IMPROVING PRODUCTIVITY AND REPRODUCTIVE EFFICIENCY OF SMALLHOLDER DAIRY COWS IN KENYA

BY

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1. Dr. Claire Windeyer (External examiner)
2. Dr. Jason Stull (Chair)
3. Dr. John VanLeeuwen (Supervisor)
4. Prof. George Gitau
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Abstract

The aims of this research were: 1) to gain a better understanding of the productive and reproductive outputs of the smallholder dairy farms (SDF); 2) to determine the factors and prevalence of selected reproductive diseases potentially affecting these outputs; and 3) to assess the use of sexed semen and reproductive hormones in improving reproductive efficiency of these cows. The research was done in the Meru area of Kenya.

A cross-sectional study was conducted to assess farm management, milk production and reproductive performance of 314 lactating cows. Two hundred smallholder farms were randomly selected from the entire sampling frame of active members of Naari Dairy Farmers Co-operative Society. Farms were visited once within the three month study period, a questionnaire administered to the farmers for risk factors associated with milk yield, and animals were examined for physical and reproductive health. A multivariable mixed linear regression model of log milk/cow/day was constructed; accounting for confounding, cow clustering within farms and days in milk. The average body condition score and milk produced were 2.4 and 6.7 kg/cow/day, respectively, with 43.4% of the cows having been bred or pregnant at the time. Almost a third of all lactating cows were anestrous, and the average days in milk was 300. In the multivariable model, log of milk yield was positively associated with increasing weight of the animals, feeding concentrates (dairy meal) on the last month of gestation, and increasing percentage of land allocated for growing fodder for dairy cows. The indigenous breed category was negatively associated with log of milk yield. Cycling and confirmed pregnant cows both had higher milk production than early pregnant and anestrous cows.

Another cross-sectional study was carried out investigating the seroprevalence of *Neospora caninum* and bovine viral diarrhoea virus (BVDV) and risk factors associated with

seropositivity. Blood from 470 cows and heifers over 6 months of age from the same 200 farms described in the first study was collected and serum was tested using enzyme linked immunosorbent assays. A face-to-face risk factor questionnaire was also administered, animals examined and appropriate information on cow and farm factors obtained during the time of visit. With no history of vaccination of any of the two diseases in this area, the antibody seroprevalence of *N. caninum* was 35.1% (165/470), and that of BVDV antibody and antigen were 47.1% (152/323) and 36.2% (169/467), respectively. There was an 18.5% (87/469) prevalence of co-infection which was taken as animals testing positive of *N. caninum* and also positive on either one of the BVDV tests. Logistic linear and mixed models were used to assess important risk factors, accounting for clustering and confounding.

The final multivariable logistic regression model of seropositivity to *N. caninum* included introducing milking cows often and lending of cattle between farms, while farm dogs eating aborted bovine fetuses and dogs whelping in the farm compound formed an important interaction in this model. Direct contact of pigs was associated with six times higher odds of BVDV antigen seropositivity while age of the test animals formed important interactions with introducing new calves into farms, and whether or not other visiting dairy farmers had access to the cow shed. Parity of the cows was the only important positive risk factor associated with BVDV antibody seroprevalence. Risk factors associated with co-infection of both of these pathogens included parity of the cows, direct contact of dairy cows with dogs and goats, and introducing new milking cows into the farms. The BVDV test results may be partly a function of classical swine fever virus or border disease virus interactions.

In a randomized controlled trial to assess the effective use of sexed semen in various contexts of cows in smallholder dairy farms, we utilized 100 randomly selected farms from the farms used in the previous two studies. The farms were randomly allocated to 5 intervention groups receiving the following interventions: 1) reproduction only; 2) nutrition only; 3) reproduction and nutrition; 4) education only (quasi-control); or 5) nothing (control). Reproductive interventions included hormone therapy for estrus induction (if needed) and education on reproduction while nutritional interventions included supplementation of cows with leguminous shrubs and education on nutrition. Percentages for conception risk, service risk and pregnancy risk were evaluated, and factors affecting hazards of conception were determined using a multivariable Cox proportional hazard model. In groups 1 and 3, breeding using sexed semen was allowed up to two times per cow, once certain breeding criteria related to body condition score, days in milk, ovarian function and vulvar mucus were achieved. In groups 2, 3 and 5, farmers were given one dose of sexed semen to use on any cow with/without meeting breeding criteria.

Overall conception percentages (defined here as conception success for all inseminations done) of 44.0% in cows and 54.5% in heifers were obtained with sexed semen. Conception percentages of 57% and 79% were obtained using sexed semen on cows induced to heat with GnRH (n=98) and prostaglandin F2α (n=29), respectively. Cows in the control group had the lowest service percentage at only 8.5%, and the reproduction group recorded the highest service percentage at 35.5%, along with the highest pregnancy percentage at 12.7%. A multivariable Cox proportional hazards model was fit for calving-to-conception interval, and higher hazards of conception were associated with average body condition score, cows on farms where farmers attended dairy related training, and cows inseminated following spontaneous heat (versus hormone induced heat). In an important interaction in this model, when cows were supplemented with concentrates (dairy meal) during the last month of gestation, higher relative hazard of conception was achieved in cows on farms where leguminous shrubs were used than on farms where no leguminous shrubs were used. Using sexed semen was associated with 2.0 times higher hazard of conception over using conventional semen and this was partly a function of the reproductive interventions assisting cows to be bred sooner with sexed semen when they met the breeding criteria. Overall, sexed semen had lower conception risk than conventional semen, but over time (because of the higher CCI in the conventional semen group), the conception hazard was higher with sexed semen.

These results suggest that farmer education on nutritional and reproductive management (e.g. use of leguminous shrubs, sexed semen and hormone therapy), and biosecurity measures and BVDV vaccination may assist farmers in addressing low milk production, poor reproduction and infectious diseases of SDF in Kenya.

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Dedication

To my son Jordan and husband Gerald, this work is dedicated.

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List of Abbreviations

AI	Artificial insemination
BCS	Body condition score
BVDV	Bovine viral diarrhea virus
CCI	Calving to conception interval
CI	Calving interval
CMT	California mastitis test
CP	Crude protein
CSF	Classical Swine Fever
DIM	Days in milk
ELISA	Enzyme Linked Immuno-sorbent Assay
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
IGF	Insulin growth factors
LH	Luteinizing hormone
NDFCS	Naari dairy farmers cooperative society
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
OD	Optical density
OR	Odds Ratio
PI	Persistently infected
QES	Queen Elizabeth scholar
RFM	Retained fetal membranes
S/D	Sample/Positive ratio
SAG-1	Surface antigen 1
SDF	Smallholder dairy farms
SHD	Smallholder dairy
SRS-2	Surface antigen-related sequence 2
TI	Transiently infected

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Chapter 1 Introduction

1.1 Introduction & Overview

Despite significant progress in reducing global hunger over the last few decades, undernutrition and food insecurity remain serious problems in many countries especially in Africa and Asia (IFPRI, 2016; FAO, 2017). In Sub-Saharan Africa, the number of under-nourished people is even increasing (Foley et al., 2011). Most of the food in Sub-Saharan Africa is produced by smallholder farmers who are ironically, the most affected by food insecurity. An estimated two-thirds of these resource-poor rural farmers keep some type of livestock for different reasons, including producing food, generating income, providing manure, producing power, serving as financial instruments and enhancing social status (Randolph et al., 2007). Dairy production in Kenya accounts for an estimated 33% of the agricultural Gross Domestic Product, and is dominated by smallholder farmers. These smallholder dairy farms have fewer than five cows, are mainly zero-grazed due to small land parcels owned, and are challenged in terms of growing and preserving fodder, leading to sub-optimal milk outputs (Bebe et al., 2003). Several factors have hampered the productivity, growth and development of the dairy industry in Kenya, namely feeding, husbandry, animal diseases, marketing and reproductive disorders (Okumu, 2006).

Attempts to upgrade the dairy breeds in developing countries have been through cross-breeding with exotic high-yielding breeds to increase milk production, but intensive nutritional management has remained a big drawback to this genetic upgrade (Usman et al., 2013). Smallholder farmers on small parcels of land have been noted to feed their cows with crop by-products which are of less nutritional value, and an increasing reliance on purchased feeds, both concentrate and forage, has in turn made it harder for these resource-constrained farmers to afford high milk production. Dairy-based Cooperative groups have supported farmers in terms of resources, knowledge on dairy farming, veterinary and extension services, credit facilities, and stable market prices for their milk. However, reproductive performance of the cows has continued to remain poor, with a majority of the farmers not achieving a calf/cow/year, and milk production has remained under 10 kg/cow/day (VanLeeuwen et al., 2012).

The low productivity and output of Kenyan smallholder dairy cows are associated with constraints such as diseases, low quality and quantity of nutrition and poor management (Msangi, 2001). Several diseases have also been associated with reproductive wastages, among them being bovine viral diarrhea and neosporosis (Yang et al., 2012), with other contributing factors being geographical and environmental constraints and poor management (Fordyce et al., 2014). All of the above factors have made it hard to achieve the reproductive goal of a calf/cow/year hard in the smallholder dairy setting in Kenya. In order to achieve this goal, SDF will require strategies related to cost-effective farm-grown feeds (such as leguminous shrubs and crops) and education on feed preservation, especially silage and hay-making.

Information on reproductive effects of pathogens in Kenyan cows is still limited. Current scientific literature on nutrition and management practices for smallholder dairy farms is mainly observational in nature, and little has been done in research trials in the field setting. Similarly, there is a big need for replacement heifers in these SDF as the cows are not reproducing as often as they should, and the male calf is undesired in this dairy setting. Use of sexed semen may be one way of replenishing the population of heifers.

Therefore, the objectives of this research thesis on smallholder dairy farms in Kenya were: (1) to determine the production (milk yield) and reproductive performance of SDF cows, and their factors; (2) to assess the seroprevalence and risk factors of Bovine Viral Diarrhea Virus and *Neospora Caninum* in smallholder dairy cows and heifers; and (3) to determine the feasibility of use of sex-sorted semen on reproductive efficiency enhancement in SDF and to inform on its criteria of use to maximize pregnancies on such cows.

1.2 Literature review

The literature review is devoted to specific aspects of small-scale dairying in Kenya related to the thesis objectives, with some focus on the highland region of Mount Kenya where the research took place. It consists of subchapters dealing with an overview of smallholder dairy farming and its impact on poverty reduction in Kenya, productive and reproductive performance of SDF in Kenya, reproductive wastages and its causes (body condition score, endometritis and other infections such as BVDV and *N. caninum*), and methods to replenish the pool of replacement heifers, and in particular, through the use of sexed semen.

1.2.1 Smallholder dairy farming and poverty reduction in Kenya

Poverty may be regarded as a lack of sufficient means or income for a minimum level of living: food, shelter, and clothing (Ravallion & Lokshin, 2005). In Kenya, the proportion of the population living in poverty is higher in rural areas, at 49.1%, compared with 33.7% in urban areas (Haughton & Khandker, 2009). National surveys set the rural poverty line at Kenya shillings (KShs) 1,562 per adult per month, and a corresponding poverty line of 2.930 KShs per adult per month for the urban areas in 2005 (Suri et al., 2008). At the current exchange rate, the national poverty line for the rural areas is lower than the international poverty line of one dollar a day.

Kenya took great strides towards achieving the millennium development goal on poverty reduction, which required it to reduce the number of those living in poverty by half by the year 2015. Among the ways devised to achieve this goal was focusing on improving agriculture and rural livelihoods. Reports indicate that even though this goal has not been achieved yet, due to the constantly increasing population and erratic weather changes affecting the seasons, there has been a notable reduction of those living in abject poverty from 41.4% to 32.5% by 2017 (The World Bank, 2018). One of the strategies that have been employed has been improvement of the dairy industry.

Smallholder dairying contributes significantly to poverty alleviation and reduction of malnutrition, particularly in rural areas. Income from milk sales is of particular value as it is generated daily and more likely to be used for increased family wellbeing than the periodic incomes derived from other agricultural activities (Mngofi, 2009). Crop-dairy farming systems, in which crop and dairy production is integrated on the same farms, is an important support to a lot of rural families and has added a great contribution to the domestic milk production supply (Delgado et al., 2001). At the household level, dairy production contributes to food security and spreading of risks and generated income, it supports crop production through traction and manure, and it is a means to accumulate capital assets for emergency cash needs (ILRI, 2000). Livestock are often the only livelihood option available for the landless (Nicholson et al., 2004). Kenya is prominent among developing countries for integrating dairy cattle into smallholder farming systems, particularly in the highlands where the climate is suitable for dairy cattle, but milk market opportunities vary, depending on the size of local human populations and marketing infrastructure.

Livestock ownership currently supports and sustains the livelihood of an estimated 675 million rural poor people in the world (FAO, 2003). The smallholder dairy industry in Kenya, with 1-2 milking cows per farm typically, represents the fastest growing source of farm income, with farms earning US$300 annually, on average, from dairying (Haggblade et al., 2004). With an estimated annual per capita milk consumption of 145 liters, Kenya's milk demand is five times higher than neighbouring East African countries. Dairy products also make up the largest share of food expenditure in Kenyan household budgets. Milk in Kenya is primarily produced from an estimated 1.8 million smallholder dairy farmers, making them the largest dairy industry in Sub-Saharan Africa. With the proportion of arable land in Kenya reported to be only 10.2%, dairy production areas are characterized by high population densities and small farm sizes (Mngofi, 2009). These small farm sizes create expansion constraints on the farmers, therefore improvements to household income must come from improved productivity, as discussed in the next section.

1.2.2 Production performance of smallholder dairy cows in Kenya

The Kenyan dairy cattle population is estimated at 4.3 million with an estimated milk production of 3.43 billion liters annually (Ministry of Agriculture, 2015). Eighty percent of these dairy cattle are owned by smallholder farmers and produce over 56% of the total milk volumes. The remaining 20% of cattle consists of cattle from large dairy farms and indigenous herds, mainly kept by the pastoralists and produce 44% of the total milk volume (Odero-Waitituh, 2017). The estimated milk production per cow per year has been reported to be as low as 1300 kg/cow/year (Omore et al., 1999) and as high as 4575 kg/cow/year (Mugambi et al., 2014) in the high potential areas, depending on the time, place and population assessed. This higher estimate of production was attributed to the availability of high quality feeds, exotic animals breeds, and well-managed production systems, however even the higher estimate is low compared to the 9000 kg/cow/year often observed in developed countries of the world (Odero-Waitituh, 2017).

Several general categories of dairy farming have been defined for smallholder dairy farms in Kenya, according to production and husbandry characteristics. Open grazing with Zebu cattle, which uses very little purchased inputs or services and with very little milk yields realized from this system, is similar to a grazing beef production system. Another system is open grazing using crosses between indigenous and exotic cattle and some inputs, such as minerals and veterinary services, to increase milk yields and protect the animal's health. Partial grazing systems use grazing to supplement stall feeding of Napier grass or other green feeds. This system allows the animals some time of the day where they are grazed on the field and confined the rest of the time. The fourth system is ''zero-grazing" which is practiced in areas with high human population density where land is scarce. Cows are fed through a cut-and-carry system in which all the feed material is brought to cows kept in stalls. With control over feeding management, this fourth system has the highest milk yields recorded, and the farmer has to manage the manure which is in turn used on crop farming (Randolph et al., 2007). Some of the Kenyan smallholder dairy farmers sell part of the daily milk, although others keep a cow solely to provide for family consumption.

Development of smallholder dairy production systems in the Kenyan highlands has been marked by declining farm size, upgrading to exotic dairy breeds, adapting to partial grazing and zero-grazing, and increasing reliance on purchased feeds, both concentrates and forage (Staal et al., 1997). Growing sufficient fodder for cows is becoming increasingly challenging with reducing land sizes due to subdivisions through intra-family inheritance, causing many farmers to prefer to grow crops to feed the family first. This has led to cattle being increasingly fed on community pasture or crop wastes (e.g. dry maize stovers, bean pods, green gram pods and banana stalks) that typically have high fibre content and very low energy and crude protein content (VanLeeuwen et al., 2012).

The performance of cattle in the smallholder dairy industry in Kenya can be characterized in terms of low milk yield constrained by a number of factors, primarily feeding, genetics and diseases (Muriuki, 2003). Good quality forage and improved pasture may only provide sufficient nutrients for maintenance and production of approximately 5 kg/day of milk (Ngongoni et al., 2006). The high cost of concentrates and the declining milk-to-concentrate price ratio makes it financially difficult for smallholder dairy farmers to feed adequate concentrates regularly, often resulting to low productivity (Ngongoni et al., 2006). Growing and storing cattle feeds which have good nutritional value, such as leguminous shrubs of the genus *Calliandra* or *Sesbania*, would be a good alternative to improving milk production with minimal need for purchased feeds, however there is limited awareness among dairy farmers about this option (Richards, 2017).

Higher lifetime milk production ensures better economic results per cow. Economic efficiency is mostly as a result of achieved milk production and longevity (Novakovic et al., 2014). Age at first calving, the length of productive life and milk yield in certain lactations are factors that affect lifetime milk production (De Vries, 2006). The lactation curve of a cow is made up of changes in daily milk yield during early (increases) and later (decreases) periods of lactation. Factors such as breed, nutrition, calving season, farming system, genetic predisposition of individual animals, mammary gland diseases, frequency of milking and length of the previous dry season all affect the time it takes for a cow to reach peak yield and the shape of the lactation curve (Litwinczuk et al., 2016). Delayed age at first calving, lower peaks of milk produced and longer calving intervals have made the lifetime milk production in the smallholder dairy cows in the tropics to be less profitable. It is beneficial to both milk yield and productive life for heifers to calve at less than two years of age but not earlier than 21 months (Nilforooshan & Edriss, 2004). King et al. (2006) recorded annual milk yield in smallholder dairy cows of Kenya as 1525 kg and reported that all the profits realized came from culled animals. Increased feed quality results in higher milk yield, increased productive life by reducing age at first calving and days open of crossbred cows feed tropical forages (Vargas et al., 2001).

Also, few studies have looked into cow comfort and its effects on milk production in the smallholder dairy settings (Kathambi, 2018; Richards, 2017). Small alleyways, low neck position, sharp objects in the cow shed and dilapidated cow sheds were all found to contribute to poor cow welfare and subsequently led to cows having injuries in their bodies in Kenyan intensive dairy farms (Aleri et al., 2012). In Malawi, Kawonga et al. (2012) found that cows affected by poor welfare had reduced milk productivity, lower body condition scores and long calving intervals.

1.2.3 Reproductive performance in smallholder dairy cows in Kenya

Reproductive health problems result in considerable economic losses to the dairy industry and are the main cause of poor productive performance in smallholder dairy farms (Kumar et al., 2014). Both infectious and non-infectious causes of reproductive failures exist. Among the major problems that have a direct impact on reproductive performance of the dairy cows are abortions, dystocia, vaginal and/or uterine prolapse, retained fetal membranes, endometritis, pyometra, anestrus, silent heat, cystic ovarian disease, and repeat breeder (Ibrahim, 2018). Other causes contributing to the economic losses from reproductive problems arise from high costs of medication, lost milk production, reduced calf crop and early culling of potentially useful cows.

Age at first calving, number of services per conception, pregnancy percentage and calving interval are important reproductive parameters which are crucial for determining the profitability of dairy productions. In particular, prolonged calving intervals and age at first calving for heifers have resulted in a lot of reproductive wastages on Kenyan smallholder dairy farms , and most cattle farmers in Kenya do not realize the extent of economic losses that can occur through reproductive failure in their cattle (Ibrahim & Olaloku, 2000). These parameters will be discussed in turn.

A substantial delay in the attainment of sexual maturity may mean a serious economic loss, due to an additional, non-lactating, unproductive period of a cow over several months (Mukasa-Mugerwa, 1989). Puberty can be delayed due to environmental and nutritional factors. The reduction of luteinizing hormone (LH) secretion when feed is severely restricted can be reversed with proper feeding, leading to the return in normal cyclicity (Marie, 1998). A wide range of age at puberty has been recorded in *Bos indicus* cattle in the tropics (16-40 months) (Mukasa-Mugerwa, 1989) depending on feeding and environmental conditions; there has been a recorded association between age at first calving with seasonality whereby seasonal fluctuation of feed quality and availability affects the rates of growth of heifers from a young age. Animals born in the dry season are weaned in the wet season in which there is abundant and good quality pastures, hence these weaned calves get better nutrition and grow faster than those born in the wet season and are weaned in the dry season (Chenyambuga & Mseleko, 2009). With good nutrition, it is expected that heifers would exhibit fast growth and attain higher weight at relatively younger ages. The optimal age at first calving under tropical conditions is between 24-

36 months, and these heifers achieve the highest first lactation yield, highest estimated life-time production, and highest proportion of total life spent in active milk production (Wathes et al., 2008).

Good calf rearing practices is an integral part in realizing availability of future replacement stock as these practices directly impact on their survival and future milk production. It is recommended that preweaned calves be fed on milk quantities equivalent to 10-15 percent of their birth weight on a daily basis but this recommendation is not achieved by most Kenyan farmers, with an equivalent of 7-10 percent birth weight being recorded, leading to poor growth rates of these calves (Situma, 2013, Lanyasunya et al 2006). Calf management was not explored in more detail in this thesis because it formed a major part of a companion thesis that is published in University of Prince |Edward Island by Dennis Makau. Calving interval (CI), the period between two consecutive calvings expressed in days or months (Flores, 1971), has been reported as the best summary indicator of a cow's reproductive efficiency. Calving interval is a consequence of a voluntary waiting period, active breeding period, and gestation period (Stevenson, 2001). If anything were to lengthen any of the above-mentioned periods, then CI is prolonged. To achieve the recommended 12-month CI, cows need to be inseminated shortly after their peak milk production, however at this time, cows are challenged metabolically. With increases in milk production during the first few months after calving, the risks of fertility problems such as silent heat and ovarian cysts increase (Harrison et al., 1990). The recommended calving interval usually is not accomplished by most smallholder dairy cows in Kenya (Msangi et al., 2005). In the Kenyan smallholder cows, calving intervals of 568-681 days have been recorded (Odima et al., 1994), while Bebe (2004) recorded 355 average days open in another population of Kenyan smallholder dairy cows.

Pregnancy percentage (commonly known as “pregnancy rate”) is defined as the percentage of eligible cows that become pregnant within a given time frame (usually 21 days). Pregnancy “rate” is expressed as a percentage (Overton, 2009), and therefore is not a rate but a risk or percentage, and therefore is subsequently called “pregnancy percentage”. Pregnancy percentage is dependent on the percentage of inseminations that result in a pregnancy (commonly known as “conception rate” but is not a rate but a percentage, so it is subsequently called “conception percentage”) and the percentage of cows eligible to be bred and to show signs of estrus and are inseminated (commonly known as “estrus detection rate” but is not a rate but a percentage, so it is subsequently called “service percentage”). Service percentage is obtained as the proportion of cows that were inseminated from the cows that were available for insemination, conception percentage is obtained as the proportion of cows that conceived from those that were inseminated. Reduced service percentages (<50%) can emanate from fewer cows coming in heat or farmers spending less time observing heat and this will in turn negatively affect the pregnancy percentage achieved in these animals (Smith et al., 2012). A conception percentage above 50% is considered good when using AI, and therefore a pregnancy percentage of 25% is also considered good (50% * 50%).

The number of services per conception is defined as the number of times an average animal is inseminated to obtain a successful conception (Radostits et al., 2001) and it depends largely on the breeding system used. Services per conception are higher when uncontrolled natural service is used and low when hand-mating and AI is used. A study in large scale farms in one peri-urban area of Kenya recorded 2 services per conception in cows in the study (Gitonga, 2010). No administration of postpartum treatment and absence of postpartum disease were associated with less than 2 inseminations per conception, while cows that had a uterine diameter of more than 5 cm was associated with more than 2 inseminations in the same study. Averages of 3 services per conception have also been recorded in Kenyan cows in the smallholder dairy production systems (Odima et al., 1994; Mutiga et al., 1994; Mutasa & Munyua, 1992)

Overall, the performance of adult cattle in the smallholder dairy industry in Kenya can be characterized in terms of late age at first calving, low pregnancy percentages, and long calving intervals, which have been attributed to low levels of nutrition and management (Ongadi, 2014; Omore et al., 1998).

In Tanzania and Kenya, poor nutrition, low body weight, mineral deficiency, and high levels of dystocia and retained placenta have been found to cause long calving intervals (Lanyasunya et al., 2006; Swai et al., 2005). Diseases and poor management factors, such as failure to detect heat, were also associated with longer calving intervals in a later study (Swai et al., 2007). These factors are discussed in the next three sections.

1.2.3.1 Uterine disease leading to poor reproductive performance in SDF

Retained fetal membranes (RFM) is a lack of expulsion of fetal membranes within the first 24 hours after calving (Bekana et al., 1994; McNaughton & Murray, 2009). Puerperal metritis is characterized by an enlarged, flaccid uterus, a fetid, watery red-brown discharge and usually fever and other signs of systematic illness within 21 days post-calving, whereas clinical metritis is typically a function of purulent uterine discharge but no systemic signs of illness during this time frame (Sheldon et al., 2006). Endometritis is an inflammation of the endometrial wall, which can be either clinical or subclinical. Clinical endometritis is defined by the presence of purulent vaginal discharge detectable at 21 days or more postpartum, or mucopurulent discharge detectable in the vagina after 26 days postpartum (Dubuc et al., 2010a). Subclinical endometritis is characterized by inflammation of the endometrium, measured by the relative presence of polymorphonuclear leukocytes in a uterine sample in the absence of clinical disease (Sheldon et al., 2006). In dairy cows, the incidence of RFM usually ranges from 5-15% (Gilbert, 2005), and metritis (puerperal and clinical) ranges from 15-20% (Gilbert, 2016), while that of clinical endometritis varies between 10-20% (LeBlanc et al., 2002a; Dubuc et al., 2010b). The incidence of subclinical metritis depends on the cut-off for diagnosis and the time after parturition, and varies between 37-74% in animals (Gilbert et al., 2005).

Abortions, dystocia, twinning, induced/early parturition, male offspring and stillbirths have all been recorded as risk factors contributing towards RFM, among other factors (Lima, 2018). Retained fetal membrane is a risk factor for puerperal metritis, clinical endometritis and pyometra. Metabolic disorders, such as ketosis and selenium, calcium and phosphorous mineral deficiencies, reduced feeding in the last 3 weeks of gestation, and increased negative energy balance (NEB) were all found to be contributory towards RFM, metritis and clinical endometritis (Lima, 2018). Proper nutrition during the transition period and hygienic calf delivery have been advocated to reduce the losses of productive and reproductive life of the cows from RFM (Ibrahim, 2018).

Although manual removal of the RFM remains a common practice in Kenya by animal health professionals, many studies have failed to show any benefit of this approach on reproductive performance or milk production, and more severe uterine infections occurred when compared with more conservative treatment (Bolinder et al., 1988). In a review by (Gilbert, 2016), gonadotrotropin releasing hormone (GnRH), oxytocin and prostaglandin F2α were not found to be beneficial to placental release or future reproductive performance, and in turn, Eiler & Hopkins, (1993) found that collagenase might aid in the detachment of the caruncle-cotyledon bonds in cows with RFM. The challenge in the Kenyan dairy industry is the education level of the different types of animal health practitioners that attempt manual removal of the placenta at three days postpartum, and the widespread belief among farmers that removal of the placenta at this time is helpful. Attempts at removal of RFM causes damage in the uterus as a result of the forced detachment of non-detached caruncle-cotyledon units, leading to bleeding and additional pain. Even though cows diagnosed with RFM are more likely to develop metritis, the results of intrauterine antimicrobial use to treat otherwise healthy cows with RFM remains controversial (Lima, 2018) and in Kenya, intrauterine antibiotics at the time of attempted RFM removal are often used as a way to mitigate or prevent the possible sequelae of metritis. Monitoring temperature, behaviour, appetite and milk production of a cow with RFM until the placenta is expelled from the uterus, usually at 7-10 days postpartum, is the recommended practice for dealing with RFM, with parenteral antibiotics and supportive therapy being provided to those cows developing systemic signs of metritis (Gilbert, 2018)

A wide variety of therapies for endometritis have been reported, including systemically or locally administered antibiotics, or systemically injected Prostaglandin F2α. The general principle of therapy of endometritis is to reduce the load of pathogenic bacteria and enhance the uterine defence and repair mechanisms, and thereby halt and reverse inflammatory changes that impair fertility (LeBlanc, 2008). Intrauterine administration achieves higher drug concentrations in the endometrium but little penetration to the deeper layers of the uterus. Intrauterine tetracycline, penicillin, cephapirin, gentamicin, spectinomycin, sulfonamides, diluted lugol's iodine, povidine iodine and chlorhexidine have all been used, even though most of them are not approved for intrauterine use (Sheldon & Noakes, 1998; Thurmond et al., 1993; LeBlanc et al., 2002). Lugol's iodine and oxytetracycline are irritating and are reported to cause coagulation necrosis of the endometrium (Gilbert & Schwark, 1992). Use of intrauterine infusion of lugol's iodine has been a common practice in cases of clinical endometritis and pyometra in Kenya because it has no withdrawal period for milk, and it is cheap, easily accessible and easy to administer. To exert an antiseptic effect, a high concentration of free iodine released from the iodine is essential. Two percent povidone iodine was tested and found to clear the uterine infection in cows with severe clinical endometritis in Japan although it rendered no significant differences in reproductive parameters (days to first artificial insemination (AI) service, first AI service conception percentages, number of AI services per conception and days to conception) between treated and untreated group (Mido et al., 2016). Infusion of 1% lugol's iodine pre- and post-service was found to improve conception percentages, reduce services per conception and reduce days open in repeat breeder cows in Sudan (Faisal & Elsheikh, 2014). Intrauterine cephapirin improved first service pregnancy risk in cows that had metritis and both clinical and subclinical endometritis compared to non-treated cows in Canada (Denis-Robichaud & Dubuc, 2015). In Kenya, penicillin and tetracycline intrauterine pessaries are available, and most animal health practitioners use them but with poor evidence of effectiveness towards reducing days to conception.

1.2.3.2 Ovarian causes of poor reproductive performance in SDF farmers

There are a number of reasons related to ovarian function that can lead to poor reproductive performance. True anestrus can be defined as period of ovarian quiescence. This condition can be a result of insufficient release or production of gonadotropins to cause folliculogenesis, or it may reflect the failure of the ovaries to respond to the gonadotropins. During anestrus, transrectal ovarian palpation reveals small, flat, smooth ovaries. A cow in anestrus will have virtually unchanged ovaries in subsequent examinations, whilst a cow in late diestrus or early metestrus will eventually have a distinct palpable CL when palpated over time (Abraham, 2017). Equine chorionic gonadotropin hormone, GnRH, progesterone and estrogens have been used somewhat successfully to treat anestrus in dairy cattle (Cunningham, 2002).

Silent heat is also a possible reason for poor reproductive performance. For silent heat, there is normal cyclic ovarian activity but the cow is not showing the normal behavioural signs of estrus. In the author's experience, sub-estrus or silent heat is a common finding in Kenyan smallholder dairy cows. Most farmers indicate only seeing the mucus string hanging on the vulva, which complicates determination of the start of standing heat and thus the time of insemination. Transrectal palpation of the ovaries at this time can be useful to determine whether the cow has ovulated and if it is still a candidate for insemination, or to advise the farmer on when next to observe for the successive heat if hormones are not used to induce heat. When ovulation occurs in the absence of observed estrus signs, it is more likely to be a result of failure of observation due to short duration of estrous behaviour rather than poor detection. Poor heat detection will be discussed in the next section.

The primary reason for anestrus and silent heats is negative-energy balance postpartum (Madhuri et al., 2017). Development of a pre-ovulatory follicle from early antral stage to ovulation takes at least 40 days in the cow. Negative energy balance can affect follicular and luteal development and the quality of the oocyte. Ideally, a cow should ovulate for the first time around 20-30 days after calving, which then allows time for one or two normal estrous cycles before insemination prior to the end of a 60 day voluntary wait period. Thus, most of the follicles that are due to ovulate at the start of the service period will undergo their early development during the NEB nadir. A prolonged NEB period has been associated with a greater incidence of irregular cycles that can increase the interval to first service and reduce conception percentages

(Wathes et al., 2003; Taylor et al., 2004). The initiation of follicular waves after calving occurs regardless of NEB status, but development of the pre-ovulatory size and likelihood of ovulation is decreased when body condition is poor (Wathes et al., 2007). Insulin growth factor (IGF) 1 and 2 enhance the ovarian action of gonadotropins and stimulate follicle cell proliferation and steroidogenesis. Circulating IGF-1 concentrations decrease sharply in the first week after calving and the extent of these decline influences both the interval to first ovulation and interval to conception. Insulin acts on the antral granulosa cells to help in estradiol production. Severe undernutrition can cause insulin levels to fall below normal physiological levels, leading to adverse effects on fertility (Wathes et al., 2007). Undernutrition has also been linked to the inability of the hypothalamus to sustain high frequency LH pulses by the pituitary gland. Luteinizing hormone pulse frequency was shown to be positively correlated with energy balance and negatively correlated with blood non-esterified fatty acids (NEFA) concentration (Kadokawa et al., 2006). Higher NEFA concentrations in blood have been shown to hamper oocyte maturation and developmental competence in-vitro (Leroy et al., 2005).

In Kenya, unless supplemented with a high protein and high energy concentrate, dairy cattle grazing natural pasture typically lose body condition postpartum, with subsequent ovarian activity ceasing when the cow loses 20-30% of their mature weight due to undernutrition (Kaitho et al., 2001). However, keeping cows in stalls (zero-grazing) was also associated with longer days open compared to cows that were partially grazed or freely grazed, unless the feed provided to the zero-grazed cows had good protein and energy content (Bebe, 2004). Mineral deficiencies, particularly phosphorus, have also been associated with anestrus and silent heats (Yasothai, 2014).

Cycling cows can also have impaired conception percentages through timing of breeding relative to ovulation – either delays in ovulation or improper breeding timing. Delayed ovulation happens as a result of delayed luteinizing hormone surge, and is generally assumed to be one of the causes of failure of conception in cyclic non breeders (Abraham, 2017). Transrectal examination can help in the diagnosis of this condition, whereby a single large follicle is detected in the same ovary on two successive examinations, one at peak estrus and another 24-36 hours later. Gonadotropin releasing hormone administration at the first signs of heat can address delayed ovulation because it causes a rapid rise in follicle stimulating hormone (FSH) and LH within 30-60 minutes of administration, and then these two hormones return to pre-injection levels within four hours. Improper timing of breeding is addressed in the next section.

1.2.3.3 Estrus management and reproductive performance

Estrus detection has been cited as the most important factor affecting the reproductive success of artificial insemination programs (SelectSires, 2012). Failure to detect estrus is the most common and costly problem of AI programs and the major limiting factor of reproductive performance on many dairy farms. As heat detection in early lactation can be difficult due to short heats/night heats/silent heats, a diagnosis of failure to cycle has to be investigated by transrectal palpation to differentiate between heat detection problems and anestrus problems from inactive ovaries (Baumgard et al., 2015).

Proper identification of the timing of estrus is difficult since peak estrus activity often occurs at night, early morning, or late evening, and determination of the actual onset of standing heat may be difficult without 24 hour observation. Careful checking of heat during these times is therefore required and additional checks during the day to allow a 90% heat detection percentage. A cow standing to be mounted is the most accurate sign of estrus. It is considered the primary sign of estrus in cows (SelectSires, 2013). Secondary signs of estrus are caused by elevated levels of estrogen on the day the animal is in estrus. They include reduced milk production, clear mucus from the vulva, swollen vulva, bellowing and frequent urinations, grouping together, rubbed marks and dirt on the back of the cows and chin resting and rubbing (SelectSires, 2012). These secondary signs can sometimes also be caused by events other than estrus and thus a single secondary sign of estrus should not be enough to make the decision to inseminate. It is advisable to inseminate the cow 12-15 hours after standing heat.

There is a weak antagonistic relationship between milk yield and expression of heat, with higher yielding cows showing slightly weaker signs of heat than lower yielding herd mates (Gavelis et al., 2018). Cows with sore feet or legs or that have poor structural conformation exhibit less mounting activity. Lame cows may stand to be mounted even when they are not on heat because it may be too painful to escape (Diskin & Sreenan, 2000). Cows have been seen to be on estrus longer and to have more total mounts and stands when they were observed on dirt than when observed on concrete floors, demonstrating the importance of non-slip flooring (Britt et al., 1986).

Most of the estrus signs are better expressed when the cows are grouped together and are in contact with other dairy cows. Cows that are themselves in heat, coming in heat or were recently in heat are most likely to mount a cow in heat compared to cows in mid-cycle or pregnant cows, although the latter cows can show interest and mount cows in heat. Housing arrangements where adequate space allows cow-to-cow interaction and a density that allows normal heat activity should be encouraged (Diskin & Sreenan, 2000). Observations of estrus are more difficult on smallholder dairy farms in the tropics due to small farms having only one cow, and from anestrous resulting from poor nutrition and/or intensive suckling. Also, the estrus period has been demonstrated to be short (10-12h), and signs of estrus are less pronounced and mainly at night when farmers are less likely to be observing the cows (Goopy & Gakige, 2016).

1.2.3.4 Diseases leading to early embryonic death or abortion

Embryo survival is a big factor affecting production and economic efficiency in all systems of meat and milk production by ruminants. The majority of embryonic losses occur in the first 3 weeks of pregnancy, particularly between days 7 and 16 of pregnancy (Minten et al., 2013). The main reproductive diseases associated with abortion in cattle are: brucellosis (which has been eradicated in some countries), vibriosis *(Campylobacter fetus)*, leptospirosis, trichomoniasis, pestiviruses (bovine viral diarrhea virus) and neosporosis. Good record-keeping of abortion patterns and reproductive performances of the cows in a farm is one way of being able to identify the problem that is causing the losses. Neosporosis, brucellosis and leptospirosis cause mid gestation abortions, while early embryonic losses (often recognized only when cows are found open on pregnancy testing) occur as a result of pestiviruses, vibriosis and *Tritrichomonas*. Sporadic abortions may indicate that the herd is partly immune to the disease and that the disease has been present for some time. An abortion storm is more likely to indicate neosporosis or brucellosis or a recent introduction of a pestivirus to a previously naïve farm. In industrialized countries, a specific diagnosis is obtained for only 23-46% of the aborted fetuses submitted to diagnostic laboratories (Walker, 2005).

In Kenya, most veterinary laboratories only test aborted fetuses for brucellosis, and therefore the specific diagnosis of the cause of abortion being brucellosis hovers around 10% (Okumu, 2014). Also, getting an aborted fetus to the laboratory can be a challenge due to poor access to a central laboratory, as well as the cost of tests, and the challenges of proper preservation and transport of the fetus. As a result of these logistical issues, only a very small percentage of aborted fetuses in the SDF in Kenya ever receive a diagnosis of the cause of abortion.

1.2.3.4.1 Neospora caninum

The protozoan parasite known as *Neospora caninum* has only become recognized as an important cause of fetal death, mummification and abortions in cattle in the last couple of decades. It had for many years been misdiagnosed as *Toxoplasma gondii* due to morphological similarities, and was ignored as a potential abortifacient for cattle until the last two decades (Silva et al., 2007). *Neospora caninum* is primarily a disease of cattle and dogs and is not considered zoonotic, whereas toxoplasmosis is a serious disease of humans, sheep and many other warm-blooded animals. Wild and domestic canids are the definitive hosts of *N. caninum*, and cattle are among the list of possible intermediate hosts, which also includes other wild and domesticated ungulates (Dubey & Schares, 2006).

Cattle become infected with *N. caninum* by ingesting feed and water contaminated by oocysts shed in dog feces or by congenital infections (Dubey & Schares, 2011). This parasite has been reported to be the most important cause of abortion in beef and dairy cattle populations worldwide (Dubey & Lindsay, 1996; Silva et al., 2007; Collantes-Fernández et al., 2006; Boger & Hattel, 2003). Abortions in cattle due to *N. caninum* occur after 3 months of gestation, though they are most common from 5-6 months of pregnancy. There could also be fetal resorption, mummification, autolysis and stillbirths. Calves born alive can be either apparently healthy but persistently infected (Dubey & Schares, 2006) or can have neuromuscular defects. Incidence of abortions is often repeated in subsequent pregnancies, and congenital transmission from seropositive dams to their offspring is considered the primary method of transmission in the epidemiology of neosporosis on most farms (Dubey et al., 2007). Cows congenitally infected with *N. caninum* have been demonstrated to have increased calving-to-conception interval and in turn, a longer calving interval (Canatan et al., 2014).

For a definitive diagnosis of *Neospora* infection, a necropsy of the fetus or calf is necessary. Samples from the brain, heart and liver should be collected and examined for histopathologic changes. Since there are no pathognomonic gross or histopathologic lesions of neosporosis, the presence of *N. caninum* on the tissue must be identified through immunohistochemistry for diagnosis to be established. Serology can reliably test for exposure and infection, looking for the major antigens of *N. caninum* (e.g. surface antigen-1 (SAG-1)) and surface antigen-related sequence 2 (SRS-2), which differentiates this organism from related apicomplexan group protozoa (*Neospora hughesi* and *Toxoplasma gondii*) (Okumu, 2014).

In Sub-Saharan Africa, the prevalence of *N. caninum* antibodies in dogs in Tanzania was 22% (Barber et al., 1997), while in the same study, no *N. caninum* antibodies were detected in dogs in Kenya. In a more recent study (Okumu, 2014), 26.0% and 17.9% of cows and farm dogs, respectively, were recorded as test-positive in Kenya. In that same Kenyan study, 51.6% of 29 cows that had pregnancy losses also had four-fold increase of antibodies for *N. caninum* when pre- and post-loss sera were compared, suggesting that *N. caninum* was involved in the pregnancy loss. *N. caninum* antibodies have also been detected in 31 wild carnivore species from Maasai-Mara reserve in Kenya (Ferroglio et al., 2003), but studies looking at prevalence of *N. caninum* and its associated risk factors in smallholder dairy cows in Sub-Saharan Africa are very limited.

