afimilk-silent herdsman and AfiAct II (Afimilk, Kibbutz Afikim, Israel)) and have been applied on-farm for monitoring aspects of cow health and welfare (e.g., heat detection, calving, and illness such as ketosis, lameness, and mastitis) (Afimilk, 2019). For the purpose of monitoring NCD, accelerometer data could be programmed to upload data at specific intervals, or as calves visit the automated calf feeder, to a collective database alongside data pertaining to milk

feeding and lying behaviours. Algorithms could then be developed to interpret the data contained within such a database and trained to send automated alerts directly to farmers to notify them of animals displaying early signs of disease. Such an alert system would facilitate decision making abilities for farmers enabling them to monitor, treat and isolate sick animals sooner to prevent the spread of disease. Such capabilities would help to minimise the impact of disease, consequently resulting in improvements for calf health and welfare and reducing the economic impact of disease.

Based on the results presented in Chapter 3, the side was determined as the optimal anatomical location for collecting infrared images for early disease detection. As this finding was based only on the collection of manual images it would be worthwhile for future studies to develop the capability to automatically collect images from this region to further confirm its suitability. Potentially, algorithms similar to those developed in this thesis for the eye and cheek regions could then be developed to automatically detect and analyse infrared images of the side region. The suitability of combinations involving the side region for monitoring calf health and welfare could then also be assessed.

Although this thesis has focussed on the detection of NCD, there is potential for the findings and developments made during this research to have further application in adult cattle or other species for the purpose of detecting or monitoring other diseases and aspects of animal health and welfare. The eye and cheek detection algorithm developed in this thesis, for example, could potentially be modified for use on adult cattle or other species and then applied to monitor animals for signs of stress, pain and disease based on a change in temperature. For application in older cattle and

other species, IRT systems could be incorporated into other platforms such as robotic milking systems, milking bails, raceways, or water stations. In New Zealand, as dairy is our largest export industry and has had the greatest uptake of automated systems so far, discussions between agricultural technology companies (e.g., LIC, Lely, De Laval) and the dairy industry will be important for identifying future research priority areas and determining what applications would be of benefit to the industry. As systems are developed and become more affordable, there are wider applications for other livestock industries.

Animal welfare and economic implications

The research conducted throughout this thesis identified and developed a number of behavioural and physiological measures to promote early disease detection. The diagnosis of NCD is reliant upon the presence of overt clinical signs. This is a major challenge with the incidence of NCD, as by the time calves present with clinical signs substantial internal damage to the intestines has already occurred (intestinal damage results in diarrhoea). The optimal behavioural and physiological measures identified and the automated systems developed throughout the course of this thesis provide the capability to overcome this challenge by enabling NCD to be detected prior to clinical signs before such extensive damage can occur. This capability could help farmers to reduce the impact of disease and prevent the spread of disease by enabling earlier treatment and isolation of diseased animals. Reducing the impact of NCD would be beneficial in improving animal welfare, by reducing the severity of disease and consequently the amount of time an animal suffers and experiences lethargy, diarrhoea, dehydration, and reduced appetite and body weight. Reducing the severity of disease will also reduce mortality rates.

In addition to promoting early disease detection, this research also has the potential to reduce economic costs associated with the incidence of disease. The short term economic benefits of early NCD detection would arise from reduced spread of disease to other animals and calf losses, and reduced labour and treatment costs required to treat sick calves (de Graaf et al., 1999; Bazeley, 2003; Smith, 2012). Other longer-term economic impacts associated with NCD include reduced weight gain, stunted growth, increased age to first calving and decreased milk production during the first lactation (Waltner- Teows, 1986; Wittum et al., 1994; Svensson and Hultgren, 2008).

The results of this research suggest that the provision of water and high milk allowances may improve calf comfort and contentment by preventing animals from experiencing hunger and thirst. Ultimately, providing water and greater milk allowances may be beneficial for improving welfare. The finding that calves consume water within the first week of life suggests calves should be provided with water at all ages. Calves consuming water at such a young age in this research contradicts the anecdotal opinion held by some farmers that calves do not make use of a water source at this age, or that they receive adequate water through the consumption of milk. Further, the opinions on whether there are any benefits in providing preweaned calves water are varied (Kertz et al., 1984), however, this research suggested that water provision was beneficial in increasing the number of visits to the hay feeder and drinking speed and reducing the number of unrewarded visits to the automated calf feeder. Additionally, the finding that water intake was associated with ambient temperature further highlights the importance of providing water during warmer weather conditions, when it is beneficial for reducing thermal load.

Early detection of disease could also reduce the threat to human health, by helping to reduce the risk of zoonotic infection; pathogens associated with NCD can be transmitted from calves to humans (Cho and Yoon, 2014). Minimising the impact of disease is further beneficial in reducing stress on farmers and farm workers. In the case of NCD, reducing the impact of disease at stressful times on-farm, for example during seasonal calving periods, would reduce the added stress that comes with dealing with sick calves. The ability to improve animal welfare through early disease detection also enables farmers to meet animal welfare requirements. Livestock industries are under increasing pressure to improve husbandry practices and animal welfare, largely due to public perceptions regarding the way in which animals should be treated. This, alongside ever increasing regulatory requirements regarding the environment, climate change and animal welfare can be both mentally and economically challenging to keep up with for farmers. Therefore, providing tools to support them in meeting these requirements are highly important for our livestock industries.

Final conclusions

The findings of this research suggest that milk feeding and lying behaviours, and additionally infrared temperatures (eye and cheek) for calves on a low milk allowance provided the strongest composite measure for early disease detection. Through identifying and developing a number of behavioural and physiological measures and automated systems, this research has made significant steps to progress the development of a prototype system for automated early disease detection in calves. Further testing in commercial farm situations are needed to take the next steps towards a fully integrated monitoring system, to ensure a reliable, non-invasive system for on-farm monitoring of calf health and welfare. The

findings of this research have also highlighted the importance of the provision of water and greater milk allowances for preventing experiences of thirst and hunger and ultimately improving calf health and welfare. The integration of behavioural and physiological measures into an automated on-farm system could promote early disease detection to minimise the severity of disease and the amount of time an animal suffers. Early disease detection could enable earlier treatment and isolation of diseased animals to prevent the spread of disease, by alerting farmers to diseased animals earlier than is currently possible based on overt clinical signs. In addition to facilitating decision-making abilities for the farmer, the development of such a system would reduce costs on-farm and to the industry as a whole, reduce potential for the spread of zoonotic diseases and would ultimately improve calf health and welfare.

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Appendix One

Validation of an automated system for monitoring water intake and drinking behaviour in dairy calves



Authors note: Appendix one is presented in the style of the Proceedings of the New Zealand Society of Animal Production where it was presented at the annual conference in Rotorua, New Zealand, June 2017 and published as:

Lowe, G. L., K. E. Schütz, M. A. Sutherland, J. R. Waas, M. T. Wilson, and M. Stewart. 2017. Validation of an automated system for monitoring water intake and drinking behaviour in dairy calves. Proceedings of the New Zealand Society of Animal Production. 77:133-136.

Abstract

The provision of palatable drinking water *ad libitum* for cattle is often neglected on farm and is considered difficult and impractical to monitor, particularly on an individual animal basis. The objective of this study was to validate an automated system for monitoring individual water intake and drinking behaviour of dairy calves. Nineteen dairy calves were observed over five days for eight hours/day. Calves were individually identified as they visited a water trough within a narrow chute where the number and duration of visits were recorded using an overhead photoelectric sensor, and water intake was recorded using a flow meter. Data from the automated system were compared to behavioural observations from video recordings across the same time period. The automated system was highly correlated with behavioural observations for both the number of visits ($R^2=0.96$, $P<0.001$) and visit durations ($R^2=0.94$, $P<0.001$), but showed no correlation for water intake ($R^2=0.01$, $P>0.1$). This system could be used as a reliable automated method for recording drinking behaviour in terms of the number and duration of visits. However, alternative systems for measuring water intake need to be investigated and incorporated into the automated system. This information could be used to investigate a number of questions regarding water use and quality, and their effects on calf health and welfare and could be integrated into other automated systems used on farm for daily animal monitoring.

Keywords: water intake; drinking behaviour; dairy calves; automated monitoring

Introduction

Although water is acknowledged as an essential requirement for animals (Drackley 2008), compared to other essential nutrients (carbohydrates, fats, proteins, minerals and vitamins), its provision and quality are often neglected on farm (Beede 2005). Previous studies have shown that for adult dairy cattle, partial water deprivation results in reduced feed intake and milk production (Little et al., 1978; Little et al., 1980), behavioural problems such as increased aggression (Little et al., 1980), physiological changes (Hogan et al., 2007), and altered urine and faecal concentrations (Hogan et al., 2007). Compared to adult cattle, few studies have been undertaken to investigate water intake in calves.

Traditionally, as with feeding behaviour, it is common for drinking behaviour to be monitored manually from live observations or video recordings (Huzzey et al., 2005; Mitlohner et al., 2001), and intake to be monitored by measuring residuals (Morris et al., 2010). However, these methods are often labour intensive, difficult and impractical to conduct, particularly in order to monitor individual animals. Previous research using these manual methods has often been carried out using animals housed in individual pens, tie stalls or trained to access purpose-built feeding bins, and may not be representative of animals that are group housed (Chapinal et al., 2007; Chizzotti et al., 2015). The development of an automated water system that can reliably monitor water intake and drinking behaviour would provide an alternative to the labour-intensive methods which are currently used, and could also enable monitoring of animals group-housed or managed on pasture. There has been an increasing reliance on automated systems on farm in response to the increasing need to reduce labour costs, increasing herd sizes, and fewer

experienced stockpeople in the industry. An automated system that could provide information regarding water intake, integrated with other automated data collected on farm could allow for daily monitoring of animal health and welfare.

Research is required to investigate the drinking behaviour of calves (e.g., at what age they begin to consume water, number of visits to the trough, and water intake) to determine water requirements and the effects of water intake on calf health, welfare and productivity. The aim of this study was to validate the use of an automated system capable of monitoring individual water intake and drinking behaviour of dairy calves. The study was part of a larger project investigating automated methods for early disease detection.