1.2.3.4.2 Bovine Viral Diarrhea Virus

Bovine viral diarrhoea virus (BVDV) is a very important viral infection of cattle in many countries because of its relatively high prevalence of infection, its broad array of detrimental health effects on cattle (e.g. reproductive, gastrointestinal, pulmonary, and immunosuppressive), and its ease of transmission. Bovine Viral Diarrhea Virus is easily transmitted by direct contact with bodily fluids and milk of infected cattle or by *in utero* infection of fetuses (Khodakaram-Tafti & Farjanikish, 2017).

Reproductive losses may be the most economically important consequence associated with BVDV infection, and there are indications that the incidence of BVDV-related reproductive losses are increasing in the United States (Evermann & Ridpath, 2002). In addition to reduced reproductive efficiency, BVDV uses the reproductive system to maintain and spread itself in the cattle population by inducing immuno-tolerance following fetal infection by non-cytopathogenic strains between 45- and 125-days gestation, resulting in the birth of calves persistently infected (PI) with the virus. Persistently infected cattle are the major source of virus spread both within and between farms (Grooms, 2006).

Infection of naïve pregnant cows and heifers in different stages of fetal development leads to different sequelae. Infection from day 9-45 of gestation results in reduced apparent conception percentages and infertility, early embryonic death, and infertility. Infections between days 45-125 of gestation result in fetal death, abortions, mummification and birth of persistently infected (PI) calves, and to a limited degree, some teratogenesis, intrauterine growth retardation and calves with congenital defects especially of the nervous system. Mid-gestation infections (day 125-180) often are characterized with high incidence of congenital abnormalities presenting as alopecia, pulmonary hypoplasia, thymic aplasia, ataxia, cerebellar hypoplasia and other central nervous system defects, or simply retarded growth and increased average age at first calving in affected herds. Late gestation transplacental infections are not associated with a significant level of congenital deformities (Fray et al., 2000). This stage of infection is usually followed by the birth of a clinically normal calf with high levels of pre-colostral neutralising antibodies (Grooms, 2006). Bovine Viral Diarrhea Virus has also been shown to cause depressive effects on ovarian function in infected heifers by disrupting gonadal steroidogenesis and impairing the quality of oocytes produced (Silva-Cardoso et al., 2017).

Bovine Viral Diarrhea Virus sero-prevalence of up to 70-81% have been reported in South American dairy cattle herds (Gogorza et al., 2005; Stahl et al., 2006) and between 21-98% in European and American unvaccinated herds (Waldner, 2005; Ahmad et al., 2011). The infection has also been detected in a variety of domestic and wild ruminants; in small Zebu cattle in the coastal area of Kenya, a prevalence of 45.8% was reported (Kenyanjui et al., 1994). Okumu (2014) found BVDV infection to be the most common abortifacient pathogen in dairy cows in the Rift Valley area of Kenya, at 79.1%. However, no research has been conducted on BVDV in the Mount Kenya region of Kenya where a large portion of Kenyan smallholder dairy farmers are located, and there is little known on the risk factors to BVDV infection on smallholder dairy farms in Sub-Saharan Africa.

1.2.4 Managing reproduction in smallholder dairy cows through body condition score and nutrition, sexed semen, hormones, and antibiotics

Body condition score (BCS) has been widely used to assess cattle nutritional status. Body condition score is a visual and/or tactile assessment based on the classification of animals into categories according to the coverage of muscle and fat on the body (Da Costa et al., 2011). Unlike body weight measures, BCS analysis is reliable for all cattle, regardless of the size and the physiological status of the animal (Lukuyu et al., 2016). According to Wright & Russel, (1984) when an animal feeds, nutrients are partitioned according to the priority of needs which are scaled as follows; 1) basal metabolism, 2) mechanical activities, 3) growth, 4) basic bodily reserves of energy, 5) ongoing maintenance of pregnancy, 6) lactation, 7) extra reserves of energy, 8) estrous cyclicity, ovulation and early pregnancy , and 9) excess reserves. Reproductive function in terms of the partition of nutrients is not prioritized and will be shut down in cases where the animal does not have enough nutrients (Da Costa et al., 2011).

Cows with low BCS at 7-10 weeks postpartum take longer to conceive and there is a significant influence of body weight loss in the first 6 weeks of lactation on calving to conception interval (Westwood et al., 2002). Idris et al. (1998), after conducting two surveys in cows in the peri-urban areas of Sudan, identified underfeeding in late pregnancy and presence of diseases as the most likely constraints of fertility in this setting. Farmer education on the importance of improving nutrition in late pregnancy is needed in order to improve fertility, as well as to identify the diseases affecting fertility and controlling for them at the same time.

Wathes et al. (2007) suggested an additional way that NEB can affect conception percentages adversely, through its ability to hinder uterine recovery following a delay in clearance of puerperal pathogens. A poor energy balance status was associated with a greater degree of uterine inflammation following calving and a slow repair process. It is well known that RFM, metritis and endometritis all negatively affect fertility, and these conditions are common due to inadequate nutrition among transition cows, particularly due to insufficient calcium, Vitamin E and selenium in the diet (Chassagne & Charcornac, 1994). Improved nutritional management is vital to preventing RFM. Timely treatments with Prostaglandin F2α and/or appropriate intrauterine antibiotics can assist in the return of a satisfactory uterine environment for a conceptus. However, there is need to look into SDF cows and the effect of NEB, mineral deficiencies, and inappropriate treatments of RFM, metritis and endometritis in relation to postpartum uterine conditions and effects on fertility.

In order to enhance the chances of getting a heifer calf for dairy farms, sexed semen has been developed and has been commercially available for a number of years. However, pregnancy percentages for sexed semen have been demonstrated to be lower than regular semen due to lowered concentrations of sperm cells in the sexed semen straws and the dead or injured sperm cells from the sperm sorting process (Bodmer et al., 2005).With the lower pregnancy percentages and the higher costs of sexed semen compared to regular semen, it has been recommended that in dairy cattle, sexed semen should be used mainly on heifers due to their greater uterine and ovarian fertility compared to postpartum cows with possible endometritis and ovarian abnormal function due to NEB (De Vries et al., 2008; DeJarnette et al., 2011; Healy et al., 2013; Norman et al., 2010; DeJarnette et al., 2009). However, efforts have been taken to maximize the pregnancy percentages of cows inseminated with sexed semen; the insemination procedure must be impeccable - from detection of estrus, semen thawing, timing of insemination and the location of deposition for the sperm itself (Saacke, 2008; Kurykin et al., 2007; Andersson et al., 2004; Dalton et al., 2001; Sá Filho et al., 2010).

Reproductive strategies aimed at enhancing fertility with exogenous hormones (e.g. GnRH) have been used to enhance the use of sexed semen in dairy breeding (Sá Filho et al., 2010). Although these strategies can produce acceptable results in terms of pregnancy per AI, incorporation of timed AI following hormonal estrus induction, along with sexed semen, has been studied extensively and shown to also produce acceptable pregnancy percentages (Macedo et al., 2013). However, in the Kenyan setting, hormone use in dairy cows has not been explored much. A study by Tsuma et al. (1996) showed marked improvement in fertility of repeat breeder and anestrous cows and heifers in Kenya after AI was done following a timed-AI protocol with GnRH and Prostaglandin F2α. Furthermore, heifers in Kenya are often not fed as well as cows on SDF because they are not providing milk income, and poorly fed heifers would not likely be good candidates for sexed semen. However, there is no research on the use of sexed semen or the best criteria for appropriate use of sexed semen on cows (or heifers) on SDF in Kenya.

1.3 Research rationale and objectives

There are currently limited controlled trials in the field setting on smallholder dairy farms in Kenya that have evaluated the effectiveness of use of sexed semen in the tropics of Africa. For these studies to be possible there is need to first determine the status of reproduction of the smallholder dairy cows and assess the possible risk factors affecting reproductive performance of these cows. There is very limited literature on the current state of the productive and reproductive performance of dairy cows in the smallholder setting. With high levels of BVDV and *N. caninum* recently recorded in dairy cows in one part of Kenya in larger scale farms (Okumu, 2014), there is need to assess and document the prevalence and risk factors surrounding these two important reproductive diseases and their effects on reproduction. All of these factors can lead to lowered conception percentages and fertility and in turn lowered overall output of the SDF enterprises, leading to reduced income for these farm families in impoverished areas. Specific recommendations for smallholder farmers are needed in order to improve productivity in a way that is suited to smallholder dairy farming in specific areas.

The objectives of this research were: (1) to determine the production (milk yield) and reproductive performance of SDF cows, and their factors; (2) to assess the seroprevalence and risk factors of Bovine Viral Diarrhea Virus and *Neospora Caninum* in smallholder dairy cows and heifers; and (3) to determine the feasibility of use of sex-sorted semen on reproductive efficiency enhancement in SDF and to inform on its criteria of use to maximize pregnancies on such cows.

1.4 Study location and Context

Naari Dairy Farmers Cooperative Society (NDFCS) is a dairy group located in the Naari area, in Meru County, located in eastern Kenya and was the site of the research described in the subsequent chapters. Meru County covers an area of 6,936 square kilometers and lies on the North Eastern highland slopes of Mount Kenya at an elevation of 17,053 feet (5,199meters) above sea level and constitutes a large area stretching northward to the volcanic Nyambene Hills. The wide range of altitude in the area (300-5199m) creates a variety of ecological zones ranging from extremely fertile, well-watered agricultural areas to low-lying semiarid lands. The rainfall pattern is bimodal with long periods of rain occurring from October to December and short periods of rain occurring from mid-march to May. The climate is generally categorized as cool or warm; daytime high temperatures range between averages of 16 degrees Celsius during the cold season and 27 degrees Celsius in the hot dry season. Meru County receives an average rainfall of between 500-2600mm per year.

Naari/Kiirua ward has been recorded to have a population of approximately 27,299 people and covers 118.6 square kilometers (KNBS, 2010), and most of them practice subsistence farming for food and income. Naari Dairy Farmers Co-operative Society has over 550 active farmers after its reactivation following a collapse in the dairy processing industry in the 90s and early 2000. As members of NDFCS, farmers are able to purchase feed for cows, obtain veterinary services, and access credit to purchase these items and other non-dairy farming activities and food stuff for their families from the society's store. Additional resources available to the members include rental of machinery for silage-making, banking services, and access to education through seminars and education materials. This location was chosen for its agro-ecological sustainability of dairy farming, as well as a good working relationship between the NDFCS, the University of Prince Edward Island, and Farmers Helping Farmers (a Canadian non-profit organization which was a partner of this work).

1.5 Thesis hypotheses and structure

There were three hypotheses on the thesis research, related to the three objectives: 1) use of sexed semen improves production and reproduction performance of small holder dairy cows in Kenya; 2) Bovine Viral Diarrhea Virus and *Neospora caninum* are found among SDF, with a number of management risk factors that could be controlled; and 3) using sexed semen and uterine antibiotics in smallholder dairy cows will lead to faster replenishment of the herd, when controlling factors that have deleterious reproductive effects in smallholder dairy cows, such as poor body condition, endometritis, and BVDV and *Neospora caninum* infections.

The thesis is presented in research paper format, with a separate introduction, methods, results, discussion, and conclusion for each substantive chapter. Objectives one, two and three are presented in chapters two, three and four, respectively. The final chapter reviews the main findings, and links the results together, providing recommendations and directions for future research.

There is a companion thesis with two randomized controlled trials looking into quantification of use and impacts of selected leguminous shrubs on milk production in cows and average dairy weight gain in calves in the same study population. The companion thesis was carried out parallel to the work described in this thesis and published in University of Prince Edward Island library by Dennis Makau.

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Chapter 2 Cross-sectional study of productive and reproductive traits of dairy cattle in smallholder farms in Meru, Kenya[1].

2.1 Abstract

A cross-sectional study was conducted to determine the farm management and milk production and reproductive performance of dairy cattle in smallholder dairy farms in eastern rural areas of Kenya, and to determine farm- and cow-level factors associated with milk production. A total of 200 farms were randomly selected from a list of the farmers shipping milk to a local dairy society. Structured questionnaires were used for data collection on management and demographic information, and farm visits occurred where the lactating cows on the farm received a physical examination. A mixed linear regression model with a random effect for farm was fit to determine associations with the natural log of daily milk production. The majority of the farmers had one to three milking cows (mean = 1.40), with an average milk production of 6.70 kg/cow/day from the 314 lactating cows on the 200 farms in the study. At the time of the study, 43.4% of the lactating cows were bred and/or pregnant, with 28.7% of the cows being confirmed to be over three months pregnant. The cows that were cycling and non-pregnant (n=74) had a mean of 304 days-in-milk (DIM), while those cows that were anestrous (n=95) had a mean of 201 DIM.

[1] Muraya J, VanLeeuwen J A, Gitau G K, Wichtel J J, Makau D N, Crane M B, McKenna S L B and Tsuma V T 2018: Cross-sectional study of productive and reproductive traits of dairy cattle in smallholder farms in Meru, Kenya. *Livestock Research for Rural Development. Volume 30, Article #171.* Retrieved October 29, 2018, from http://www.lrrd.org/lrrd30/10/jmura30171.html

Explanatory cow- and farm-level variables in the final milk production model were reproductive status of the cow, breed type, weight, DIM, concentrates (dairy meal) fed during the last month of pregnancy and land allocated for growing fodder for dairy cows. Exotic breed crosses, producing 6.80 kg of milk per day, on average, had higher milk production than the indigenous breeds, producing an average of 4.90 kg of milk per day. Heavier animals yielded more milk on the day of the visit; cows that weighed over 550 kilograms had twice as much milk production as those that weighed 250kg and less. The study categorized the cows into different reproductive statuses (early pregnancy/anestrus, pregnant, and cycling) and noted an increase in milk produced by cows in these different groups, with the cows that were cycling recording a 19.8% higher daily milk production over those in early pregnancy or anestrus. Milk yield reduced as DIM increased beyond the first hundred days. Milk production from cows that received concentrates (dairy meal) dairy meal in the last month of gestation was 34.3% higher compared to those that did not receive any. The percentage of land allocated to growing fodder for dairy cows was positively associated with the cow's milk yield per day, with a 15.6% increase for every 25% increase in land set aside for growing fodder.

We conclude that, even though smallholder dairy farmers in this area of Kenya have made attempts to improve their animals by cross-breeding them with exotic breeds, the milk production was still low. This can likely be largely attributed to poor feeding (especially as young-stock and during the transition period) and reproductive management. A more detailed cohort study or trial is recommended that can examine all the changing cow and management factors over time, providing necessary recommendations for farmers that account for these changes over time.

Key words: Days in milk, Risk Factors, Nutrition, Pregnancy, Reproductive performance, Transrectal palpation.

2.2 Introduction

The smallholder dairy (SHD) sub-sector in Kenya accounts for 80% of the total number of cattle in the country, contributing to 70% of the total milk output (IFAD, 2006; Odero-Waitituh, 2017). Irrespective of the large numbers of animals, per cow milk productivity of the dairy sector is still very low. The SHD farmer is faced with limitations to achieving optimum milk production, including poor management, poor nutrition, lack of desirable breeds, infertility, reproduction disorders, animal diseases and a poor marketing system (VanLeeuwen et al., 2012). The reproductive performance of the herd or animal is a key indicator of sustainability of a dairy farming system (Swai et al., 2007). In the North American dairy sector, if a cow cannot show heat promptly, conceive at an optimal time, and deliver a calf per year, lifetime milk production is suboptimal and the enterprise is not considered very profitable or sustainable (Hare et al., 2006).

Assessment of reproductive performance depends on composite parameters, with the main indices being average Calving Interval (CI) and days open. Average days open has been advocated as the most appropriate measure of current reproductive performance (Radostits et al., 2001), but for SHD farms, this measure is too variable with the small herd size. In order to achieve the optimal CI of 12-13 months, a Calving-to-Conception Interval (CCI) of 85-110 days is recommended (Radostits et al., 2001). These intervals are negatively influenced by biological (postpartum diseases, delayed resumption of heat, and cystic ovarian disease) and management factors (poor nutrition, heat detection problems, poor breeding techniques, and long voluntary wait periods) (Radostits et al., 2001).

Reproductive performance in smallholder dairy enterprises in Kenya has been described as poor (Odima et al., 1994; Bebe et al., 2003; Owen et al., 2005) . It is characterized by long calving intervals of about 633 days (Bebe et al., 2003). These low reproduction indices, together with high young stock mortality rates, have resulted in farmers being unable to produce enough replacement heifers. In order to overcome these and many reproductive constraints facing farmers, effective input services are required. Interventions from the government in terms of service provision and subsidies, and also strengthening of farmers' cooperative societies, are ways of achieving these reproductive goals. Romney et al. (2000) reported that in the Kenyan highlands, farmers were willing to purchase supplemental feeds when given access to credit facilities.

Previous studies on reproductive performance in Kenya have been done in the 1990s and early 2000s (Odima et al., 1994; Bebe et al., 2003; Owen et al., 2005), and there have been efforts to improve reproductive performance since those reports. These studies however were done in the peri-urban and urban areas surrounding Nairobi, and management, ecological and production factors in these peri-urban areas are not similar to those of rural settings of Kenya, such as the Meru highlands.

Due to Kenya's steady population growth, progressive land subdivision has been ongoing and that has rendered these small portions of land too small for subsistence crop agriculture (Asoka et al., 2013). Small-scale farmers in these rural areas have now intensified dairy production as their main source of income, and this has opened a door for the need to improve their production and reproduction. For this intensification to happen, studies are needed to determine the state of the industry and the challenges farmers are facing, and to make feasible recommendations that will lead to the improvements needed. This study was therefore designed to determine the production and reproductive performance of dairy cattle in smallholder dairy farms in the Naari area of Meru, and to determine associations between reproductive status (main predictor of interest) and milk production (outcome of interest), while investigating other important variables and controlling for confounding. The results of this study provide a baseline assessment for a larger project that involved a dairy cooperative society, a Canadian non-governmental organization called Farmers Helping Farmers, and supplementation of nutritional and reproduction interventions for their animals.

2.3 Materials and methods

2.3.1 Study area

The study was conducted within 10-15 km radius around the rural area of Naari in Meru County, Kenya. Meru County is located in the eastern parts of Kenya (longitudes 37^o 18’ 37” to 37^o 28’ 33” east and latitudes 00^o 07’ 23” to 00^o 26’ 19” south), approximately 270 km north of Nairobi, and has a population of approximately 1.5 million people, of whom, 84% reside in the rural areas (Mutarari, 2010). The precipitation in this county is bimodal, with short rains around the months of March to May and long rains around the months of October to December. The highest amount of rainfall approaches 2200 mm in the highest altitude areas of this county, while only 500 mm may fall in the lowest altitude areas of the county. Average daily temperatures in the highlands range between 14^oC to 17^oC while those of the lowlands are between 22^oC to 27^oC. Agriculture is one of the main economic activities in Meru County, with both cropping and livestock being common. According to the welfare monitoring report by the government of Kenya, the percentage of households living below the poverty line in 2008 in Meru North was

44.7%, with this number expected to rise (GoK Meru-North distict Development Plan 2004-2008).

2.3.2 Study farms and cows

The sampling frame for the study consisted of 568 farms that were identified from the Naari Dairy Farmers Cooperative Society (NDFCS or Naari Dairy) database as active members shipping milk to the Dairy in the month of February 2015. A total of 200 farms were randomly selected from the database for the study.

In computing the necessary sample size, a confidence of 95% and power of 80% were assumed using Epi-Info version 6.04b (CDC Atlanta USA 1996) to detect associations between the main dependent variable (milk production) and the main independent variable (reproductive status – pregnant or not), based on a mean milk production of 5.52 kg/cow/day in pregnant cows and 6.69 kg/cow/day in non-pregnant cows, with a combined standard deviation of 1.41 kg/cow/day (Melaku & Gurmessa, 2012). Sample size estimation results indicated 200 farms were necessary from the sampling frame of 568 smallholders for the study. Farms were estimated to have 1-3 milking cows (2 cows on average) for this sample size to be considered adequate. All milking cows from the selected farms were included in the study.

2.3.3 Data collection

The farms were visited once (cross-sectional study) during the period of May-August 2015, and a questionnaire was administered to collect all the relevant information. This involved taking records of all milking cows on the farm, and examination of any written records, if any, so that all ages of the cattle, calving dates, history of reproductive diseases and conditions around parturition, such as mastitis cases, were recorded accordingly. Other information collected through the questionnaire included details on feeding and mineral supplementation, whether the cattle owner had attended any dairy husbandry training, herd size, awareness and monitoring of heat signs, age of the cows and source of animals.

The animals were examined physically, and the following information was collected: live weights using a weight measuring tape around the girth area, height at the withers, body condition scored on a 5 point scale where 1 represented very thin and 5 represented grossly overweight, using half point increments (Nicholson & Butterworth, 1986), and any clinical abnormalities. A California mastitis test (CMT) was performed, and pregnancy status and ovarian status were obtained by way of transrectal palpation.

2.3.4 Definition of reproduction parameters

Days open was calculated as the period between the last calving and conception if the cow was pregnant, or the visit date if the cow was open. It was hard to get the actual days open for all cows since farmers did not practice good record-keeping, and their recall of dates when the cows were inseminated or last delivered a calf was approximate to the nearest month. Therefore estimates of days open used the 15^{th} day of the month reported for calving and breeding where there was no physical record of them.

Days in milk (DIM) was defined as the number of days during the current lactation that a cow had been milking, beginning with the last date of calving to the current date. Abortion was defined as the expulsion of one or more calves <271 days after natural mating or artificial insemination. Foetal membranes were considered retained if they remained unexpelled for at least 24 hours after calving or abortion. Dystocia was considered to occur if parturition was assisted either by the farmer or by a veterinary field officer.

2.3.5 Statistical analysis

Data were entered and organized in an Excel spreadsheet (Microsoft, Sacramento, California, USA). The unit of analysis was the individual lactating cow in the farm at the time of visitation. Descriptive statistics for the animal- and farm-level variables and analytical statistics were carried out using STATA/IC 13.0 (StataCorp LLC, College station, Texas, USA).

For the analytical statistical analyses, the main outcome (dependent) variable investigated was the reported natural log of kilograms of milk produced per cow per day for the day prior to the visit. Due to the lack of records kept by most farmers, leading to possible measurement error, continuous variables of age, days open and DIM were modified into categorical variables to minimize information bias for the analytical statistics. Farm was included as a random effect because cows on one farm are not statistically independent of one another (Kristula et al., 1992).

In the first step of the modeling, relationships between each independent variable and the outcome variable were individually investigated. Fishers test was used to test for associations between the outcome of interest and other predictors. In the second step, any variables that were associated at the $p<0.15$ level were eligible to be included in multivariable models. Correlation matrices between variables meeting the cut-off level ($-0.3<r<0.3$) were examined to determine correlations among these variables. Both forward stepwise and backward elimination regressions were used to identify the most parsimonious model in which all independent variables retained at the $p< 0.05$ level. Other variables not in the final model were examined for confounding of the variables in the final model, as recommended (Dohoo et al., 2009). Interactions between variables in the final model were investigated. Model fit was examined by checking the standard residual diagnostics, performing predictions, and checking shrinkage of the model used.

2.4 Results and discussion

2.4.1 Farm characteristics and management

Since all 200 selected farms agreed to participate in the study, there was a 100% response rate. The principal farmers were primarily women (52.5%), although there were instances where both the male and female jointly considered themselves as principal farmers (16.5%). Most of the principal farmers were married (79.5%), but a few of them were young people who were single and establishing themselves as dairy farmers (9%). The majority of the principal farmers had either none or primary level of education, whether male (56%) or female (57%), indicating the low literacy levels among the farmers, and leaving a huge need for training on dairy farming matters. The mean (±SE) household size recorded in this study was 3.78 ± 0.12 with a minimum of 1 person and a maximum of 11. Higher man's education was positively associated with log of milk production as an ordinal variable in the univariable regression analyses ($p<0.05$), with 31.9% and 7.0% of male farmers having completed secondary and tertiary education, respectively (Table 2-1).

Among the farmers interviewed, 61% of them indicated that other than dairying, they also practiced crop farming, which supported their source of income and food, while the crop residues were used as feed for their cows. Only 13% of the farmers had wage or salaries coming to either them or their spouse, while 10% of the farmers had no other source of income other than the dairy cows. It has been reported that cattle production plays an important role in improving the livelihood for farmers in Kenya (Thornton, 2010), and our research would corroborate this assertion. In our study region, cattle were mainly kept for food and cash income (milk and/or meat), but also for draught purposes and farm manure/fertilizer. Source of income met the eligibility criteria for multivariable regression modeling (Table 2-1), being marginally associated with natural log of milk production as an ordinal variable ($p<0.05$).

The mean total land holdings owned by the respondents was 2.04 ± 0.17 acres, although the farmers indicated having access to other pieces of land in the form of leasing it, borrowing it, or using part of the nearby government-owned forest which was leased to them for some time. These additional portions of land were small and made up a mean of 0.41 ± 0.06 acres. A majority of the respondents (51%) indicated that, of all the combined land pieces, they allocated between 25-50% of the farmland to growing feed for their dairy cows, and this was because dairy farming was considered the major source of income. Land allocated for dairy use was positively associated with natural log of milk production as an ordinal variable (Table 2-1) in the univariable regression analyses.

Half (51.2%) of the farmers indicated that they had obtained their milking cows through purchasing them as adult cows, as compared to those who purchased them as young stock (28.4%) or raised them on their farm (20.4%). The animals were reported to be obtained from the neighbouring smallholdings within the greater Meru County, as buying from large-scale establishments in Rift Valley and Central provinces of Kenya was considered expensive and those animals were less adaptable to the local challenging feeding management. Purchased animals had also been indicated as a common source of cows in a study in the Kenyan highlands nearby (Kiambu, Machakos, Kirinyaga, Maragua, Nakuru, Nyandarua and Narok former districts) for supplying milk to Nairobi (Bebe et al., 2003). However, the farmers also indicated a preference for raising heifers born on their farms as replacement stock as it was considered cheaper than purchasing an animal, and the fertility and production history was known for home-raised heifers.

Artificial insemination services were readily available, and offered by private practitioners, government veterinary officers and veterinary technicians. However, 13% of the study farmers still preferred to use bulls for breeding. Even among the farmers that used AI for breeding their animals, a majority did not have the knowledge to choose which sire to use on their cows, with the majority allowing the AI or veterinary technicians to choose which bull to use, or to advise them on what bull to use, even though most of them had attended some form of dairy training. A few of the farmers were specific in their answers, saying they used "imported" or "Canadian" sexed semen on their cows, and reporting "good results" with that semen as well. This semen is usually highly priced in Kenya, and so most farmers shy away from using it, especially due to reported perceived low conception percentages compared to regular semen (Norman et al., 2010), potentially leading to a repeat service. Kenya's Animal Genetic Resource Centre (KAGRIC) is responsible for keeping the AI bulls and distributing semen in the country, as well as in neighbouring countries (Wakhungu et al., 2000). There is also a presence of imported gametes, in terms of semen and embryos, that come into the country through the veterinary services office, and lately, sexed semen from different countries has been made available through this avenue (APSK, 2015).

The basal dairy cattle feeds in our study were based on natural pastures and home-grown fodder, mainly maize stovers, Napier grass and crop residues. Of the 200 farms, 73% zero-grazed exclusively, while the remainder utilized cattle grazing on their land at least some of the time. Napier grass contains moderate crude protein (CP) content (6-12%) when it is fed at 1- 1½ meters in height, but declines to less than 5% when it is fed at 2½ - 3 meters in height (Njoka-Njiru et al, 2006). When natural pastures and other cultivated pastures are available during the rainy season, Napier grass is usually not fed to animals but instead is left to grow tall and then fed during the dry season, usually leading to milk production dropping substantially.

During the dry season in our study, maize stovers was a common crop fed to cows; over 80% of the farmers reported using it. Although dry maize stovers are important sources of roughage, they have low nutritive values with CP as low as 2.5% of dry matter, and neutral detergent fibres exceeding 70% of dry matter, making them a poor choice for lactating dairy cow feed. Crop residues that were available and sometimes fed to the study cows included relatively nutritious cowpea pods (7.10%), bean pods (63.7%), and sweet potato vines (15.5%). However, poor storage methods practiced by the farmers predisposed the crop residues to rains and sunlight, likely resulting in further deterioration of the nutritive quality of the feed.

To counteract some of this diminishing quality of feeds, concentrates were usually fed to cows, with dairy meal being the principal commercial supplement offered. Milling by-products, such as wheat or rice bran, wheat pollard and maize germ, have also been used as they are seen as a quick cheap source of energy for the cows. All of these products were available in the Naari Dairy consumer shop or at feed stores located in the local shopping centres. Farmers indicated their preferences to using the Naari Dairy consumer shop since they had access to credit there as Naari Dairy members. About 84% of the farmers indicated feeding dairy meal to their lactating cows, while only 58% were giving dairy meal to dry cows during the transition period. The 16% of farmers that did not give dairy meal to their cows cited high cost as the main constraint, and they were occasionally feeding the cheaper milling by-products mentioned. Dairy meal fed to cows on the farm in last month of gestation was positively associated with natural log of milk production as a dichotomous variable in the univariable regression analyses ($p<0.05$).

Mineral supplements in the form of powdered salts, blocks or molasses were available to farmers in this area. A total of 88% of farmers fed mineral powders to their cows, and 48% of those not giving the powders indicated using mineral blocks. Molasses was only used in the dry season and mixed with dry fodder to increase its palatability.

The quantity of mineral and concentrate supplements fed was generally low, and in most cases, a fixed amount was used throughout the lactation without adjustments according to the amount of milk produced. For example, 33% of farmers were giving cows a 1 kg can of dairy meal twice per day to lactating cows (equivalent to 1.3 kg of dairy meal per day), regardless of milk production or desire to get the cow pregnant. Similar findings had been reported in the central highlands of Kenya (Rufino et al., 2009) and in the semi-arid areas of eastern Kenya (Njarui et al., 2011). It was clear that most farmers were unaware that not providing the required amounts of mineral and concentrates supplementation to lactating dairy cows will lead to lower milk production and delayed conceptions (Moran, 2005).

2.4.2 Cow variables

The 200 farms had 316 total milking cows at the time of the study. There were two cows that were very hostile, and therefore a transrectal palpation was not carried out to confirm their reproductive status, although they were reported to be open. Therefore, reproductive status results are based on 314 cows.

Mean and median milk yield of the 316 lactating cows was 6.7 ± 0.23 and 6.0 kg/cow/day, respectively, with 35% of the farms producing less than 5 kg/cow/day at the time of the study, while the upper 10% produced over 12 kg/cow/day of milk, on average. Milk yield was not normally distributed, and 3.8% of the farms produced more than 15 kg/cow/day. As reported elsewhere, this low average milk yield could be attributed to underfeeding of lactating cows and giving poor feed quality, since most of the farms in smallholder dairy farming in Kenya rely on Napier grass as the main roughage, which can be very poor in quality if it is allowed to grow to 2 metres or more (Omondi & Njehia, 2014).

Dairy stock kept included *Bos taurus* crosses (Friesian, Ayrshire, Jersey, and Guernsey) and *Bos indicus* crosses (Zebu, Boran). A majority (48.1%) of the respondents preferred Friesian to Guernsey (31.9%) or Ayrshire or Jersey crosses (12.3%), due to the perception that Friesian cows have a higher milk production. The Friesian-Holstein crosses produced an average of 7.50 kg of milk per day, which was the highest production of all the breeds. Guernsey crosses gave 6.24 kg/cow/day, which was higher than the Ayrshire or Jersey crosses (5.38 kg/cow/day). The least common breed (Zebu or other indigenous crosses) only produced 4.90 kg of milk per day on average. However, DIM and other factors affecting milk production are not taken into account in these means, and therefore multivariable model coefficients that control for other production confounders should be examined to provide valid breed comparisons. Although the preference for the Zebu breed was low in this area (7.59%), their positive attributes of easy-keeping cattle with high resistance to disease, better adaptation to harsh climates and powerful draught abilities were still anecdotally recognized by those owners who had them. Breed was negatively associated with natural log of milk production as an ordinal variable in the univariable regression analyses, according to the order of breeds presented above ($p<0.05$).

For this study, the largest proportion of cows (45.6%) was relatively young between two and five years of age. Age was not recorded as one of the major reasons farmers culled their milking cows, with the oldest cow encountered in the area being 17 years old. There were 37.3% of cows between the ages of 5 and 8 years, while 13.3% of the cows were over eight years old. These age trends were seen as a result of heifers taking a long time before they reached breeding sizes due to nutritional deficiencies that slowed their growth rates (Makau et al., 2018). Younger animals that were less than five years old produced an average of 6.48 kg/day of milk and this increased to 7.05 kg/day for the middle-aged cows (5-8 years) and then the mean dropped (6.15 kg/day) for cows older than 8 years old. This pattern follows the natural trend of milk production when cows are expected to reach maximum production around 5-6 years of age and at the third parity (Lee & Kim, 2006).

The lactating cows had an overall mean and median DIM of 300 and 243 days, respectively, and were categorized into various lactational stages (e.g. early, mid, late, extended, and very long, for cows with DIM of ≤100, 101-200, 201-300, 301-400, and >400 days). There were 44, 78, 33, 53 and 108 cows in early (13.9%), mid (24.7%), late (10.4%), extended (16.8%), and very long lactation (34.2%), respectively. Days in milk was negatively associated with natural log of milk production as a continuous variable in the univariable regression analyses (Table 2-1).

The 314 lactating cows that were palpated were categorized into various reproductive states (e.g. anestrus, cycling, possible early pregnancy (no diagnosis on transrectal examination), and pregnant confirmed by transrectal examination). At the time of the study, 43.4% of the lactating cows were bred and/or pregnant, with 28.7% of the cows being confirmed to be over three months pregnant. According to ovary palpation findings, 30.6% of the lactating cows were in an anestrous phase at the day of the rectal examination, with no palpable structures identified from both ovaries. This group of cows had a mean of 201.2 DIM. The cows that were cycling and non-pregnant (n=74) had a mean of 304 DIM. Since these cows were not yet pregnant, these estimates of days open were expected to increase until the cows conceived. With the poor records kept by farmers, it was not possible to determine calving intervals or days open for previous lactations. It was also not possible to reliably determine first service conception percentages or number of breeding per conception. Cow reproductive status was positively associated with natural log of milk production as an ordinal variable in the univariable regression analyses (Table 2-1), according to the order of states presented above.

The proportion of all the milking cows currently with subclinical mastitis based on CMT $\geq$ 1 was 44.0%. Cow current mastitis status was negatively associated with natural log of milk production as a dichotomous variable in the univariable regression analyses (Table 2-1).

The overall mean and median weight of all the milking cows was 388.4 and 362.0 kg, respectively. There were 5.38%, 64.6%, 28.1% and 1.89% cows that weighed <250, 251-400, 401-550, and over 550 kg, respectively. Cow weight was positively associated with natural log of milk production as an ordinal variable in the univariable regression analyses (Table 2-1).

The overall mean body condition score (BCS) for the lactating cow was 2.44 ± 0.31. No cow was recorded to have a score of 1 or over 4. A majority of the cows (59.4%) had a body condition score less than or equal to 2.0, which is below the desired body condition score, and this could have been due to the time the cross-sectional study was carried out, with many cows being examined months into a dry season, likely leading to insufficient quantities of low quality feed being available to most farmers for feeding. Fisher's exact test revealed that there were strong differences in body condition for different reproductive status groups, and the body condition differed in different lactation stages. There were also differences (p=0.004) in BCS in cows on those farms that had received training on dairy husbandry (BCS=2.49) versus those who had not received training (BCS=2.26). Imparting knowledge on the farmers was done through farmer training days by extension officers. When the farmers were asked about the topics on which they had received training, cow feeding regimes seemed to be the most common topic (23.1%) that most farmers could recall.

2.4.3 Factors associated with milk yield

In the first step of the modeling of factors associated with the natural log of milk yield, nine variables were found to be associated with the outcome variable at ($p<0.15$) when individually investigated (Table 2-1). The correlation matrix did not indicate any serious correlation among these variables; all correlation coefficients were lower than 0.17.

Table 2-2 shows the results of the final mixed model: one farm characteristic, four cow variables and one farm management factor were strongly associated with the natural log of daily milk yield, while controlling for the other variables in the model. Many of the expected factors of milk production were in the final model, and we start the model description with them.

Breed type of the cow was associated with milk yield. The indigenous crosses (e.g. Zebu) showed a 23.7% lower milk yield when compared to exotic crosses, which was the baseline as shown in Table 2-2. Milk yield within these two breed groups did not differ in the final model when the model controlled for other confounding variables, such as weight, DIM and reproductive status, indicating that these other variables were primarily responsible for breed differences in milk production within the two model categories. These results corroborate other findings in Kenya that the low performance of dairy herds on smallholder dairy farms in the region are associated with the type of breeds kept (Omondi & Njehia, 2014). The predominance of exotic crossbreeds in this study is an indication of attempts by these farmers at higher milk production, even though other factors hindered production. Farmers that preferred keeping Jersey cows indicated their preference to a smaller cow that was not feeding as much as the other exotic breeds, even with their perceived low milk production.

Heavier cows were found to have yielded more milk on the day prior to the visit. Cows that weighed over 550 kilograms yielded over twice much milk as those that weighed below 250 kilograms. Heavier cows were more likely to have an adequate body condition (BCS$\geq$2.75) and were reflective of good feeding management in terms of quality and quantity of feeds, explaining a higher milk yield. Emaciated cows that weighed less than 250 kilograms had the lowest daily milk yield (3.24 kg/cow/day), that was way lower than the means of all the other weight groups. Body weight changes are also affected by the parity of cows, and higher-parity dairy cows often lose more body weight in early lactation compared to lower-parity cows (Roche et al., 2007a). The relationship between parity and post-partum body weight changes could not be explored in this cross-sectional study because parity records were not kept on the cows, but cows estimated to be over eight years of age did weigh more than cows less than five years of age, although there was no difference in milk yield between the three different age groups (p=0.269).

Cows in early lactation had 26.4% more milk than cows in mid-lactation (101-200) days. The differences in milk production increased as we moved farther towards cows in later DIM categories; with 55.1% lower milk production in the cows above 400 days in milk, compared to early lactation cows. When all the categories above 100 DIM were compared to the baseline, all the means were clearly different from the baseline. With non-overlapping 95% confidence intervals between 101-300 DIM and >400 DIM (Table 2-2), cows in this very long DIM category had lower milk production than the mid-lactation and late-lactation cows. Farmers should rebreed their cows sooner to avoid long DIM to ensure good utilization of the animal's productive life and better milk production. Long DIM is indicative of animals not coming into noticeable heat, getting inseminated in a timely manner, conceiving and/or retaining a pregnancy. Lactation stage was also associated with milk production elsewhere, as reported by Baul et al. (2012).

For this study, reproductive status was categorized into groups, namely anestrus, cycling, early pregnancy and pregnant. Nearly a third (30.6%) of the cows in the present study were anestrus, defined as milking, not pregnant and not cycling at the time of examination. Our study showed an increase in milk produced by the cows in different reproductive status groups compared to those cows that were in early pregnancy, which was set as the baseline. Cows that were open and cycling had a 28.9% higher milk yield ($p<0.05$) compared to cows in early pregnancy. It is hypothesized that open and cycling cows were more likely to be in a positive energy balance, while pregnancy above 3 months of gestation can also draw on energy and protein intake. Unfortunately, during the time of this study, cows were found to have extended days open sometimes over 600 days, a situation that was likely brought about by long periods of drought and poor feed storage and management.

There were a few other variables associated with milk production that were interesting. Feeding dairy meal during the last month of gestation led to increased milk yield such that 35.5% more milk was obtained from the cows that had been received some extra supplementation with high protein concentrate (dairy meal) during the transition period compared to those that had not received any. It has been demonstrated that supplementing dairy cows with 0.5 to 2 kg of dairy meal concentrate per day before parturition, with increasing amounts as parturition approaches has been associated with cows attaining higher levels of milk production during the early days of lactation (Richards et al., 2015; Richards et al., 2016). Our results confirm that the impact of this management factor may have a lasting effect beyond the first 2 months of lactation. Farmers should be encouraged to practice this management recommendation.

The percentage of land allocated to dairy feed was positively associated with the cow's milk yield per day. A 25% increase in the land allocated to growing dairy feeds was associated with a 15.0% increase in milk produced, holding all the other factors constant. Due to a constantly increasing population in this area of Kenya, land holdings per owner have decreased by more than half over the past few decades, mainly because of subdivision through family inheritance (Bebe et al., 2003). Farmers indicated owning a mean of 2.04 acres of land, leading to competition between growing food for people and feed for the cows. The study showed that with more land allocated to growing feed for their cows, more milk yield could be obtained.

Another factor that has been found to affect the amount of milk produced by cows in the tropics is suckling calves. Some farmers in the tropics still practice restricted suckling in any of its three forms namely: (i) the calf may initiate milk letdown, the cow is milked and the calf sucks residual milk; (ii) the calf is allowed to suck one quarter; or (iii) the calf may suck the residual milk once milking is completed. Restricted suckling has been associated with many advantages over bucket rearing, including increased milk production, increased persistence of lactation and extended lactation, reduced incidence of mastitis, and increased calf growth and survival (Preston & Vaccaro, 1989; Little et al., 1991; Agyemang et al., 1993; Msanga & Bryant, 2004; Juhlin, 2013). The greatest disadvantage of this practice is said to be its adverse effects on reproduction (Little et al, 1991). In the current study, the aspect of restricted suckling was not explored as farmers rarely allowed calves to suckle cows other than for colostrum. Calf rearing and calf management in this study population was described and reported in a separate publication (Makau et al., 2018)

The intraclass correlation of 0.246 indicated that there was substantial correlation of cows within farms, confirming the need to adjust for clustering of cows within farms using a random herd effect. There was no confounding of model variables among the other variables not in the final model, and no interaction between model variables. The R^2 of the final model was 0.468, suggesting that 47% of the milk variation was explained by the model.