Materials and methods

All animal procedures were approved by the University of Waikato Animal Ethics Committee under the New Zealand Animal Welfare Act 1999 (Protocol #985).

Animals and experimental design

The study was undertaken at the Tokanui research farm, AgResearch Ltd, Te Awamutu, New Zealand, between July and October 2016. Data from 19 (managed in a group of 35 calves) mix-breed heifer calves (average 20 days old), were obtained over five days for eight hours/day (00:00-04:00h and 20:00-24:00h). Calves were housed in a barn in a pen (7.5x14.0 m) constructed on a dirt floor with walls on all four sides. Within the pen, an area (100 m²) of woodchip (25 cm deep) was provided as bedding material. Calves were fed using two automated calf feeders (RFID Calf Feeder, A&D Reid, Temuka, New Zealand) and provided

access *ad libitum* to meal (Superior, OSP Stockfeeds, Auckland, New Zealand), water and straw.

Automated water system

The automated system consisted of a water trough (10 L) that was positioned inside a narrow chute (0.4x1.2m), designed to allow one animal at a time to enter. As individuals entered the chute they were identified using radio frequency identification (RFID) ear tags and an RFID reader (G03121, Gallagher, Hamilton, New Zealand) attached to the side of the chute. The automated system determined the number and duration of visits to the trough based on the beam from an overhead photoelectric sensor (WTB27-3S1511, SICK, Germany) being broken and then reconnected as the calf entered and backed out of the chute. A flow meter (SPX-075, Seametrics, USA) was connected to the trough which recorded water intake during each visit.

Behavioural observations

Behaviour at the water trough was recorded continuously in real time (30 frames per second) using security cameras (DS-2CD2332-1, Hikvision, China) secured to the ceiling of the calf barn at a height of 2 m above the ground. Infrared lights built in to the cameras allowed behavioural observations to be carried out at night without affecting the calves' behaviour. Video footage was analysed continuously for each animal to investigate water intake and drinking behaviour including visit frequency and duration. The start of a visit to the water trough was defined as being when the poll of the calf's head moved in front of the overhead sensor. The end of a visit to the water trough was defined as being when the calf moved backward such that its

entire body was situated behind the overhead sensor. A drinking event was defined as being when the calf's head was situated over the water trough and the water was seen to move. This could not provide information regarding the volume of water the calf had consumed during a given visit; but it was used as an indicator for when it would be expected that a change in water volume may be detected by the flow meter. This was compared to a non-drinking event, defined as being either when the calf did not place its head over the trough or if the calf's head was placed over the trough, but the water was not seen to move. These non-drinking events were used as indicators for when it would not be expected for the flow meter to detect any change in water volume.

Video footage was analysed using Adobe Premiere Pro CC (version 10.4 Good Buddy). Each calf had a numbered ear tag, was fitted with a coloured collar (Calf neck bands, Shoof International Ltd., Cambridge, New Zealand) and also had photographs of its coat pattern taken to allow for individual identification during video analysis.

Statistical analysis

Using Microsoft Excel 2016 (Microsoft Corporation, Washington, USA), data recorded from the automated system were regressed against data gathered from behavioural observations from the video recordings in order to assess the level of agreement between the two methods for recording water intake, number of visits and visit duration.

Results

Based on the 51 visits observed during the observation period, the recordings from the automated system were highly correlated with behavioural observations for the number of visits ($R^2=0.96$, $P<0.001$, Fig. 1). For the number of visits there is marginal evidence that the sensor detected slightly more visits than recorded via behavioural observations. For five of the animals the automated system detected one more visit than the behavioural observations; the exact binomial test had a P value of 0.063. There is not significant evidence that the bias changes across the range of values. Lin's concordance value is 0.968. Recordings from the automated system were also highly correlated with behavioural observations for visit durations ($R^2=0.94$, $P<0.001$, Fig. 2). A Bland Altman graph of differences plotted against the means showed no evidence of any change in bias across the range of values and the average bias was not significant (0.13 ± 0.072 (mean difference \pm SEM)). Lin's concordance value is 0.966. Of the 51 visits observed, nine visits were recorded as being non-drinking events based on behavioural observations and correctly were not detected by the flow meter. However, in contrast the remaining 42 visits were all recorded as drinking events but only two of these drinking events were detected by the flow meter. Overall, there was no correlation between water intake measured from the automated system vs. behavioural observations ($R^2=0.01$, $P>0.1$).