The quartile plot of the standardized residuals did not indicate any serious deviations from the normal distribution, and the residual plot did not reveal any serious concerns after the data were log transformed. Based on the residual and leverage diagnostics, farm 43 had somewhat high values, though within the acceptable range of 3 and -3. The magnitude and the influence of the residuals for farm 43 did not reveal any problems; when the model was analysed without this farm, there was little change in the variables, and thus farm 43 was retained in the final model.

Research limitations included a language barrier, especially with aged farmers who could only communicate in the native Kimeru language. This needed an interpreter who was fluent in the native language to relay the message and convey the respondents' answers to the research team. Suspicion and mistrust were also noted among some respondents, particularly with details surrounding their personal life, and this got in the way of data collection. It was however mitigated by assuring them that the information given would be treated with utmost confidentiality, respect and professionalism. There were a few uncooperative and unfriendly respondents, but this situation improved when word went around the community about the project and its objectives.

Data quality was considered to be good by the researchers because collection was carried out by a well-trained team, and the questionnaire used was adopted from a previous study carried out in a different part of Kenya, and thus it had been pretested and modified. Participants of this study were randomly selected to avoid any selection bias, and of the 200 farmers that were selected for the study, they all agreed to participate and provided the requested data. Physical, clinical and rectal examinations were done by qualified veterinarians and veterinary students under the supervision of veterinarians.

Since this was a cross-sectional study, we cannot use results herein to determine causality of the model factors, but the results obtained were used to as a guide in the randomized control trials that were to follow the project. A more detailed cohort study or trial is recommended to test hypothesized model factors, to document and examine all the changing cow and management factors over time, and to provide the necessary evidence for recommendations for farmers, in turn improving the output from their dairy enterprises.

2.5 Conclusions

1. The mean and median milk yield for the study cows in this study was 6.7 ± 0.23 and 6.0 kg/cow/day respectively.
2. The principal farmer was female in 52.5% of the farms, and had either none or only the basic level of formal education (grade 8), leaving a need of training in area that would lead to improvement of the dairying enterprise.
3. A third of the farmers practiced crop farming as a way to supplement their income. As the mean total land holdings owned by the respondents was small (2.04 acres), the farmers were in dire need to find ways to support their families, including improved efficiency with the land they had.
4. Reproductive performance among the study cows was sub-optimal, with over half of the milking cows being open at the time of the study and had an average of 253 DIM among these open cows. Only 28.7% of the milking cows were confirmed pregnant at the time of the study.

5. The sub-optimal milk production of the dairy cows recorded in this area was associated with cow-level factors including; breed type, weight, reproductive status, and lactation stage as well as farm-level factors including; education levels of the male farmer, percentage of land allocated to growing fodder for dairy cows, and whether or not the farmers provided high protein supplements to dry cows before parturition
6. Extension services for training smallholder dairy farmers on best management practices associated with record-keeping, dairy cattle management (especially nutrition and reproduction), and fodder growing and conservation should be improved to enhance milk production on these farms.

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Table 2-1: Univariable mixed linear regression results of variables meeting the p<0.15 cut-off for eligibility for multivariable modeling of the natural log of daily milk production (kg/cow/day) for 316 cows on 200 Kenyan smallholder dairy farms in 2015

Variable	**Variable type**	**Coefficient**	**95% CI**		**p value**
Man's education	Ordinal	0.167	0.062	0.271	0.002
Income source	Ordinal	-0.067	-0.157	0.022	0.147
Land allocated for dairy use	Ordinal	0.240	0.144	0.337	0.001
Dairy meal fed to cows on the farm in last month of gestation	Dichotomous	0.374	0.205	0.544	0.001
Cow Breed	Ordinal	-0.117	-0.187	-0.045	0.001
Cow Reproductive status	Ordinal	0.072	0.028	0.116	0.001
Cow current mastitis status	Dichotomous	-0.225	-0.350	-0.100	0.001
Cow Weight	Continuous	0.333	0.217	0.449	<0.001

Table 2-2: Final multivariable mixed linear regression model of variables associated with the natural log of daily milk production (kg/cow/day) for 314 cows on 200 Kenyan smallholder dairy farms in 2015

Variable	**Coefficient**	**95% CI**		**P value**	**Exponentiated Coefficient**[1]
Cow breed					
1. Exotic crosses	Baseline				
2. Indigenous crosses	-0.270	-0.486	-0.054	0.014	0.763
Cow weight (kg)				<0.001*	
1. < 250	Baseline				
2. 250-400	0.303	0.0414	0.568	0.023	1.354
3. 401-550	0.608	0.329	0.887	<0.001	1.837
4. > 550	0.734	0.235	1.233	0.004	2.083
Cow days in milk				<0.001*	
1. 0-100 days in milk	Baseline				
2. 101-200 days in milk	-0.306	-0.504	-0.108	0.002	0.736
3. 201-300 days in milk	-0.352	-0.601	-0.104	0.005	0.703
4. 301-400 days in milk	-0.539	-0.761	-0.317	<0.001	0.583
5. Over 400 days in milk	-0.802	-1.011	-0.593	<0.001	0.449
Cow reproductive status				0.039*	
1. Early pregnancy	Baseline				
2. Anestrus	0.123	-0.069	0.315	0.209	1.131
3. Pregnant	0.208	-0.023	0.392	0.028	1.231
4. Cycling	0.254	0.070	0.438	0.007	1.289
Dairy meal fed to cows on the farm in last month of gestation					
1. No	Reference				
2. Yes	0.304	0.171	0.436	<0.001	1.355
% land allocated for dairy use	0.140	0.063	0.216	<0.001	1.150

P-value*: Global P-value

[1]*Exponentiated coefficient used to determine percent change for each variable or level of categorical variable. For example, for cows that were fed some Dairy meal on the last month of gestation, the percentage change would be 1.355-1.0= +0.355 indicative of a 35.5% increase in milk output and for days in milk, the percent change would be 0.736 – 1.0 = -0.64 for a 26.4% less milk produced by cows between 101-200 days that those in the first 100 days of lactation.*

Chapter 3 Seroprevalence and risk factors of *Neospora caninum* and Bovine Viral Diarrhea Virus in smallholder dairy cattle in Meru, Kenya

3.1 Abstract

Neospora caninum and Bovine Viral Diarrhoea Virus (BVDV) are among the most important pathogens of dairy cattle. Very little is known of their occurrences or risk factors in Kenya. This study was carried out to document the seroprevalence and risk factors of these pathogens, and recommend possible management practices, in smallholder dairy farms in eastern Kenya.

A total of 470 serum samples from dairy cattle over six months of age were collected from 158 randomly selected farms in Meru County, Kenya, and analysed for antibodies to *N. caninum* and antibodies and antigens to BVDV through Enzyme-Linked Immunosorbent Assays (ELISA) tests. Risk factor data were obtained through a face-to-face interview with the farmers. Generalized mixed logistic regression models accounting for cattle clustered within farms were used to identify significant risk factors.

The antibody seroprevalence of *N. caninum* was 35.1% (165/470), and that of BVDV antibody and antigen were 47.1% (152/323) and 36.2% (169/467), respectively. There was an 18.5% (87/469) seroprevalence of co-infection with both pathogens (current and/or previous infection). Animals that tested positive for *N. caninum* and positive on either of the BVDV tests were considered positive for co-infection. There was no history of vaccination for *N. caninum* or BVDV in this area of Kenya.

The final multivariable logistic regression model of risk factors associated with higher odds of seropositivity to *N. caninum* included introducing milking cows into the farm, lending of cattle between farms, farm dogs eating bovine aborted fetuses, and dogs whelping in the farm compound, with an interaction between the last two variables. Direct contact of dairy cattle with pigs was associated with higher odds of BVDV antigen seropositivity. In this BVDV model, age of the test animals formed two significant interactions with introducing new calves into farms, and whether or not other visiting dairy farmers had access to the cow shed. Being a cow and not a heifer was the only risk factor associated with higher odds of testing seropositive for BVDV antibodies. Risk factors associated with co-infection included parity of the cow, direct contact of the dairy cattle with dogs and goats, and introducing new milking cows into the farms. The BVDV antibody and antigen test results may be partly a function of classical swine fever virus or border disease virus interactions.

It was concluded that *Neospora caninum* and BVDV infected animals are present and widespread in the smallholder dairy farms in the Meru area of Kenya. Farmer education on biosecurity measures addressing the identified risk factors is required, and BVDV vaccination of animals in this area is needed.

Keywords: *Co-infection, Abortion, Infectious diseases, Biosecurity.*

3.2 Introduction

Raising livestock is a major agricultural activity in Kenya and even though the cattle population is large, the performance of the industry in terms of production is low (ICPALD, 2013). Smallholder dairy farm production dominates the highlands and peri-urban areas, while the arid areas are primarily inhabited by pastoralists. Suboptimal productive and reproductive performance has been recorded in these farms, with a lot of reproductive wastages attributed to abortions, early embryonic deaths and stillbirths. Very little is known about the different risk factors of this wastage in Kenya (Okumu, 2014). Bovine viral diarrhea virus (BVDV) and *Neospora caninum* protozoa are known to be among the most common infections associated with reproductive disorders worldwide (Asmare et al., 2013).

Neospora caninum is an apicomplexan protozoan parasite that can infect a variety of animal species and has a worldwide distribution. Dogs (*Canis lupus familialis*), coyotes (*Canis latrans*), dingoes (*Canis lupis dingo*) and the grey wolf (*Canis lupus*) are considered the definitive hosts for the parasite. Several livestock and wild animal species have been identified as the intermediate host (Dubey & Schares, 2011; Donahoe et al., 2015). Horizontal transmission of *N. caninum* in cattle occurs through cattle ingesting contaminated pastures and water by oocytes from infected dog feces, or vertically by infection of fetuses *in utero* from infected dams (Kamali et al., 2014). Direct reproductive losses occur following abortions other reproductive losses, such as early embryonic death and stillbirths. Loss of milk yield in aborting cows and increased cow culling due to abortions from *N. caninum,* as well as reduced growth and feed efficiency in calves born alive, are among the direct losses associated with *N. caninum* (Dubey et al., 2007; Dubey & Schares, 2006).

Bovine viral diarrhea virus is a flaviviridae pestivirus, and is arguably one of the most widespread cattle pathogens worldwide. The virus is endemic in many countries, with as high as 60-85% of unvaccinated adult cattle being antibody positive, demonstrating exposure to the virus (Lindberg & Houe, 2005). The virus is responsible for reproductive problems, diarrhea, immunosuppression and respiratory disease syndrome and therefore has enormous importance financially to the cattle industry (Yeşilbağ et al., 2017). The virus has great efficiency with which it crosses the placenta of susceptible pregnant females, and this intrauterine transmission can increase the prevalence of antibodies in young stock or persistently infected calves with no detectable antibodies (Fray et al., 2000). A majority of acute BVDV cases in cows and heifers are subclinical but if a female is pregnant, the fetus can become infected in animals with no antibodies to protect the fetus (through natural infection or vaccination). Fetal infections can occur at any time a fetus is exposed, but the results of infection vary with the strain of the virus and the stage of pregnancy. Infections can cause poor conception and in the first half of gestation, could lead to early embryonic death, abortions or birth of persistently infected (PI) calves, while infections during the second half of gestation could result in abortions, birth defects, still births, weak calves or apparently normal calves, but not persistently infected calves (Khodakaram-Tafti & Farjanikish, 2017). These PI animals typically represent between one and two percent of the cattle population and continuously shed infectious virus through bodily secretions and excretions (Fray et al., 2000).

Neospora caninum and BVDV are both important abortifacient pathogens on their own, but synergistic effects of concurrent infections in causing abortion in cattle has been reported in previous studies (Björkman et al., 2000; Weston et al., 2012). The concurrent infection effects had been thought to be due to the immunosuppressive effects of BVDV, which increase the chances of fetal infection by *N. caninum* in pregnant cows, resulting to *N. caninum* abortions. However, limited attention has been paid to these infections in Sub-Saharan Africa (Handel et al., 2011b; Yeşilbağ et al., 2017; Kenyanjui et al., 1994; Okumu, 2014; Callaby et al., 2016). Ferroglio et al. (2003) reported *N. caninum* antibodies in Kenyan wildlife. Prevalence of 18.8% and 25.6% was recorded for *N. caninum* and BVDV in farms in large farms in Rift Valley part of Kenya (Okumu, 2014). Over 80% of the cattle seropositive of *N. caninum* were also seropositive for BVDV in the same study. Only being an animal older than 49-96 months was marginally associated with BVDV seropositivity in the Kenyan study and no other risk factor was identified in this study with association to *N. caninum* occurrence (Okumu, 2014). Kenyanjui et al. (1994) in his study with smallholder Zebu cows in the Kenyan coastal area recorded a prevalence of 45.8% for BVDV although his study did not identify any associated risk factors. Neosporosis is common in farm dogs in the Nakuru area of Kenya, as reported by Okumu (2014). However, it is unclear how widespread the infection is in Kenya, especially within smallholder dairy farming systems (which constitute 80% of farms in the dairy industry in Kenya), or what factors are associated with infection. The objective of this study was to characterize the prevalence of these two pathogens and the factors associated with their occurrence in smallholder dairy farms in Eastern Kenya in order to come up with control strategies for them.

3.3 Materials and methods

3.3.1 Study area

The study was carried out in 2016-18 in the Naari sub-location of Meru County, Kenya. This study area is located in the north-eastern slopes of Mount Kenya and borders the great Mount Kenya forest, approximately 2000 meters above sea level. It has daytime high temperatures ranging from as low as 16^0C during the cold season (July-August) and as high as

35^0C in the hot season (January-February), and receives an average rainfall of between 500 to 2600 mm each year (worldweatheronline.com). The study area is well-suited for small-scale farming since it usually has sufficient precipitation and fertile soils, falling within the agro-ecological zones 2 and 3. This study was part of a larger randomized control trial on nutrition and reproductive management that ran for a year and a half between May 2016 and December 2017.

3.3.2 Selection of study farms and animal management

The complete list of member farmers of the Naari Dairy Farmers Cooperative Society (NDFCS) was obtained, and from these farms, a list of 200 farmers was generated randomly through computer generated random numbers. For the current study, eligible farms among these 200 farms also had to be currently shipping milk to the NDFCS at the time of contact for the study, or currently not shipping milk due to cows being dry (but not from shipping milk elsewhere). The area covered by the NDFCS is divided into eight regions, and sampling was proportional to the total number of farms per region, producing a stratified random sample of farms.

3.3.3 Data and sample collection

Participating farms were visited in September-October 2016 and March-April 2017 and blood samples were collected from all the cows and heifers over the age of six months on the farm on the date of the farm visit. Blood sampling was done in two batches in order to capture a more complete population of the study farms; the second visits allowed for sampling heifers that were too young on the first visit, and other cattle that were not on the farm on the first visit (e.g. on community pasture, lent to another farm, or purchased since the first visit). For farms with more than three milking cows and/or three youngstock, blood sampling was restricted to only 3 cows in milk and 3 heifers greater than six months of age per farm so that the overall results represent typical smallholder farmers in Kenya, without a bias toward larger farms.

Blood was collected from the coccygeal vein into sterile redtop blood collection tubes. The blood samples were kept on ice during transport, and in the evening, allowed to stand and clot undisturbed at room temperature for clot contraction. The serum was transferred into serum vials and frozen at -20^{o}C until the time of laboratory analysis.

At the time of blood collection, a risk factor questionnaire was also administered to the farmer, and the information obtained included: animal and farm demographics (e.g. breed, age, breeding method, parity, and herd size), animal reproductive history, and other hypothesized risk factors (e.g. possible exposures to other animals). On farms where cattle were sampled during both visits, the questionnaire was re-administered during the second visit for confirmation of the answers given during the first visit. Where the data were different, the farmer was asked for the response that better represents the normal practices or circumstances of the farm.

3.3.4 Laboratory methods

Serum samples were tested for antibodies to *Neospora caninum,* and antibodies and antigen to BVDV, according to the tests and standards set in the OIE manual (www.oie.int, search for *N caninum* and BVDV) using commercial Enzyme Linked Immunosorbent Assays (ELISA). Specific methods of the ELISA tests are described below.

Antibody ELISA tests were carried out for *N. caninum* exposure using the CHEKIT Neospora ELISA test kit (IDEXX Laboratories, Zurich, Switzerland). Sample preparation was done by diluting the serum 100-fold with sample diluent (5 µl of sample and 500µl of sample diluent). A 100 µl aliquot of the undiluted negative and positive controls were then dispensed in wells A1-A2 and A3-A4, respectively. A 100 µl aliquot of the diluted test sample was dispensed into the appropriate wells and the plate incubated for 30 minutes at room temperature. The plates were then rinsed off with 300µl of phosphate buffered wash solution four times. A hundred microliter aliquot of anti-bovine horse radish peroxidase conjugate was dispensed into each well and the plate incubated for a further 30 minutes at room temperature. Wells were again rinsed off and patted dry, and 100µl of the substrate was added, and then the plates were incubated for another ten minutes at room temperature. The final step was to dispense 100µl of stop solution to stop the reaction and immediately read off the optical density (OD) at 650nm wavelength using the spectrophotometer (MR-96A, Shenzhen Mindray Biomedical Electronics Company Limited, Shenzhen, China) that had been blanked on air. The average readings of the negative control wells (A1 and A2) and positive controls (A3 and A4) were obtained by calculating the means of the two respective well readings. The negative and positive control mean OD was used to validate the test as described on the manufacturer's guidelines. The positive wells appeared a hue of yellow in color. For the assay to be valid, the positive mean OD minus the negative mean OD had to be >0.15 and the negative mean OD had to be <0.2. The sample/positive (S/P) ratio was calculated using the absorbance obtained with the test sample and a positive control, corrected for the absorbance of the negative control, using the formula provided in the kits. All samples with S/P ratios equal to, or above, 0.5 were considered positive according to the manufacturer's guidelines. This kit is reported to have a sensitivity of 90% and specificity of 95%.

The presence of BVDV antigen was tested with E_{ms}-based Ag ELISA (HerdChek Ag/Serum test kit, IDEXX Laboratories, Switzerland). The sera, the plate and the reagents were thawed to room temperature. After recording the sample position on the worksheet, 50 µl of the detector antibody was added into each sample well, and 50 µl of the positive and negative control were then dispensed in wells A1-A2 and A3-A4, respectively, followed by 50 µl of the thawed serum sample into their respective wells. The contents of the wells were gently mixed by tapping, and the plate was incubated for 60 minutes at 37^{o}C. The plates were then rinsed with the wash solution provided in the kit 4 times and 100 µl of the conjugate was added into each well and incubated for 30 minutes. The plate was rinsed again, and 100 µl of tetramethylbenzidine (TMB) substrate was added into each well, and then plates were incubated for 10 minutes at room temperature. This step was followed by 100 µl of stop solution being added into the wells in the same order the substrate was added, and the optical density (OD) was read at 450 nm in the same spectrophotometer as described above. The positive wells appeared a hue of blue in color. The S/P ratio was calculated and samples with S/P ratios above 0.3 were considered positive according to the manufacturer's guidelines. This kit is reported to have a sensitivity of 98.7% and specificity of 95%, and is able to detect the majority of BVDV 1 and 2 antigens.

For BVDV antibody testing, IDEXX total antibody ELISA kits from IDEXX Laboratories, Switzerland were used. Plates and all the reagents were brought to room temperature together with all the reagents. One hundred µl of sample diluent was added into each well. Then 25 µl of positive and negative control samples were added into the respective labelled duplicate wells, and 25 µl of each serum sample were added into each respective sample well. Mixing of the samples in the wells was done by gently tapping the plate, and the plate was then incubated for 90 minutes at 18-26 degrees Celsius in a humid chamber after the plate was hermetically sealed. The solution was then removed, and each well was rinsed as described above and 100µl aliquot of anti-horse radish peroxidase conjugate was then added and the plate was incubated for 30 minutes at 18-26^{0}C. The plate was rinsed again, as described above, and TMB substrate was added. The plate was then incubated for 10 minutes at 18-26^{o}C and 100 µl of stop solution was added. A yellow color was generated and the absorbance was measured using the same spectrophotometer as described above at 450 nm wavelength. The S/P ratio was calculated and S/P ratios equal to, or above, 0.3 were considered positive according to the manufacturer's guidelines. This kit is reported to have a sensitivity of 100% and specificity of 95%, and is able to detect the majority of BVDV 1 and 2 antibodies.

3.3.5 Data management and analysis

Data were entered and organized in an Excel spreadsheet (Microsoft, Sacramento, California, USA). The unit of analysis was the seropositivity for the two pathogens on each individual animal in the farm at the time of sampling. Descriptive statistics for the animal- and farm-level variables and analytical statistics were carried out using STATA/IC 13.0 (StataCorp LLC, College station, Texas, USA).

Significant risk factors associated with infections with *N. caninum* and BVDV were determined through multivariable logistic regression models. In total, four models were fit to four outcome variables for this study: 1) risk factors of *N. caninum* antibody seropositivity; 2) risk factors of BVDV antibody seropositivity; 3) risk factors of BVDV antigen seropositivity; and 4) risk factors of co-infection of both pathogens (antibody positive to *N. caninum* and positive to antibodies and/or antigen to BVDV).

In the first step of the modeling, relationships between each independent variable and the outcome variable were individually investigated. In the second step, any variables that were associated at the $p<0.15$ level were eligible to be included in multivariable models. Correlation matrices between variables meeting the cut-off level ($-0.3<r<0.3$) were examined to determine correlations among these variables that would be important during the multivariable regression analyses. Both forward stepwise and backward elimination regressions were used to identify the most parsimonious model in which all independent variables remained significant at the $p < 0.05$ level. Other variables not in the final model were examined for confounding of the variables in the final model, as recommended by Dohoo et al. (2009). Interactions between significant variables in the final model were investigated. Model fit was examined by checking the standard residual diagnostics, performing predictions, and the predictive ability of the models. Clustering of cattle within farms was assessed through intra-class correlations. Seropositivity with BVDV antigen test had a 27.4% intra-class correlation, and therefore this model was run as a mixed logistic regression model with a random effect included for cattle clustered within farms, while the other regressions were run as ordinary logistic regression models with robust errors.

3.4 Results

3.4.1 Seroprevalence results

A total of 470 cattle from 158 farms were sampled and tested for Neospora and BVDV infections. Reasons for some of the 200 farms not being in this study included: no available milking cows, unwillingness to participate in the project and lack of availability due to death in the family or relocation out of the study area.

The seroprevalence for BVDV antibody was 47.1% (152/323) (95% CI=40.7% - 51.6%). Seroprevalence for BVDV antigen was 36.2 % (169/467) (95% CI=31.9% - 40.6%). There were 25.1% (81/323) of cattle positive for both BVDV antibody and antigen, which is 53.3% of the 152 cattle testing positive for antibody (81/152). Of the 163 cattle testing positive for BVDV antigen and having an antibody test result, 49.7% were also antibody positive, suggesting that half of antigen-positive animals were transiently infected (TI). Conversely, of the 163 cattle testing positive for BVDV antigen and having an antibody test result, 82 were antibody negative, suggesting that half of antigen-positive animals were either transiently (early stage before antibodies develop) or persistently infected (PI). These results would suggest that of the 470 animals sampled, there is evidence that 82 (17.4%) were potentially PIs. From the physical examinations carried out on the all cattle, only 7.9% were clinically ill at the time of sample collection. For logistical reasons, 144 samples tested for BVDV antigen were not available for BVDV antibody testing.

The seroprevalence of *N. caninum* in this area was 35.1% (165/470) (95% CI=30.9% - 39.5%). There were 18.5% (87/469) (95% CI=15.2% - 22.3%) of the samples testing positive for both *N. caninum* and BVDV when either of the BVDV tests was positive. Of the 165 animals found positive for *N. caninum*, 20.6% (34) had a reported abortion in the last five years. Twenty percent (34/169) of the cows reported seropositive for BVDV antigen had reported an abortion while this number was just slightly higher for BVDV antibodies at 23.6% (36/152). Of the 87 animals with co-infection of both these pathogens, (23% - 20/87) had a reported abortion in the last five years.

3.4.2 Demographic and univariable risk factor analysis results

Univariable associations to prevalence of *N. caninum* and/or BVDV were assessed at a cut-off point of $p<0.15$. Sixteen, 13, 15 and 17 predictors were found to be individually associated with *N. caninum*, BVDV antigen, BVDV antibody and co-infection (antibody positive to *N. caninum* and positive for antibodies and/or antigen to BVDV), respectively. The seroprevalence of *N. caninum*, BVDV antibody, BVDV antigen, and co-infection with *N. caninum* and BVDV antibody and/or antigen, by categories for the categorical variables that met the cut-off point, are shown in Tables 3-1 (cow-level) and 3-2 (farm-level).

With regards to age of the animals sampled, 58.1% (273/470) were between 3 and 8 ½ years old, while 26.2% (123/470) were fairly young, between ½ and 3 years of age. Only a small proportion was equal or over 9 years old, at 15.7 % (74/470), with an overall mean age of 5.6 ± 0.15 years for the whole sample population. The age range of the sampled animal was between 0.5-17 years. Compared to older cattle, young stock ≤ 3 years old had a significantly lower seroprevalence of BVDV antibodies and co-infection with both *N. caninum* and BVDV (either antibody and/or antigen) (Table 3-1).

Parity ranged from 0 to 9, with a mean of 2.2. The biggest proportion (61.7%) of cattle was in the one-to-three parity group (290/470), while nulliparous animals had a slightly lower proportion (17.9% - 84/470) than cows with four parities and above (20.4% - 96/470). Similar to age, compared to other parity groups (Table 3-1), nulliparous cattle had a significantly lower seroprevalence of BVDV antibodies and co-infections with both *N. caninum* and BVDV (either antibody and/or antigen).

Friesian crosses were the most common breed in the sample population (Table 3-1), with a proportion of 46.0% (216/470) of the sampled animals. The rest of the breeds found in the sample population included Guernsey crosses at 28.3% (133/470), Ayrshire crosses at 14.0% (66/470), Jersey crosses at 3.6% (17/470) and Zebus at 8.1% (38/470). The exotic breeds in the area were not pure breeds and therefore are all indicated as crosses, with a smaller body than expected but color markings that indicated the primary breed reported. Friesians and Zebus had a significantly higher seroprevalence of BVDV antigen than the other breeds (Table 3-1).

In this area, just less than half of the animals tested were fully zero-grazed (44.9%; 211/470) while 55.1%; (259/470) of the cattle were allowed to roam free for some hours of the day to graze in the household compound (partial grazing) or were grazed out on communal pastures along the roadside or in the forest (Table 3-2). There was no significant difference in the seroprevalence of the pathogens among the different management groups, although for BVDV antibody, this feeding management practice met the cut-off point for multivariable modeling.

There was a strong univariable association at $p<0.15$ between farmers that indicated buying and introducing milking cows into their farms with seropositivity of *N. caninum*, BVDV antigen and co-infection of the two pathogens (Table 3-2). A similar observation was made for cattle on farms where farmers allowed direct contact of their cattle with pigs. Cattle in farms that had a designated kennel for bitches to whelp in had a 41.5% proportion test positive to *N. caninum* and 29.5% to BVDV antigen. These two proportions showed a strong univariable association with BVDV antigen seroprevalence. A fifth (20.4%) of the farmers indicated buying and introducing calves into their farms. Smaller proportions of cattle in farms not introducing new calves were positive for *N. caninum* (22.9%) and BVDV antigen (21.9%) seroprevalence and formed important univariable associations. Over half (59.8%) of the farmers indicated that they allowed their cattle out to community pastures where they had contact with other dairy cattle and this variable formed important univariable association with *N. caninum*, and BVDV antigen and antibody seroprevalence. Also, a total of 7.87% of the farmers indicated lending animals to other farmers and within these farms, 56.8% 54.1% and 32.4% of the cows were positive for *N. caninum*, BVDV antigen and co-infection of the two pathogens, respectively.

3.4.3 Multivariable risk factor analyses

For the *N. caninum* multivariable model (Table 3-3), five farm level factors were shown as important factors at $p<0.05$, while no cow level variable was significantly associated with the risk of seropositivity in the final model. Cattle belonging to farmers that had bought and introduced cows to their farms in the last 12 months (OR=2.1) and farmers that lent animals out to other farmers (OR=3.1) were associated with increased odds of testing positive for *N.*

caninum. Cattle belonging to farmers that bought and introduced calves in the last 12 months of the study were associated with significantly lower odds of infection than farmers that had not introduced any calves (OR=0.4). Farms whose bitches had no designated whelping area and farms where dogs had access to aborted bovine fetuses were both significant risk factors in this model. However, the results in Table 3-3 for these last two variables are not shown because they were part of an interaction term in the model where the effect of one variable depended on the effect of the other variable (Figure 3-1). Cattle on farms whose bitches had no designated birthing area were nearly three times more likely to test seropositive for *N. caninum* when farmers allowed their dogs to access aborted fetuses compared to cattle owned by farmers who did not allow their dogs to access aborted fetuses.

When the model was fit incorporating BVDV as a risk factor (due to its ability for immunosuppression), the odd ratios of the above predictors and the interaction term changed very little. Furthermore, there was little evidence that BVDV was an important confounder in the model, with a non-significant p value of 0.317 for the 1.2 odds ratio. In this model with BVDV infection, direct contact with pigs was associated with three times higher odds of seropositivity (Table 3-3). However, since there is no indication in the literature of contact with pigs being a risk factor for *N. caninum* infection, and BVDV was not significant in the model or a confounder to other significant variables in the model, the model without BVDV was chosen as the final model for *N. caninum* seropositivity.

The Pearson goodness-of-fit test for this final model without BVDV showed that the model fit the data very well (p=0.58). One covariate pattern was found to be highly influential due to the fact that all 12 cattle in the covariate pattern were from farms that were lending animals, had introduced milking cows and had no designated birthing area for their bitches.

When observations with this covariate pattern were left out of the model, coefficients for all the predictors increased substantially, with the highest change recorded in the coefficient for lending animals. Therefore, the observations were retained in the final model, albeit with a note of caution for interpretation. The pseudo R^2 for the final *N. caninum* model was 0.072.

For the BVDV antigen multivariable mixed logistic model analysis, cattle on farms where they had direct contact with pigs had six times higher odds of testing positive for the antigen, compared to farms that did not allow this contact, while accounting for clustering of cattle within farms (Table 3-4). Cattle on farms that had bought and introduced open heifers (OR 0.14), and farms whose bitches had no designated birthing place (OR 0.4) were associated with lower odds of testing positive for BVDV antigen. Age of the animals was dichotomized at a cut-off point of 5.5 years (since the mean was 5.6 and median was 5.8), and when age was dichotomized, it formed two important interactions. On farms where no visiting dairy farmer entered the cow shed in the last year, older animals had a higher probability of testing seropositive for BVDV antigen than younger animals (Figure 3-2). Similarly, older animals had a reduced probability of testing positive for BVDV antigen when the farmers had introduced new calves to the farms but not when farmers had not introduced new calves (Figure 3-3). There was quite a bit of clustering of cattle within a farm in this model, indicated by an intra-class correlation of 27.4% for cattle within farms, confirming the need to control for clustering of cattle within farms. The Pearson goodness-of-fit showed that the model fit the data very well (p=0.265). For this model, the residuals at the farm level were normally distributed and the model was adopted as such.

The BVDV antibody multivariable mixed logistic model revealed that being a cow was associated with 11 times higher odds of testing seropositive over being a nulliparous heifer when all the other factors were held constant (Table 3-5). Cattle on farms that allowed contact of dairy cattle to other cattle through community pastures, and cattle on farms that allowed dairy cattle contact with pigs were associated with lower odds of testing positive for BVDV antibody compared to those cattle on farms that did not allow such contact. The Pearson goodness-of-fit showed that the model fit the data well (p=0.13). There were only nine covariate patterns in this model and thus dropping any of them would have had a big influence on the coefficients of the predictor variables. The pseudo R^2 of the final model was 0.117.

When risk factors for co-infection with *N. caninum* antibodies and BVDV antibodies and/or antigen were assessed (Table 3-6), parity was the only cow-level predictor found to be a risk factor (nulliparous cattle had lower odds of co-infection compared to cows with 3 or fewer parities and cows with 4 or more parities). Farm level risk factors in the final model included direct contact of dairy cattle with dogs (OR 1.7), direct contact of dairy cattle with goats (OR 2.4), and introduction of new milking cows (OR 1.8). The Pearson goodness-of-fit showed that the model fit the data well (p=0.20). The pseudo R^2 for the model was 0.07. Two covariate patterns were influential but not outlying and they were left in the model since dropping them meant dropping 76 observations.

3.5 Discussion

3.5.1 Neospora

Our study is the first of its kind to test for both BVDV antigen and antibody, along with *N. caninum* antibody, in a random sample of cattle in Kenya. Neospora caninum has been reported as the most important cause of bovine abortions in dairy populations worldwide and in this study, a seroprevalence of 35.1% was recorded. Worldwide prevalence has been estimated to range between 7.6% and 76.9% in America (Sousa et al., 2012; Cedeño & Benavides, 2013), 10.7% and 25.6% in Africa (Ibrahim et al., 2012; Okumu, 2014, 5.7% and 43% in Asia (Koiwai et al., 2006; Nazir et al., 2013), 0.5% and 27.9% in Europe (Bartels et al., 2006; Imre et al., 2012), and 10.2% in Oceania (Hall et al., 2005), which are comparable to the findings of the present study. In Kenya, *N. caninum* sero-prevalence has been recorded in dairy cattle on large-scale farms in the Rift Valley area (Okumu, 2014) with a prevalence of 25.6%. This research, being a second study carried out in Kenya on *N. caninum*, confirms the threat of this under-documented disease in Kenya, and likely many other developing countries.

Our study also found significant risk factors associated with *N. caninum* seropositivity relevant to the smallholder dairy farm context of Kenya. Farms that purchased and introduced milking cows were associated with higher odds of *N. caninum* (OR 2.1) compared to those that kept a closed herd (Table 3-3). In Croatia and Ethiopia (Asmare et al., 2014; Beck et al., 2010), purchasing animals for replacement raised the probability of acquiring infection by five times and two times, respectively. This association emphasizes the importance of biosecurity measures to prevent and reduce introduction of infected animals into farms. Purchasing animals from herds with unknown serological status is a common practise in this area of Kenya, and there is virtually no quarantine and testing done when the cattle are introduced into a new farm; naive cattle on the farm will be at risk of infection.

In this study, cattle on farms that reported lending animals had 3.1 higher odds of being seropositive than cattle on farms that were not lending animals (Table 3-3). Use of bulls for draught purposes (e.g. tilling of the land and hauling farm produce to the market) is a norm in this area of Kenya, and bulls are rented within the community for these reasons and other purposes. Although the majority of new infections for *N. caninum* are from vertical transmission *in utero*, horizontal spread is common through consumption of oocysts shed by canids (Dubey et al., 2007), and therefore movement to other farms or community pastures could increase the risk of exposure to these oocysts. There is need to educate farmers on the dangers of having animals move freely between farms and what they can do to reduce this risk.

Whelping area for farm dogs and farm dogs eating aborted bovine fetuses formed an interaction term in this study. Cattle on farms with bitches that had no designated birthing area and on farms where dogs had access to aborted fetuses had 3 times higher odds of likelihood of testing seropositive for *N. caninum* compared to cattle on farms that had a designated kennel for whelping and had dogs that ate aborted fetuses. In horizontal transmission of *N. caninum*, dogs become infected after eating tissues of intermediate hosts with the parasite, especially the placentas and nervous tissues of aborted fetuses, and they can intermittently shed the oocysts in their feces. Outside the host, the oocysts undergo sporulation in 24-72 hours and develop two sporocysts, each of which contains four sporozoites, which renders them orally infectious to the intermediate hosts (Dubey et al., 2007). Cattle grazing on oocyst-contaminated fodder can get infected, keeping the cycle going (Dubey et al., 2007; Williams et al., 2009). In this study, the absence of birthing kennels on some farms could be taken to mean that these dogs were free-roaming or were chained in a spot around the compound and thus their feces would in turn contaminate the cattle environment. These two factors together synergistically potentiate the likelihood of cattle on these farms testing seropositive. Complete inaccessibility of dogs to bovine placenta materials, dead calves, fetal membranes, aborted fetuses, raw/undercooked meat, and preventing the dogs from defecating in the compound, feeders, water sources and pastures are among the major ways suggested to curtail the infection and keep susceptible animals free of *N. caninum* (Silva & Machado, 2016). Using correct placenta disposal methods that limit them from being eaten by canids has been found to lower the prevalence of *N. caninum* in dairy cows

(Bruhn et al., 2013). However, in our study, placenta disposal method was not significantly associated with *N. caninum* seropositivity, perhaps because there were few farms (5.5%) that used correct placenta disposal methods (incineration or burying in a deep hole).

Interestingly, this study indicated that introducing new calves into the farm was an important protective factor against *N. caninum* seroprevalence (OR 0.4). As most farmers in this area of Kenya are resource-constrained and are still faced with the problem of replacement heifers, there is a preference of purchasing weaned heifer calves and raising them to adulthood over purchasing pregnant heifers or adult cows which would be expensive. It may be possible that when calves are purchased, those calves do not survive to become pregnant and have an abortion for horizontal spread, or give birth to a vertically infected calf. Conversely, purchased infected adult cows could quickly lead to horizontal or vertical transmission when those cows become pregnant, which would likely be much sooner than the young calf. Furthermore, if the purchased calves are infected bull calves, they cannot spread *N. caninum* vertically, and would only contribute to horizontal spread if the animal dies and part of it is consumed by canids. For both these reasons, purchased calves could appear to be protective, even though purchasing animals would generally be considered a risk factor for spread of infections. As only calves from six months and older were tested in this study (i.e. there was no pre-colostral testing of newborn calves), it is not easy to conclude about the level of congenital transmission of *N. caninum* in this area or the seroprevalence of calves born with the infection. However, of the nulliparous heifers in our study, 27.8% were seropositive, suggesting the need for a more detailed cohort study that would look at the level of congenital transmission of *N. caninum* in this area.

3.5.2 Bovine viral diarrhoea virus

The overall apparent prevalence of BVDV in cattle in this study area was 36.1% on antigen ELISA and 46.1% on antibody ELISA. As vaccination against BVDV has never been carried out in this area, the presence of antibodies indicated a natural exposure to BVDV in the past, while the presence of antigen indicated the presence of animals transiently or persistently infected with BVDV. There is minimal documentation of BVDV as a cause of reproductive wastages in Kenya; in large-scale farms in Rift Valley, Kenya, an antibody prevalence in dairy cows of 79.1% was recorded (Okumu, 2014). In Zebu cows in the Coastal area of Kenya, a 45.8% prevalence was recorded (Kenyanjui et al., 1994), but only 19.8% of Zebu cows were positive in western Kenya (Callaby et al., 2016).

Since BVDV is easily transmitted between cattle through body secretions, and BVDV antibodies developing from transient infections can remain in circulation for long periods of time (Lindberg & Houe, 2005), it was not surprising that our prevalence of BVDV antibodies approached or exceeded these other reports. Having a third of tested cattle testing positive for BVDV antigens was surprising, suggesting that a substantial proportion of cattle had either transient or persistent infections of BVDV at the time of blood sampling, despite showing little or no clinical signs of BVDV disease. However, these BVDV test results may be partly a function of test cross-reaction with classical swine fever (CSF) virus because CSF is found in Kenya, CSF virus is in the same pestivirus family as BVDV, BVDV can infect pigs, and others have found cross-reaction between CSF on BVDV tests (Loeffen et al., 2009; Gatto et al., 2018). Furthermore, there is very recent preliminary evidence for possible cattle infection with CSF virus in China and India (Giangaspero et al., 2017), which could further complicate the interpretation of our results if the long-held belief that CSF only infects swine is confirmed to be untrue. Project funding did not include testing for CSF virus. Therefore the BVDV results reported for this project are based on the test results obtained with the BVDV tests, but they should be interpreted with caution. Future research should explore the relationship between BVDV and CSF virus in cattle and pigs in Kenyan SDFs.

In an infected herd, there are principally two sources of BVDV, animals that are persistently infected and animals that undergo a transient infection (TI). Persistently infected cattle generally play a substantially larger role in transmitting the virus than transiently infected cattle. They are always viremic (virus-positive and usually antibody–negative), are often asymptomatic, and continually shed large amounts of BVDV in all body secretions (Lindberg & Houe, 2005). A similar situation of being antigen-positive and antibody–negative is seen in animals that are very early in the transient infection stage where there is an infection but the antibody response in their bodies has not formed yet. Even though PI cattle are usually seronegative to BVDV antibodies once maternal antibodies have cleared, an immune response can be elicited to a heterogeneous strain of BVDV, turning them seropositive to antibody. This antibody response can follow either natural or vaccine exposure (Tinsley et al., 2012). These dynamics should be kept in mind when interpreting these prevalence results.

In terms of risk factors for BVDV antigen, direct contact of dairy cattle with pigs was in the final model (Table 3-4). Farmers allowing contact between these two species of animals put their dairy cattle at six times higher odds of testing seropositive for BVDV antigen than cattle on farms where this contact is not allowed. Farmers that kept a few pigs on the farm would frequently have the pig sty constructed just next to the cow shed, allowing very close contact of these two species of animals at all times. Bovine viral diarrhoea virus has been reported to cross from cattle to pigs, and cause disease in both of these species (Liess & Moennig, 1990). Bovine viral diarrhoea virus has recently been found in sheep, goats, pigs, buffaloes and other wildlife, although the role of these species in BVDV transmission to and from cattle has not been experimentally proven (Khodakaram-Tafti & Farjanikish, 2017). The role that pigs play as risk factors to bovine BVDV occurences remains unclear and may be a function of cross-reactions of tests between BVDV and classical swine fever virus, BVDV transmission between pigs and cattle, and/or CSFV transmission between pigs and cattle, as mentioned above (Loeffen et al., 2009; Gatto et al., 2018).

Age of the test animals formed important interactions with other variables in BVDV antigen model. On farms where no visiting dairy farmer entered the cow shed in the last year, older animals had a higher probability of testing seropositive for BVDV antigen than younger animals (Figure 3-2). Also, older animals had a reduced probability of testing positive for BVDV antigen when the farmers had introduced new calves to the farms but not when farmers had not introduced new calves (Figure 3-3). In Ireland, BVDV was reported to have higher seroprevalence in adult cows than in calves less than 9 months old (Sayers et al., 2015). An increase in seroprevalence from 10% in heifers to 75-85% in cows aged 10 years has been reported, possibly due to an increase in cumulative risk of having been exposed over time (Daves et al., 2016). Farmers that indicated having other farmers visit and access their cow sheds tended to belong to local dairy-based self-help groups that were organizing some training sessions on dairy management. Since the exposure of other dairy farmers in the two animal age subgroups differed, it can only be speculated that the above trend could have emanated from the fact that farmers keeping older cows and involved in this self-help groups had had their cattle exposed at some point, and the cattle had recovered and had immunity build up in their systems, leading to reduced risk of reinfection with a related strain of virus. This result requires a more detailed study whereby these relationships can be explored and explained better.

The present study also indicated that buying and introducing open heifers and calves into new farms were significant in the final model and were associated with reduced risk of BVDV antigen seropositivity (Table 3-4). This result could be explained by the fact that younger cattle had a higher probability of not being infected compared to older milking cows, and thus the older cattle would have higher odds of testing positive if they were to be tested at purchase. This result emphasizes the need for testing purchased animals (if purchased animals are needed) as one of the major ways of keeping disease-free herds that way. In this study area, it is a common practice to exchange animals through purchase or lending without any testing for any diseases or executing any quarantine practice. Purchase and exchange of animals has been identified as a classic risk for the occurrence and dissemination of infectious organisms (Fèvre et al., 2006). In a study in Brazil, the only farms that did not have any seropositive animals were those that did not have a history of purchasing or exchanging animals (Marques et al., 2016).

For the BVDV antibody seropositivity final model, cows were 12 times more likely to test seropositive for BVDV antibodies then heifers (Table 3-5). Our results differ with what was obtained in Danish dairy herds by Houe & Meyling in (1991) who reported that the risks of BVDV infection were approximately similar in all age groups. The dissimilarities may be due to farm size and the fact that our study was carried out in smallholder settings where cows are rarely culled for their age or low production, and therefore can remain on the farm for as long as the farmer will have them (the oldest cow in the study being 17 years old). Age as a risk factor is probably due to the fact that BVDV antibodies from infections (versus maternal antibodies) can last a long time, and perhaps even a lifetime, therefore, the older the animals are, the higher the probability that it has been infected during its lifetime (Talebkhan et al., 2009). It was common to find cows older than 10 years (8%) in our study, with 41.4% of these older cows being seropositive for BVDV antibody.

This model has shown that direct contact between dairy cattle and pigs was protective towards BVDV antibody seropositivity and was associated with an OR of 0.4. This result would indicate that cattle on farms without pigs were more likely to be BVDV antibody positive than cattle of farms with pigs. One explanation for this finding could be that CSF is spread from pigs to cattle on farms with both, and the CSF virus is offering some protection against BVDV infection. Antigen seropositivity can be a function of either persistently or transiently infected cattle, and would only be found in transiently infected cattle if the blood sample were taken during early infection before an immune response is mounted to clear the infection.

A surprising factor that was found to be protective in this BVDV antibody model was contact of dairy cattle with other dairy cows in community pastures. In this setting in Kenya, community pastures were defined as either grazing along the roadsides, school and/or church yards, or portions of open land in the forest. It was noted that animals that were grazed in the school and/or church yards were driven to the yards in the morning and collected in the evening and there was a fee attached to grazing in these areas that the farmers were required to pay. This type of community pasture grazing usually limited the number of farmers grazing their cattle in these areas and would therefore lead to limited or no contact with other dairy cattle. Cattle driven into the forest were mainly left there for longer periods of time. Farmers that reported constantly grazing their cattle in the forest usually had a few cows that were considered “zero-grazed” and were usually left behind in the homestead, and those cattle remaining behind were the ones that were sampled. There was certainly a chance that there would eventually be nose-to-nose contact between the cattle that were grazed in the forest and those that were left behind on the farm, and this relationship would need to be explored further. There were also a number of farmers that indicated grazing their cattle along the roadside especially during the dry season. Nose-to-nose contact between cattle from different farms would be possible through this type of community pasture. With the different types of community pasture possible, future research should differentiate the type of community pasture utilized to be able to more clearly identify which types present risk of infection and which types could be protective or do not present risk of infection.

3.5.3 Bovine viral diarrhea virus and *Neospora caninum* co-infection

From our sample population, 18.2 % (87/469) of the animals were positive for both *N. caninum* (antibodies) and BVDV (antibodies and/or antigens). It has been suggested that concurrent infection with BVDV could be a possible contributory factor in *N. caninum* abortion outbreaks; due to BVDV's immunosuppressive nature, it increases a hosts' susceptibility to other infectious agents (Björkman et al., 2000). A statistically significant association between antibodies against *N. caninum* and BVDV was found in Swedish cattle, and had a direct association to abortions (Björkman et al., 2000). In a previous study in Kenyan cattle, 83.3% of the *N. caninum* positive cattle were also positive for BVDV, and more abortions were reported in cattle that had sero-positivity for more than one abortifacient pathogen compared to cattle positive for only one abortifacient pathogen (Okumu, 2014). This result supports the observation that BVDV and *N. caninum* infections complement each other in causing abortions, but further investigation with a larger sample size in a longitudinal study would provide more evidence to confirm this observation. In terms of risk factors for *N. caninum* and BVDV co-infection, a higher OR was recorded for cows between parity 1 and 3, and also over 3 parities, compared to those that had not delivered yet (Table 3-6). Parity was also shown as a risk factor of BVDV infection elsewhere (Muñoz-Zanzi et al., 2003).

Dairy cattle contact with dogs was associated with an OR of 1.7 indicating that it was a risk factor for co-infection of *N. caninum* and BVDV (Table 3-6). Otranto et al. (2003) reported a higher seropositivity for *N. caninum* in farms with two or more dogs than in those with one dog or none. For this study, it was not possible to draw definitive conclusions on the involvement of dogs in transmission of bovine infection since no samples were collected for testing from dogs on the farm or in the area. In a study carried out in the Rift Valley part of Kenya (Okumu, 2014), a 17.9% seroprevalence of *N. caninum* was reported in dogs found on farms, compared with a 0% prevalence in feral dogs in a study carried out 17 years ago in Kenya (Barber et al., 1997). A better understanding is needed to show the contribution of farm dogs and the risk of their close contact with dairy cattle, especially in cases of cross-infection with *Neospora* and another abortifacient.

Dairy cattle contact with goats was another risk factor to co-infection. It is known that sheep and goats can be infected by BVDV-1, BVDV-2 and border disease virus, producing similar clinical signs of BVDV infection to cattle (Kim et al., 2006). Transmission of BVDV infection between small ruminants and cattle have been demonstrated, although usually it is from cattle to sheep or goats, and cattle can become infected with border disease virus (Handel et al., 2011b). There are also reports of *N. caninum* abortions in goats in Brazil (Varaschin et al., 2012) acting as intermediate hosts in the lifecycle of *N. caninum.* Therefore, goats' contributions to the seroprevalence of the two pathogens should not be ignored. In Kenya, small ruminants are usually herded together with cattle or freely grazed out in the farm compound. Purchase and introduction of milking cows was a risk factor as had been seen in the *N. caninum* model above.

3.6 Study Limitations and Future Research

Serological investigations with a cross-sectional design have both advantages and disadvantages as methods to establish the prevalence and risk factors of infection. Cross-sectional studies for antibodies to pathogens provide good prevalence estimates when the pathogens and/or antibodies are persistent. For BVDV, animals are generally seropositive for at least several years after the infection (Fredriksen et al., 1999), while *N. caninum* infections are retained for life (Dubey et al., 2007). Therefore, a cross-sectional study for antibodies to test for BVDV and *N. caninum* should have produced relevant estimates of infection prevalence in the study area of Kenya where vaccines for these two diseases are rare. However, identifying risk factors for prevalence of infection is not as helpful as identifying risk factors for incidence of infection because prevalence is a function of both incidence and duration, complicating the interpretation of the statistical results (Dohoo et al., 2009). Future research on risk factors of incidence of infection would be helpful to tease out which prevalence risk factors are also incidence risk factors. Risk factors of incidence may also clarify some of the unexpected risk factor results.

Interpreting BVDV test results can be confusing and complicated. Given the logistics of the project, we did our best with testing for antibodies and antigens, assuming that if they were antigen positive and antibody negative, they were likely either transiently (in early stages of infection before antibodies develop) or persistently infected. However, taking a second sample a month after the first one that was test-positive would have been helpful but that was logistically impossible because the test kits had to be imported into the country once all samples were taken and the number of samples to be tested known. Furthermore, where pigs and goats are kept in close proximity to cattle, the role of classical swine fever virus (Giangaspero 2017) and border disease virus (Kim et al., 2006) should be considered, in terms of study design (testing) and interpretation of results. For logistical reasons, we were unable to test our sera for these other viruses.

There were 147 samples that were not run for the BVDV antibody test after they were tested for BVDV antigen test. This difference in sample numbers for BVDV testing was purely logistical where not enough antibody kits were available at the initial time of antigen testing. Then, a lab problem meant the samples were no longer available for testing when the antibody kits did arrive. It is unlikely that a bias was introduced from the difference in sample numbers because the reason for the difference in sample numbers was not related to the purpose of the study.

The risk factor analysis of co-infections utilized an outcome variable definition that included cattle that were antibody-positive for *N. caninum* and also antibody-positive and/or antigen-positive for BVDV. The reason for the inclusivity of the BVDV results was to ensure that we included all cattle exposed to BVDV, either current or historical, since we were using all cattle exposed to *N. caninum*, regardless of when they became infected. We could have defined co-infection to include just cattle that were either antibody-positive or antigen-positive for BVDV, but that would have led to a different interpretation of the results. Using only antigen-positive cattle would include only current TI cattle and PI cattle, but not previously exposed TI cattle. Using only antibody-positive cattle would include only previously exposed TI cattle. We chose to be inclusive in our definition.

3.7 Conclusions and recommendations

The seroprevalence of *N. caninum*, BVDV antibody, BVDV antigen, and co-infection with *N. caninum* and BVDV antibody and/or antigen in the study area were 35.1%, 47.1%, 36.2% and 18.5%, respectively. For *N. caninum* infection, lending of cattle between farms and farmers buying and introducing milking cows to their farms were risk factors, while introducing calves into the farms was a protective factor. In an interaction variable, cattle on farms whose bitches had no designated whelping area were more likely to test seropositive for *N. caninum* when farmers allowed their dogs to access aborted fetuses compared to cattle owned by farmers who did not allow their dogs to access aborted fetuses. For BVDV antigen seropositivity, buying and introducing open heifers (versus cows) and farm dogs having a designated birthing kennel were protective factors, while direct contact of cattle with pigs was a risk factor. Age was involved in two interaction variables. On farms where no visiting dairy farmer entered the cow shed in the last year, older animals had a higher probability of testing seropositive for BVDV antigen than younger animals. Similarly, older animals appeared to have a reduced probability of testing positive for BVDV antigen when the farmers had introduced new calves to the farms but not when farmers had not introduced new calves. For BVDV antibody seropositivity, age of the cows (cows versus heifers) was a risk factor while direct contact of dairy cattle with pigs and with other dairy cows in community pastures appeared to be protective factors. For co-infections between *N. caninum* and BVDV antibody and/or antigen seropositivity, parity, direct contact of dairy cattle with dogs and with goats, and buying and introducing milking cows into farms were all significant risk factors in the final model.

There is no record of vaccination against BVDV in Kenyan smallholder dairy cattle. Identifying ways to have BVDV vaccinations introduced by a government vaccination protocol and/or industry-led program would go a long way in curtailing new infections that are likely happening, especially in areas where livestock have close contact to other livestock and wildlife. Farmer education on the prevalence of infections of reproductive importance and methods of their control through biosecurity need to be emphasized in this and other areas of Kenya.

Recovery and testing of aborted foetuses as well as placental tissue would go a long way in enhancing the diagnosis of the actual causes of abortion in this area. Farmers and animal health providers should be informed on the importance of submitting these samples in cases of abortion. More research should also be carried out to determine the effects on production, as well as economic impacts of abortifacient pathogens in the dairy cattle industry in this country.

3.8 References

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Table 3-1: Descriptive statistics and univariable associations of categorical cow-level risk factors for seroprevalence of *Neospora caninum* and BVDV on 158 smallholder dairy farms in Kenya in 2016-17

Categories of certain hypothesized risk factors	**Positive (%) to *Neospora caninum* (n=470)**	**Positive (%) to BVDV antigen (n=467)**	**Positive (%) to BVDV antibody (n=323)**	**Co-infection (%) to BVDV & *N. caninum* (n=469)**
Age in years				
• ≤3.0	38/123 (30.9)	35/122 (28.7)	19/83 (22.9)	12/123 (9.76)
• 3.5-8.5	100/273 (36.6)	107/273 (39.2)	103/189 (54.5)	55/273 (20.1)
• ≥9.0	27/74 (36.5)	27/72 (37.5)	27/51 (52.9)	20/74 (27.0)
P-value	0.523	**0.129**	**<0.005**	**0.006**
Parity				
• 0	23/84 (27.4)	18/83 (21.7)	6/59 (10.2)	6/84 (7.14)
• 1-3	108/290 (37.2)	118/290 (40.7)	108/200 (54.0)	56/290 (19.3)
• ≥4	34/96 (35.4)	33/94 (35.1)	35/64 (54.7)	23/96 (24.0)
P-value	0.254	**0.006**	**<0.005**	**0.006**
Breed				
• Friesian	72/216 (33.3)	88/214 (41.1)	70/153 (45.8)	42/216 (19.4)
• Ayrshire/Jersey	30/83 (36.1)	21/83 (25.3)	28/55 (50.9)	14/83 (16.9)
• Guernsey	49/133 (36.8)	42/132 (31.8)	38/88 (43.2)	20/133 (15.0)
• Zebu	14/38 (36.8)	18/38 (47.4)	13/27 (48.1)	9/38 (23.7)
P-value	0.905	**0.022**	0.705	0.400

Table 3-2: Descriptive statistics and univariable associations of categorical farm-level risk factors for seroprevalence of *Neospora caninum* and BVDV on 158 smallholder dairy farms in Kenya in 2016-17.

Categories of certain hypothesized risk factors	**Positive (%) to *Neospora caninum* (n=470)**	**Positive (%) to BVDV antigen (n=467)**	**Positive (%) to BVDV antibody (n=323)**	**Co-infection (%) to BVDV & *N. caninum* (n=469)**
Feeding practice				
• Zero grazed	77/211 (36.5)	81/211 (38.4)	58/140 (41.3)	41/211 (19.4)
• Not zero grazed	88/259 (33.9)	88/256 (34.4)	91/183 (49.7)	46/259 (17.8)
p value	0.570	0.369	**0.084**	0.643
Farmers lending animals				
• No	144/433 (**33.3)**	149/430 (34.6)	138/229 (46.2)	75/433 (17.3)
• Yes	21/37 (56.8)	20/37 (54.1)	11/24 (45.8)	12/37 (32.4)
p value	**0.004**	**0.018**	0.883	**0.023**
Introducing new milking cows into farms				
• No	42/152 (27.3)	69/154 (54.4)	31/79 (39.2)	21/154 (13.6)
• Yes	123/316 (38.9)	100/315 (31.8)	118/244 (48.4)	66/316 (20.9)
p value	**0.013**	**0.004**	0.196	**0.058**
Dairy cows contact with dogs				
• No	95/298 (31.8)	110/297 (37.0)	89/202 (44.1)	46/298 (15.4)
• Yes	70/172 (40.7)	59/170 (34.7)	60/121 (49.6)	41/172 (23.8)
p value	**0.054**	0.614	0.282	**0.024**
Dairy cows contact with pigs				
• No	154/451 (34.2)	157/449 (35.0)	144/306 (47.1)	79/451 (17.5)
• Yes	11/19 (57.9)	12/18 (36.2)	5/17 (29.4)	8/19 (42.1)
p value	**0.034**	**0.006**	0.315	**0.007**
Whelping areas				
• Kennel	84/274 (30.7)	112/274 (40.9)	91/193 (47.2)	48/274 (17.5)
• Compound/feed-store	81/196 (41.3)	57/193 (29.5)	58/130 (44.6)	39/196 (19.9)
p value	**0.017**	**0.012**	0.868	0.512
Introducing new calves to farms				
• No	143/374 (38.2)	148/371 (39.9)	105/238 (44.1)	72/374 (19.3)
• Yes	22/96 (22.9)	21/96 (21.9)	44/85 (51.8)	15/96 (15.6)
P value	**0.005**	**0.001**	0.444	0.414
Community pastures contact				
• No	59/189 (31.2)	76/189 (40.2)	99/127 (52.0)	32/189 (17.0)
• Yes	106/281 (37.7)	93/278 (33.5)	83/196 (42.4)	55/281 (18.5)
p value	**0.147**	**0.136**	**0.126**	0.470

Table 3-3: Final model of risk factors associated with seropositivity of *N. caninum*, with (n=470) and without (n=469) controlling for BVDV antigen as a confounder, in dairy cattle on 158 smallholder dairy farms in Kenya in 2016-17.

	While controlling for BVDV antigen			**Without controlling for BVDV antigen**		
Variable	**OR**	**95% CI_{OR}**	**P-value**	**OR**	**95% CI_{OR}**	**P-value**
BDVD antigen						
• Negative	Baseline					
• Positive	1.25	0.81-1.94	0.317			
Milking cows introduced into the farm						
• No	Baseline			Baseline		
• Yes	2.17	1.38-3.43	0.001	2.11	1.35-3.29	<0.001
Lending cattle						
• No	Baseline			Baseline		
• Yes	3.03	1.31-6.98	0.009	3.12	1.37-7.07	0.006
Calves introduced into the farm						
• No	Baseline			Baseline		
• Yes	0.36	0.20-0.63	<0.001	0.35	0.20-0.62	<0.001
Dairy cattle contact with pigs						
• No	Baseline					
• Yes	3.08	1.04-9.15	0.043			
Farm dogs access to aborted fetuses	Baseline			Baseline		
•	a	a	0.698	c	c	0.816
Whelping area						
•	Baseline			Baseline		
	a	a	0.095	c	c	0.150
Interaction						
•	Baseline			Baseline		
	b	b	0.030	d	d	0.035

a&c- variables which are part of an interaction so coefficient of main effects is best reported using a graph
b&d- interaction variables which have many cross-tabulated categories for main effects (not shown) so the coefficients are best reported using a graph

Table 3-4: Final model of risk factors associated with seropositivity for BVDV antigens in 467 dairy cattle on 158 farms in Kenya in 2016-17.

Variables	OR	95% CI_{OR}	P-value
Direct contact of dairy cows with pigs			
• No	Baseline		
• Yes	6.14	1.29-29.2	0.022
Open heifers introduced into the farm			
• No	Baseline		
• Yes	0.14	0.06-0.29	<0.001
Whelping area			
• Kennel	Baseline		
• Cow compound/feed storage	0.37	0.20-0.71	0.003
Age of the animals			
•	a	a	<0.005
Other dairy farmers accessing cowshed			
•	a	a	0.600
Age / dairy farmers accessing cowshed interaction			
•	b	b	0.008
Calves introduced to farms			
•	c	c	0.586
Age / calves introduced interaction			
•	d	d	0.002

a&c -variables which are part of an interaction so coefficient of main effects and best reported using a graph
b&d- interaction variables which have many cross-tabulated categories for main effects (not shown) so the coefficients are best reported using a graph

Table 3-5: Final model of risk factors associated with seropositivity for BVDV antibodies in 323 dairy cattle on 158 farms in Kenya in 2016-17.

Variable –Antibody titres	**OR**	**95% CI_{OR}**	**P-value**
Parity			
• Nulliparous heifers	Baseline		
• Cow (≥ 1 parity)	11.86	4.85-29.05	<0.001
Direct contact of dairy cows with pigs			
• No	Baseline		
• Yes	0.35	0.13-0.98	0.045
Contact of dairy cows with other cattle through community pasture			
• No	Baseline		
• Yes	0.58	0.34-0.95	0.032

Table 3-6: Final model of risk factors associated with seropositivity for *N. caninum* and BVDV co-infection in 469 dairy cattle on 158 farms in Kenya in 2016-18.

Variable	OR	95% CI_{OR}	P-value
Parity			*0.004
• 0	Baseline		
• $\geq$1 but $\leq$3	3.02	1.23-7.36	0.015
• >3	4.31	1.63-11.38	0.003
Dairy cattle contact with dogs			
• No	Baseline		
• Yes	1.67	1.03-2.70	0.037
Dairy cattle contact with goats			
• No	Baseline		
• Yes	2.43	1.37-4.28	0.002
Milking cows introduced into the farm			
• No	Baseline		
• Yes	1.77	1.04-3.01	0.035

P-value*: Global P-value

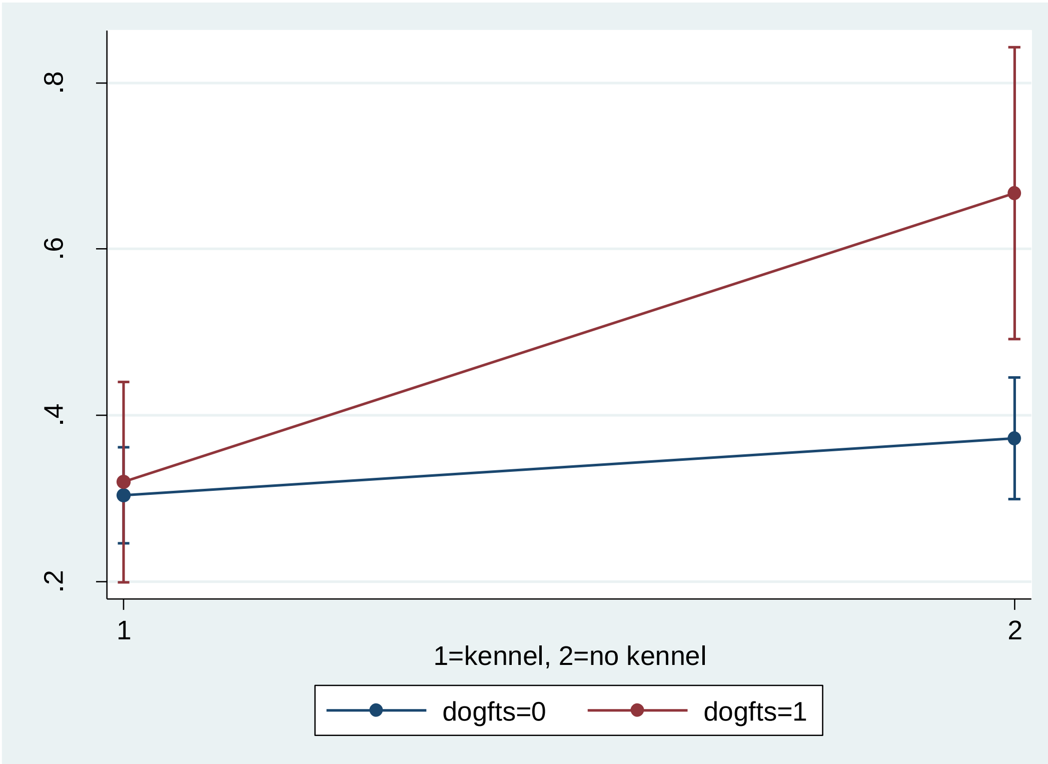

Figure 3-1: Interaction plot of birthing areas for bitches and dogs eating aborted fetuses (dogfts), for the Neospora seropositivity final risk factor model in 469 dairy cattle on 158 smallholder dairy farms in Kenya in 2016-17.

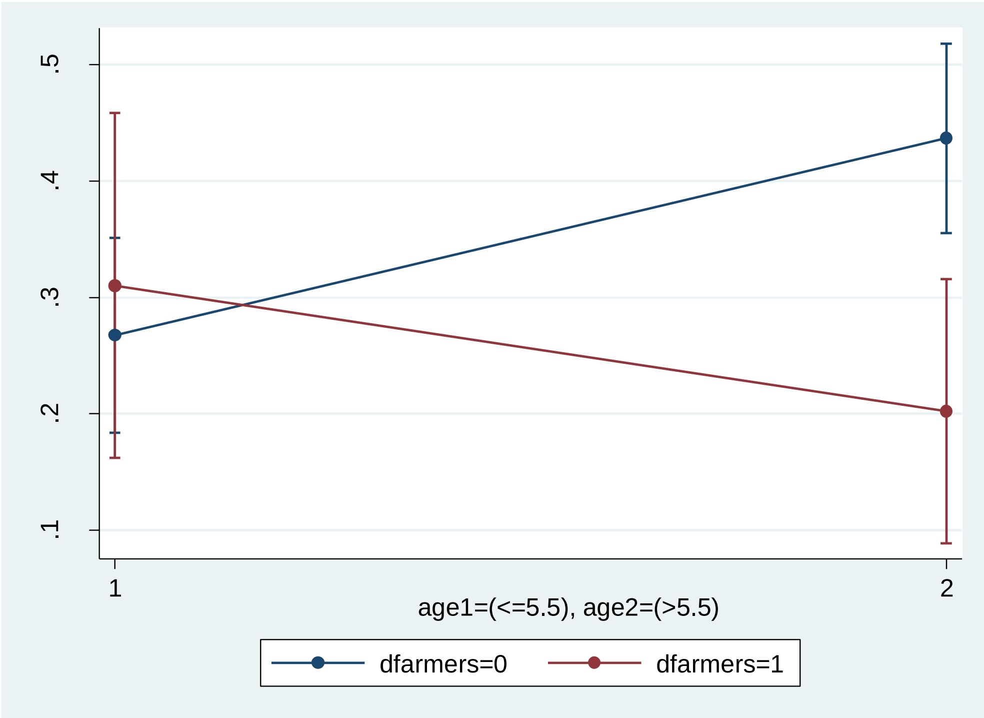

Figure 3-2: Interaction plot between age of the test animals and other dairy farmers accessing the cow shed (dfarmers) in 467 dairy cattle on 158 farms in Kenya in 2016-17.

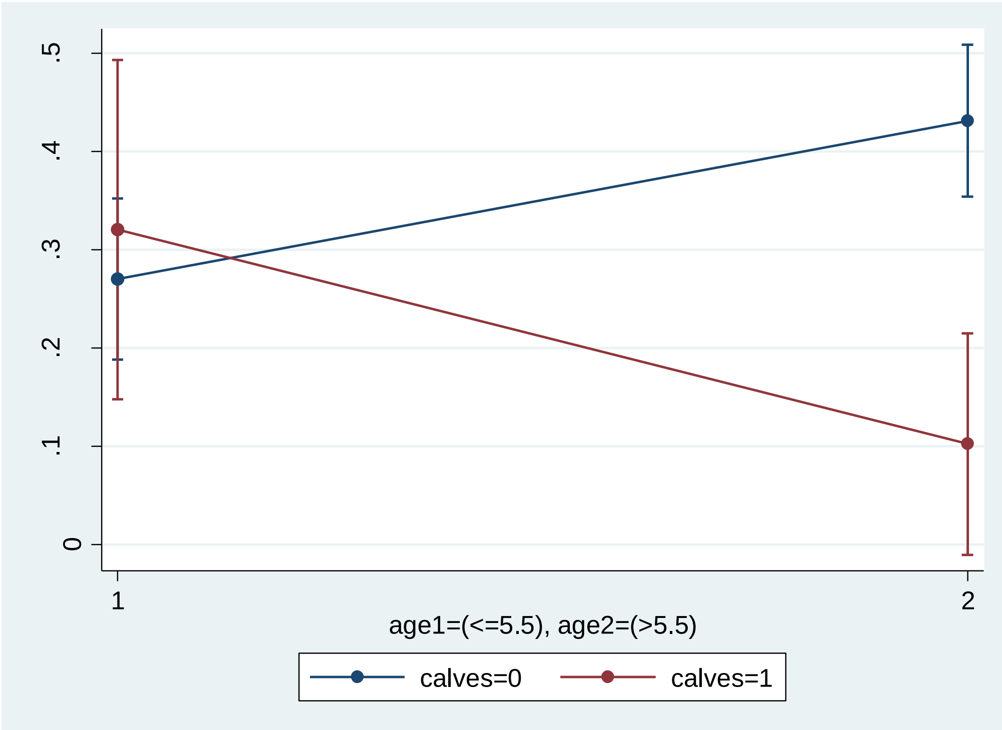

Figure 3-3: Interaction plot between age of the test animals and introducing calves into the farms in 467 dairy cattle on 158 farms in Kenya in 2016-17.

Chapter 4 Randomized controlled trial on impacts of using sex-sorted semen and reproductive hormones in smallholder dairy cows in Eastern Kenya.

4.1 Abstract

Lack of replacement heifers is one of the major limitations facing the expanding smallholder dairy industry in Kenya. The goal of one calf born per cow per year is rarely achieved in Kenya, thus getting a female calf when cows deliver is important for the pool of needed replacement heifers. To date, few studies have examined the utilization of sexed semen in dairy cattle in the subtropical regions. The primary aim of the present study was to assess the effective use of sexed semen in various contexts of smallholder dairy cows in Kenya.

A hundred farmers were randomly selected and randomly allocated to five intervention groups receiving: 1) reproduction only; 2) nutrition only; 3) reproduction and nutrition; 4) other cow management education only (quasi-control); or 5) nothing (control). Reproduction interventions included provision of prostaglandin F2α and gonadotropin releasing hormone to induce heat in cows, as needed, and education on reproduction, whereas nutrition interventions included provision of leguminous shrubs in addition to education on nutrition. In groups 1 and 3, breeding using sexed semen was allowed up to two times per cow, once breeding criteria were achieved: 1) body condition score (BCS) $\geq$2.25 or BCS$\geq$2.0 and rising; 2) 60-300 days in milk; 3) functioning ovaries; and 4) mucus at previous heat as observed by the farmer was clear. In groups 2, 3 and 5, farmers were given one dose of sexed semen to use on any cow with/without meeting breeding criteria. Cattle were examined approximately monthly (groups 1-3) or bimonthly (groups 4-5) and data were collected on cattle and farm management characteristics

on these visits for 17 months in total. A multivariable Cox proportional hazards model was fit for calving-to-conception interval.

The overall conception risk achieved with sexed semen was 44.0% in cows and 54.5% in heifers. Twenty six percent of the enrolled cow records reported an acceptable BCS. Among the cows, the control group had the lowest service percentage at only 8.5%, and the reproduction group recorded the highest service percentage at 35.5%. In the final Cox proportional hazards model, accounting for time to conception, each unit increase in the average body condition score of the cows was associated with 3.5 times higher hazard of conception. Using sexed semen was associated with 2.0 times higher hazard of conception over using conventional semen and this was partly a function of the reproductive interventions assisting cows to be bred sooner with sexed semen when they met the breeding criteria. Overall, sexed semen had lower conception risk than conventional semen, but over time (because of the higher CCI in the conventional semen group), the conception hazard was higher with sexed semen. Cows on farms where farmers had attended any dairy related training other than what was provided by the researchers in this study had 1.8 times higher hazard of conception than cows in farms where owners had not attended any dairy related training. Cows that were inseminated following spontaneous heat had 1.8 higher hazard of conception over cows that were inseminated following heat after induction using reproductive hormones. There was a significant interaction in the study between the intervention given and cows that had been supplemented with dairy meal concentrate during the last month of gestation. When cows were supplemented with dairy meal in the last month of gestation, higher relative hazard of conception was achieved in cows on farms where leguminous shrubs were used than on farms where no leguminous shrubs were used.

This study concludes that sexed semen has potential for providing much needed replacement heifers in the smallholder dairy setting in the tropics, but BCS management needs to be practiced to give cows the best chance of conception. Drought-resistant leguminous shrubs should be promoted as a useful way of supplementing cows with a higher protein diet to improve CCI.

4.2 Introduction

Efficiency of calf production on smallholder dairy farms in the tropics is low due to the late sexual maturity of heifers and long calving intervals of cows (Centurión-castro et al., 2013). This situation is caused by many stressors such as heat and humidity (Khorshidi et al., 2017), diseases, parasites, poor nutrition and loss of cow's body condition score during lactation (Centurión-castro et al., 2013). Primary reproductive factors that extend calving intervals are a delay in the resumption of cycling in cows after calving, a weak estrous expression, and low detection rates of estrus (Kurykin, 2017).

The peri-partum period is critical to subsequent health and fertility. In early lactation, dry matter intake does not catch up with the increase in milk yield after calving (Butler, 2000), resulting in a negative energy balance (NEB) and a decrease in BCS caused by mobilization of accumulated body fat. A severe NEB suppresses the LH pulse frequency, leading to ovarian quiescence, which extends the period from calving to first ovulation (Beam & Butler, 1999). Negative BCS changes in early lactation lead to delayed days to first estrus as a result of delayed ovarian activity, infrequent luteinizing hormone pulses, poor follicular response to gonadotropins, and reduced functional competence of follicle (Chagas et al., 2007). These postpartum BCS losses can be inversely related to BCS at calving; if BCS at calving is too high, this may limit feed intake postpartum and predispose cows to a high rate of BCS loss. Conversely, if BCS at calving is too low, the cow will calve with limited body reserves, and in this case, BCS remains low. In the majority of the Kenyan smallholder cows, body condition is often low at calving and continues to be low into the postpartum period, leading to postpartum anestrus and overly long open periods (Gitonga, 2010). This anestrus is also a result of inadequate quality and quantity of feeding materials, particularly during the dry season when there is unreliable feed availability (Bebe, 2004).

In the tropics, various strategies have been used to decrease the length of the calving interval, including the use of hormones to induce estrus in cows (Centurión-castro et al., 2013). Prostaglandin F2α alone requires a functional corpus luteum (CL) between days 7-16, lysing it to induce heat in approximately 3 days. Alternatively, gonadotropin-releasing hormone (GnRH) can cause ovulation of a large follicle to start a new follicular wave, and is found to be useful for the treatment of delayed puberty in heifers and prolonged postpartum anestrus in cows (Islam, 2011). Together, these two hormones have been used widely for estrus synchronization and for timed AI programs such as Ovsynch (Colazo & Mapletoft, 2014). However, appropriate nutritional management is essential for successful implementation of any hormone therapy programme in both cows and heifers (Islam, 2011).

Sexed semen is now available globally, and many dairy producers are using it to get larger number of heifer calves with high potential for future milk production. However, despite the availability of the sexed semen even in Africa, the high cost per straw (Othieno, 2016) and potentially low conception percentages associated with sexed semen (Joezy-Shekalgorabi et al., 2017), have hampered its widespread use among the resource-constrained smallholder dairy farmers in sub-Saharan Africa. Due to the reduced conception percentages reported for sexed semen, its use is usually promoted in virgin heifers where conception percentages are historically higher and intrauterine infections are less common compared to cows. Also, using sexed semen on the second or later services could reduce conception compared to the first service (Seidel, 2007; DeJarnette et al., 2011). Since oocyte quality is a function of nutritional status (Ashworth et al., 2009), BCS may also be a factor of conception success. Semen companies are also trying to address other fertility factors that are under their control, including optimizing semen donor selection, sorting procedures, semen processing and handling procedures (Schenk et al., 2009), and sperm numbers per straw (Seidel & Schenk, 2008).Time of insemination in relation to ovulation has been found to be critical for sexed semen, where a delay of 6 hours compared to the time used for conventional semen has been recommended (Sá Filho et al., 2010a; Seidel et al., 1999).

To facilitate the use of sexed semen with a resulting maximum benefit, the need exists for reproductive strategies optimizing the quality of the egg, the intrauterine environment, the detection of estrus and the timing of insemination (Sá Filho et al., 2010a). Control of ovulation through induced heats and/or timed AI may also optimize the use of sexed semen in cattle breeding (DeJarnette et al., 2010a). This study sought to identify the feasibility of interventions used to enhance sexed semen use on dairy cows in the semi-commercial smallholder dairying enterprise.

4.3 Materials and methods

4.3.1 Study area

Approval was sought and granted from the Research Ethics Board and the Animal Care Committee of the University of Prince Edward Island, the Naari Dairy Farmers Corporative

Society (NDFCS) and a partner non-profit organization, Farmers Helping Farmers. Signed consent to join and participate in the study was obtained from all the participating farmers after the project had been fully explained.

The study was carried out between August 2016 and January 2018 in the Naari sub-location of Meru County, Kenya. This study area is located in the north-eastern side of Mount Kenya and borders the Mount Kenya forest, approximately 2011 meters above sea level. Meru County has daytime high temperatures ranging from 16^0C during the cold season (July-August) and 35^0C in the hot season (January-February), and receives an average rainfall of between 500 to 2600 mm each year (worldweatheronline.com). The study area is well-suited for small-scale farming since it usually has sufficient precipitation and fertile soils, falling within the agro-ecological zones 2 and 3 (Jaetzold & Schmidt, 2006).

4.3.2 Selection of study farms and animal management

The complete list of member farmers (n=558) belonging to the NDFCS was obtained, and from these farms, a list of 200 farmers was generated randomly through computer generated random numbers for a related study (Chapter 2). For the current study, 100 farms from the 200 farms were then randomly selected, with the following inclusion criteria: they had to be currently shipping milk to the NDFCS, or currently not shipping milk due to all cows being dry; have 3 or fewer milking cows; and zero-grazing their cattle. The area covered by the dairy society is divided into eight regions, and sampling was proportional to the number of farms in these eight areas to reflect the total number of farms per region.

4.3.3 Study design

This study was a randomized control trial with four intervention groups and one additional comparison group, where random allocation of farms was blocked by herd average days in milk (DIM) of cows on the farms within the groups. A total of 20 farms were randomly allocated to each group at the beginning of the study.

The first intervention group ("Reproduction") received hormone treatment, where needed (see below), and Artificial Insemination (AI) using sexed semen. The breeding criteria used were BCS, vulvar mucus, evidence of ovarian cyclicity, and DIM as shown in Figure (4-1). Body condition score was estimated by using the five point scale with 0.25 increments, with 1 representing emaciated cows and 5 representing obese cows (Ferguson, et al, 2006: Ferguson et al, 1994). Body condition score was assessed on each farm visit and categorized as acceptable (≥2.25 out of 5) or low/unacceptable (<2.25 out of 5). The cows categorized as acceptable (acceptable BCS, but also cycling and clear vulvar mucus) were enrolled to receive the sexed semen if they were 60-300 DIM and still open (see breeding criteria in Figure 4-1). The cows were allowed two services using the sexed semen to increase the chances of conception with sexed semen. If a cow already had two sexed semen services and was still open, it would no longer be eligible for more sexed semen even with acceptable BCS and DIM and was characterized as censored in the Cox proportional model. Cows that were categorized as unacceptable by the breeding criteria were reassessed during the next visit. Cows in this group that failed to meet the breeding criteria by 300 DIM were subsequently inseminated with conventional semen at the owner's discretion.

The second group ("Nutrition") received leguminous shrubs and face-to-face nutritional training and education materials. Two high protein leguminous fodder shrubs were used namely *Calliandra calothyrsus* and *Sesbania sesban* (150 shrubs of each kind distributed to each farmer) which were distributed to the farmers as seedlings seven months before the trial started, and the farmers were taught how to plant and care for them. The farmers were instructed on how to harvest the leaves of the shrubs for the lactating cows as a supplement. Two types of leguminous shrubs were used since there was a large difference in altitude among the farms in the study area, and it was unclear which type of shrub would be best on the farms. *Sesbania* is known to be hardier at higher altitudes than *Calliandra* but has slightly lower protein content than *Calliandra* (Wambugu et al., 2006). Cows in this group could be bred without meeting the breeding criteria and were inseminated using conventional semen, except at the end of the trial (see below).

The third group ("Combined") included farms that received both reproductive and nutritional interventions. Sexed semen was again used to serve the cows for up to two services per cow during the entire time of the study once they met the same breeding criteria, similar to the reproduction group. Again, cows in this group that failed to meet the breeding criteria by 300 DIM were subsequently inseminated with conventional semen at the owner's discretion.

The fourth group ("Education only") was an education-only comparison group that did not receive any of the resources of the nutrition or reproduction interventions (e.g. fodder shrubs or hormones) but did receive education to help them with reproductive and nutritional management (a quasi-control group). Cows in this group could be bred without meeting the breeding criteria and were inseminated using conventional semen, except at the end of the trial (see below).

The education provided to the farmers in the above four groups included face-to-face training from the researchers during every visit. Training was tailor-made to the needs of the particular farm and included husbandry practices for more milk production, enhanced reproduction and better feeding practices for improved BCS of their cows. To augment the face-to-face training, similar educational materials, including a smallholder dairy training manual, was issued to all the farmers in these four groups.

The fifth group ("Control") was a true control group that did not receive any intervention or training or education material of any kind for the entire study period. All farmers in all 5 groups had their animals dewormed as needed – typically twice per year. Cows in this group could be bred without meeting the breeding criteria and were inseminated using conventional semen, except at the end of the trial (see below).