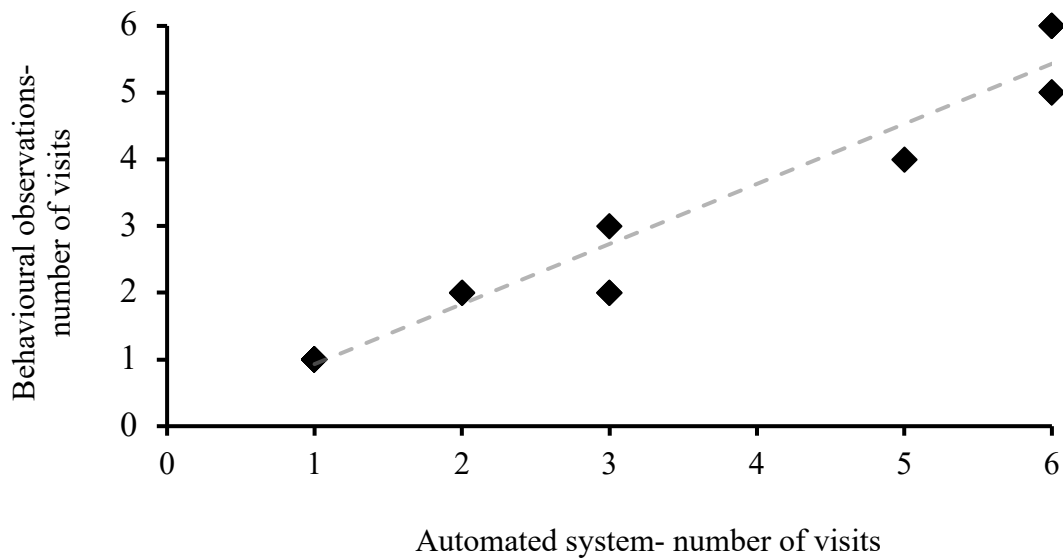


Figure 1. Correlation between the number of visits made by calves to the water trough obtained through behavioural observations from video recordings compared to the number of visits as recorded by the automated system for a total of 51 visits.

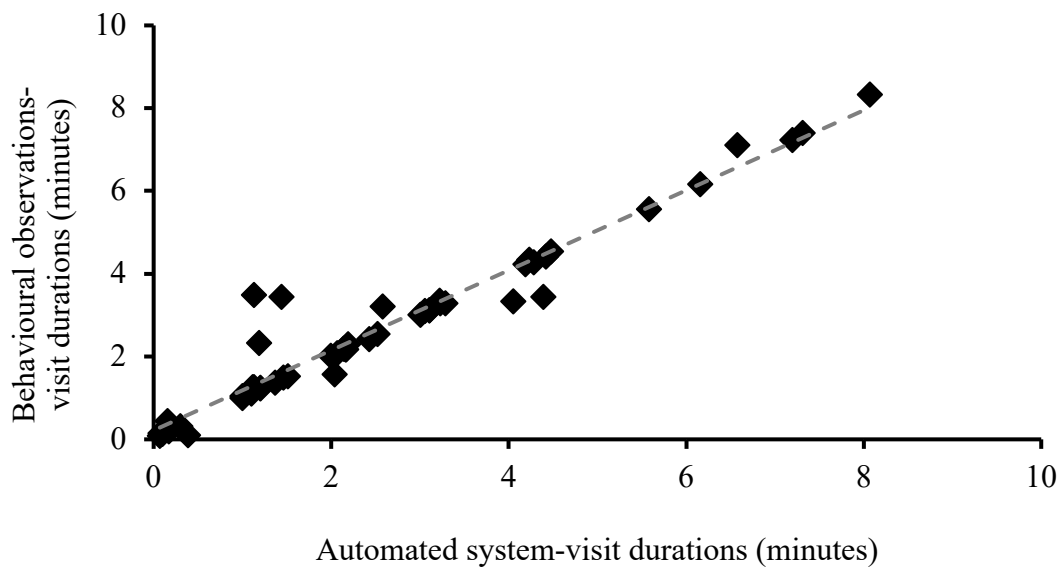


Figure 2. Correlation between visit durations (minutes) by calves to the water trough obtained through behavioural observations from video recordings compared to visit durations as recorded by the automated system for a total of 51 visits.

Discussion

The results show that this automated system can reliably record drinking behaviour in terms of the number and duration of visits. However, the poor correlation between the two methods when measuring water intake suggests that the flow meter used in this study was not sensitive enough to accurately and reliably measure the small volumes of water that were being consumed by the calves during each visit. In a previous study, Chapinal et al. (2007) validated the use of the Insentec monitoring system (Insentec, Marknesse, Netherlands) as an automated method of measuring both feed and water intake of adult cattle. Chapinal et al. (2007) found that durations of feeding and drinking visits and feed and water intake per visit recorded by the Insentec system were highly correlated with direct observations ($R^2 \geq 0.99$). This is similar to the present findings for the duration of drinking visits, however, the Insentec system used by Chapinal et al. (2007) showed a much stronger correlation with water intake recorded per visit. Compared to the flow meter used in the current study, the Insentec system instead used a weigh scale to measure water intake, which was likely to have been more sensitive and reliable than the flow meter and could explain the inconsistency between these two studies. Another explanation for the inconsistency is that Chapinal, et al. (2007) investigated the water intake of adult cattle, which are likely to have consumed larger volumes of water per visit in comparison to the low consumption of calves in this study and would have been easier for the system to detect.

In conclusion, the system was able to reliably, automatically record drinking behaviour in terms of the number and duration of visits. However, an alternative system to measuring actual water intake that is sensitive and accurate enough to

measure small volumes of water intake needs to be investigated and incorporated. This system could be used to investigate a number of questions regarding water use and consequent effects on animal health, welfare and productivity. Additionally, the development of an automated water system, integrated into other automated data collection on farm, could enable continuous animal health and welfare monitoring.