Reproductive hormones were used to induce heat in recruitable animals in the reproduction intervention groups (group 1 and 3). A dose of cloprostenol (500μg; Estrumate®) was injected intramuscularly into cows that had a palpable corpus luteum at the day of the visit. A subsequent dose was given 11 days later if the cow had not come in estrus yet. Cows with no palpable ovarian structures and ovaries greater than one and a half centimeters in diameter received 100μg of gonadorelin (Fertiline®) intramuscularly. In all the groups, animals that had retained placenta and subsequent metritis were treated with a single dose of cephapirin benzathine uterine suspension (500mg; Metricure®) after day 25 postpartum.

The farms for groups 1-3 were visited every month, while the farms for groups 4-5 were visited once every two months since they required less follow-up. At these visits, animals were examined, including physical, clinical and rectal exams, California Mastitis Tests (CMT), and body condition scores. Questionnaires were administered by the research team through a face-to-face interview to collect information on cow and farm management and history since the last visit. Sections of questions included cow and farm demographics and management, disease status, and milk production. On each visit, any cows with DIM>25 days that were not pregnant were eligible for data collection for the study.

The breeding criteria (Figure 4-1) included: 1) body condition score (BCS) $\geq$2.25 or BCS$\geq$2.0 and rising; 2) clear mucus at previous heat as observed by the farmer; 3) evidence of ovarian activity; and 4) DIM is 60-300 days (or 250 DIM if BCS$\leq$2.0 at 250 DIM). In order to have some cows bred with sexed semen that were not restricted by the breeding eligibility criteria, farmers in groups 2, 4, and 5 were given one dose of sexed semen to use on any cow during the last 3 months of the trial, regardless of the breeding criteria. Farmers in these three groups did get advice on which cows would be more suitable for their dose of sexed semen, but it was left to the discretion of the farmer to choose which cow would receive the dose of sexed semen.

Due to lower than expected numbers of cows being inseminated using sexed semen (due to lower than normal rainfall), during the last 6 months of the study, nulliparous heifers of breeding age and size in the groups receiving sexed semen throughout the trial (group 1&3) were also examined for ovarian activity and body condition score to determine if they were eligible for sexed semen. This additional group of cattle provided an interesting comparison for sexed semen conception risks, but was not included in the regression modeling explained below. The sexed semen was provided to the farmers at a subsidized cost, the price of conventional AI semen.

For cows and heifers enrolled in the CCI part of this study, blood was collected from the coccygeal vein into sterile redtop blood collection tubes with clot activator. The blood samples were kept on ice during transport, and in the evening, allowed to stand and clot undisturbed at room temperature for clot contraction. The serum was siphoned into serum vials and frozen at -20^{o}C until the time of laboratory analysis. Serum samples were tested for antibodies to *Neospora caninum*, and antibodies and antigen to BVDV, according to the tests and standards set in the

OIE manual (www.oie.int, search for *N caninum* and BVDV) using commercial Enzyme Linked Immunosorbent Assays (ELISA). Specific details on methods can be found in Chapter 3.

4.3.4 Definition of reproductive terms

Service percentage in this study is defined as the percentage of the animals inseminated from all the recruited animals. Service percentage is usually defined for a 21-day time period. As farm visits in this study did not happen every 21 days to coincide with heat cycles (for logistic reasons), animals that were recruited to receive sexed semen at monthly visits (after meeting the breeding criteria) represent the heat availability parameter and the denominator for the percentage of cows inseminated. Conception percentage is defined as the percentage of inseminated cows that became pregnant at each service in a given time period. Pregnancy percentage is defined as the product of both service percentage and conception percentage. With similar application across groups, this modified pregnancy percentage is a useful and familiar way of comparing the reproductive performance of the cows across groups.

4.3.5 Data management and analysis

Field data were entered into MS Excel 2010 (Microsoft, Sacramento, California, USA). Statistical analyses were done using Stata13.0 software (StataCorp LLC, College station, Texas, USA). There was a hierarchical nature to the data, with visits clustered within cows, and cows clustered within farms.

The outcome used in establishing the feasibility of using sexed semen was calving-to-conception interval (CCI). Date of conception was taken as the date of service preceding a positive transrectal pregnancy diagnosis. A Cox proportional hazard (survival analysis) model was used to analyze these data. Descriptive statistics (means, s.d., and medians, and percentages, as applicable) were calculated for the conception risk and the predictor variables, including farmer demographic data, and farm and cow management data.

Since the primary study objective was to determine if the reproduction interventions contributed to better conception using sexed semen on those farms receiving the intervention, the initial data analysis included comparisons of conception risks among intervention groups and among types of semen used, and also a univariable Cox proportional hazards model with only intervention group as the predictor. This initial data analysis assumed that the random allocation to groups balanced out the other factors affecting CCI between groups. Demographic and other known factors of conception risk were compared by group using ANOVA analyses to confirm that groups were not different for these variables and the success of the random allocation at balancing these factors among groups.

Because the study had only 20 SDFs per group, and only 1.5 cows/farm, on average, the random allocation may not have succeeded in balancing out the groups, or the study may not have had enough power to detect significant differences between groups. Therefore, a second set of analyses were planned, multivariable Cox proportional hazard analyses, including all possible significant and confounding variables.

For this second set of analyses, some of the variables were modified. Body condition was assessed every month (given on a scale of 1-5 with 0.25 increments) and for this analysis, an average BCS was obtained for all the animals in the dataset (adding all the scores and dividing by the number of visits per animal). As well, lowess smoothed curves were obtained to assess the probability of conception and the different body condition scores of cows on the visit prior to each service for all the cows in the study. This was done with the outcome of the service (conceived or not) for sexed semen service, conventional semen service and overall service.

For this second set of analyses, univariable associations with $p<0.25$ were initially conducted, and then a final model was built with any confounders and variables significant at $p<0.05$. The final model was assessed for proportional hazards, assumption of independent censoring, overall fit of the model, functional form of the predictors, and presence of outliers and influential points. The Efron method was used to handle ties between two or more events in the model and model fit assessed.

4.3 Results

At the start of the study, there was a 100% response rate of the 100 farms to all invitations to participate. However, at the end of the study 17 months later, 21 farms had dropped out for various reasons: 8 sold the cows, 3 relocated from the area, 5 did not want to participate anymore, 3 had a death of the principal farmer, and 2 had grown too large within the course of the study and therefore were no longer qualifying as small-scale farms. In addition to these drop-out farms, 3 farmers were not available for questioning on the initial visit and the person left to care for the animals was not able to answer questions. Therefore, the results will include a variable number of herds depending on when the farms left, and whether they were around for long enough to provide sufficient data for their cows to be included in the CCI results.

However, some of these cows were on farms that started the study but did not remain in the study. Even on the farms that remained in the study, some cows left the study because they were sold, died, were lent to a neighbour, or were not on the farm at the time of the visit because they were grazing on a community pasture. Therefore, the results also include a variable number of cows and visits per cow, depending on whether or not the cows were on the farm for assessment at a visit (some missing data), and whether they were around long enough to provide sufficient data to be included in the CCI results. The CCI model includes records from 191 cows that were in the study long enough to provide good enough data for analysis.

4.4.1 Descriptive statistics

There were 473 cow records in total for 191cows across the five groups who were recruited for the study. From this group, 378 cow records were enrolled at some point in time during the study for possible service with sexed semen because they were in the study breeding stage (60-300 DIM) used for sexed semen. This group included mostly cows in the reproduction and combined groups, and a small proportion of cows in the other three groups. These 378 cow records enrolled were made up of 277 cow records that were already past 60 DIM at the time they were enrolled and an additional 101 cow records that were in the 25-60 DIM window at the time of their enrollment. Sixty-two percent of enrolled cow records (234/378) reported an acceptable BCS ($\geq$2.25), rendering them eligible for sexed semen service if they came on heat according to the breeding criteria. A proportion (43.6%) of the eligible cows (102/234) was cycling at the visit when they had BCS$\geq$2.25. With the intervention groups, there was an average of 22 cows entering or in the breeding stage (past day 60 postpartum) at any visit, with a range of 10-45 cows per visit.

Of the 378 cow records from 191cows that were enrolled in the study, only 75 services were actually provided with sexed semen (19.8% service percentage), leading to 33 conceptions (44% conception percentage). Combining the service percentage and conception percentage, the overall pregnancy percentage was 8.7%. The service, conception and pregnancy percentages (and 95% confidence intervals) for each of the intervention groups are shown in Table 4-1. Out of the 33 conceptions, 10 calves had been born by the end of study, and all the calves born were female and of good physical and clinical health. Cows in the reproductive only group recorded the highest pregnancy percentage.

Twenty-six heifers also met the breeding criteria and were enrolled for service with sexed semen during the last 6 months of the study among the nutrition and combined intervention groups. Among the 26 heifers, 11 were inseminated, with 6 being confirmed pregnant (conception percentage of 54.5%). The combined conception percentage for sexed semen in the cows and heifers altogether was 50.1% (39/86). Heifers recorded higher service percentage (42.3%) than any of the intervention group cows. Among the cows, the control group had the lowest service percentage at only 8.5%, and the reproduction group recorded the highest service percentage at 35.5%. The other groups had service percentages that were around 15% because some farmers were not very interested in utilizing the subsidized sexed semen on their farms. The overall conception achieved by conventional semen was 72% in cows and 79% in heifers.

Thirty cows had received a single dose of Metricure® for intrauterine infections during the study time. A total recovery was reported in 23 of these cows, 2 of them were culled due to subsequent pus in the uterus, and 5 had been treated for other concurrent infections, with 3 dying and 2 recovering.

A total of 98 cows received GnRH for induction of cyclicity during the study period, recording an average of almost 6 cows per month being treated. The number of cows that received at least one injection of prostaglandin F2α was 29, for an average of almost two cows per month. Estrus was reported in 39.8% (39/98) of the cows that received GnRH and 58.9% (17/29) of the cows that received prostaglandin F2α. From the animals that displayed estrus following GnRH administration, 14 were inseminated with sexed semen and 8 became pregnant resulting in a 57.1% conception percentage, while 22 were inseminated with conventional semen resulting in a 77.3% (17/22) conception percentage. Three animals were not inseminated for various reasons. Out of the 17 cows that showed signs of estrus after prostaglandin F2α, 14 were inseminated with sexed semen resulting in a 78.6% (11/14) conception percentage, while 3 were inseminated with conventional semen from which 2 became pregnant for a 66.6% conception percentage.

In total, one-third of tested cows participating in this part of the study were test-positive for *N. caninum,* while half of the tested cows were positive for BVDV antigen and half of the tested cows were positive for BVDV antibody (Table 4-2). For logistic reasons, not all samples tested for BVDV antigen were tested for BVDV antibody – of the 110 cows tested for both BVDV antigen and antibody, 55 (57.3%) were positive for both. For *N. caninum* and BVDV antibody, the highest proportion of positive animals was in the control group, with 53.6% and 81.3% positive, respectively. For BVDV antigen, the reproductive group had the highest proportion of test positives, at 64.3%.

For the heifers, 8 of the 26 heifers (30.8%) tested were positive for *Neospora,* while 9 (34.6%) were positive for the BVDV antigen test. Due to a limited number of BVDV antibody tests available (for logistical and budgetary reasons), only the 9 antigen-positive heifers were tested for BVDV antibody to determine if they were likely to be persistently infected or not. Only one of the 9 antigen-positive heifers for BVDV tested positive for BVDV antibody. None of the heifers tested positive for both Neospora and BVDV.

An average of 226.9 (S.E. 10.7) days to conception was recorded in the cows with a pregnancy in this study. Days to conception for the entire study population ranged between 27-947. In the cows that were inseminated with sexed semen (43/191), a mean of 205.3 days to conception were recorded with a median of 205 days and an interquartile range of 56-449 days.

The mean of the CCI for cows inseminated with conventional semen (136/191) was 238.9 days, with a range of 29-946 days and a median of 249 days. Twelve cows had been inseminated naturally using a bull and recorded a mean CCI of 270 days with a 45-774 range of CCI. There were no significant differences in the median and means of CCI among these groups.

4.4.2 Univariable Cox proportional hazards model for days to conception analysis

Descriptive statistics of the categorical variables univariably associated ($P<0.25$) with the Cox proportional hazards model for days to conception are shown in Table 4-3. While nearly a quarter of study cows received sexed semen, a majority of study cows received conventional semen (as part of the education only and control groups, and cows in the other groups who did not meet the sexed semen eligibility criteria). There were still some farmers using bulls to inseminate study cows. Almost a third of the cows had come into estrus after induction using hormones and had recorded a median CCI 63 days longer than the cows coming into heat spontaneously. Only 7.8% of the farmers indicated owning a smartphone (as a sign of wealth) and a quarter of farmers indicated supplementing their cows with dairy meal in the last month of gestation. A majority of the farmers had attended dairy related training other than what was offered by the research teams during the time of visit and among these.

The mean primary land holdings in this area were small, with most farmers having less than 2 acres of land (Table 4-4). The farmers indicated that nearly half of the land they owned was being used for growing fodder for their dairy cows. The average body condition score indicated that the study cows were generally thin.

The prediction of conception of services with sexed semen, conventional semen and overall prediction according to different body condition scores (using a lowess curve) are shown in Figure 4-2. A gradual increase in the probability of conception of cows was observed between BCS 1.75 to 3.5 with sexed semen and overall (top 2 plots). With sexed semen, the BCS needs to be at least 2.5 in order to achieve a 50% probability of conception. A threshold was observed in conventional semen service around BCS 3.0, but there were not many cows inseminated with BCS over 3.5.

The following eight variables were univariably associated with CCI at $p<0.25$: type of semen used, hormonally induced estrus, farmer owned a smart phone, dairy meal fed during the last month prior to calving, and farmer attended dairy training, primary land size owned, percent of land used to grow fodder for cows, and average BCS of the cows. The five-level intervention variable was not significantly associated with CCI in the univariable Cox proportional hazards model, and therefore the multivariable Cox proportional hazards model for CCI was built.

For the multivariable Cox model, the five intervention groups were re-categorized into three groups by combining the two groups that received fodder shrubs (group 2 and 3) and the two comparison groups (group 4 and 5), and comparing them with the group receiving only reproductive interventions (group 1), based on the similarity of interventions applied and what was observed in terms of CCI in the five intervention groups (Table 4-3).

4.4.3 Final multivariable Cox proportional hazards regression model for days to conception analysis

In the final multivariable Cox proportional hazards regression model, 3 cow-level and 3 farm-level variables were significantly associated ($p<0.05$) with days to conception, and two of these variables formed an important interaction in the final model (Table 4-5). A one unit increase in the average BCS of cows led to a 3.5 times higher hazard of conception (hazard ratio = 3.5). In a Cox proportional hazards regression model, this hazard ratio of 3.5 is the ratio of the hazard risk corresponding to the average BCS and 1 unit higher than the average BCS, with the hazard risk being the risk of conception given that the animal had not conceived up to that specific time. Therefore, the hazard ratio interpretation relates to the risk of conception, adjusted for days to conception.

Use of sexed semen was also in the final multivariable Cox proportional hazards model, and it increased the hazard of conception by 2.0 times over that of conventional semen use, while use of a bull for service had a non-significantly lower hazard of conception compared to conventional semen. Cows that were inseminated during a hormonally induced estrus had decreased hazard of conception for that service compared to cows that came into heat spontaneously (inverse of 0.57 is 1.8- the hazard ratio for spontaneous heats versus induced heats). Cows on farms where the farmers indicated having attended dairy training other than that offered by the research team had a 1.8 times higher hazard of conception compared to cows in farms where farmers had no form of dairy related training. An interaction was discovered between intervention group and cows that were fed dairy meal in the last month of gestation. Cows on farms that fed dairy meal supplementation in the last month of gestation had a higher hazard of conception in the groups that fed leguminous shrubs, and this association was not seen on farms that did not feed leguminous shrubs (Figure 4-3).

4.4 Discussion

This is the first study to test the effect of a breeding program that includes a specific set of breeding criteria meant to enhance the success of using sexed semen relevant to the normal circumstances among semi-commercial smallholder dairy farmers in Kenya. Conception percentage and CCI were used as the outcome of interest. The study results showed that the overall conception percentages were 44% and 54.5% for cows and heifers (Table 4-1), which was higher than that reported by Norman et al. (2010) of 24% in cows and 39% in heifers. Silva et al. (2009) reported 49-63% conception percentages in heifers and 21% in cows. Continued and recent advances improving the fertility of sexed semen may account for these differences seen. Although the conception percentage for conventional semen was higher than for sexed semen overall, the mean and median CCI were lower for sexed semen due to the breeding criteria and efforts of the reproductive intervention, which was also demonstrated by the 2.0 hazard ratio for sexed semen relative to conventional semen in the CCI survival model (Table 4-5). With this information, smallholder farmers in Kenya interested in spending the higher cost for sexed semen and applying BCS breeding criteria should expect reasonable conception success.

Reproductive performance has been found to be significantly affected by BCS and its changes during lactation (Roche et al., 2007a). The amount of energy reserved during late gestation, parturition and early lactation influences the length of postpartum anestrus and the probability of successful mating (Roche et al., 2007a). Low body condition at any time during the early lactation is associated with prolongation of initiation of ovarian activity, low frequency of LH pulses, poor follicular response to gonadotropin stimulation, and a decrease in the functional competence of oocytes (Diskin et al., 2003). Because the cow puts on fat more efficiently while lactating, she should go dry with a BCS of 3.5 to 4.0. If a cow is in good body condition at dry-off, she should calve at approximately the same body condition. When milk production peaks and the energy requirement exceeds its intake, cows go into negative energy balance when they mobilize their lipid reserves, getting thinner and losing their body condition score (Aeberhard et al., 2001; Mishra et al., 2016).

While BCS is an important indicator of cow health, related to better milk production and reproduction, it has no effect on the sex of an *in utero* calf, and on dairy farms, female calves are clearly preferred as future milk producers on the farm. Sexed semen allows farmers to significantly skew the sex ratio of their calves, thus accelerating genetic gain, improving herd biosecurity, and reducing the number of superfluous bull calves and their associated dystocias (Healy et al., 2013). This study did show a gradual rise in the probability of conception with rising BCS, with a steadier rise observed when BCS were over 2.0. For sexed semen to achieve a 50% probability of conception cows need a BCS of at least 2.5 and this justified our cut-off point of 2.25 for sexed semen use.

The major constraints to using sexed semen are the lowered conception percentages, requiring more services before the cow is confirmed pregnant, and the higher cost of sexed semen, which is especially a concern to the resource-constrained smallholder farmers. Sexed semen has been associated with 75-80% relative fertility compared to that of unsorted semen (DeJarnette et al., 2011). Our results showed 72% and 79% conception percentages for heifers and cows, respectively, for conventional semen, whereas 44.0 and 54.5% were the conception percentages using sexed semen, respectively. Due to differences in the parameters of use of sexed and conventional semen in this study (i.e. the timing of the conventional semen was later in lactation than the sexed semen, when NEB would likely be reduced), their relative fertility cannot be directly compared.

The Cox proportional hazards models also indicated that the hazard of cows conceiving was 2.0 times higher when sexed semen was used compared to when conventional semen was used in this area (Table 4-5). However, we suspect the reason for this result is not because sexed semen has better conception (our results would indicate otherwise), but because the cows receiving sexed semen were put through the rigorous breeding criteria that allowed good BCSs, clean uteri and better estrus observation while this was not done for animals receiving conventional semen. Some cows being inseminated with conventional semen may not have been on standing heat and were not in the optimal BCS, and could have had infected uteri leading to a reduced hazard of conception. By optimizing the use of the stated breeding criteria for cows receiving sexed semen in this study, conception percentages could be increased, and in turn, we could increase the number of replacement heifers produced in smallholder farm settings. This randomised controlled study should be replicated in another area with SDFs in Kenya to determine the generalizability of the results.

Sexed semen is recommended for use on heifers because a heifer uterus is usually more fertile (i.e. less likely to have infection) than a cow, and they are genetically superior on average compared with older cows of previous generations (Garner & Seidel, 2008). However, heifers with delayed puberty were a common finding in our study that emanated from poor feeding management of young stock. In this study area, a related study looking at calves and heifers indicated that the mean age of open heifers was 23 months, with open heifers over 36 months old being commonly found (Makau et al., 2018). The average daily gain for calves under 15 months was 0. 482 kg (s.d.=0.4) against a recommended 0.4-0.5 kg (Lukuyu et al., 2016), while that of heifers between 15-36 months was substantially lower at 0.364 kg (s.d. 0.15) (Makau et al., 2018). There is ample opportunity for improved management of heifers on smallholder dairy farms in this area of Kenya since there were nulliparous heifers older than 36 months in our study population who had not shown signs of heat, as described in an companion thesis in the same project (Makau et al., 2018)

Conception percentages achieved through sexed semen have also been known to vary significantly across studies, which emphasizes the influence of number of on-farm factors on conception. Conception percentages are affected by parity, age, body condition, sire selection and accuracy of estrus detection (Healy et al., 2013). Low conception percentages with sexed semen have been associated with reduced quality of the sexed semen following the sexing process and other factors that affect conception risk with artificial insemination such as improper heat detection, inseminator's technique, infection of the reproductive system, heat stress, and other diseases that affect the reproductive system (Donovan et al., 2003).

Increasing sperm numbers from the recommended two million sperm cells to ten million sperm cells in a straw of sexed sorted semen did not seem to improve the conception percentages (DeJarnette et al., 2011; Seidel & Schenk, 2008). In our study, sperm concentrations of 1.2 million per straw were used, but it is unclear whether this lower sperm number impacted the conception percentages.

Cows in farms where the farmers had received some form of dairy related training were at increased hazard of conception compared to cows in farms where farmers had not had any training at all. In this area of Kenya, farmer training took the form of group-targeted teachings organized by dairy-related non-governmental organizations in the area, farmer seminars and workshops from selected individuals on selected topics, as well as farm visits to other better producing farms and dairy cooperatives in this and other regions to learn better management procedures. This training program provides continuous education opportunities to these farmers, and keeps them up-to-date with new techniques in dairy management and fodder production and conservation. A majority of rural dairy farmers in Africa have experience in rearing animals, but relying on traditional husbandry practices may be a contributory factor to the low production and productivity (Vaarst et al., 2007). Studies that encompass farmer training methods have yielded positive results in adoption of new techniques and could in turn lead to on-farm benefit to the smallholder dairy farmers and their families (Warriach et al., 2018). Smallholder farmers who adopted the recommendations shared with them during the extension programme observed a wide range of positive impacts.

The hazard of conception in cows that came on heat after hormonal use was 0.6 times lower than those cows coming on heat spontaneously. Reproductive hormones have been used to induce estrus (if the farmer knows what to expect and how best to observe for estrus) or to enable AI on a known date and time (referred to as estrus synchronization or timed AI). In both these cases, hormones can be used as soon as the cow is eligible for breeding, or alternatively be used if the cow has not been inseminated by the end of a certain period of time (Perry, 2005). The use of hormones in both induction or synchronization of heat has been associated with better conception and pregnancy percentages in cycling and anestrous cows in several studies (Lemaster et al., 2001; Perry, 2005; Lucy et al., 2004). Hormonal treatments are quick and easy to implement, but routine use may diminish the need to tackle root causes of poor fertility, and this may have productivity, health and welfare implications for the herd (Perry, 2005). Hormone use in our study was associated with lower hazard of conception, and this could be due to the fact that cows that received the GnRH were those that had small, smooth ovaries that were over 1.5cm in diameter and had not been seen on heat. Similarly, there may have been an underlying problem of estrus detection among the farmers, especially in cows that had a functional CL, requiring the use of prostaglandin F2α with instructions on when to observe for heat signs to help resolve this problem. Donovan et al. (2003) has associated prostaglandin F2α heat synchronization with reduced conception percentages in Holstein heifers. Due to the relatively low numbers of cows being bred after the use of hormones, the results of this study should be used with caution to advice on use of reproductive hormones based on conception risks. However, if farmers are having difficulty with cows not cycling or showing heat signs, hormone therapy may be helpful since conception percentages obtained with hormone use in this study are considered good (57-79%).

In an interaction term, supplementing cows with some high energy concentrates during the last month of gestation was associated with a higher hazard of conception, especially when farmers gave leguminous shrubs (Figure 4-3). The transition period in dairy cow management extends from the last 3 weeks of gestation (dry period) through the first two weeks of lactation (early fresh period). During this time, the cow moves from a low maintenance phase to a high production phase when energy and protein requirements increase. Supplementation with concentrates during the few weeks prior to calving allows the rumen flora and papillae to adapt to increasing grain fermentation and nutrient absorption. Failure to provide concentrate during the weeks prior to calving can also lead to loss of body condition as the fetus rapidly grows, leading to NEB before calving, and then worse NEB after calving, leading to a longer anestrus period before normal reproduction function can be regained (Block, 2010). Therefore, proper feeding of concentrates to the transition cow is of utmost importance, not only for milk production but also for the reproductive health of the cow for the next pregnancy. However, the additional protein provided from the leguminous shrubs being fed appears to have provided a synergistic effect on CCI with the concentrate being fed pre-partum. Alternatively, these two groups receiving leguminous shrubs had also received extensive teaching on cow nutrition and thus they were expected to have better nutritional management both pre- and post-partum, improving the likelihood of a lower CCI. With the limited extension services and cattle nutrition knowledge among smallholder dairy farmers, transition cow feeding is not widely known. The very long CCI recorded in the study demonstrates a need for enhanced management, focused on both nutrition and reproduction.

The study period ran for a year and a half and posed a lot of challenges, especially with extreme changes in weather patterns. Prolonged drought periods were experienced in the year 2017, due to lower than expected rainfall during the long rains, and therefore farmers were faced with major challenges of feeding appropriate quality and quantities of forage to the cows. Body conditions dropped during this time and it took a while before the animals recovered BCS to pre-drought levels. Even though the farmers have been taught how to conserve fodder, especially through silage-making, some farmers indicated that it was costly to make silage since they did not own a chopping machine so they had to hire one and also hire the labour to pack the silage. As an intervention of the NDFCS, more chopping machines were procured and rented out to the farmers at affordable prices but only after the life of the project.

Accurate data collection in the smallholder dairy setting can also be challenging; even with the small number of cows they had, farmers had trouble recalling the reproductive events of the past calving and most farmers did not keep proper records. Therefore, the farmers were supplied with writing books that were labelled to show them when and how to record the dates of heat, service, drying off and parturition for each cow in the farm. Some of the farmers were still missing this information due to illiteracy or poor compliance, while other farmers misplaced the books, and so farmers were advised to carefully store the inseminators' records and produce them to the research team on the next visit for data collection.

The biggest challenge during the study was the very high turnover of animals. Cows in this area are deemed as assets that could be easily liquidated in cases where there is need of money, such as in cases of death or disease in the family, need for school fees, or even animals given out as a form of dowry. This high turnover led to many study animals being lost to follow-up at different stages of the study.

A number of logistical challenges led to fewer services with sexed semen than expected. First, despite much education, some smallholder dairy farmers still had a problem correctly detecting heat or reporting it in good time. Also, farmers sometimes chose not to breed their cows for varying reasons, e.g. lack of funds, planned to sell the cow, or planned to skip a heat before service.

Secondly, inseminations in this study area were carried out by private veterinary practitioners and private AI technicians on cash basis, along with an AI technician employed by the NDFCS obtained on credit to the members of the dairy. Therefore, there was a lot of competition in the area amongst the AI providers who were sometimes blood relatives to some of the study farmers. These farmers would sometimes feel obliged to use their relatives for AI rather than the NDFCS AI technician. Also, for some of the AI providers who had been in the area for long periods, they were also able to provide credit facilities to the farmers, making the farmers consider them as well. Only the NDFCS AI technician was supplied with the subsidized project sexed semen for logistical reasons.

Thirdly, there was competition from other subsidized sexed semen in the area during part of the study period. With a national Kenyan election on August 2017, Meru county government wanted to help improve local cattle breeds by supplying low-priced sexed semen, and this semen was actually rolled out a few months before our project began. The semen was very competitively priced and there was a lot of ground work done by the government to alert the farmers on its use such that it was a challenge to convince the farmers in the study area to comply with using our project sexed semen. The project utilized sexed semen from only two bulls with similar historical conception parameters to minimize the effect of different bulls to conception percentages. Using sexed semen from other bulls from other AI providers would add another confounder to our study.

Fourthly, it was reported at the focus group discussion at the end of the study that it was common practice among some of the AI technicians in the area that if cows did not conceive on the first service, the second service was at a discounted price where the farmers did not pay the cost of the semen for the repeat service. However, the semen quality of that second AI would be doubtful with this practice – although the practice was appealing to the farmers. Since our project had already subsidized semen, repeat services could not be subsidized further for budgetary reasons, and we did not want farmers to believe that the second AI service was done with inferior semen. However, as a result, farmers indicated that they felt they were not willing or able to pay for the sexed semen for a second time on the same cow, and with the lower conception percentage of sexed semen, possibly have to pay for a third service.

Finally, the NDFCS AI technician was the only one doing the AIs with sexed semen for the project during the study period. However, being the only AI technician employed by the NDFCS, the technician was unavailable over some weekends, leading to some cows in heat being missed or leading to farmers seeking AI services elsewhere, but not using the project sexed semen. Future research projects should engage a larger number of smallholder farms and/or multiple AI technicians to ensure a higher sample size of cows bred using sexed semen.

4.5 Conclusions and recommendations

Sexed semen has a lot of potential in providing much needed replacement heifers in the smallholder dairy setting in the tropics. However, an adequate body condition score needs to be attained before sexed semen should be used for good conception. Feeding cows concentrate during the month prior to calving and utilizing drought-resistant leguminous shrubs should be promoted as a useful ways of supplementing cows with a better diet to improve BCS and CCI. Animal health personnel and extension officers should provide smallholder dairy farmers with additional training aimed at improving nutritional and reproductive performance.

4.7 References

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Table 4-1: Descriptive statistics for use of sexed semen in 191 cows on 97 smallholder dairy farms in Kenya in 2016-18

Group	**Recruited**	**Inseminated**	**Pregnant**	**Service percentage (95% CI)**	**Conception percentage (95% CI)**	**Pregnancy percentage**
Reproduction	110	39	14	35.5 (26.7-45.2)	35.9 (21.7-52.9)	12.7
Combined (Reproduction+Nutrition)	107	15	8	14.0 (0.08-0.22)	53.3 (27.4-77.7)	7.5
Nutrition	62	10	6	16.1 (0.08-0.28)	60.0 (27.4-86.3)	9.7
Education	52	7	3	13.5 (0.06-0.26)	42.8 (11.8-79.8)	5.8
Control	47	4	2	8.51 (0.03-0.21)	50.0 (9.19-90.8)	4.3
Total	**378**	**75**	**33**	**19.8 (0.16-0.24)**	**44.0 (32.7-55.9)**	**8.7**
Heifers	26	11	6	42.3 (23.9-62.8)	54.5 (25.6-81-9)	23.1

95% CI – 95% confidence interval

Table 4-2: Distribution of *Neospora* caninum and bovine viral diarrhea virus (BVDV) seropositivity, by intervention group, in 191 cows on 97 Smallholder dairy farms in Kenya in 2016-18.

Group	***Neospora caninum* antibody percent positive**	**BVDV antigen percent positive**	**BVDV antibody percent positive**
Reproduction	37.2 (16/43)	62.8 (27/43)	64.5 (18/28)
Combined (Reproduction+Nutrition)	16.8 (7/42)	54.8 (23/42)	39.1 (9/23)
Nutrition	26.2 (11/42)	52.4 (22/42)	50.0 (14/28)
Education only	36.1 (13/36)	38.9 (14/36)	33.3 (5/15)
Control	53.6 (15/28)	50.0 (14/28)	81.3 (13/16)
Total	32.5 (62/191)	52.4 (100/191)	53.6 (59/110)
p-value	**0.640**	**0.925**	**0.268**
Heifers	30.8 (8/26)	34.6 (9/26)	11.1 (1/9)

Table 4-3: Descriptive statistics of categorical variables eligible (P<0.25) to be included in the multivariable Cox proportional hazards model of days to conception for 191cows on 97 smallholder dairy farms in Kenya in 2016-18.

Categorical Variable	**Count**	**Proportion (%)**	**Median DIM at conception[1]**	**P value**
Type of semen used				0.0240*
• Conventional AI	136	71.2	249	Baseline
• Sexed AI	43	25.1	203	0.013
• Bulls	12	7.23	190	0.405
Hormonally induced estrus				
• No	135	70.6	215	Baseline
• Yes	56	29.3	278	0.099
Farmer owned a smartphone				
• No	176	92.1	228	Baseline
• Yes	15	7.85	243	0.211
Concentrate fed last month of gestation				
• No	147	77.0	246	Baseline
• Yes	44	23.0	215	0.167
Farmer attended dairy training				
• No	47	92.8	254	Baseline
• Yes	144	7.22	216	0.137
Intervention group				0.475*
• Reproduction	43	22.5	261	Baseline
• Reproduction & Nutrition	42	22	222	0.596
• Nutrition	42	22	215	0.227
• Education	36	18.8	195	0.248
• Control	28	14.6	277	0.631
Intervention group (re-categorized)				0.604*
• Reproduction	43	22.5	261	Baseline
• Combined & Nutrition	84	44.0	222	0.325
• Comparison and Control	64	33.5	246	0.655

[1]*For cows which conceived (cows not conceiving or were lost to follow-up are not included in this median)*
*Global p value for the categorical variables

Table 4-4**:** Descriptive statistics of continuous variables eligible (P<0.25) to be included in the multivariable Cox proportional hazards model of days to conception for 191cows on 97 smallholder dairy farms in Kenya in 2016-18.

Variable	**Mean**	**Range**	**95% CI**	**P value**
Primary land size owned (acres)	1.86	0.25-21.0	1.5-2.14	0.065
Percent land used for dairy fodder	46.6	20-95	43.6-49.6	0.182
Average Body Condition Score	2.32	1.33-3.54	2.27-2.37	<0.001

Table 4-5: Final Cox proportional hazards model of calving-to-conception interval (measured in days) for 191cows on 97 smallholder dairy farms in Kenya in 2016-18.

Variable	Hazard ratio	95%CI	P-Value
Average Body Condition Score	3.47	2.07-5.81	<0.005
Type of semen used			
• Conventional AI	Baseline		0.0207*
• Sexed AI	1.96	1.21-3.16	0.006
• Bull	0.87	0.41-1.84	0.717
Hormonally induced estrus			
• No	Baseline		
• Yes	0.57	0.37-0.86	0.008
Farmers attended dairy training			
• No	Baseline		
• Yes	1.76	1.13-2.74	0.012
Concentrates fed last month of gestation			
• Yes/no	a	a	0.845
Intervention group			
• Reproduction	Baseline		
• Leguminous trees	a	a	0.983
• Comparison	a	a	0.202
Interaction			
• Dairy meal fed last month of gestation * intervention group	b	b	0.0143

a-variable is part of an interaction so coefficients of the main effects are best reported using a graph
b-variable is part of an interaction with many cross-tabulated categories for interacting main effect categories (not shown) so coefficients are best reported using a graph
*Global p value for the categorical variable

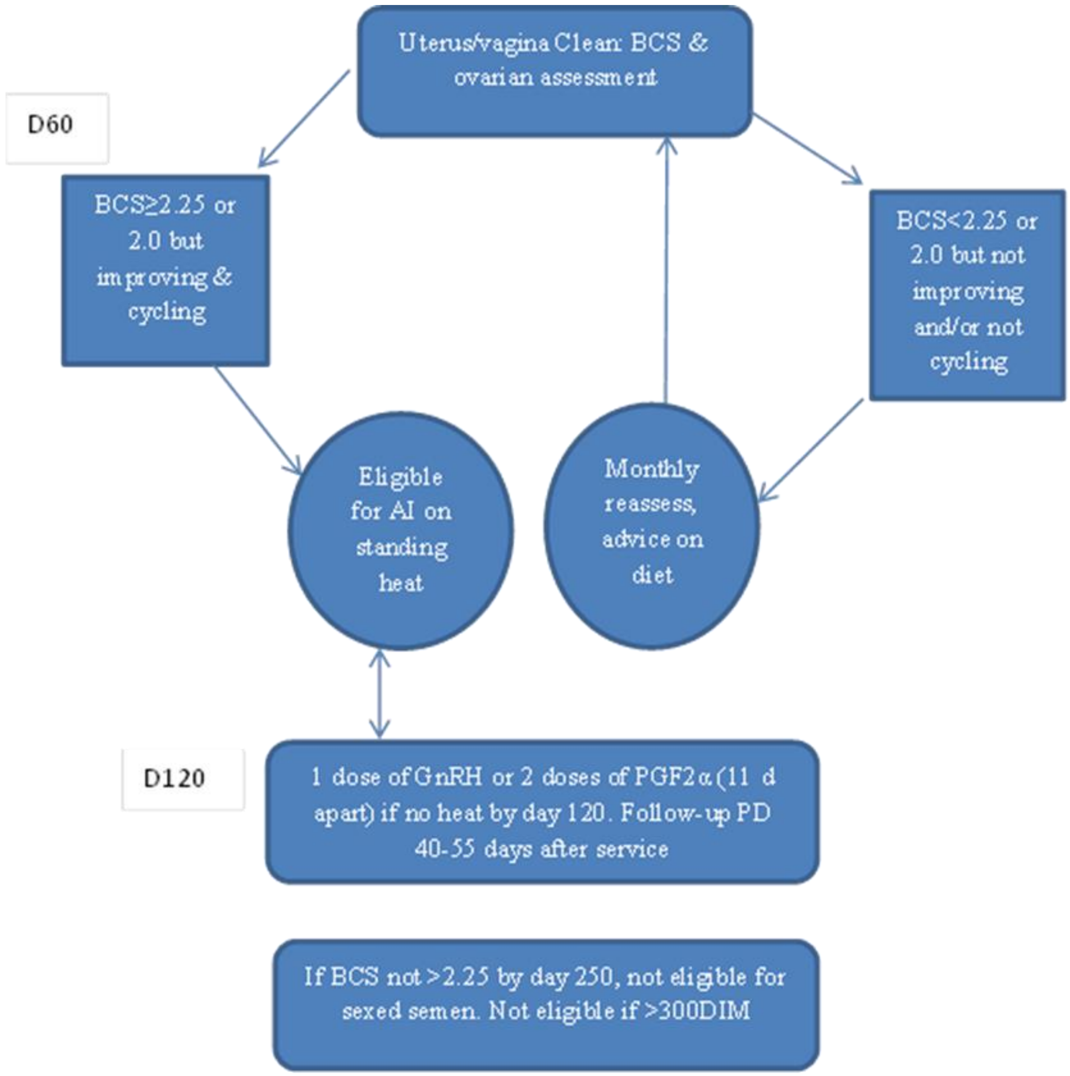

Figure 4-1: Chart demonstrating the selection criteria for cows recruited for service using sexed semen.

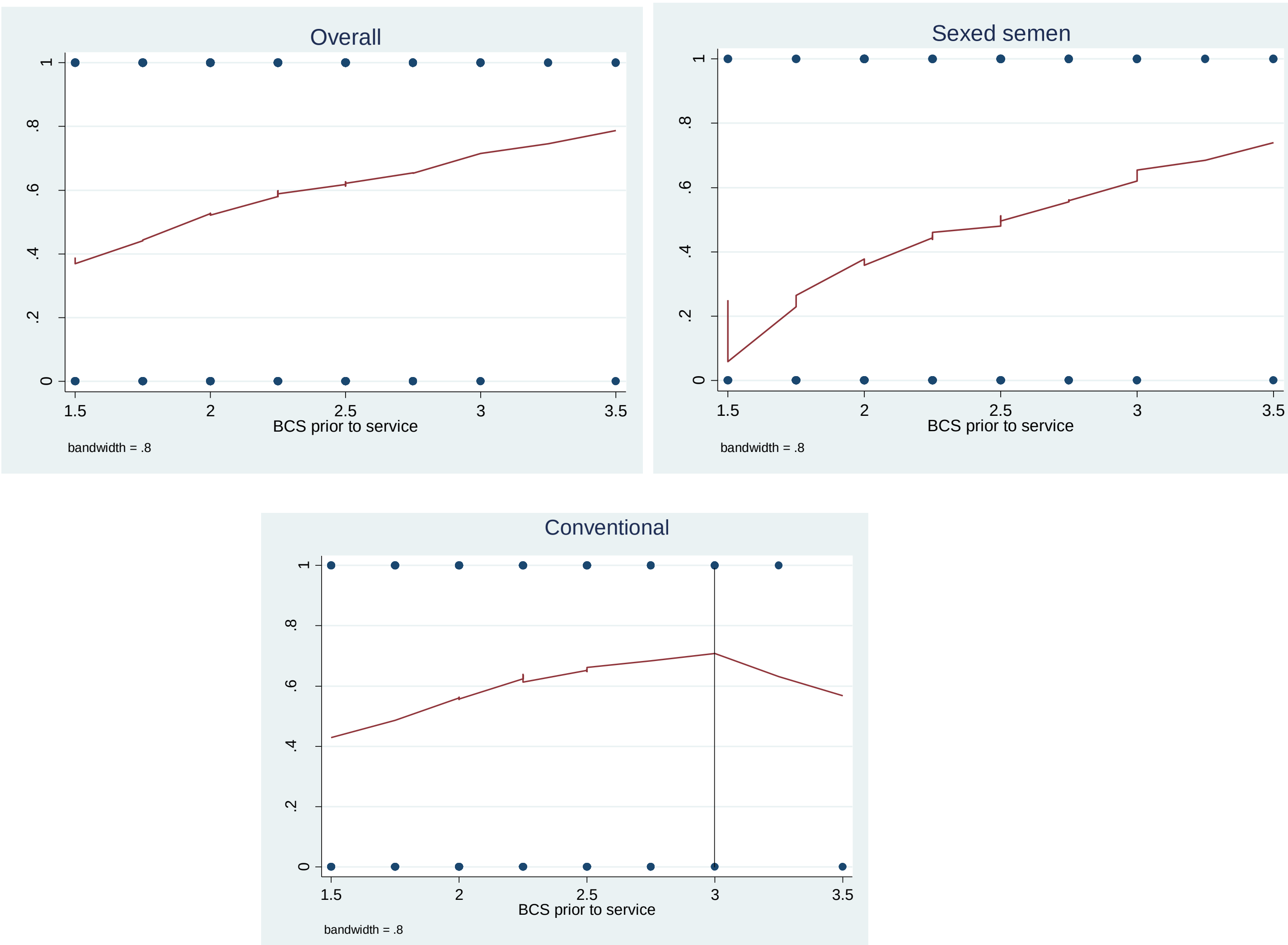

Figure 4-2: Prediction of probability of success of conception with service with Sexed and Conventional Semen (top left), Sexed Semen only (top right) and Conventional Semen only (bottom).