Acknowledgements

The authors gratefully acknowledge the assistance from Ariane Bright, Jessica Roberts (InterAg) and Shona Broughton (AgResearch). We also thank Alvin Reid for the use of the automated feeders. The study was funded by DEC International, Hamilton, New Zealand and AgResearch Ltd Core Funding.

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Appendix Two

ISAE 2018 conference abstract and poster presentation



Authors note: Appendix two is an abstract published in the proceedings for the 52nd ISAE conference, Prince Edward Island, Canada, 2018, along with the accompanying poster presentation.

Behavioural and physiological responses as early indicators of disease in New Zealand dairy calves.

Lowe, G. L.¹, Sutherland, M. A.², Waas, J. R.¹, & Stewart, M.³.

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Proceedings of the 52nd Congress of the International Society for Applied Ethology, Prince Edward Island, Canada, August, 2018, p142

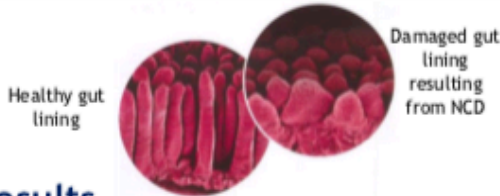
Automated systems are needed to monitor animal health and welfare, and detect disease before overt clinical signs are evident. This study was part of a larger project investigating automated methods with a focus on the use of infrared thermography for early disease detection of neonatal calf diarrhoea (NCD). This part of the study investigated physiological and behavioural responses associated with NCD onset in calves experimentally infected with rotavirus; and assessed the suitability of these responses as indicators for early disease detection. Forty-three calves were either: (1) infected with rotavirus (through an oral drench (40 ml water and 6 ml faeces (from rotavirus positive calves) at 6 days of age (n=20), or (2) acted as uninfected controls (n=23). Control and infected animals were housed separately but handled in the same manner. Daily assessments of coat condition, gut fill, faecal consistency, rectal temperature and dehydration levels were conducted. Once exhibiting clinical signs (e.g., scouring and dehydration), faecal samples were collected to verify NCD as the cause of illness and calves were treated with antibiotics and electrolytes. Lying behaviour was recorded continuously using accelerometers. Respiration rate (RR) was recorded daily by observing flank movements. Drinking behaviour at the water trough was filmed continuously to determine the number and duration of visits. An unexpected outbreak of *Salmonella* (NCD causing pathogen) meant all calves developed NCD; therefore, treatment was ignored, and each animal was analysed as its own control with data analysed relevant to when each calf displayed

clinical signs of NCD regardless of the causative pathogen. A sign test measured the significance of changes between periods (days -7 to -4 to days -3 to 0 and days -7 to -1 (pre) to days 0 to 6 (post) relative to clinical signs (day 0)) and the standard error of the difference (SED) measured variability. There was no change in RR or lying time prior to clinical signs of disease, but both decreased following clinical signs of disease (34.9 ± 7.3 vs 29.9 ± 9.1 breaths/min, 1085.3 ± 61.2 vs 1041.5 ± 91.0 min/day respectively: $P < 0.001$). The number of lying bouts decreased (16 ± 2.9 vs 14.7 ± 3.3 bouts/day: $P = 0.017$) and bout duration increased (71.0 ± 16.1 vs 79.1 ± 19.0 min/bout: $P < 0.001$) prior to and following clinical signs of disease (15.5 ± 2.9 vs 13.4 ± 3.1 bouts/day, 74.5 ± 15.5 vs 85.3 ± 21.2 min/bout respectively: $P < 0.001$). There was no change in the number of visits to the water trough, but visit duration increased prior to clinical signs of disease (22.0 ± 16.0 vs 27.0 ± 14.0 sec/visit: $P = 0.027$). In conclusion, number and duration of lying bouts, and duration of water trough visits, show potential as early indicators of disease. Integrating these measures into an automated system has the potential to alert farmers to disease onset, enabling earlier treatment and isolation of diseased animals. Such technology has the potential to reduce production costs and improve calf welfare.



Introduction

- Neonatal calf diarrhoea (NCD) is an enteric disease affecting beef and dairy industries worldwide.
- Intestinal damage resulting from NCD often occurs prior to overt clinical signs (e.g. diarrhoea and dehydration).



Objectives

- Investigate behavioural and physiological responses to NCD and assess their suitability as indicators for early disease detection.

Methods

- Daily health checks identified calves displaying clinical signs of NCD (n=43); faecal samples collected for verification of NCD.
- Measures: lying and drinking behaviour, and respiration rate prior to and following clinical identification of disease (day 0).