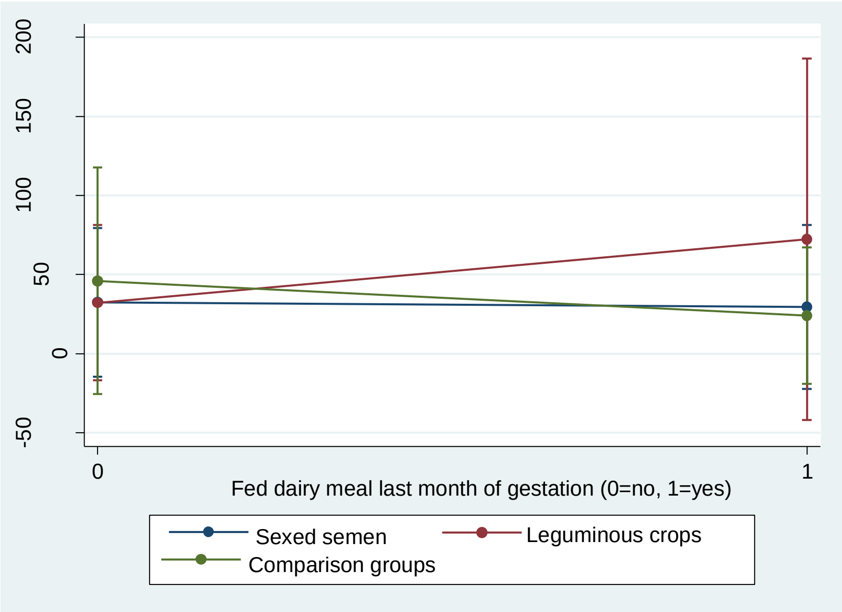

Figure 4-3: Interaction plot of high energy concentrate supplementation during the last month of gestation and intervention group on the hazard of conception in 191 smallholder dairy cows on 97 farms in Kenya in 2016-18.

Chapter 5 Conclusion

5.1 Introduction

This research was carried out with a purpose to address the need for quality replacement heifers and improve the reproductive output of the cows in the Kenyan smallholder dairy industry. The research was undertaken in collaboration with Naari dairy Farmers cooperative society, Farmers helping farmers, Atlantic Veterinary College and University of Nairobi with the financial help of the Canadian government through the Queen Elizabeth II Diamond Jubilee Scholarships that are managed by the Rideau Hall Foundation, Community Foundations of Canada and Universities Canada. The overall hypotheses of this thesis were that 1) there is suboptimal production and reproductive performance of SDF cows in the Meru area of Kenya; 2) the prevalence of *Neospora caninum* and BVDV in the Meru area would be high; and 3) use of sex-sorted semen and reproductive efficiency in the SDF of Meru is feasible and will contribute to availability of replacement heifers.

An observational study was used to characterize the nature of production and reproduction outputs of the SDF cows. Milk yield for the day prior to the visit was analysed and factors affecting this output were also determined (Chapter 2). Prevalence of two reproductively important pathogens, *Neospora caninum* and bovine viral diarrhea virus (*N. caninum* and BVDV), were also established through a cross-sectional study, and relationships among management, nutritional, farmer and cow-based factors to these pathogens also established (Chapter 3). A randomized controlled trial was then carried out, assessing the impacts of using sex-sorted semen and reproductive hormones as reproductive interventions, leguminous shrubs as nutrition interventions and farmer education on the CCI of the same SDF cows upon service (Chapter 4). Factors associated with CCI were assessed in the same study and are reported in Chapter 4.

This chapter outlines the linked discussion and conclusion of what was found during this thesis, and outlines future research areas that can be carried out to further improve the SDF output in this and other areas of Kenya.

5.2 Cross-sectional study of productive and reproductive traits of dairy cattle in smallholder farms in Meru, Kenya

This study was carried out to first assess the milk yield of the dairy cows, determine their reproductive outputs, and characterize the cow and farm level factors associated with their recorded milk and reproductive performance. The members list of the 550 farmers shipping milk to the NDFCS in the month of April 2015 was used as the sampling frame to randomly select 200 farms that would be used for this study. The principal farmer was primarily female (52.5%), and among them, the majority had either none or primary level of education (57%). The mean household size was 3.78 persons, and mean total land holdings owned by these farmers were 2.04 acres. Half of the farmers indicated that they allocated 25-50% of the total land owned or rented to grow fodder for their dairy cows.

A total of 316 cows were utilized for this study and they recorded an average and median milk yield of 6.7 and 6.0 kg/day, respectively, for the day prior to the visit. Around four percent of the cows in this area reported producing over 15 kg of milk the previous day, while the upper 10% had produced over 10kg of milk. Subclinical mastitis was analysed by way of a California Mastitis Test done on the farm and 44% of the cows tested positive (>+1) on one or more quarters. These cows recorded an average body condition score (BCS) of 2.44, with a majority of them (59.4%) being assessed as BCS≤2. The timing of this study was months after a dry season had started, which was likely to have led to low quality and quantity of feed availability.

Since the distribution of the milk yield was not normal, a log transformation was done on the outcome. A linear mixed model was fit to assess the factors that affected milk output, adjusting for clustering within farms. An intraclass correlation of 24.6% was recorded in this model and there was no interacting or confounding variable in the final model. Four cow variables (breed, weight, days in milk and reproductive status), one farm characteristic (percent of land allocated for growing fodder) and one farm management factor (farmers who fed dairy meal to their cows in the last month of gestation) were associated with the natural log of daily milk yield when other variables were controlled for in the model.

Overall mean and median days in milk in this study were 300 and 243 days, respectively. Long lactation of over 400 days was found with 34.2% of the cows being in this category, and a third of them not being pregnant by 400 days. Nearly a third of the cows in this study were milking and anestrous. As expected, days in milk (DIM) was negatively associated with the log of daily milk yield, especially since it has been shown that cows have a limited peak in milk production at 2 months postpartum (Richards et al., 2016). However, our study found reproductive status was associated with milk production, while controlling for DIM. We categorized the cows into different reproductive statuses (early pregnancy/anestrus, pregnant, and cycling) and noted a steady increase in milk produced by cows in these different groups, with the cows that were cycling recording a 19.8% higher daily milk production over those in early pregnancy or anestrus.

Artificial insemination services were readily available in this area, although 13% of the farmers still preferred to use natural bull service on their cows. A major problem reported by the farmers in this area was their inability to choose the bulls for AI, and they relied heavily on the recommendations of the AI technician, or used the semen that was within their price range. Therefore, efforts of improving the genetics of cows in this area have been slow since most low-priced semen was from bulls of questionable quality.

Farmers in this study area preferred exotic crosses because of their high milk production, with the Friesian crosses being kept by 48.9% of the farmers and they did seem to record the highest milk output with an average of 7.5 kg/day. Holstein-Friesian crosses have been the breed of choice for Kenyan smallholder zero-grazing units. In Britain, this breed of cattle has been recorded to produce >10,000 kg of milk per 305-day lactation, while in Kenya, the highest annual milk yield has been recorded as 4575 kg/cow (Mugambi et al., 2014).

In our study, heavier cows were also associated with more milk yield, after controlling for breed in the model. Poor feeding management of young stock continue to hinder the production potential of adult cows. It has been shown that lifetime productivity of a dairy system can be improved by increasing feed intake through adding a good quality supplement to the poor basal diets (Rufino et al., 2009). Farmers need to be educated more on target feeding to meet the nutritive requirement of the animals to be able to optimize on the returns for longer periods.

Farmers who fed dairy meal during the last month of gestation, and percent of land allocated for growing fodder were positively associated with natural log of milk yield, demonstrating the importance of nutritional management on milk production in smallholder dairy farming systems. Milk production from cows that received dairy meal in the last month of gestation was 34.3% higher compared to those that did not receive any. The percentage of land allocated to growing fodder for dairy cows was positively associated with the cow's milk yield per day, with a 15.6% increase for every 25% increase in land set aside for growing fodder.

Quality and quantity variability of forages fed to the smallholder cows differs depending upon season, forage management (species, variety, planting practices, timing of cutting, chopping), income and labor availability. There have been consistent gains in terms of milk production and better BCS, and in turn better reproductive outcomes associated with supplementation of cows in the SDF with dairy meal, but the major constraint to dairy meal supplementation still remains the cost (affordability). Leguminous crops are a good source of protein and energy that can and have been associated with high daily milk production, and has been used as good alternatives to commercial high protein supplements like dairy meal (Franzel et al., 2014). In our study area, knowledge on leguminous trees for dairy consumption was very minimal.

5.3 Seroprevalence and risk factors of *Neospora caninum* and Bovine Viral Diarrhea Virus (BVDV) in smallholder dairy cattle in Meru, Kenya

The specific aims of this cross-sectional study were to determine the seroprevalence of *N. caninum* and BVDV and to characterize the risk factors contributing to their occurrences in smallholder dairy farms in the eastern part of Kenya. A total of 470 serum samples were obtained from all cows and heifers above six months old on the same 200 randomly selected farms in the Naari area involved in the first study. In the laboratory, the samples were analysed for *N. caninum* antibodies and for BVDV antibodies and antigens using ELISA tests. At the time of blood collection from the cows, a face-to-face questionnaire was administered to the farmers to assess the risk factors associated with the seroprevalence of these two pathogens. Over half of the sampled animals were between three and eight and a half years old with an arithmetic mean of 5.6 years of age recorded for the cows in the study. Forty-four percent of the animals tested were from zero-grazed dairy units while 55% of the tested cows were allowed to graze around the compound as they were in semi zero-grazed units.

Of the 470 samples tested, 165 were seropositive of *N. caninum*, yielding a 35.1% prevalence of the *N. caninum* infection in cattle in this area. This prevalence was high compared to other studies that reported prevalence of 18.8% (Kenyanjui et al., 1994) and 25.6% (Okumu, 2014) in Kenyan cows. Work in other parts of Africa identified lower prevalence as well, including 13.3% in Ethiopia (Asmare et al., 2013a), 10.7% in Sudan (Ibrahim et al., 2012), 17.9% in Senegal (Kamga-Waladjo et al., 2010) and 19.6% Algeria (Ghalmi et al., 2012).

Buying and introducing milking cows as well as lending and borrowing of cows amongst farmers were two farm level factors that were associated with increased odds of seroprevalence of *N. caninum,* as seen in Ethiopia (Asmare et al., 2013b), Greece (Sotiraki et al., 2008) and Croatia (Beck et al., 2010). Purchasing cows from unknown sero-status or moving animals between farms of unknown sero-status are likely to be important contributors to the prevailing high seroprevalence of *N. caninum* in the area.

Farm dogs eating aborted fetuses and dogs whelping in the farm compound were also associated with higher odds of seropositivity to *N. caninum,* with an interaction between the last two variables. Cattle on farms whose bitches had no designated birthing place were nearly three times more likely to test seropositive for *N. caninum* when farmers allowed their dogs to access aborted fetuses compared to cattle owned by farmers who did not allow their dogs to access aborted fetuses.

Eliminating the possibility of dog access to bovine aborted placenta and fetuses and dog faecal contamination of cattle feed and water sources by infected dogs should be recommended as a major method of breaking the lifecycle and ensuring control. Several other studies have found higher odds of disease with lack of confinement of farm dogs and canine neosporosis (Lopez-Sicupira et al., 2012). Prevalence of *N. caninum* was also reported to have been higher in rural dogs than in urban dogs in both Kenya (Okumu et al., 2016) and

Brazil (Lopez-Sicupira et al., 2012), probably due to greater likelihood of them encountering cattle offal, placenta or fetus.

N. caninum is one of the most important agents associated with infectious bovine abortions throughout the world (Dubey et al., 2007; Quinn et al., 2002; Kul et al., 2009). There is not a lot of research carried out on risk factors of *N. caninum* in most African countries; therefore our data on risk factors can go a long way in advising farmers on what to do to keep the prevalence low. A study in large scale farms in the rift valley area of Kenya did not identify any management risk factors significantly associated with *N. caninum* seroprevalence (Okumu, 2014).

A 47.1% seroprevalence of BVDV antibodies and 36.2% of BVDV antigens was found in this study. This antibody prevalence closely compares to the antibody prevalence of 48.5% in Kenyan coastal Zebu cows (Kenyanjui et al., 1994), and is lower than the 79.1% that was reported for larger dairy farms in the Nakuru area of Kenya (Okumu, 2014). For cows with positive antigen and antibody results in our study, 49.7% of the animals were identified as transiently infected (both antigen and antibody positive), while 50.3% were identified as potentially persistently infected (antigen positive but antibody negative). Additional testing was not possible for logistic and financial reasons, and therefore it remains unclear if half of the cattle that tested positive for antigen were truly persistently infected. Based on studies elsewhere, we would expect this prevalence of possibly persistently infected cattle to be substantially lower than 50% (Scharnböck et al., 2018). However, these BVDV test results may be partly a function of test cross-reaction with classical swine fever virus or border disease virus because they are both found in Kenya, and are in the same pestivirus family as BVDV and border disease virus is known to infect cattle (Handel et al., 2011). BVDV can infect pigs, and others have found cross-reaction between CSF on BVDV tests (Loeffen et al., 2009; Gatto et al., 2018). Furthermore, there is very recent preliminary evidence for possible cattle infection with CSF virus in China and India (Giangaspero et al., 2017), which could further complicate the interpretation of our results if the long-held belief that CSF only infects swine is confirmed to be untrue. Project funding did not include testing for CSF virus. Therefore the BVDV results reported for this project are based on the test results obtained with the BVDV tests, but they should be interpreted with caution. Future research should explore the relationship between BVDV and CSF virus in cattle and pigs in Kenyan SDFs.

Older cows had eleven times higher odds for being seropositive of BVDV antibodies compared to heifers. High seropreprevalence (>40%) to BVDV infection in heifers is an indicator of the presence of PI animals (Mainar-Jaime et al., 2001). The low seropositivity within the younger group would suggest the absence of PI animals, unless most of the young stock were PI animals and, therefore, seronegative for antibody (Mainar-Jaime et al., 2001). Of the 51 heifers that had an antigen and antibody test result, 31.4% (16/51) were identified as potentially persistently infected, after having a positive antigen test and a negative antibody test. The constant shedding of the virus by these PI animals and the absence of physical barriers between the younger and adult cows would contribute to the observed high seroprevalence in the older age groups

The role that pigs play as risk factors to bovine BVDV occurences remains unclear. There may be cross-reactions of tests between BVDV and CSF virus, BVDV transmission between pigs and cattle, and/or CSF virus transmission between pigs and cattle, as mentioned above (Gatto et al., 2018; Giangaspero et al., 2017). Further research is required to clarify how BVDV and CSF virus interact with each other among pigs and cattle in Kenyan SDFs.

The main method of prevention and control of diseases in a farm is through good biosecurity (Lindberg & Houe, 2005; Laanen et al., 2013). Biosecurity can be both external (not to bring the pathogen into a clean herd) or internal (to reduce spread within the farm between sick and healthy animals). Vaccination is often used as the lone control measure and it is suggested that this approach does not result in a decrease of BVDV prevalence (O'Rourke, 2002). Moreover, due to incorrect use of vaccines, cattle may not be fully protected (Meadows, 2010). Nonetheless, BVDV vaccination may be useful as an additional control measure in a systematic control scheme alongside biosecurity (Lindberg et al., 2006).

Age of the animals formed important interactions with two farm management variables in the BVDV antigen model. On farms where no visiting dairy farmer entered the cow shed in the last year, older animals had a higher probability of testing seropositive for BVDV antigen than younger animals. Similarly, older animals had a reduced probability of testing positive for BVDV antigen when the farmers had introduced new calves to the farms than when they had not. Older animals have been associated with higher seroprevalence of BVDV elsewhere, possibly due to an increase in cumulative risk of having been exposed over time (Daves et al., 2016). Farmers moving within farms and not taking necessary measures to disinfect between cohorts of animals would potentiate transmission of this virus from infected to naive animals.

5.4 Randomized controlled trial on impacts of using sex-sorted semen and reproductive hormones in smallholder dairy cows in Eastern Kenya.

A randomized controlled trial was designed to assess the impacts of using sexed semen and reproductive hormones in the same study population of Naari dairy farmers. Five groups of twenty farms each that had been randomly selected from the farms used in the two above chapters were then randomly allocated into the five intervention groups and treated as follows; 1) reproduction only; 2) nutrition only; 3) reproduction and nutrition; 4) other cow management education only (quasi-control); or 5) nothing (control). The reproduction intervention included reproduction advice and provision of prostaglandin F2α and gonadotropin releasing hormone to induce heat in cows, as needed, whereas the nutrition intervention included nutritional advice and provision of leguminous shrubs. In groups 1 and 3, breeding using sexed semen was allowed throughout the trial up to two times per cow, once breeding criteria were achieved: 1) BCS $\geq$2.25 or BCS$\geq$2.0 and rising; 2) 60-300 days in milk; 3) evidence of ovarian cyclicity; and 4) clear estrus mucous at previous heat and were visited once a month. In groups 2, 4, and 5, farmers were given one dose of sexed semen to use on any cow with/without meeting breeding criteria. Cattle were examined approximately monthly (groups 1-3) or bimonthly (groups 4-5) and data were collected on cattle and farm management characteristics on these visits for 17 months in total.

Cows and heifers that met the breeding criteria for sexed semen were enrolled in the study, and inseminated on the next observed heat, with or without the assistance of hormone therapy, depending on the DIM and heat detection (Figure 4-1). Service percentage was calculated, based on the proportion of enrolled cows that were subsequently inseminated during the time period. Conception percentage was calculated as the proportion found pregnant on transrectal palpation at 40-70 DIM from the total number of animals inseminated. Pregnancy percentage was calculated by multiplying service percentage by conception percentage. A multivariable Cox proportional hazards model was fit to determine variables significantly associated with calving-to-conception interval.

An overall conception risk for sexed semen for the cows and the heifers combined was 50.1% which was quite encouraging considering it was the first trial conducted with sexed semen in smallholder dairy farms in Kenya. The conception percentages for sexed semen were better in heifers (54.5%) than in the cows (44.0%). Heifers are known to have better conception risks than cows due to a higher likelihood of having an infection-free uterus

(Norman et al., 2010), but it was unclear if this would also happen in Kenya where heifers may be fed with poor quality feed in times of feed shortage (drought) so that milking cows can receive better quality feed. However, since the heifers needed to follow the same breeding criteria as the cows, nutritional status and BCS were unlikely to be substantially different between cows and heifers. The results can be used to advise SDF on the use of sexed semen so they can to replenish the replacement heifers faster, as all the calves born from sexed semen (n=11) by the time the study was ending were female and of good health. The prediction of conception risk from services with sexed semen, conventional semen and overall prediction, by different body condition scores are shown in Figure 4-2. The trends in the figure indicate a gradual increase in probability of conception with increasing BCS between 1.75 and 3.0 for all of the curves, suggesting that there was no improvement in conception with higher BCS when BCS was below 1.75 and above 3.0, although there was some improvement in conception risk for sexed semen between BCS 3.0 and 3.5.

Among the different intervention groups in this study, service percentage was better in the reproduction group only (35.5%) compared to all the other groups (8-16%), and this difference could be a result of the ways the reproduction only group had been intensively trained on estrus detection methods. It was expected that better service percentages would be obtained in the intervention group receiving both reproduction and additional nutrition intervention (fodder shrubs) as the BCS of the cows in this group were expected to be better and thus increase the service percentages. There were no significant differences in the BCS averages obtained among the intervention groups, although the nutrition only group did have slightly higher BCS averages. Some farmers had some difficulty with growing and managing the leguminous shrubs, limiting the amounts that they could feed to the cattle.

The conception risk obtained in the reproduction group for sexed semen was the lowest (35.9%) among all the groups, indicating that fertility among this group of inseminated cows was not as good as the fertility of inseminated cows in other groups (43-60%). With the much higher service percentage, the reproduction only group still had the best pregnancy percentage, despite the below average conception percentage.

With the highest average BCS, the nutrition only group also recorded the highest conception percentage for sexed semen (60%), showing that intensive education and support on nutrition can yield positive results. Intensive education on estrus detection and other related topics surrounding reproduction also seemed to pay off. Farmers seemed to understand and act upon one specific message and yield better results as compared to when they were taught multiple messages (reproduction and nutrition intervention).

Among the animals included in this study, *Neospora caninum* and BVDV status were assessed as risk factors of reproductive performance. Even though they were not significantly associated with CCI in the final model, numerical differences in the proportions of positive animals within the intervention groups were found. N. *caninum* and BVDV are primary abortifacients, and BVDV can exacerbate the abortion potential of other abortifacient pathogens through its immunosuppressive effects, therefore these pathogens would not likely demonstrate their full effect on CCI since abortions would occur after conception. For *N. caninum,* infection prevalence ranged from 17 to 54% by group. For BVDV, the prevalence of antigen-positive animals ranged from 39 to 63%, whereas the prevalence of antibody-positive animals ranged from 33 to 81%. Farmers in this area had very little or no knowledge of the two diseases. Training on what they are, how they are transmitted and the economic repercussions of these pathogens is required.

A multivariable Cox proportional hazards model was used to identify associated cow- and farm- level factors with CCI for 191 cows enrolled in this study. The average BCS was significantly positively associated with the risk of pregnancy in the final model. Most cows lose body condition in early lactation as a result of high demands from the peak milk production, especially if their diet is not meeting their energy and protein requirements. High producing multiparous cows experience deeper NEB nadirs and take longer days postpartum to regain zero or positive energy balance than lower producing and younger cows (Ferguson, 2005).

In our study, the hazard of conception was lower in the cows that had come on heat after induction with hormones than the cows coming into heat spontaneously. The cows receiving hormones were both cows that were anestrus but had larger ovaries that received GnRH as well as cows that had a functional CL and in turn received Prostaglandin F2α. A conception percentage of 57.1% was reported for cows that responded with estrus following GnRH treatment and service with sexed semen, compared to 77.3% for those inseminated with conventional semen in this group. Conversely, the conception percentage reported for cows that responded with estrus following induction with prostaglandin F2α, was 78.6% following sexed semen service, and 66.6% following conventional semen service, demonstrating good conception risks on services induced by hormone therapy, if the cows came into heat and were inseminated. However, of the cows receiving injections of GnRH and prostaglandin F2α, only 39.8% and 58.9% showed signs of heat, likely due to nutritional deficiencies in their diets.

There is very little research carried out on the use of reproductive hormones for either estrus induction or estrus synchronization in Kenya. Tsuma et al. (1996) investigated the use of Prostaglandin F2α and GnRH in anestrous cows and those classified as repeat breeders in zero-grazed dairy farms in Kenya and found that the overall fertility of these two groups was improved by hormone use; however these cows do not represent all cows. Mass hormonal estrus synchronization was attempted in Ethiopian smallholder dairy farms by use of prostaglandin F2α followed by insemination of cows that came on heat and they noted a 97.7% response to the hormone and a 57.7% pregnancy percentage for inseminated cows, after pregnancy diagnosis (Tegegne et al., 2016). Our results cannot be used to promote or rule out the use or reproductive hormones as that was not the main study goal (therefore, there were small numbers, and lack of cow-level random allocation). However, with proper and adequate training of the farmers on heat detection techniques, hormone therapy could contribute to reduction of days open for cows on SDF in Kenya, especially anestrus and problem breeder cows.

Since the variable for the intervention formed a significant interaction with a management variable (supplementation of dairy meal to cows in last month of gestation), interpreting its effects in this study can only be done together. Farmers that received the leguminous shrubs and also supplemented their cows with concentrates (dairy meal) during the last month of gestation recorded the highest hazard of conception compared to those that did not supplement their cows or did not receive any leguminous fodder shrubs. Management of cows, especially high milk producers, during the transition period should ensure the best conditions to meet nutritive requirements. Provision of leguminous fodder crops has been one way to provide a high protein diet to the smallholder dairy cows since they can be planted along the fence-line and thus reduce the use of land that is in short supply on these SDF.

5.5 Linked conclusions

Farmers in this study area have a challenge of raising enough of their own replacement heifers, as a result of long inter-calving intervals that reduce the productive life of the cows and, in turn, lead to an absence of female heifers, since conventional semen has a 50% chance of yielding bull calves that are not useful to dairy farmers. Over half (51.2%) of the farmers indicated that they had obtained their milking cows through purchasing them as adult cows, as compared to those who purchased them as youngstock (28.4%) or raised them on their farm (20.4%). Introduction of milking cows in the farms was also an important risk factor in both *N. caninum* and co-infection of *N. caninum* and BVDV.

Long days in milk were found in the cows in our cross-sectional study, due to low body conditions and nutritional deficiencies leading these cows to go into long periods of anestrous phase that would not be reversed until the cows were on a good energy balance and better body condition scores. This slow return to ovarian cycling was also observed in our intervention trial, where trial cows also recorded long days to conception, despite efforts to improve the CCI in three intervention groups who received hormone therapy, better nutrition and reproduction advice and/or leguminous shrubs. Some improvements in CCI were seen among the three intervention groups, but the carry-over effect of the low BCSs of cows entering the trial was not something that could be overcome during the trial period. A longer trial period could have produced CCIs close to the recommended number (80-110 days) for a calving interval of 12-13 months

Farmers in this study area are also challenged by low milk production, and indicated owning very small land sizes (average of 2 acres) that severely constrained their farm management and nutrition options. During the dry season when feed shortages are common, farmers indicated lending some of their dairy stock to their neighbors or relatives who had larger pieces of lands and were able to feed the cows, and in turn, the neighbours/relatives would milk and sell the milk obtained during this period as their own. The original owner would get the cow back once they had enough feed to feed the cow again, avoiding the need to sell the cow or see the cow deteriorate in condition and perhaps even die.

Farmers also indicated having access to other pieces of land from leasing it from other farmers or leasing government–owned nearby forest, especially during the dry season. However, lending cows or utilization of community pastures, including cows driven to the forest, will lead to increasing contact between cows from different farms, and this could increase the exposure of cattle to *N. caninum* or BVDV. The model of supplying the farmers with leguminous fodder shrubs that they could plant along the fence-line could assist in providing additional forage, which could reduce the need for accessing land off the farm, and so reduce exposure to pathogens. Due to the small farm sizes, farmers were reluctant to allocate a separate piece of land to grow the fodder trees permanently.

In an attempt to counteract some of the diminishing quality and quantity of feeds of SDF, concentrates have been used in milking cows, with the main ones used in Kenya being dairy meal or milling by-products such as maize germ, pollard, or rice bran. Sixteen percent of the farmers did not use dairy meal at all, and they cited high prices as the major limitation, as the milk obtained from their cows was not enough to offset the cost of the concentrate. In the CCI study, an interaction between intervention group (growing leguminous shrubs) and farmers that supplemented their cows with dairy meal in the last month of gestation recorded better hazard of conception, and this interaction clearly shows the importance of these cows being fed a high protein diet, even in the last month prior to calving. Since the quality of the commercial concentrates vary considerably in Kenya, there is need to educate farmers on how they can plant, cultivate and preserve nutritious fodder crops, which would ensure a good quality high protein diet for all the cows at all times.

Farmers in this area preferred keeping Friesian crosses in a bid to increase productivity, as the Holstein is known to be bigger than the indigenous cattle, and produce more milk than other breeds of cattle. As well, it was found that cows with heavier weights produced more milk and cows with better BCS had better hazard of conception in the CCI Cox model. High BCS and weight are indicative of good nutritional management on the farm, which would in turn lead to better performance for the cows. Therefore, farmers who want improved milk production should continue to breed cattle with exotic breeds, such as Friesians, and should search for and obtain more training on good nutrition, which should improve BCS and weight, and ultimately improve milk production and reproductive performance.

Future research in this area should focus on larger populations to allow for greater representation of other dairy breeds beside Friesian and different farm management methods (zero-grazing and semi zero-grazing systems) in order to better determine their roles in milk production and reproduction on SDF. Cohort studies could be designed and carried out that will provide better causality of the factors contributing to suboptimal milk production and reproduction, and the factors to control for infectious diseases such as *N. caninum* and BVDV. A cohort study in relation to *N. caninum* and/or BVDV incidence should be planned, since identifying and reducing risk factors associated with incidence of diseases is more likely to yield better results than a study identifying risk factors associated with prevalence. Where pigs and goats are kept in close proximity to cattle, the role of classical swine fever virus (Giangaspero 2017) and border disease virus (Kim et al., 2006) should be considered, in terms of study design (testing) and interpretation of results. For logistical reasons, we were unable to test our sera for these other viruses. Conception risks of sexed semen yielded encouraging results in this study, and there is need to look into how sexed semen can be used in other contexts of smallholder dairy farming to ensure availability of replacement heifers.

5.6 Overall recommendations

From the results of this study, we can recommend that for the smallholder dairy farm setting in Meru County of Kenya (and farms elsewhere with closely related management):

1. Some of the identified factors leading to the suboptimal production and reproduction recorded in this study can be reversed. Farmers should endeavour to maintain good BCS of the cows through better nutrition during the month before calving and during the postpartum period until conception since their reproduction efficiency is greatly

affected by poor BCSs. Breed improvement by use of sexed semen can be encouraged, as this will replenish the pool of replacement heifers in the area faster, but should only be considered when cows meet appropriate breeding criteria; 1) clear vaginal mucous; 2) ovaries cycling; and 3) BCS$\geq$2.25. An economic analysis of short- and long-term costs and benefits could clarify if the use of sexed semen is financially appropriate, depending on the cost of the semen and other inputs into the analysis.

2. Nutrition-related interventions, such as the leguminous shrubs used in this study, have a lot of potential for improving the BCSs of cows in smallholder dairy farms, and in turn, improve the milk production and reproductive efficiency of the cows. Planting leguminous shrubs (e.g. *Calliandra* and *Sesbania* species) along the fence lines will optimize the use of the diminishing land sizes in this area and provide the much needed high-quality high-protein forage to cows.
3. Due to their small land areas, farmers should zero-graze dairy cows and utilize mixed crop farming systems in this area to increase the farmers' economic outputs from their small farms. With better land utilization, farmers should be able to grow and conserve enough good quality fodder (i.e. through hay or silage) for feeding their animals all year without compromising on the productivity of the animals during long drought periods. With zero-grazing, there would be better biosecurity with less exposure to animals from other farms.
4. Cows on farms where the farmers had attended some dairy related training had better hazard of conception. Since government-sponsored extension services are not uncommon, the model practices in this dairy society (seminars, workshops and farmer visits to other high-producing dairy farms and dairies) should continue and be emulated in other areas to increase the farmer knowledge on new techniques of dairy

farming. Topics on nutrition and reproductive diseases and their effects on reproduction need to be addressed.

5. Identifying ways to have BVDV vaccinations introduced by a government vaccination protocol and/or industry-led program would go a long way in curtailing new BVDV infections that are likely happening, especially in areas where livestock have close contact with other livestock and wildlife.
6. Recovery and testing of placental tissue and aborted bovine foetuses will enhance the diagnosis of the actual causes of abortion in Kenya. Farmers and animal health providers need to be informed on the importance of submitting samples in cases of abortion, where farmers can afford it, particularly when laboratories start testing for BVDV and *N. caninum*. Communications within the livestock farming communities and government livestock services units should be coordinated so that one group is not waiting for the other group to act on these recommendations. Government veterinary services will only want to provide these diagnostic services if farmers submit samples, and farmers will only want to submit samples if they know that government veterinary services will test for these diseases.
7. More research should be carried out to determine the effects of abortifacient pathogens on milk production, reproductive performance and the economic impacts on the Kenyan dairy cattle industry.
8. For better success in use of sexed semen in this and similar smallholder dairy settings, a body condition score of at least 2.5 is recommended.
9. Women should be encouraged to take leadership roles on the farm and in the community, with a focus on dairy farming, which is likely to improve milk production and reduce CCI.

5.7 Reference

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Chapter 6 Appendices

This thesis research shared the study population and trial groups with another Ph.D. thesis by Dr. Dennis Makau, part of which is included as appendices 6.1 and 6.2, which have been published. I am a co-author on those papers because Dennis and I worked together closely on the farm visits and data collection and consulted with each other during the data analysis and chapter writing phases of our theses. Therefore, these published articles are included as appendices to this thesis.

Since this research was closely related with another Ph.D. thesis, some of the unpublished chapters of that thesis have also been mentioned in this thesis. These unpublished chapters highlight some determinants of nutritional performance of dairy cows in smallholder dairy farms in the study area in rural Kenya. Dennis Makau's thesis can be found by searching for the title of his thesis "Enhancing Productivity and Livelihoods of Smallholder Dairy Farmers in Kenya through Agroforestry and Cellphone-Mediated Training".

Appendix 6.3 and 6.4 provide the data collection tools utilized in this thesis. Appendix 6.3 was for the initial 200 farms, and Appendix 6.4 was for the monitoring of 100 farms during the trial.

6.1 Animal and management factors associated with weight gain in dairy calves and heifers on smallholder dairy farms in Kenya.

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Abstract

Calf growth is an important determinant of dairy herd productivity, particularly in countries where the dairy industry is expanding, such as Kenya. Our objective was to determine factors associated with weight gain in randomly selected dairy calves and heifers in smallholder dairy farms (SDF) in Kenya.

A cross-sectional study on a census of 321 calves and heifers (dairy calves and heifers up to 36 months of age), sampled from 200 randomly selected SDF in Naari, Kenya, formed the study population. Youngstock management was recorded using a questionnaire. Biodata were obtained through subsequent physical examination and heart girth measurement. Descriptive statistical analyses were conducted, and mixed model regression was used for identification of factors associated ($p<0.05$) with the natural log transformation of estimated average daily weight gain (ADG).

Median and mean ADG of the youngstock were 360 and 443 (s.d.=375) g day^{-1}, respectively. In the final model, ADG was highest in pre-weaned calves and declined with age. Supplementing with quality hay during the dry season at least weekly was associated with increased ADG. There was an interaction between breed and historical disease on ADG such that disease was associated with decreased ADG in *Bos taurus* breeds, while ADG in *Bos indicus* breeds was not affected by disease. There was a significant interaction between education levels of the husband and wife caretakers; when the man's education was low (having less than or equal to primary school), ADG was highest when the woman had not completed primary school, but was lower when the woman had completed primary, secondary or tertiary education, possibly because such women worked off-farm more often.

General growth performance of animals on these farms was lower than benchmarked standards recommended for optimum dairy production but within previously reported ranges for the East African region. Supplementation of diets (with hay and/or concentrates) is recommended for optimum growth in calves and heifers, especially in the dry season. If nutritional management of *Bos taurus* youngstock is not improved, crosses of *Bos indicus* could be better suited for the current nutritional management systems in SDF in Meru having

calf disease problems. Interventions to support educating women (the primary caretakers of the cattle) and men in the community on calf management should be initiated, preferably with shared on-farm responsibilities. Training in better management, even for learned farmers, would be critical to better calf growth.

Key words: smallholder dairy farms, calves, heifers, average daily weight gain, Kenya

Introduction

Good calf management is the cornerstone of dairy cattle productivity, particularly in countries where the dairy industry is expanding, such as Kenya (Odero-Waitituh, 2017). Milk production in Kenya in 2011 was estimated at 4.8 million tonnes of milk, 4.6 million tonnes from cows (Dairy Farming in Kenya, 2011). This volume was produced by approximately 3.5 million cows with exotic blood lines (Friesian, Ayrshire, Jersey and Guernsey breeds and their crosses), and 9.3 million indigenous cows (Muriuki, 2011). More than 80% of the milk produced in Kenya comes from 2 million smallholder dairy farms (SDF) concentrated in the moderately productive areas of Kenya (Dairy Farming in Kenya, 2011).

One very important constraint to expansion of production in SDF in Africa is suboptimal feeding (Steven et al., 2014). It has been documented that most nutritional problems occur during the dry seasons in Africa (Smith, 2000). The most common constraints on optimal nutrition include insufficient water, inadequate knowledge and technology on feed conservation, and deficient quantities and quality of forages used in nutritional management of dairy animals (Lukuyu et al., 2011). Although feed conservation is practiced to some extent in Kenya, Napier grass and crop residues are often of very poor nutritional quality due to late harvesting, and the quantities are frequently inadequate (Njarui et al., 2011). Kenyan farmers often resort to buying feed from neighboring farms or renting grazing land during the dry season (Njarui et al., 2011). Other high quality feeds, such as hay, can be either expensive and/or unavailable to the Kenyan SDF (Bii, 2017).

A reasonable growth benchmark for weight gain in calves on SDF is 400 g day^{-1} (Lukuyu et al., 2012). For bigger breeds, such as Holstein-Friesian, the ideal age at weaning is 12 weeks, or when the animal reaches 80kg body weight (B Lukuyu et al., 2012). In the early stages of life (1-2 months), nutritional management is aimed at ensuring proper ruminal development through papillae and increase in size (Ueno et al., 2014). Post-weaning, when the rumen is well-developed, provision of high quality feed is key to maintaining healthy growth rates, and therefore poor quality feed in the dry season can affect skeletal growth and weight gain (Lukuyu et al., 2012). The optimum weight gain for heifers to achieve first calving before the age of 27 months was estimated to be 500-700 g day^{-1} (B Lukuyu et al., 2012). Krpálková et al., (2014) reported that the group of Holstein calves that had ADG between 850 g day^{-1} and 949g day^{-1} between the ages of 5 to 14 months had the highest milk production in their first lactation.

Nutrition is closely associated with disease. Calf-hood diseases impact subsequent heifer survival and productivity, and affect the economic value and welfare of animals in a production unit (Windeyer et al., 2014). Studies in Kenya have reported on disease and mortality in dairy calves on SDF (Gitau et al., 1994), but there is limited recent information on factors, including disease, associated with weight gain in dairy calves/heifers in SDF.

In this cross-sectional study, we examined a random sample of young-stock (dairy calves and heifers up to 36 months of age) to identify factors associated with young-stock weight gain in smallholder dairy farms (SDF) in Meru County, Kenya.

Materials and methods

Description of study area

This cross-sectional study was carried out in Naari sub-location of Meru County, Kenya, at 0°6'0" N and 37°35'00" E. Meru County is located on the eastern slopes of Mount Kenya and is about 270 kilometers north of Nairobi, the capital city of Kenya. The Naari sub-location is located in the high agricultural potential region within an altitude of approximately 2,000m above sea level, and the main agricultural activities include dairying, horticulture and lumbering. Farmers grow food crops such as maize, beans and Irish potatoes. The study area was purposively selected since this research was part of a larger study involving dairy farmers in the area (Figure 1a). A non-governmental organization, Farmers Helping Farmers, and the University of Prince Edward Island had an existing developmental partnership with the Naari Dairy Cooperative Society, which provided a strong foundation for the work and the entry point to the community.

Sample size and data collection

The farmers included in the study were from Naari Dairy Cooperative Society, a dairy group with an active membership of about 500 farmers who regularly deliver milk to the dairy. A sample size of 200 farms (Figure 1b) was randomly selected from the registry of active members between January and May 2015 using software-based random number generation. The sample size was determined based on a need to identify 80 farms with specific characteristics for a related intervention study. Farm-level inclusion criteria included active membership with the Naari Dairy, zero-grazing, and <4 milking cows.

Principal farmers consenting to participate in the study were visited in May-August 2015, and they answered a semi-closed questionnaire covering various management factors on their farms. The questionnaire had 58 questions with three main sections covering farm management, youngstock health and productivity, and farmer training and demographic information.

Calves/heifers were included in the study if they were male or female animals less than or equal to 15 months old, or female and more than 15 months but less than 36 months of age and had not given birth or had a miscarriage/abortion. None of the young stock was excluded because of having had an abortion/miscarriage. A total of about 300 eligible calves and heifers were targeted from the 200 participating farms. Additional information on the health of each eligible calf/heifer on each participating farm was collected from a physical examination of the calves/heifers, and the weight was estimated with a heart girth tape.

Data management and analysis

For each calf/heifer, the ADG was calculated as the difference between weights observed in the study at examination and average recorded weights of calves less than three days old in the area divided by the age at examination. For breeds where this information was not available, birth weights from published studies were used (Hickson et al., 2015).

Statistical analyses were done using Stata13.0 software. Descriptive statistics s included means, medians, distributions, and proportions, where applicable. Data collected were analyzed using both univariable and multivariable regression analysis as detailed below, with

ADG as the outcome of interest. Tests for normality of ADG, using the Shapiro-Wilk test, and Box-Cox analysis for suitable transformations were explored. These tests found that ADG was right-skewed and a natural logarithm of ADG was normally distributed.

A univariable mixed linear regression model (restricted maximum likelihood) was built for each of the variables to ascertain associations with natural logarithm of ADG at $p \leq 0.4$ in order to determine eligible variables for the multivariable model-building process. Farm identification was utilized as a random effect in the models, to account for possible clustering of calves/heifers within farms.