Results

Prior to clinical signs:

Lying Behaviour

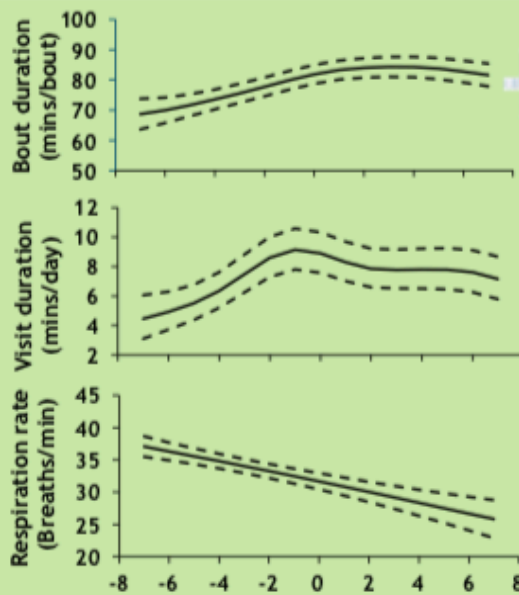
- No significant change in lying time.
- ↓ Number of bouts (P=0.014).
- ↑ Bout duration (P<0.001).

Drinking Behaviour

- No significant change in drinking visits.
- ↑ Visit duration (P=0.017).

Respiration Rate

- No significant change in respiration rate.



Conclusions

- Number and duration of lying bouts and duration of drinking visits show potential suitability as early indicators of disease.
- The next step for this research is to investigate integration of these measures into automated systems that could alert farmers to calves with early signs of disease.

Acknowledgements

The authors gratefully acknowledge the assistance from Neil Cox, Ariane Bright, Tania Blackmore, Melissa Hempstead and Richard Laven. The study was funded by DEC International, Hamilton, New Zealand. G. Lowe thanks ISAE CAF and the Ron Kilgour Memorial Trust for travel funding.

Appendix Three

ISAE 2019 Australasia-Africa regional conference abstract and poster presentation



Authors note: Appendix three is an abstract published in the proceedings for the ISAE Australasia-Africa regional conference, Wellington, New Zealand, 21st-22nd November 2019, along with the accompanying poster presentation.

Influence of milk allowance on the suitability of automated behavioural and physiological measures as indicators of neonatal calf diarrhoea (NCD)

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Early disease detection is essential for promoting earlier diagnosis and treatment to minimise the impact of disease. As automation increases in the livestock industry, it would be beneficial for early disease indicators to be incorporated into automated systems to support remote, reliable monitoring of animal health and welfare. An enteric disease, neonatal calf diarrhoea (NCD) is a leading cause of calf morbidity and mortality worldwide. A major challenge diagnosing NCD is the reliance on overt clinical symptoms which often do not arise until significant intestinal damage has already occurred. We investigated the influence of milk allowance on the suitability of behavioural (feeding, drinking and lying behaviours) and physiological (infrared temperatures and respiration rate) responses, individually and in combination as early NCD indicators. 120 heifer calves were randomly assigned to 5 L/d or 10 L/d milk allowances and monitored daily for naturally occurring NCD. Automated feeders recorded milk feeding behaviour, and infrared cameras automatically recorded eye and cheek temperatures. Accelerometers recorded lying behaviour, and water drinking behaviour was recorded using an automated water system and video observations. Respiration rate was determined from flank movements. Calves diagnosed with NCD (n=112) were used as their own controls, with data analysed relative to clinical identification (d 0) using the t-test and Theil-Sen estimator. Prior to clinical signs (across d -6 to 0), feeding behaviours typically only changed for calves on the 10 L/d milk allowance with

increased total ($P<0.001$) and rewarded visits to the feeder ($P=0.013$), and decreased milk consumption ($P=0.011$). Infrared temperatures only changed significantly for calves on the 5 L/d milk allowance, with a decrease in eye ($P=0.013$) and cheek ($P=0.006$) temperatures. Regardless of milk allowance, lying time ($P=0.028$, 5 L/d; $P=0.011$, 10 L/d), number of lying bouts ($P<0.001$, 5 L/d; $P=0.007$, 10 L/d), and average bout duration ($P<0.001$, 5 L/d; $P=0.002$, 10 L/d), all changed significantly. The only change in water drinking behaviour was an increase in total trough visit duration for calves on the 5 L/d milk allowance ($P=0.029$). Respiration rate showed no change regardless of milk allowance. For calves on the 10 L/d and 5 L/d milk allowances, total number of visits to the feeder ($P<0.001$) and number of lying bouts ($P<0.001$), were the most suitable indicators of disease, respectively. Regardless of milk allowance, combinations of feeding and lying behaviours, and additionally infrared temperatures for calves on the 5 L/d milk allowance, provided the strongest composite indicators of disease. The lack of change in feeding behaviour for calves on the 5 L/d milk allowance, likely reflects a reduced ability to alter feeding behaviours in response to disease whilst still ensuring enough consumption to maintain function. Occurring as the animal generates a fever, decreased infrared temperatures only for calves on the 5 L/d milk allowance, likely reflects a lower metabolic rate. Results indicate milk allowance should be considered when determining which measures will act as the optimum early indicator/s of disease. Assessing these measures, we aimed to facilitate the development of non-invasive, automated systems capable of identifying animals displaying early signs of disease, enabling earlier treatment and isolation, to prevent the spread of disease. Resulting systems could improve decision making abilities for farmers, decrease economic costs and ultimately improve calf health and welfare.