Multivariable linear regression was later performed using a mixed model (maximum likelihood) with natural logarithm of ADG as the outcome. Again, farm identification was utilized as a random effect in the models, to account for possible clustering. The p-value was set at 0.05, and interactions between significant parameters were explored. Tests for collinearity (Pearson correlation coefficient) among all parameters meeting the regression modeling cut-off ($p<0.4$) were determined to aid decision-making on collinear variables to be included in the model building. Wald test was used to test overall significance of categorical parameters with more than 2 categories. Assessment of linearity between ADG and continuous variables was done using a lowess plot. Model building used a backward elimination technique and models were compared using likelihood ratio tests for significance of dropped parameters. Testing for confounding by age and other possible confounders was done in the final model by comparing changes in coefficient estimates with and without the suspected confounder. Identification of extreme and influential observations was done by sorting and graphing the standardized residuals and comparing changes in coefficient estimates and their significance when modeling with and without influential observations. Therefore, all observations were retained in the model.

Model evaluation was done to confirm that normality and homoscedasticity assumptions on both random and fixed effects were met. Tests for normality of residuals were done using the Shapiro-Wilk test. Predictions of ADG were performed using the margins command on lnADG and subsequently back-transformed to the original scale of g day^{-1} for ease of interpretation.

Results

From the 200 sampled farms, a total of 321 calves and weaned heifers were examined. Twenty animals were excluded from the analysis for not meeting the inclusion criteria; eight were male cattle over 15 months old, 12 were heifers over 36 months old. None of the heifers under 36 months had a calf/abortion/miscarriage. There were 41 farms that did not have an eligible calf or heifer, and therefore the final dataset consisted of 301 animals from 159 farms. The mean ADG of the 301 animals was 0.443 kg (s.d. = 0.375) with a median of 0.360 kg. The calves under 15 months of age had a mean ADG of 0.482 kg (s.d. = 0.441), while the heifers over 15 months of age had a mean ADG of 0.364 kg (s.d. = 0.151).

Descriptive statistics and univariable analyses between ln of ADG and various factors.

The mean age of calves and heifers combined was 12.5 months (s.d. = 9.5), with a median of 12 months. The population was comprised of 202 calves and 99 heifers over 15 months of age. There were 123 female calves and 79 male calves. Animal-level variables that met the $p<0.40$ univariable analysis cut-off are presented in Table 1.

Age was a highly significant factor of ADG, as expected, in the univariable analyses. Sex of the calf was however not associated with ADG at $p<0.40$. A total of six cattle breeds were kept on these farms (Table 1) with more than half of the dairy cattle population comprised of Friesian crosses.

Most of the calves and heifers had no history of disease, with only a quarter of them having suffered from navel ill, diarrhea or pneumonia. Among this population of 79 calves and heifers with history of disease, pneumonia was the most common disease affecting 63.3% of them. Farmer demographic variables that met the $p<0.40$ univariable analysis cut-off are in Table1. Women were more often the principle farmer than men, and more women than men had completed at least primary education. Higher levels of formal education (university/college) were not common among both women and men. More than half (60.4% of women, 57.2% of men) of participating farmers had only obtained a primary school education, or less.

A number of farmer demographic variables were included in the questionnaire but did not meet the ADG univariable analysis cut-off of $p<0.40$. The mean age of women and men in dairy farming in the area was 47.1 years (s.d. = 13.6) and 51.7 years (s.d. = 14.3), respectively. The mean land size owned in this area was 2.3 acres (s.d. = 2.9) and, on average, 40.3% (95% CI: 39.5% - 44.2%) of land owned was used for dairy production. Household sizes were, on average, 4 persons, with a s.d. of 2 people.

The following farm management variables met the $p<0.40$ univariable analysis cut-off (Table 1). Most of the farms housed their young animals in a dirt-floored pen, whereas 10.1% had wooden floors, while few (2.5%) had concrete floors. Most farmers dewormed their young cattle 3 months after the last deworming. Feed changes among young-stock were common on two-thirds of farms, especially when the season changed. At the time of this study, feed shortage was a problem in this area. Only one-third of farmers had adequate feed for their young cattle within the 12 months prior to the commencement of the project.

Nearly all, 98.1%, of the farms fed calves and heifers on Napier grass. Twenty-two farms fed calves and heifers on silage, with 90.9% (20/22) of these animals being fed on maize silage, while the others were given grass silage. Farms feeding maize and grass silage were combined for regression analysis and met the $p<0.40$ univariable analysis cut-off (Table 1).

Other significant farm management factors included supplementation with concentrates for heifers and calves at least once a week; with half of farms having some form of concentrate feed in their calf/heifer diets. The three most commonly used concentrate supplements on the 82 farms were dairy meal 64.6%, maize germ 20.7%, and calf pellets 14.6%. A quarter of the farmers fed hay as a daily supplement during the dry season. A third of the farms occasionally fed calves and heifers on banana leaves and non-leguminous tree foliage. Slightly more than half of the population of calves and heifers in these farms always had access to clean drinking water.

In summary, factors associated with AGD ($p<0.4$) included calf age, breed, and history of disease, principal farmer gender, education level of both farmers, and the following management factors: deworming frequency, feed adequacy in last 12 months, feed changes, feeding of silage, hay, tree and banana foliage, concentrates and access to clean drinking water.

Other farm-level management variables were included in the questionnaire but did not meet the univariable analysis cut-off of p<0.40. Few farmers fed high protein forages (desmodium/lucerne) and leguminous trees/shrubs (*Calliandra*/*Sesbania*/mulberry) to calves and heifers, at 4.4% and 8.2%, respectively. The use of sweet potato vines as fodder was practiced, among 25% of the farmers, feeding them to calves and heifers at least once a week. Although 74.8% of farmers had received some form of training on dairy management, most (70%) farmers incorrectly indicated that the calves consumed at least 4 liters of first colostrum only after 24 hours. However, free suckling of colostrum by newly born calves was practiced by nearly two-thirds of farms, making it difficult to know exactly how much colostrum calves consumed within the first 12 or 24 hours. Vitamin and mineral supplementation was common, with 74.2% of these SDFs providing some form of mineral and vitamin supplementation to calves and heifers on the farms, but again, there was no difference in ADG between farms supplementing vitamins and minerals versus those farms who did not supplement.

Multivariable analysis between ln ADG and various factors

The highest correlation observed between variables meeting the cut-off p-value was 0.3028, between weekly hay feeding and weekly concentrate feeding. Of the 83 calves supplemented with hay at least weekly, 62 (74.7%) were also supplemented with concentrates at least weekly. Of the 151 calves supplemented with concentrate at least weekly, 62 (41%) were also supplemented with hay at least weekly. Therefore, weekly concentrate feeding was not retained in the final model in favour of weekly hay feeding, since both correlated variables could not remain in the final model and be statistically significant.

In the final multivariable linear mixed model, age, breed and history of disease were animal-level variables that were significantly associated with ADG (Table 2). Since the relationship between ADG and age appeared curvilinear, a quadratic form was used for age as a continuous predictor. Weekly supplemental feeding of hay, education level of both the man and the woman, and gender of the principal farmer were farm-level variables that were significantly associated with ADG. There was a significant interaction between breed and disease, and a significant interaction between man's education level and woman's education level, and thus the final model used for this analysis was:

Ln_ADG= hay + age + age squared + breed + disease history + breed*disease history + gender of principle farmer + man's education level + woman's education level + man's * woman's education level

The intra-class correlation of ADG estimated among farms was 0.25 with 95%CI = 0.14 to 0.42. About 36.6% of the total variation observed in ADG was at the farm level.

Figure 2 provides a Lowess plot of the relationship between age and ADG, since it is hard to understand the curvilinear relationship from the coefficients in Table 2. There was a general decrease in predicted ADG from about 750g day^{-1} at 2 weeks of age to about 500g day^{-1}. The predicted ADG in a preweaning calf from the final model ranged from 711 - 798 g day^{-1} at 1 month of age, depending on the breed. There was a subequent decrease in ADG in older animals, dropping to approximately 350 g day-^{1} for heifers between 10 and 30 months of age. Since there was a significant interaction between breed and disease history (p<0.0005), we cannot interpret the coefficients of the main effects of breed and disease history in isolation because they depend on the level of the other variable within the interaction (Table 2, Figure 3). While controlling for age in the model, the effect of disease on ADG was most observed in the predominantly Guernsey breed among all exotic cross breeds, presenting with an ADG of about 259.1 g day^{-1} (95% CI: 214.9 to 303.3 g day^{-1}), which is substantially lower than the overall mean ADG of 443 g day^{-1}. Local and dual-purpose crosses (Others in Figure 3) were estimated to have the highest ADG of about 676 g day^{-1} when subjected to similar management conditions as the others and when disease was present (Figure 3).

For the farm-level variable "supplemented hay", when we exponentiated the coefficient, there was a 1.23 times effect, meaning that there was a 23% increase in ADG compared to calves not supplemented weekly with hay. For the principle farmer variable, ADG was lower by 20.7% when the principle farmer was female, compared to the baseline of both males and females identified as the principle farmers, whereas there was no statistically significant difference between males identifying as the principle farmer versus the baseline.

Since there was also a significant interaction between the education levels of the husband and wife with respect to ADG, their associations with ADG again depend on each other (Table 2, Figure 4). When women's education was low (having less than primary school), ADG was highest when the man had not completed primary school, but ADG was lower when the man had completed primary school, and substantially lower when the man had completed secondary school. When the women's education was high (completed college/university), the ADG was significantly lower when the men finished primary school compared to lower or higher levels of education. There were no differences in ADG, by men's education level, when women finished primary or secondary school.

Model evaluation

The model assumptions on normality and homescedasticity were well met. Scatter plots of fitted values and standardized residuals did not depict distinct patterns in the distribution of residuals, with only 4 observations that were outside 2 standard deviations, and these 4 observations were not outliers. The standardised residuals had a good fit on the normality plot, with only the 2 extreme observations mentioned earlier. A model fit with and without these observations had no difference from a model with all observations. Therefore, all observations were retained in the final model with 301 observations from 159 farms.

Discussion Conclusion and Recommendations

The descriptive findings for ADG for this study were generally in agreement with similar studies conducted in Kenya. Gitau et al., (1994) estimated that the overall median daily weight gain in similar farming systems in Kenya was 210 g day^{-1} with a range of -400 to 900 g day^{-1}. The observed mean ADG of calves and heifers in SDF in Naari was 443 ±375 g day^{-1} with a median of 360 g day^{-1}. This weight gain was within the benchmarked performance achievement for dairy farms of between 400 to 500 g day^{1} (B Lukuyu et al., 2012). However, the optimum ADG for calves and heifers within the first 5 months in dairy farms is estimated at 500 g day^{-1} to 700 g day-^{1} for heifers to achieve first calving at age 27 months or less (B Lukuyu et al., 2012). The general performance (ADG) of calves in SDFs in this area was within range but with a large standard deviation. The effects of low ADG on heifers only becomes quantifiably evident post-puberty i.e. age at first calving and milk production per lactation (Abeni et al., 2000). For calves that were at the onset of puberty (12 months), estimated ADG in these Naari production systems suggest that it is unlikely that heifers and calves included in this study would achieve the primary target of first calving at about 27 months. Krpálková et al., (2014) observed that a higher lactation productivity per lifetime had been observed in heifers/calves that had a prepubertal (12-15 months) ADG of about 850g day^{-1} suggesting that calves in this study would have limited lifetime milk-production potential.

Our study was able to identify a number of interesting animal- and farm-level variables associated with ADG, while identifying important interactions and controlling for confounding and within-herd clustering. Age was observed to be significantly associated with ADG ($p<0.0005$) in the study farms in a curvilinear manner (Figure 2). Within the first 4 months of life, the predicted ADG appeared to constantly decrease in a fairly linear manner (section A Figure 2). This trend was similar to the one observed (Gitau et al., 1994b) in SDFs in Kiambu district, Kenya. This decrease could be as a result of reduced milk consumption resulting from the sale of milk soon after the designated colostrum period of lactation (milk sales are banned for the first 2 weeks post-calving in Kenya). Therefore, the calf should be introduced to good quality solid feeds such as hay and concentrate early to allow proper rumen development and hence good absorption of any consumed feeds. In our study, for calves 6 to 12 months of age, there was a slower overall rate of decline in the ADG in the calves (section B Figure 2). After 12 months of age, growth stabilized at about 300 g day^{-1} before a decrease in trend to <300 g day^{-1} after 27 months of age. However, due to possible survivor bias affecting this study population, these ADG estimates may overestimate the true ADG because calves/heifers with very poor growth due to chronic diseases or very poor management likely did not survive to become part of this study.

Given that this was not a cohort study, it was difficult to ascertain at what point slow growth actually occurred in older heifers, but from the cross-sectional data, it is clear that older calves and heifers have lower cumulative ADG than younger calves and heifers. It could be that the older heifers had poor growth as calves and better growth as heifers, or vice versa, or even steady modest growth throughout their lives. Additionally, the farmers in this study had no standardized feeding regimes for calves or heifers according to respective ages. Therefore, it was not possible to completely ascertain the actual effects of different feeding methods used on the farms, unless a cohort study of calves and heifers were conducted.

According to most literature, calves reach puberty at 9 months of age, are considered breeding heifers at 15-16 months, and become lactating cows at about 24-27 months (B Lukuyu et al., 2012). In our study, the late maturing age of the heifers resulted in our nulliparous heifer age bracket being wide (up to 36 months of age). This wide range in age adds variability to our data since the long period of growth prior to first parturition likely includes several additional environmental and management changes in the life of the animals, such as seasonal feeds and changes in housing.

Two other animal-level variables were associated with ADG in the final model, and their interpretation in the final model is dependent on each other since they were involved in an interaction: breed and history of disease (Table 2). Calf diseases are documented to negatively affect calf and heifer survival, growth, welfare and productivity (Windeyer et al., 2014). Although disease resulted in a decreased ADG in most predominantly *Bos taurus* breeds in our study, especially in Guernsey, weight gain in other breeds was not severely affected. In general, animals that had no history of disease had higher ADG in *Bos taurus* breeds compared to those with disease, but the opposite was seen in predominantly *Bos indicus* breeds (Figure 3). Dual purpose breeds, *Bos indicus,* and their crosses, tend to be more resilient in response to diseases in Africa (Mwai et al., 2015). However, the history of disease on our participating farms was unlikely to be completely accurate due to inaccurate memory recall, and these errors may have led to misclassification bias of these results, particularly the unexpected *Bos indicus* results that were based on a relatively small number (n=19) of calves in this category.

Various nutritional and management factors were associated with ADG in our study (Table 2). Supplementation of diets with hay at least weekly was observed to result in increased weight gain in calves and heifers. The average nutritional requirements for heifers are 12-19% crude protein (CP), inversely depending on age, to maintain a good growth rate (B Lukuyu et al., 2012). In calves and heifers, the CP content in the diet is generally positively associated with weight gain (Moran, 2005). However, in our study, there was no significant association between ADG and supplementing diets with concentrates, although weekly concentrate feeding, and hay feeding were correlated, therefore retaining weekly hay feeding in the model likely prevented weekly concentrate feeding from being retained in the model. The concentrates commonly found in this study were dairy meal or calf pellets that have an estimated CP of 14 to 18% (BLGG -Wageningen University, 2013). In a controlled trial (Ueno et al., 2014) found that feeding hay during the suckling phase resulted in a significant increase in daily dry matter intake (DMI), of about 318 g, and at least 0.23% increase in live body weight in the post-weaning phase. This increased DMI was postulated to stimulate growth of ruminal papillae and development of rumen-reticulum muscles, thus increasing capacity and digestive ability. This physiological maturation then translated into better nutritional gain from the feed consumed, as well as increased volume of feed intake (Ueno et al., 2014). Our study, based on a regression model, agrees with the postulated and observed effect of hay in the SDFs in Naari, Meru, with estimated weight gain of 456.7 g day $^{-1}$ when hay is supplemented at least weekly, compared to 371.4 g day^{-1} in calves not feeding hay. Our results suggest that it is important to provide supplements to youngstock, either hay or concentrates, although feeding both may help ADG when forage supply and quality are in short supply.

The management arrangement of the farm in our study was associated with the ADG of the animals on a particular farm (p=0.0021). The estimated ADG in farms managed/run by female farmers was only slightly lower than that on farms where the principal farmer was male (427.2 g day^{-1} vs 430.4 g day^{-1} respectively. However, a synergistic effect was observed when both male and female farmers were involved in management of the farm, with an ADG of 510.5 g day^{-1} (95% CI: 388.3 to 632.6g day^{-1}). These findings were in agreement with a study done (Richards et al., 2015) in Nyeri county , Kenya, where it was observed that overall milk production of farms run by women was lower than those farms run by their male counterparts. However, there was a significant interaction between feed availability and gender on milk production in that study; farms run by men did not have decreased milk production as a result of feed shortage, but those farms run by women did experience decreased milk production, likely because women were busy with other household chores, leaving less time to search for additional cattle feed compared to men. On farms where both male and female farmers were equally involved in management, shared responsibility for searching out high quality feeds for calves, even in times of scarcity, is more likely to be successful.

The level of formal education/training of both the man and woman on the farm was significantly associated with ADG (p<0.0054 and p<0.0001, respectively), and there was also a significant interaction between the education level of the man and that of the woman (p<0.0005). When women's education was low (having less than primary school), ADG was highest when the man had not completed primary school but was lower when the man had completed primary school, and substantially lower when the man had completed secondary school. This finding might suggest he is employed off the farm and the woman's access to critical inputs is restricted due to lower involvement of the man and his ability to access resources (Doss et al., 2011). When the women's education was high (completed college/university), the ADG was significantly lower when the men finished primary school compared to lower or higher levels of education. There were no differences in ADG, by men's education level, when women finished primary or secondary school (Figure 4). The interaction could be a result of the farmer (man or woman) with better education levels preferring to get off-farm jobs, thus spending less time on farm management. This interaction could possibly be a result of more hired labor that was unsupervised on the farm, thus leading to underfeeding of animals in those farms, but these factors were not examined as part of our study.

Our selection criteria were meant to exclude any animals that had either miscarried or aborted for one reason or the other. This could be influenced by the fact that the animals were either poorly fed or of low productivity. However, although there was a risk of survival bias, none of the animals sampled were excluded on this basis. The current study was cross-sectional in nature, which normally means that identified factors associated with the outcome may not have occurred prior to the outcome. However, with the outcome being ADG, which is a function of management throughout the rearing period, this temporality issue for our study is unlikely to be a major concern. It would be helpful to carry out a cohort study to better monitor growth of calves and heifers in these smallholder dairy farms, and identify significant factors associated with superior growth. An alternative study for quantifying the benefits of certain growth factors related to management and nutrition would be a field trial, with random allocation and controlled management to reduce the effects of confounding variables.

In conclusion, growth in calves and heifers in smallholder dairy farms in Meru, Kenya, was low compared to internationally expected performance targets (post weaning and at puberty). However, compared to other SDF in Kenya, the ADG in the study area was within previously reported ranges. ADG was found to be significantly associated with age, breed, history of disease, supplementing with hay at least weekly during the dry season, gender of the principal farmer, and education levels of the farmers. Since 26% of youngstock were reported to have had at least one of the three common calf-hood diseases (navel ill, diarrhea, and pneumonia), farmers should be encouraged to feed 4 litres of colostrum within the first 6 hours of life to enhance passive immunity against these diseases. Supplementation of diets (with hay and/or concentrates) with additional protein and energy is recommended for optimum growth in calves and heifers. If nutritional management of *Bos taurus* youngstock is not improved, crosses of *Bos indicus* could be better suited for the current nutritional management systems in SDF in Meru due to their growth performance in the face of disease. With the highest ADG occurring when both genders were heavily involved in managing the farm, efforts to encourage shared responsibility of farm work could be helpful. Since secondary education was associated with higher ADG, efforts to improve secondary education attainment may be a route to greater dairy animal productivity. As higher education of the man and the woman running the farm was associated with lower ADG, perhaps due to them being heavily involved in off-farm activities, these farmers should be reminded that neglected calf management will lead to poor calf welfare and ADG and ultimately lost potential for the animals' long-term productivity. Additionally, training and capacity-building for hired help could minimize the farm impact of absences of principle farmers. Farmers and animal health professionals could use these conclusions and recommendations to advise farmers better on calf management and growth.

Declaration of interests
None.

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Figure 1a: Study area showing Naari sub-location in Meru County, Kenya.

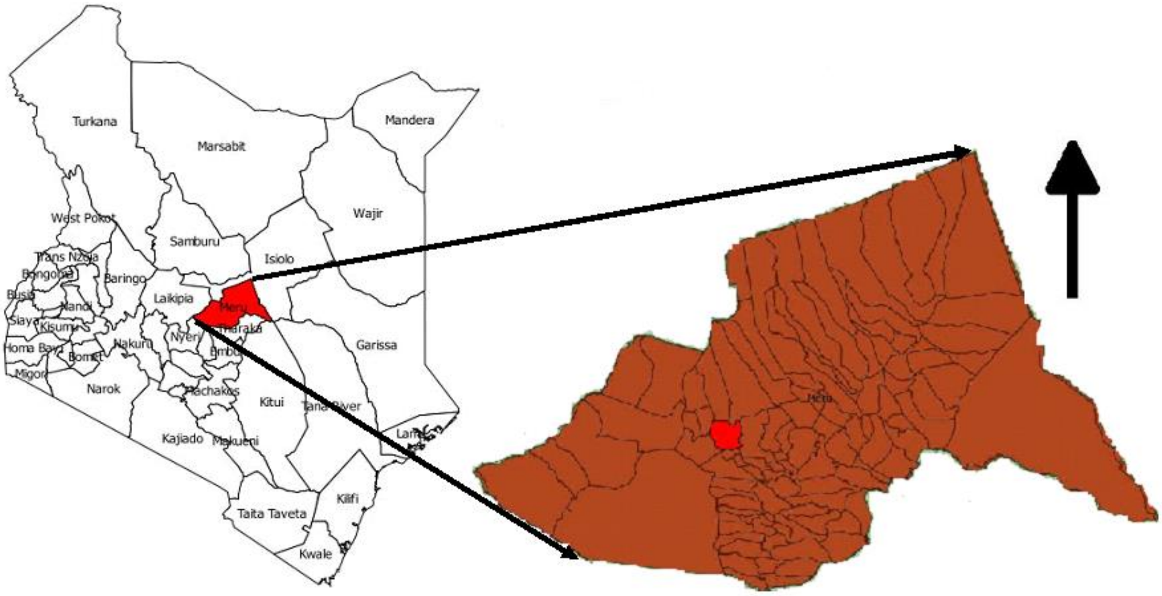


Figure 1b: Study households in the Naari Dairy Cooperative Society region

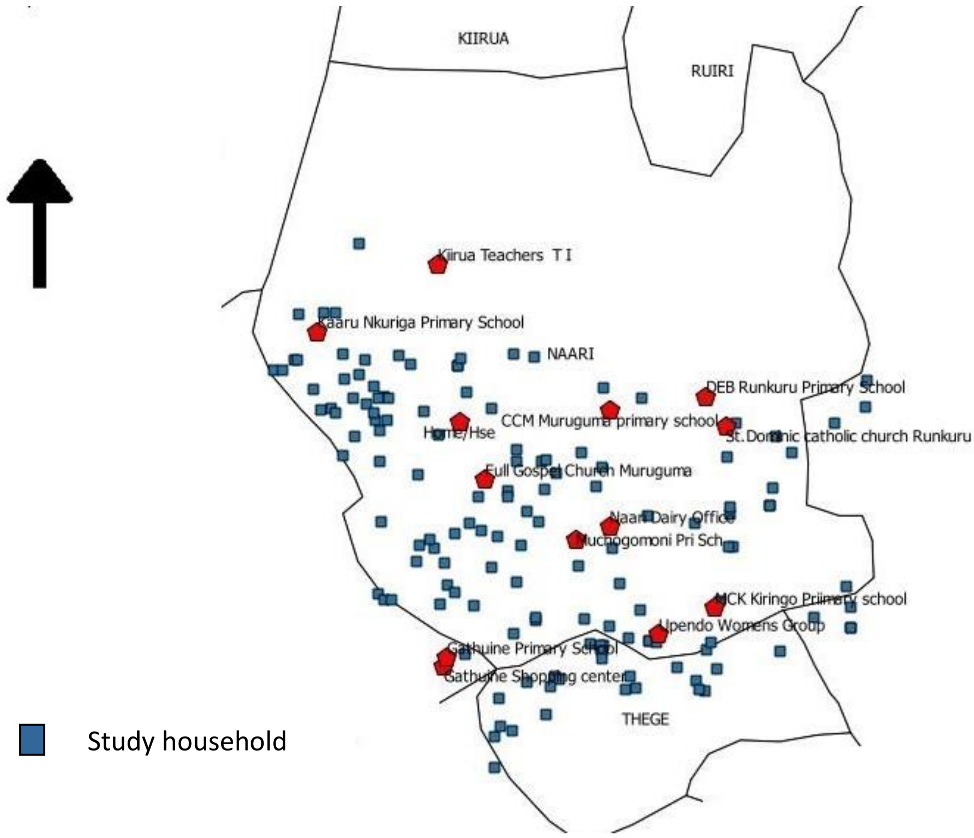


Table1: Descriptive statistics and p-values for unconditional mixed linear regressions for variables marginally (P<0.40) associated with ln of Average Daily Gain for 301 calves and heifers among 159 smallholder dairy farms near Meru, Kenya in 2015.

Variable Names and Categories	Percentage (Numerator / Denominator)	Geometric mean Average Daily Gain (grams)	p-value
Animal Factors:			
Age (months)	n/a [b]	n/a [b]	<0.001
Breed			0.278 [a]
Ayrshire	9.3 (28/301)	356.1	reference
Friesian	58.8 (177/301)	369.0	0.806
Guernsey	25.6 (77/301)	354.1	0.742
Others	6.3 (19/301)	491.9	0.085
History of disease			
No	73.8 (222/301)	381.6	reference
Yes	26.1 (79/301)	341.4	0.191
Farmer Demographic Factors:			
Principal farmer/ manager			0.066 [a]
Both (shared)	17.9 (28/159)	418.3	reference
Female	52.8 (85/159)	353.7	0.041
Male	29.2 (46/159)	374.5	0.560
Woman's education level			0.001 [a]
None	13.2 (21/159)	446.2	reference
Primary	47.2 (75/159)	329.8	0.002
Secondary	32.1 (51/159)	413.6	0.618
College/university	7.6 (12/159)	359.3	0.189
Man's education level			0.012 [a]
None	15.7 (25/159)	431.2	reference
Primary	41.5 (66/159)	333.9	0.004
Secondary	35.9 (57/159)	387.5	0.348
College/university	6.9 (11/159)	394.8	0.466
Farm Management Factors:			
Type of barn floor			
Wooden/concrete	12.6 (20/159)	409.2	reference
Dirt	87.4 (139/159)	363.7	0.281
Deworming frequency			
> 3 months	6.3 (10/159)	318.9	reference
≤ 3months	93.7 (149/159)	375.5	0.266
Farm had adequate feed in the last 12 months			
No	67.9 (108/159)	379.5	reference
Yes	32.1 (51/159)	352.4	0.179
Feed changes within 1 year			0.035 [a]
Never	30.8 (49/159)	341.7	reference
At least once a month	3.2 (5/159)	574.5	0.012
Seasonally	66 (105/159)	376.2	0.210

Fed on silage			
No	86.2 (137/159)	364.7	reference
Yes	13.8 (22/159)	405.9	0.367
Supplemented with concentrates			
No	48.4 (77/159	341.4	reference
Yes	51.6 (82/159)	402.1	0.034
Supplemented with hay			
No	74.2 (118/159)	348.9	reference
Yes	25.8 (41/159)	434.3	0.004
Fed on tree & banana foliage			
No	68.6 (109/159)	381.8	reference
Yes	31.5 (50/159)	348.8	0.249
Always access to clean drinking water			
No	49.1 (78/159)	349.0	reference
Yes	50.9 (81/159)	391.1	0.154

[a] Overall p-values for categorical variables with >2 categories.

[b] Continuous variable, therefore proportions and ADG by group not applicable

Table 2: Final mixed regression model for ln Average Daily Gain in 301 calves or heifers in 159 dairy farms in Meru County, Kenya, in 2015.

Variables and their categories	Coeff.	Coeff. Conf. [95% Interval]		P value
Animal Factors:				
Age in months	-0.057[@]	-0.074[@]	-0.039[@]	<0.0005
Age in months squared	0.001[@]	0.001[@]	0.002[@]	<0.0005
Breed				0.3799[!]
Ayrshire	reference			
Friesian [a,]	0.079[#]	-0.116[#]	0.274[#]	0.429[#]
Guernsey [b]	0.160[#]	-0.049[#]	0.369[#]	0.134[#]
Others [c]	0.012[#]	-0.271[#]	0.295[#]	0.932[#]
History of disease (no disease is reference)	-0.036[#]	-0.414[#]	0.341[#]	0.850[#]
Breed & History of disease interaction				<0.0005[!]
Ayrshire * no disease	reference			
Friesian * disease [a]	0.035 [$]	-0.371[$]	0.442 [$]	0.864
Guernsey * disease [b]	-0.484[$]	-0.910 [$]	-0.058 [$]	0.026
Others * disease [c]	0.577 [$]	0.009 [$]	1.146 [$]	0.047
Farm Management Factors:				
Supplemented hay	0.207	0.080	0.334	0.001
Farmer Demographic Factors				
Principal farmer/manager gender				0.0021[!]
Both (shared)	reference			
Female [a]	-0.232	-0.368	-0.095	0.001
Male [b]	-0.088	-0.235	0.058	0.237
Man's education level				0.0054[!]
Did not complete primary school	reference			
Completed primary school [a]	-0.575[#]	-0.915[#]	-0.235[#]	0.001[#]
Completed secondary school [b]	-1.099[#]	-1.466[#]	-0.732[#]	<0.0005[#]
Completed college/university [a]	-0.120[#]	-0.506[#]	0.265[#]	0.541[#]
Woman's education level				0.0001[!]
Did not complete primary school	reference			
Completed primary school [a]	-0.739[#]	-1.055[#]	-0.423[#]	0.000[#]
Completed secondary school [a]	-0.574[#]	-0.944[#]	-0.203[#]	0.002[#]
Completed college/university [a]	-0.516[#]	-1.026[#]	-0.005[#]	0.048[#]
Man's education * Woman's education				<0.0005[!]
Man (Did not complete primary) *Woman (Did not complete primary)	reference			
Man (Primary education) *Woman (Primary education) [a]	0.391 [$]	0.007[$]	0.775[$]	0.046
Man (Primary education) *Woman (Secondary education) [a, b, d, e]	0.403[$]	-0.051 [$]	0.856 [$]	0.082
Man (Primary education) *Woman (College/university education) [c]	-0.851[$]	-1.799 [$]	0.096 [$]	0.078
Man (Secondary education) *Woman (Primary education) [d, e]	1.116 [$]	0.692 [$]	1.540 [$]	<0.0005

Man (Secondary education) *Woman (Secondary education) [e]	1.087 [$]	0.632 [$]	1.543 [$]	<0.0005
Man (Secondary education) *Woman (College/university education) [a, b, d, e]	0.844 [$]	0.232 [$]	1.456 [$]	0.007
Man (College/university education) *Woman (Primary education)	[α]	[α]	[α]	[α]
Man (College/university education) *Woman (Secondary education)	[α]	[α]	[α]	[α]
Man (College/university education) *Woman (College/university education)	[α]	[α]	[α]	[α]
Constant	0.093	-0.268	0.455	0.613

[!] Overall P-values for categorical variables with >2 categories.

[@] Variable is part of a curvilinear relationship, and therefore coefficients cannot be interpreted in isolation but rather in combination with the other relevant coefficients for the curvilinear variable, and these combinations are best reported using a graph (Figure 1)

[#] Variable is part of an interaction variable; therefore, coefficients and P values should be interpreted with caution, in combination with the other relevant variable of the interaction, and these variables are best reported using a graph (Figure 3 & 4)

[$] Levels are part of an interaction variable with many cross-tabulated categories from the main effect variables, and therefore coefficients should be interpreted with caution relative to the other variable in the interaction, and these results are best reported using a graph (Figure 3 & 4)

[α] Interactions and pairwise comparisons for this level could not be estimated from the model.

[a-e] Different letter superscripts represent significant differences between coefficients of different levels (other than the reference level which use the category p-values) for interaction variables and categorical variables not involved in interactions when they have more than 2 levels.

Figure 2: Lowess plot indicating a curvilinear relationship between ADG kilograms day^{-1} and age of a calf/ heifer based on individual weights of 301 calves and heifers among 159 smallholder dairy farms near Meru, Kenya in 2015; *(A-preweaning period, B- Period between 6 months and onset of puberty).*

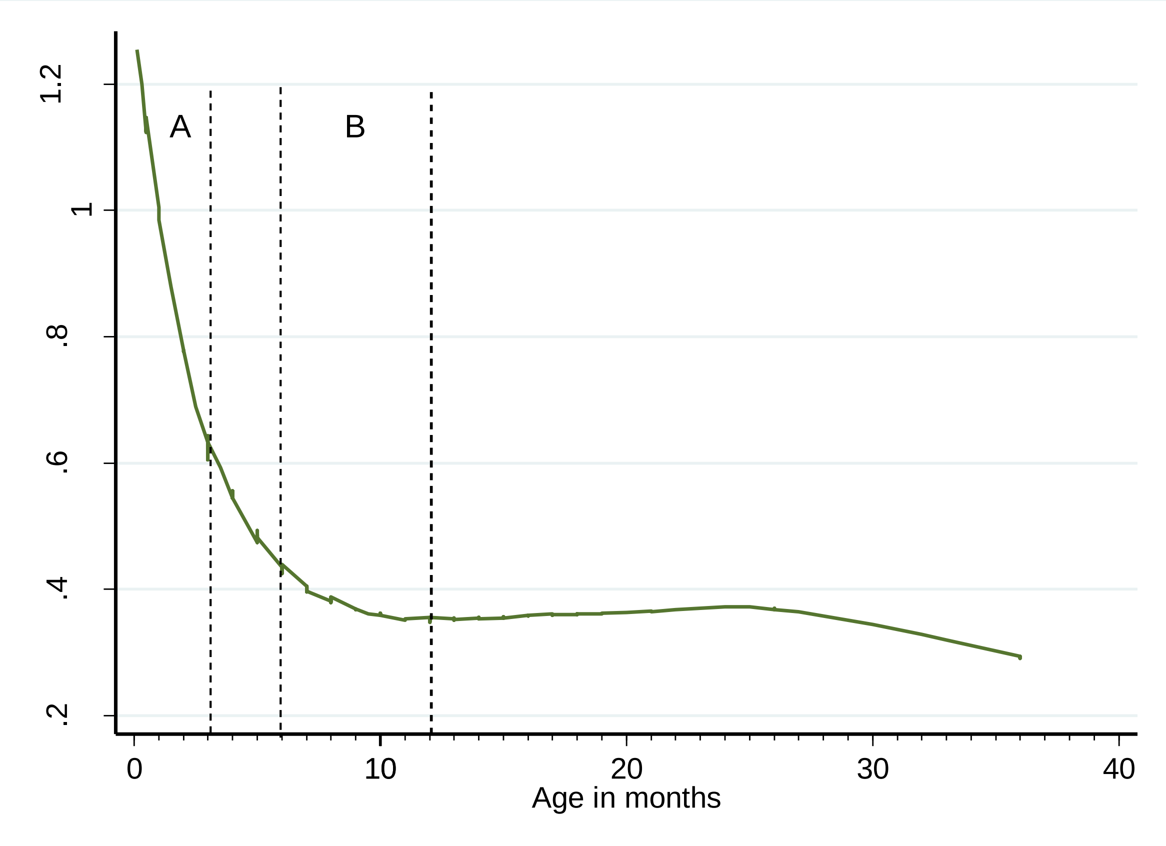


Figure 3: Predicted median ADG (g day^{-1}) and 95% confidence interval bars for average **aged** calf/heifer of various breeds when kept under basic management in farms managed by both genders with no education when affected by disease, based on the final model of 301 calves and heifers among 159 smallholder dairy farms near Meru, Kenya in 2015.

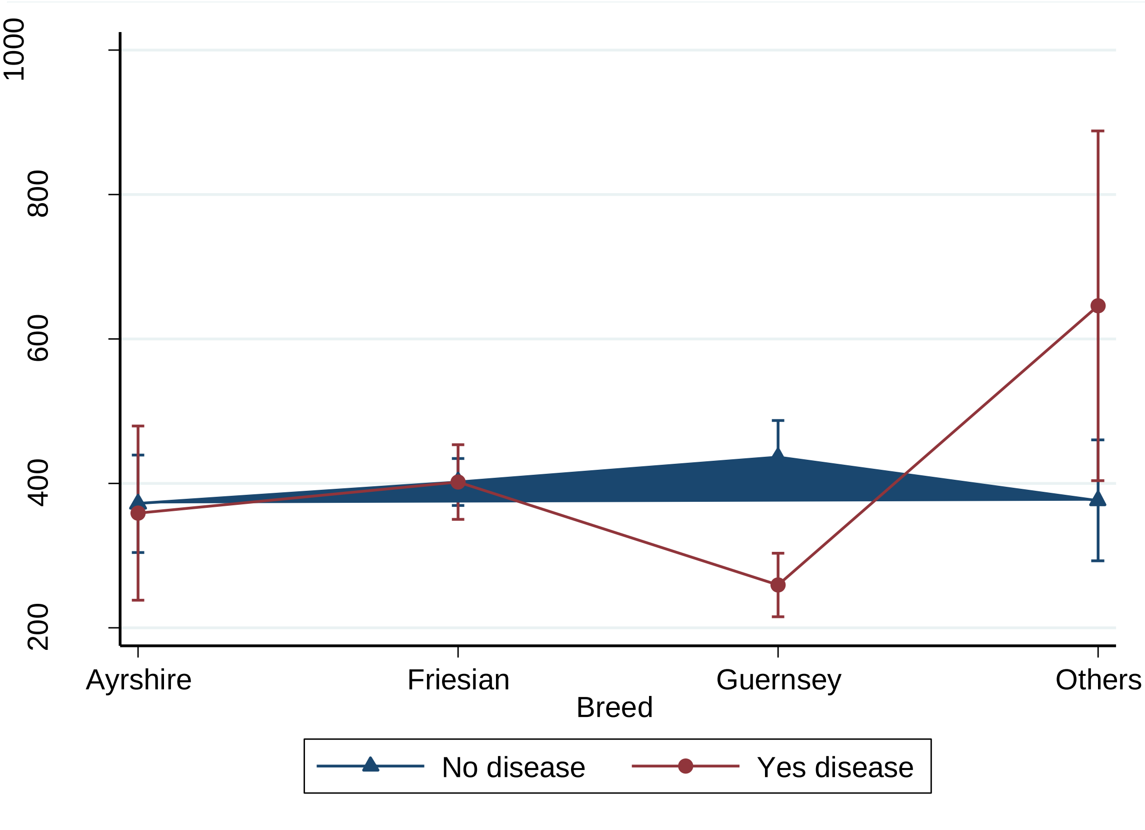
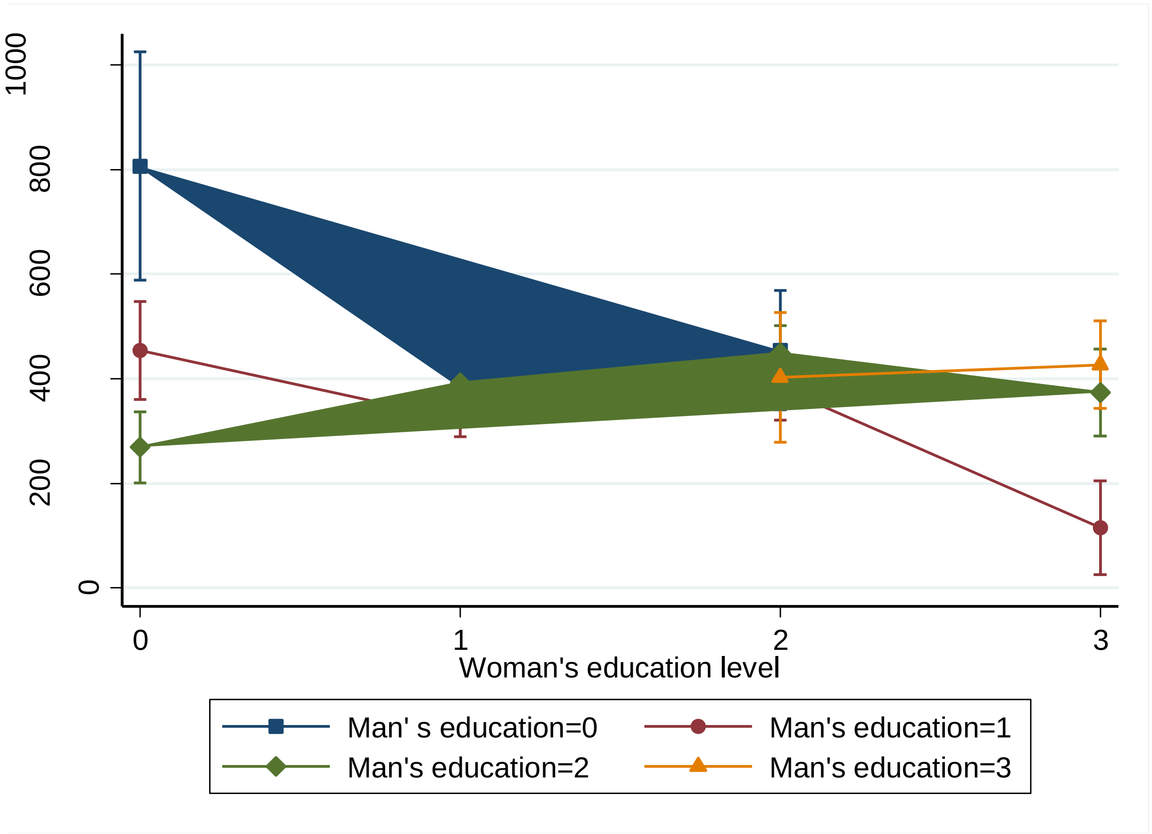


Figure 4: Results of the interaction between male and female education levels on the predicted ADG and 95% confidence interval bars for 301 calves and heifers among 159 smallholder dairy farms near Meru, Kenya in 2015; (0- Primary education not complete, 1- Primary education complete, 2- Secondary education complete, 3- Tirtiary level education).