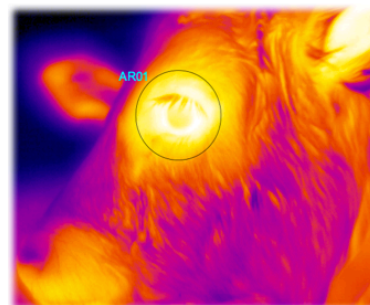
Animal ethics requirements

All procedures involving animals in these studies were approved by the AgResearch Ruakura and University of Waikato Animal Ethics Committees under the New Zealand Animal Welfare Act of 1999.



Introduction

- NCD is a leading cause of calf morbidity and mortality
- Current diagnosis relies on clinical signs which arise following significant intestinal damage
- Early disease detection would minimise the impact of disease
- Incorporated into automated systems, early disease indicators would support remote, reliable monitoring of animal health and welfare
- There is a trend towards increasing calves milk allowances from the equivalent of 10% up to 20% body weight



Objectives

- Investigate the influence of milk allowance on the suitability of behavioural and physiological responses, individually and in combination, as early NCD indicators



Methods



- 120 heifer calves were assigned to receive a 5 L/d or 10 L/d milk allowance
- Daily health checks monitored calves for naturally occurring NCD (n=112)
- Measures:
 - Feeding behaviour-** Automated calf feeders
 - Lying behaviour-** Tri-axial accelerometers
 - Drinking behaviour-** Automated water system and video observations
 - Infrared thermography-** Eye and cheek temperatures
 - Respiration rate-** Flank movements

Results

Prior to clinical identification (across d -6 to 0):

- Infrared temperatures only changed significantly for calves on the 5 L/d milk allowance
- Feeding behaviours typically only changed significantly for calves on the 10 L/d milk allowance
- Lying behaviours changed significantly regardless of milk allowance
- Combinations of feeding and lying behaviours, and additionally infrared temperatures for calves on the 5 L/d milk allowance were the optimum indicators of NCD

Suitability of individual measures as early NCD indicators

Variable	5 L/d milk allowance			10 L/d milk allowance			
	Direction of change	P-Value	Indicator ranking	Direction of change	P-Value	Indicator ranking	
Feeding behaviour	Daily total visits to feeder	↑	0.002	3	↑	<0.001	1
	Daily rewarded visits to feeder	↑	0.316	12	↑	0.013	6
	Daily milk consumption	↓	0.184	10	↓	0.011	5
Infrared temperature	Eye temperature (°C)	↓	0.013	5	↓	0.211	11
	Cheek temperature (°C)	↓	0.006	4	↓	0.120	8
Lying behaviour	Daily lying time	↓	0.028	6	↑	0.011	4
	Daily number of lying bouts	↓	<0.001	1	↓	0.007	3
	Average lying bout duration	↑	<0.001	2	↑	0.002	2
Drinking behaviour	Daily total trough visits	↑	0.139	9	↑	0.174	10
	Daily rewarded trough visits	↑	0.484	13	↑	0.273	12
	Average trough visit duration	↑	0.105	8	↑	0.132	9
	Daily total trough visit duration	↑	0.029	7	↑	0.081	7
Respiration rate	Breaths/min	↓	0.310	11	↓	0.336	13

Conclusions

- Milk allowance influenced behavioural and physiological responses during NCD onset
- Milk allowance needs consideration when determining the optimum indicator/s for early NCD detection
- Optimal indicators could be integrated into automated on-farm systems and utilised for early NCD detection
- Early disease detection would promote earlier diagnosis and treatment, ultimately improving calf health and welfare



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Appendix Four

Co-Authorship forms





Co-Authorship Form

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Chapter three is presented in the style of the Journal of Dairy Science where it has been published as:
 Lowe, G. L., M. A. Sutherland, J. R. Waas, A. L. Schaefer, N. R. Cox, and M. Stewart. 2019. Physiological and behavioral responses as indicators of early disease detection in dairy calves. J Dairy Sci. 102:5389-5402.

Nature of contribution by PhD candidate: Conceptualisation, Experimental design, Data collection, Data Analysis, Manuscript preparation, Manuscript Revision

Extent of contribution by PhD candidate (%): 85%

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Name	Nature of Contribution
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Mhairi Sutherland	Conceptualisation, Experimental design, Manuscript Revision, Supervision
Joe Waas	Conceptualisation, Experimental design, Manuscript Revision, Supervision
Al Schaefer	Conceptualisation, Experimental design, Manuscript Revision
Neil Cox	Statistical Analysis

Certification by Co-Authors

The undersigned hereby certify that:

- ❖ the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
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Lowe, G. L., McCane, B., Sutherland, M. A., Waas, J. R., Schaefer, A. L., Cox, N. R., and Stewart, M. 2019. Automated collection and analysis of infrared thermograms for measuring eye and cheek temperatures in calves. *Animals*, 10, 292.

Nature of contribution by PhD candidate	Conceptualisation, Experimental design, Data collection, Data Analysis, Manuscript preparation, Manuscript Revision
Extent of contribution by PhD candidate (%)	85%

CO-AUTHORS

Name	Nature of Contribution
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Mhairi Sutherland	Conceptualisation, Experimental design, Manuscript Revision, Supervision
Joe Waas	Conceptualisation, Experimental design, Manuscript Revision, Supervision
Al Schaefer	Conceptualisation, Experimental design, Manuscript Revision
Neil Cox	Statistical Analysis
Brendan McCane	Conceptualisation, Algorithm development

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 Lowe, G. L., M. A. Sutherland, J. R. Waas, A. L. Schaefer, N. R. Cox, and M. Stewart. 2019. Infrared thermography - a non-invasive method of measuring respiration rate in calves. *Animals*. 9: 535.

Nature of contribution by PhD candidate	Conceptualisation, Experimental design, Data collection, Data Analysis, Manuscript preparation, Manuscript Revision
Extent of contribution by PhD candidate (%)	85%

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Name	Nature of Contribution
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 Lowe, G.L., M. A. Sutherland, J. R. Waas, N. R. Cox, A. L. Schaefer, and M. Stewart. 2019. Effect of milk allowance on the suitability of automated behavioural and physiological measures as early disease indicators in calves.

Nature of contribution by PhD candidate	Conceptualisation, Experimental design, Data collection, Data Analysis, Manuscript preparation, Manuscript Revision
Extent of contribution by PhD candidate (%)	85%

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Chapter seven is presented in the style of the international journal where a majority of it is planned for submission as: Lowe, G. L., M. A. Sutherland, M. Stewart, J. R. Waas, N. R. Cox, and K. E. Schütz. 2019. Water consumption and drinking behaviour in preweaned, group housed calves provided a high or low milk allowance.

Nature of contribution by PhD candidate	Conceptualisation, Experimental design, Data collection, Data Analysis, Manuscript preparation, Manuscript Revision
Extent of contribution by PhD candidate (%)	85%

CO-AUTHORS

Name	Nature of Contribution
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Mhairi Sutherland	Conceptualisation, Experimental design, Manuscript Revision, Supervision
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Neil Cox	Statistical Analysis

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Appendix one is presented in the style of the Proceedings of the New Zealand Society of Animal Production where it was published as:

Lowe, G. L., K. E. Schütz, M. A. Sutherland, J. R. Waas, M. T. Wilson, and M. Stewart. 2017. Validation of an automated system for monitoring water intake and drinking behaviour in dairy calves. Proceedings of the New Zealand Society of Animal Production. 77:133-136.

Nature of contribution by PhD candidate	Conceptualisation, Experimental design, Data collection, Data Analysis, Manuscript preparation, Manuscript Revision
Extent of contribution by PhD candidate (%)	85%

CO-AUTHORS

Name	Nature of Contribution
Mairi Stewart	Funding acquisition, Conceptualisation, Experimental design, Data Collection, Manuscript Revision, Supervision
Mhairi Sutherland	Conceptualisation, Experimental design, Manuscript Revision, Supervision
Joe Waas	Conceptualisation, Experimental design, Manuscript Revision, Supervision
Karin Schütz	Conceptualisation, Experimental design, Manuscript Revision

Certification by Co-Authors

The undersigned hereby certify that:

- ❖ the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
Mairi Stewart		16/10/2019
Mhairi Sutherland		16/10/2019
Joe Waas		25/10/2019
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Appendix two is an abstract along with the accompanying poster presentation published in the proceedings for the 52nd ISAE conference 2018 as:

Lowe, G. L., M. A. Sutherland, J. R. Waas, and M. Stewart. 2018. Behavioural and physiological responses as early indicators of disease in New Zealand dairy calves. Proceedings of the 52nd Congress of the International Society for Applied Ethology, Prince Edward Island, Canada, August, 2018, p142.

Nature of contribution by PhD candidate	Conceptualisation, Experimental design, Data collection, Data Analysis, Manuscript preparation, Manuscript Revision
Extent of contribution by PhD candidate (%)	85%

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Joe Waas	Conceptualisation, Experimental design, Manuscript Revision, Supervision

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Appendix three is an abstract along with the accompanying poster presentation published in the proceedings of the ISAE Australasia-Africa regional conference 2019 as:

Lowe, G. L., M. A. Sutherland, J. R. Waas, A. L. Schaefer, N. R. Cox, and M. Stewart. 2019. Influence of milk allowance on the suitability of automated behavioural and physiological measures as indicators of neonatal calf diarrhoea (NCD). Proceedings of the International Society for Applied Ethology Australasia-Africa regional conference, Wellington, New Zealand, 2019.

Nature of contribution by PhD candidate	Conceptualisation, Experimental design, Data collection, Data Analysis, Manuscript preparation, Manuscript Revision
Extent of contribution by PhD candidate (%)	85%

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Mhairi Sutherland	Conceptualisation, Experimental design, Manuscript Revision, Supervision
Joe Waas	Conceptualisation, Experimental design, Manuscript Revision, Supervision
Neil Cox	Statistical Analysis
Al Schaefer	Conceptualisation, Experimental design, Manuscript Revision,

Certification by Co-Authors

The undersigned hereby certify that:

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