6.2 Effectiveness of using cellphone technology as a dairy management training tool for smallholder dairy farms in Kenya

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Abstract

There is increasing need for knowledge on the utility of information and communication technology (ICT) for improved agricultural productivity and enhanced income in smallholder production enterprises. The objective of this study was to determine the effectiveness of using cellphone technology as a training tool on smallholder dairy farms (SDFs) in Kenya.

This field trial was carried out between June and September 2017 on 40 farms randomly selected from members of the Naari Dairy Farmers Cooperative Society in Naari sub-location of Meru County, Kenya. An abridged dairy management handbook, developed by Farmers Helping Farmers and the University of Prince Edward Island, was translated into the local dialect, and disseminated as short message text. After pre-intervention knowledge and attitudes assessments on dairy management, farms were randomly allocated into intervention and comparison groups. Using an online short message service interface (because the study population all had cell phones but only 1.7% had smart phones), short messages on management practices were sent daily, for 3 months, to the phones owned by the farmers in the intervention group. Post-intervention assessment of dairy management knowledge and attitudes related to the messages was done 3 weeks post-intervention. Within and between group comparisons and net changes were determined using t-tests, Chi-squared tests where applicable.

There were no significant demographic or knowledge differences between the two groups pre-intervention. Compared to pre-intervention, trained farmers in the intervention group were significantly more informed on: mastitis prevention, disease (calf diarrhea) prevention, stall management, the role of a balanced nutritious diet on immunity and the resolution of some health conditions post-intervention. Translation of message content to the local language and using easily understandable terminology were reported to be helpful for better understanding and motivation of farmers to implement recommendations.

Cellphone technology with a short message service interface can be an effective training tool for SDFs in remote areas of Kenya located far from where seminars are conducted for dairy farmers.

Keywords: developing country; economic; education; information communication technology; livelihoods; rural farmers

Introduction

Like other developing countries, optimal production of the smallholder dairy industry in Kenya is constrained by various challenges including: inadequate feed quality and quantity, poor storage facilities for feed conservation, high cost of feed inputs and inadequate information on production approaches and technologies (Lukuyu et al 2011). Poor communication of research findings to farmers has been identified as a major stumbling block to uptake of best management practices and existing technologies for better cattle nutrition and production on smallholder dairy farms (SDFs) (Ngwira, 2003; Mwangi & Wambugu, 2003; Hove et al 2003; Franzel et al 2014; World Bank Group, 2017). Participatory education and training of farmers could enhance adoption of improved fodder crop use and establishment and efficient use of pastures in SDFs in Kenya (Mwangi & Wambugu, 2003; Lukuyu et al 2011).

Cellphones have been used in different parts of Africa by farmers and fishermen to support their businesses, with numerous benefits and challenges alike. In Ghana, cocoa farmers were able to save on various transaction costs, such as transportation and operational costs (arranging for inputs and contacting purchasing clerks), through the use of cellphones (Ofosu-asare, 2011). A study on the fishing industry (Ghana) observed that fishermen who had cellphones were able to expand their markets using cellphone communication with clients. In addition, the fishermen were able to make decisions based on current information received through their cellphones (Salia et al 2011). Other benefits highlighted by farmers in northern Ghana included improved communication with farm input suppliers, resulting in increased efficiency in farming (Alhassan & Kwakwa, 2012). However, there has been limited research on the use of cellphones for agricultural education purposes in Africa.

A study to assess the use of cellphones for dissemination of agricultural information in India concluded that farmers mostly used their phones for meeting social needs, and receiving extension messages was incidental (Sahota & Kameswari, 2014). However, a more recent study, in the same area of India, concluded that farmers had used cellphones for communication with universities and veterinary institutions on animal husbandry for more than 3 years prior to the 2014 study (Rathod et al 2016). The authors recommended that adequate measures be undertaken to promote adoption of cellphone technology for effective dissemination and use of livestock-related information (Rathod et al 2016). There has been further innovation in agriculture to increase the impact of human communication and social connections on agricultural productivity and smallholder incomes. These social connections have been achieved through specialized applications that act as conduits of information dissemination in the United Kingdom (The World Bank, 2012). These innovations are needed in Africa as well.

Over the last decade, cellphone technology has become largely accessible in even the remotest parts of Kenya (Karlsen et al 2010). Like the rest of Africa, cellphones in Kenya are used for exchange and dissemination of information such as: disease monitoring, weather monitoring, advertising, marketing, financial transactions, business promotion, credit facility, access to advice, and much more (The World Bank, 2012).

A study done on SDFs in Nakuru county, Kenya, documented significant positive association between increased milk yields and use of cellphones for provision of extension services (Smollo et al 2016). However, although use of cellphones has a huge potential for improving smallholder productivity, various factors influence the gains. These factors include: timeliness, quality and trustworthiness of the information disseminated, type of agricultural practices, skills and knowledge levels of the farmers, institutional policies and regulations (Mittal & Tripathi, 2009 ; Mutunga & Waema, 2016). As a consequence of these factors, under-utilization of animal husbandry information via cellphones has affected milk production in SDFs in Kenya (Smollo et al 2016). There is increasing need for knowledge on the utility of information and communication technology (ICT) for enhanced agricultural productivity, and subsequently improved income in smallholder production enterprises (The World Bank, 2012). However, research on the effectiveness and use of cellphones, as one method of ICT, in training farmers or disseminating extension-related information in the East African region, especially Kenya, is minimal.

The objective of this study was to determine the effectiveness of using cellphone technology as a dairy management training tool on knowledge and attitudes of smallholder dairy farmers in rural parts of Kenya.

Materials and methods

Description of study area

This randomized controlled field trial was carried out in Naari sub-location of Meru County, Kenya (0°6'0" N and 37°35'0" E). Meru County is located on the eastern slopes of Mount Kenya and is 270 kilometers north of Nairobi, the capital city of Kenya. Naari sub-location is in the high agricultural potential region with an altitude of approximately 2,000 m above sea level. The main agricultural activities include: dairying, subsistence crop farming, horticulture and lumbering. Farmers grow food crops such as maize, beans and Irish potatoes and forages for dairy cows. This study area was predetermined since this trial was part of a larger study involving dairy farmers in the area (Makau et al 2018; Muraya et al 2018). A non-governmental organization, Farmers Helping Farmers (FHF), and the University of Prince Edward Island (UPEI) had an existing developmental partnership with the Naari Dairy Farmer Cooperative Society (NDFCS). This rapport provided a strong foundation for the work and the entry point to the community.

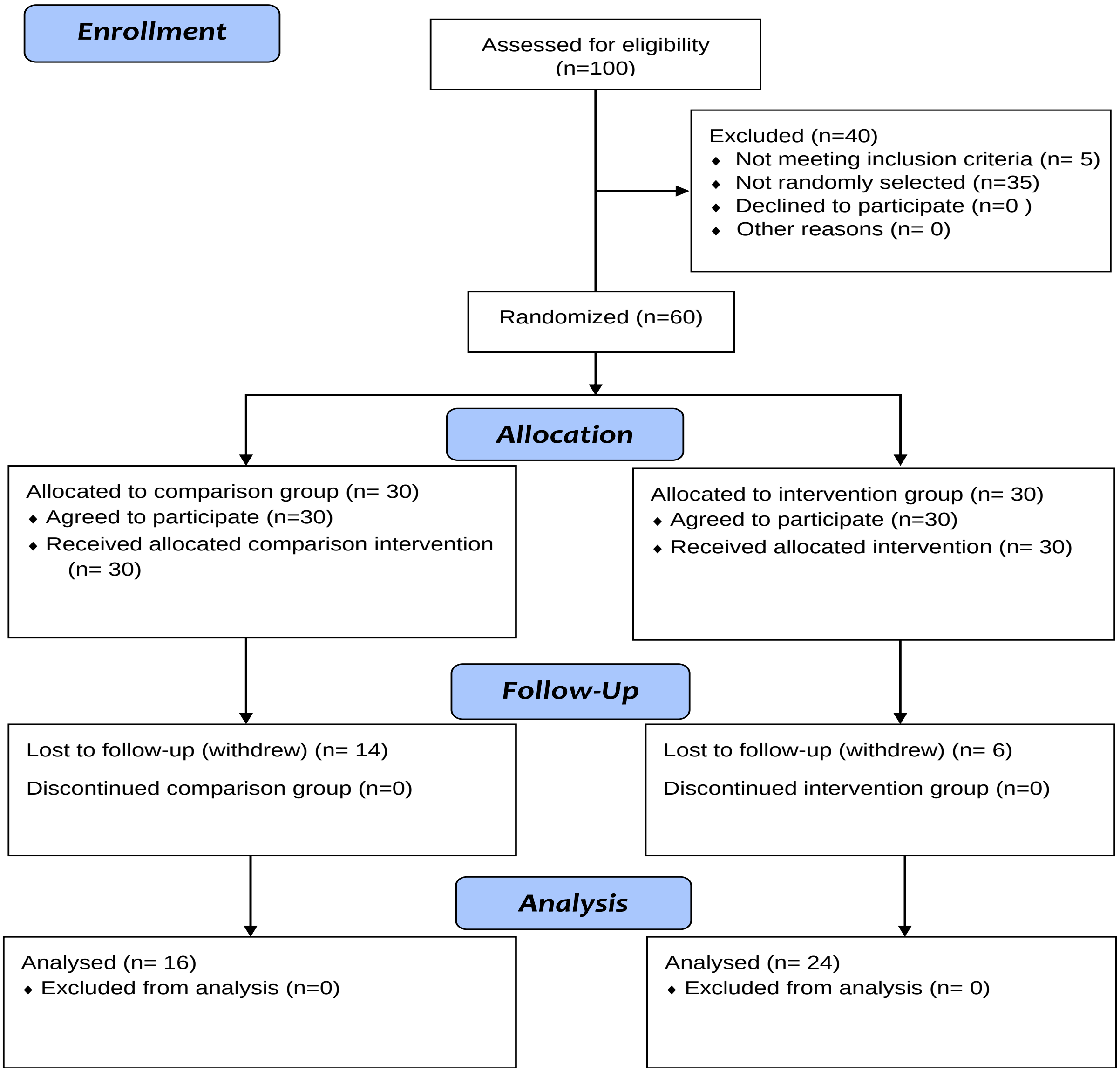
Sample size and data collection

The farmers included in the study were from NDFCS, a dairy group with an active membership of 550 farmers (active member is defined as one who regularly sold milk to the NDFCS at the time of the trial). In May 2015, 200 SDFs were randomly selected from the NDFCS registry for a related cross-sectional study using software-based random number generation. One hundred of the 200 SDFs were involved in another related intervention study, and therefore were not eligible for this trial to preserve the integrity of the intervention study. Of the remaining 100 SDFs, participants were selected if they met the eligibility criteria of: active membership with the NDFCS, possession of a cellphone, and subscription to the Safaricom carrier as the cellphone service provider. A total of 95 of the 100 SDFs met the inclusion criteria. Sixty farms were selected for this study through random number generation. Phone interviews were conducted to confirm compliance with the criteria and interest in participating in the study. When a farmer declined to participate in the study, the farm corresponding to the next random number was invited to participate as a replacement. The sample size was determined based on a need to demonstrate differences in knowledge levels between two groups of 30 farmers with respect to the cellphone training intervention, 95% confidence and 80% power.

The 60 farmers were randomly allocated into either a comparison (30) or intervention (30) group. The principal farmers for each group were invited to attend an initial meeting for their group orientation. The meeting for the intervention group was held one day before that of the comparison group. Both meetings were followed by administration of a questionnaire for collection of baseline data (pre-intervention) on knowledge and attitudes on dairy management. The questionnaire was self-administered but facilitated by a local farmer who served as a translator from English to 'Kimeru' (local language) where necessary.

Some sections of the questionnaire were borrowed from a questionnaire used in the 2015 study. The questionnaire had 37 questions with sections on farm household demographics and principal farmer's knowledge and attitudes related to: mastitis prevention and management, teat blockages, nutritional management, stall design, and neonatal calf management practices. After these two initial meetings, held on two consecutive days in June 2017, the 60 selected farmers subsequently began participating in the study (Figure 1).

Figure 1: Flow diagram of participants for a cellphone training intervention trial on dairy management in Kenya in 2017.



The farmers in the intervention group were registered in a database management system using MySQL (Structured Query Language) and content dissemination was managed through an Apache platform. Only 1.7% of the farmers in the study population owned smart phones.

Intervention

An abridged version of a dairy management handbook developed by FHF and UPEI was used to develop the content used for training the intervention group. The abridged handbook was translated into the local language (Kimeru) and compressed into short text messages of 160-200 characters. Using a XAMP server and an online integrated SMS interface, 'Africa's Talking' provided by Safaricom, the short text messages were sent daily to the cellphones owned by the farmers in the intervention group. One message was sent per day, 5 days a week for 3 months between June and September 2017.

Post-intervention data were collected during a follow-up meeting 3 weeks after completion of the intervention. These meetings were held separately for each of the groups (intervention and comparison), at different times on the same day. A local farmer (translator) facilitated the filling of a self-administered questionnaire and subsequent focus group discussions (FGD) for both groups. During these meetings data on knowledge and attitudes of the farmers on dairy management were collected. The FGD for the intervention group was aimed at assessing the overall experience and impact of the cellphone intervention and clarify any issues emanating from the training messages. The FGD for the comparison group served as an avenue to address some challenges the farmers faced on their farms related to feeding and mastitis. The themes for discussion were centered around nutrition and mastitis management questions in the questionnaire. At these meetings, participants in both groups received one-liter of cooking oil and one kilogram of dairy cow mineral supplements as appreciation for their participation. All farmers in the comparison group were subsequently provided with detailed education seminars to address some of the farm management challenges they faced.

Data management and descriptive analysis

Data from the questionnaires were keyed into MS Excel 2010 (Microsoft, Sacramento, California, USA) and checked for errors. Data were then transferred to STATA software 13.0 (StataCorp LLC, College station, Texas, USA) for statistical analysis. Descriptive statistical analysis (summarizing distributions, means, and medians) was done for continuous variables. Categorical variables were also summarized using frequencies and percentages.

Knowledge scores were calculated based on responses provided to groups of questions on feeding (3) and mastitis prevention (7). Each right answer given was allocated a value of 1 while each wrong answer was 0. Responses to all questions within a group were then summed up to provide a score for each individual respondent for that group of questions. There were no missing responses to these questions.

For continuous variables (e.g. size of land used for dairy production and knowledge scores), pairwise comparisons were carried out using two sample t-tests for between-group comparisons, and one-sample paired t-tests for before and after comparisons of the same group. For categorical variables, Pearson's Chi-square and Fisher's exact tests (if cells had fewer than 5 farmers) were used. The net change was calculated by comparing differences in scores on questions pre- and post-intervention within and between groups. For proportions, confidence intervals were used to identify significant differences between groups and within groups (Barr, 2018). Results were considered significant if p value ≤ 0.05 or confidence interval were not overlapping. Farmers agreed to the use of the data for research purposes as long as confidentiality was maintained.

Results

A total of 40 farmers participated up to the completion of the study, 20 farmers withdrew from the study (Figure 1). Their reasons for withdrawing included: ineligibility because they were no longer selling their milk to NDFCS; getting a job off the farm (making the training irrelevant and not being available for post-intervention assessment); and having a change of farming priorities (resulting in sale of animals, hence no motivation to continue to participate in the project). These reasons were not perceived to be related to the study or its objectives and therefore selection bias was expected to be minimal.

Demographics of and farm characteristics of participating SDFs.

Out of the 40 farmers who fully participated in the study, most were male, with no significant difference in gender between the intervention and comparison groups ($p = 0.34$) (Table 1).

Most of the women (78.6%) had only studied up to primary level education, while most of the men (61.5%) had studied up to secondary school level. The difference in education levels between the two genders in the study population was statistically significant ($p = 0.02$). However, there was no statistically significant difference between the education levels of the principal farmers between the two study groups (Table 1).

More than two-thirds of farmers reported that a substantial (50-75%) proportion of their total household income was earned through dairy farming (Table 1). On average, farmers had about 3.4 acres (s.d.= 2.4 acres) of land available for dairy and crop production. Most farmers (55.0%) allocated at least 50.0% of their available land to dairy production (Table 1).

Pre-intervention knowledge analysis and comparison between groups

Farmers were keen to increase their knowledge in dairy farming, with 62.5% of them having attended some form of training on dairy farming. The proportion of farmers that had attended some training (through seminars and or educational/experiential trips) on dairy production in the last one year prior to the field trial was not significantly different between the two groups ($p > 0.05$) (Table 1). However, slightly more farmers in the comparison group reported having attended training than the intervention group. A high proportion of principal farmers in both groups were not able to recall the subject of training sessions they had attended. Although not significantly different between groups, the proportion that could not remember was modestly higher among intervention group members (Table 1).

General knowledge on mastitis prevention was fairly good in the study population pre-intervention. Washing the udder prior to milking was a commonly known practice (82.5% - 33/40), but only a handful knew about using some cleaning agent in the wash water (15.0% - 6/40). Few farmers knew about post-milking teat dip (25.0% - 10/40) and dry cow therapy (30.0% - 12/40). There were no significant differences in mastitis prevention knowledge scores between the two groups pre-intervention (Table 2).

Table1: Demographic and other characteristics of 40 smallholder dairy farms participating in a cellphone training trial on dairy management in Kenya in 2017.

Variable Names and Categories	**Intervention Group (n=24)**	**Comparison Group (n=16)**	***p***
Gender			0.34
Female	29.2% (7)	43.8% (7)	
Male	70.8% (17)	56.3% (9)	
Marital status			0.06
Married	87.5% (21)	93.8% (15)	
Divorced or widowed	4.2% (1)	6.2% (1)	
Single	8.3% (2)	0.0%	
Education attained by principal farmer (regardless of gender)			1.00
Primary	50.0% (12)	50.0% (8)	
Secondary	45.8% (11)	50.0% (8)	
University/college	4.2% (1)	0.0%	
Proportion of total income from dairy			0.13
Less than 50%	8.3% (2)	31.3% (5)	
50 – 75 %	83.3% (20)	68.8% (11)	
More than 75 %	8.3% (2)	0.0%	
Proportion of land used for dairy			0.89
25% or less	33.3% (8)	25.0% (4)	
50 – 75	50% (12)	62.5% (10)	
More than 75%	16.7% (4)	12.5% (2)	
Attended any training within the last year			0.06
Yes	50.0% (12)	81.2% (13)	
No	50.0% (12)	18.8% (3)	
Subject of training if attended training within the last year			0.27
Can't remember	75.0% (9) *	46.2% (6) *	
General husbandry and feeding	16.7% (2) *	15.4% (2) *	
Silage making	8.3% (1) *	38.4% (5) *	

** Based on n= 12 and 13 in the two groups, respectively (those who attended some training)*

Feeding knowledge (and its application) was similar between the two study groups pre-intervention. One-third of farmers (13/40) knew that it was good to supplement calf diets with some concentrate and thought dairy meal would suffice, while 80.0% (32/40) of farmers knew that they needed to supplement the diet of dairy cows with dairy meal for steaming up pre-calving. There were no significant differences in nutrition knowledge score between farmers in the two groups pre-intervention (Table 2).

Table 2: Mean knowledge scores on mastitis prevention and feeding for 40 smallholder dairy farms participating in a cellphone training trial on dairy management in Kenya in 2017.

	Mean knowledge scores	**Intervention (n=24)**	**Comparison (n=16)**	***p***
Pre-intervention	Mastitis prevention	3.8 (s.d. 1.9)	4.7 (s.d. 1.1)	0.07
	Feeding	2.2 (s.d. 0.5)	2.0 (s.d. 1.0)	0.48
Post-intervention	Mastitis prevention	4.3 (s.d. 1.4)	1.8 (s.d. 0.8)	<0.001
	Feeding	2.3 (s.d. 0.7)	2.4 (s.d. 0.8)	0.69
Net change	Mastitis prevention	0.5 (s.e. 0.4)	- 2.9 (s.e. 0.3)	<0.001
	Feeding	-0.1(s.e. 0.2)	0.4 (s.e. 0.3)	<0.001

Intervention summary and feedback

All farmers in the intervention group received cellphone training messages during the 3-month intervention period. Most (70.8% - 17/24) of these farmers did not keep track of the number of messages sent to them and mentioned that they received many messages. Although a message was sent out daily for 5 days a week, the mean number of messages reported to be received by farmers was 4.4 messages per week, with a s.d. = 2.0 messages per week. Some farmers (29.2% - 7/24) estimated they had received between 4-7 messages during the entire training period, lowering the average. Most farmers reported that they always read 100% of the message (the entire message) (Figure 2-1).

From the post-intervention meeting with the intervention group, farmers generally found the content of the message understandable, except for one farmer who had some difficulty understanding the messages (Figure 2-2). On a scale of 1 (very easy to understand), 2 (easy to understand), 3 (somewhat easy to understand), 4 (difficult to understand), and 5 (very difficult to understand), the mean, s.d. and median scores for content understandability were 2.3, 0.9, and 3.0, respectively.

On a scale of 1 (very informative), 2 (informative), 3 (somewhat informative), 4 (not very informative), and 5 (not informative at all), the mean, s.d. and median scores regarding how informative the messages were comprised of 2.3, 1.0, and 3.0, respectively. More than a third of farmers reported that the messages were very informative (Figure 2-3). Over half of the farmers felt extremely or very motivated (Figure 2- 4) to practically implement the dairy cow management practices from messages such as those covering mastitis prevention and Napier grass feeding and other cow nutrition practices. Additionally, most farmers felt that the messages received (such as management of cases of retained placenta) were very effective for their dairy management systems (Figure 2-5).

Since the messaging was one-way (farmers could not ask questions for clarification), the extent of the challenge faced by the farmers regarding not being able to call back to inquire about the messages was assessed on a scale of 1 (not challenging at all), 2 (slightly challenging), 3 (challenging), 4 (very challenging), and 5 (extremely challenging). Eleven of the 24 intervention farmers were indifferent and so didn't respond to the question. The challenge of not knowing who to call back about the messages was not largely experienced among the farmers except for those who found this a big challenge (Figure 2-6), with mean, s.d. and median scores of 2.0, 1.5 and 1.0, respectively.

A third of the farmers (8/24) had some questions and concerns about some messages received in the 3-month intervention period. A few of these farmers with concerns (37.5% - 3/8) thought that the messages were not very clear and orderly for thematic continuity in each message, while 25.0% (2/8) of these farmers had concerns that some of the translations from English to the local (Kimeru) language were difficult to contextualize on their farms. However, 37.5% (3/8) of farmers with concerns chose not to articulate their concerns altogether. Some of the 8 farmers (25.0% - 2/8) that had concerns chose to ask for help from veterinarians, veterinary technicians or their neighbors to read and better understand the knowledge, while the rest chose to ignore the concerns and understand the messages as they had read them.

From the FGD, some farmers expressed a challenge not previously envisioned. Since the screen of the feature phones was small, scrolling through to read a full 160-character message took some time.

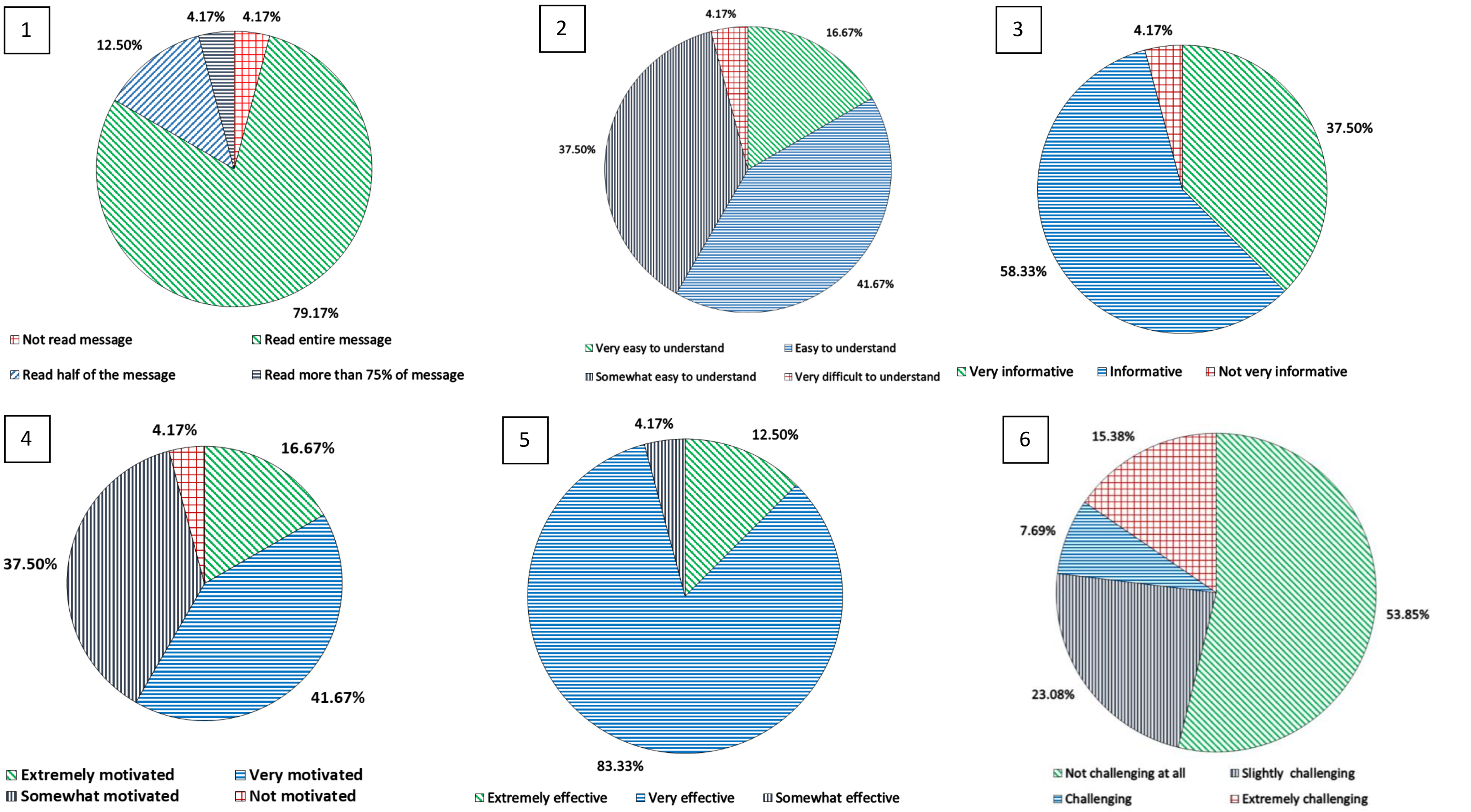
Post-intervention comparison between and within groups

Knowledge on the different practices taught as beneficial methods of mastitis control (using a different wash cloth for each milking cow, drying udder before milking with a clean cloth or paper towel, using a different drying cloth for each milking cow, using a teat dip post-milking, giving fresh feed soon after milking, using dry-cow treatment when drying cows off prior to calving, and not leaving milk in the udder to allow calves to suckle) was again assessed for the two groups post-intervention.

The mean mastitis prevention knowledge score on comparison farms decreased, but in the intervention group, there was an increase in mean score on knowledge of mastitis prevention practices, producing a net change in knowledge on mastitis prevention of 3.4 between the 2 groups, which was significant ($p < 0.01$) (Table 2). From FGD, it was evident that, although farmers in the intervention group were more knowledgeable about some of these practices post-intervention, the rationale was not always clear to them. Clarification was provided on how each of the practices was relevant in reducing mastitis occurrence on farms.

There was also a difference between groups in knowledge level on diarrhea prevention post-intervention ($p < 0.01$). Most of the intervention group (87.5% - 21/24) and (25.0% - 4/16) of the comparison group - knew that housing the calf in a clean and dry place would help reduce occurrence of calf diarrhea cases. Similarly, post-intervention, more farmers in the intervention group (66.7% - 16/24) knew that always providing dry bedding and removing manure from the stall daily was helpful in preventing diarrhea in calves compared to the comparison group at 0% ($p < 0.01$). There were no differences in diarrhea prevention knowledge between groups pre-intervention. From the FGD, it was apparent that although farmers had calves on their farms, most of them did not have conventional stalls/pens for their calves. Because of this farming practice, bedding in calf pens was not a major consideration for them.

Figure 2: Descriptive analysis of attitudes and experience of 24 farmers in the intervention group participating in a cellphone training trial on dairy management in Kenya in 2017.



On causes of teat blockage, there was a significant difference (p = 0.02) in the understanding that udder infection was a risk factor for teat blockage between the comparison (62.5%, 10/16) and intervention (91.7%, 22/24) groups post-intervention. Similarly, more farmers in the intervention group (58.3%, 14/24) than the comparison group (18.8%, 3/16) knew (post-intervention) that improper milking techniques (pulling hard on the teats during daily routine milking) was associated with teat blockage (p = 0.01). Compared to the comparison group, post-intervention, the intervention group was also more aware that teat blockage problems could be an inherited problem (0% - 0/16 vs 25.0% - 6/24, respectively) (p = 0.03). There were no differences in teat blockage knowledge between groups pre-intervention.

From the FGD, farmers indicated that pulling the teat during milking was necessary for some cows because they had small teats due to cross breeding of *Bos taurus* breeds with *Bos indicus* breeds, with the latter mostly having small teats. With this cross-breeding being common in the area, most farmers had habituated to this pulling technique of milking, even when the cows had standard size teats that could be milked easily using the squeezing technique.

The mean knowledge score on feeding practices was assessed based on the understanding of ideal height for harvesting Napier, need for dairy meal for steaming up cows pre-calving, and colostrum feeding times for newborn calves. Although there was a 0.4 increase and a 0.1 decrease in the mean knowledge score in the comparison and intervention groups respectively, these changes were not significant. There was no significant difference between the two groups post intervention. However, the net change of 0.5 in scores on knowledge about recommended feeding practices was significant (Table 2). When asked about the benefits of good nutrition post-intervention, 58.3% (14/24) of the intervention farmers were more knowledgeable (p = 0.04) on the role of a balanced nutritious diet in supporting the resolution of rain scald compared to 25.0% (4/16) in the comparison group. During the FGD, farmers from both groups mentioned that feeding cows on short Napier grass and steaming up were not very novel concepts to them since they had been trained about them in other seminars as well. However, the physiological rationale behind these practices was not clear to them.

Discussion

Analyses in this trial ultimately involved 40 farms randomly selected and allocated into intervention and comparison groups. Training the intervention group through SMS on smallholder dairy management best practices for 3 months resulted in significant increases in dairy management knowledge scores on various husbandry aspects in the intervention group. Farmers in the intervention group were more knowledgeable on mastitis prevention practices, associations between stall hygiene and calf disease, as well as some beneficial nutritional management practices post-intervention; indicative of the improvement of knowledge for better production, irrespective of previous training and formal education levels. This improvement in knowledge could be attributed to the fact that by using cellphones as a training tool, farmers could keep the information with them at their fingertips for potentially long periods of time (Martin & Hall, 2011). Moreover, the cellphone messaging as a training tool was well-received by the farmers, who read the messages and were largely motivated to implement the recommendations. In addition, the farmers considered a frequency of one message a day as a suitable and effective way of delivering training content to them.

These trial findings had some semblance to findings in other SDFs in Kenya; a study by (Staal et al 2003) highlighted a positive effect on milk production (not evaluated in this study) when farmers in Njoro sub-county used husbandry information received through mobile phones. However, a trial in Machakos County found that: 1) use of cellphones had no significant effect or influence on agricultural practices by farmers; 2) use of SMS among farmers was more likely when farmers had formal education of high school level and above; and 3) more farmers preferred use of voice to SMS since SMS was tedious (Mutunga & Waema, 2016). Nevertheless, neither of these studies was expressly designed to evaluate effectiveness of using cellphones as a training tool towards improved knowledge, which was the focus of this study.

This study population was generally representative of other SDFs in Kenya. Most of the principal farmers were female, which has been observed in other studies (Gallina, 2016). However, in the current training and research sessions, more men (65.0%) than women participated, which is likely a result of women being busy with chores at home and the patriarchal culture. Men are more frequently involved in off-farm activities, such as attending training and research sessions, than women. Some of the men attending the sessions indicated that they were representing their wives. Comparative pre-intervention analysis between the two groups showed that the groups were generally alike.

Similarly, on dairy management practices, such as feeding and mastitis prevention, there was no significant difference between the groups prior to intervention. The random allocation assisted in mitigating possible selection bias (Kahan et al 2015).

Analogous to other findings in other areas in Kenya (Richards et al 2015), dairy production was the main source of income for most (55.0%) farmers. The land acreages in this study population were small, with an average of 3.4 acres available for dairy and crop farming. The average land size of these SDFs was slightly higher than the average (2 -2.8 acres) documented in the region (Mugambi et al 2015) but within the range documented by other studies in Kenya (Omiti et al 2006; Vanleeuwen et al 2012).

Most farmers in this study were keen on dairy production and thus had attended some form of training on dairy management. This is a common happening in dairy cooperatives in Kenya where the dairy cooperative organizes seminar/extension sessions for farmers to increase knowledge and improve production (Wambugu et al 2011; Ettema, 2012). However, a short-coming of this form of farmer training has been that the knowledge retention can be relatively low among session attendants. Less than a half of the farmers in this study were able to recall details of the trainings they had attended within the last year pre-intervention.

Use of cellphone messaging for information dissemination in Kenya has increased in the last decade; in the agricultural sector, this dissemination has played a great role in enhancing information transfer between farmers, researchers and industry representatives (Kiptum, 2016). In recent years, cellphones technology has been adopted and is now used for some agricultural purposes in Kenya. Most of the innovations being prioritized include using SMS on cellphones for information access to farmers (The World Bank, 2012). Using one-way messaging in this study was done through MySQL on an Apache-based SMS gateway server which allows for transmission of uniform messages from a server to many individuals as a promotional item (Hussain, 2016). MySQL is essentially a common language for accessing databases (Oracle Corporation, 2013). Apache is one of the most widely used software for database interaction, visualization and management (Balkhi, 2009).

Overall, the farmers reported that the message reception was good. On assessing how the farmers felt about the information in the messages, the general feeling was that the messages were informative, and hence more than half of the farmers were extremely or very motivated to implement dairy management advice in the messages. This messaging encouraged discussions between farmers, as well as consultations with veterinary service providers, especially when some components were unclear. The use of cellphone messaging as a form of information dissemination has been shown to increase farmer-to-farmer training and uptake of various technologies (The World Bank, 2012). Unfortunately, most feature phones have small storage capacities and thus farmers sometimes need to delete older messages when the phone memory is full. However, farmers mentioned that they read most of the messages sent to their phones at any one moment and could retain the messages that provided new information to them and they preferred not to delete them. For this reason, receiving the messages made them happier compared to one-day farmer seminar trainings. Similar findings in relation to content retention were observed in another study (Farm Africa, 2015).

The main message-related challenge highlighted by the farmers in the FGD was that parts of the message were not easily readable on the small screen of the feature phone. For example, some farmers reported that some messages were longer than the phone screen display could handle at one time and took a long time to scroll through it at the time of receiving the message. Farmers in the FGD said they sometimes took a break in reading one long message, and then they sometimes forgot to read the rest of the message later. However, the farmers indicated that translation of messages into the local language was a welcome idea. Although some farmers had a challenge in understanding some translated words, the messages were still considered by most farmers to be easy to understand. Cellphone training has been considered a sustainable approach to support the use of media technologies for training purposes (World Bank Group, 2017).

The mastitis prevention knowledge scores in the comparison group appeared to decrease significantly, while the intervention group scores increased slightly. The relatively high pre-intervention knowledge scores for the comparison group (compared to post-intervention) may have been a result of the pre-intervention meeting for the intervention group being held a day before that of the comparison group. Farmers in the intervention and comparison groups may have discussed the contents of the questionnaires and the meeting since they were all within the same relatively small community, resulting in higher comparison group scores. This unintended dissemination was mitigated post-intervention where both groups were interviewed at different times of the same day. It is therefore recommended that reducing the interval between assessments of the study groups would reduce probability of information transfer between the comparison and intervention groups.

Knowledge scores on feeding practices, such as the amount and time of colostrum given to calves, and ideal height of Napier grass harvesting for optimum milk production, also had significant net changes between the two groups. The comparison group appeared to have increased their knowledge scores while the intervention group didn't change much. This unexpected net change in knowledge score could be attributed to the fact that such information was also communicated to the farmers by another NGO in the area and in other training forums. However, knowledge on other nutritional information (such as for some skin conditions (rain scald) which could quickly resolve when cows are fed a well-balanced diet) (Roberson et al 2012)was significantly higher in the intervention group than comparison group. Similarly, more farmers in the intervention group were knowledgeable on the benefits of hygiene in calf diarrhea management post-intervention.

A shortcoming of the current study was due to technological constraints. The basic format of content deliverable to the feature phones meant that non-text information, such as pictures and diagrams, could not be used to augment the messages for better understanding by the farmers. While smart phones are currently uncommon in rural parts of Kenya (only 1.7% of the study population had a smart phone in the family), as the price of smart phones decreases, leading to wider utilization, auxiliary material can be provided to farmers for training purposes.

Another limitation was loss to follow-up in both the intervention and comparison groups, reducing the final sample size and power of this study. The reasons for farmers not completing the trial were unlikely to be related to the study objectives, minimizing any bias from this attenuated sample size. However, a smaller sample size leads to reduced power to detect significant differences between groups. Fortunately, we were still able to find significant differences in knowledge between the groups, even with the smaller sample size.

A final limitation of the study was the unintended dissemination of knowledge during the trial, leading to farmers in the comparison group improving in their knowledge scores. A change in study design to have different populations farther apart and a shorter period between evaluation of study groups would help reduce unintended information diffusion between the groups and 'contamination' of the comparison group.

We hypothesize that the improvement in knowledge of the farmers in this study would most likely translate into better dairy management, production and improved incomes to the farmers. This effect of training was observed by (Richards et al 2016) where good use of high protein forages coupled with continuous on-farm education and training on best management practices, significantly increased daily milk production in SDFs in Kenya. While a significant difference was observed on knowledge levels of trainees, further investigation on effectiveness of this form of training on actual milk production and practices would be helpful.

A cost-benefit analysis would likely show that use of cellphones for training is a cost-effective approach for knowledge transfer from the farmer's perspective, given that in

Kenya, most farmers already have a cell phone and do not pay anything to receive messages. Therefore, a subscription fee would likely be the only real cost to the farmer, along with a slight increase in charging costs if the phone was used more. Benefits to the farmer could be substantial, depending on the improvements made on the farm. A comparative investigation of effectiveness of seminar training vs cellphone training would be informative on the impact and sustainability for such alternative farmer training methods.

With this study population, interventions implemented needed to be based on a feature phone interface. Evaluation of a smartphone application that allows a more interactive interface between the farmer and messages on smartphones could be explored in the future when smartphones become more common among rural farmers. Furthermore, a trial to compare the differences in cost and impact of training using the feature phone and smartphone would be more informative on the best cellphone interventions for SDFs in Kenya.

Conclusion and Recommendations

The results of the current study indicate that SMS technology for feature phones can be an effective training tool for farmers on SDFs in Kenya. Furthermore, the technology can be applied in remote areas located far from where regular seminars are conducted for dairy farmers. The bulk educational messaging to farmers can effectively complement other forms of farmer education.

There is demand for knowledge on good husbandry practices on SDFs in Kenya as exemplified by the high level of motivation, value of information, and willingness to implement the recommendations expressed by the farmers in this trial. Translation of message content to the local language and using easily understandable terminology were reported to be helpful for better understanding and motivation of farmers to implement recommendations.

Dairy production accounts for a sizable portion of the income in SDFs in Kenya and thus use of cellphone-based interventions to improve production could be a way to improve livelihoods and economic power of farmers in SDFs in Kenya.

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6.3 AUCC Questionnaire for Management and Feeding Practices on Naari Smallholder Dairy Farms

Farm Number: Survey Visit Date: Interviewer Initials: Farm Number

I. Farm overview:

1. How many people live in this household for more than 5 days per week? _____
2. Gender of principal farmer (person who takes care of the cows): male / female
3. Marital status of principal farmer (person who takes care of the cows):
   __ single __ married __ separated/divorced __widowed
4. Woman's education completed: ____ primary ___ secondary ____ college/university ___ n/a
5. Man's education completed: ____ primary ___ secondary ____ college/university ___ n/a
6. Woman's age: _____ years ___ n/a
7. Man's age: _____ years ___ n/a
8. a) Percent of total income coming from dairy production: ____< 50% ____50-75% ____> 75%
   b) Other sources of income _______________ __________________ __________________
9. Area of land owned: _______________ acres / hectares (circle units)
10. Area of land rented: _______________ acres / hectares (circle units)

II. Feeding - Part A – Normal feeding: 11. Some feeds are only given seasonally. Over the last year, please check which of the following you fed to your cattle (amounts not needed).

Feed name	Calves/Heifers	Cows
a. Napier Grass		
b. Silage		
c. Grass Hay		
d. Desmodium		
e. Sweet potato vines		
f. Other high protein forages – Lucerne, alfalfa, clover –identify which one(s) ___________________________		

g. Tree fodders – identify which one(s) _______________		
h. Banana leaves		
i. Other fodder ____________________________________		
j. Dairy meal		
k. Wheat Bran		
l. Maize "Jam"		
m. Vitamin/mineral powder		
n. Vitamin/mineral block		
o. Calf pellets/calf pencils. If yes, until what age?		
p. Other feeds (specify) _____________		
q. Water available (always/sometimes)	A/S	A/S
r. Source of water		

12a. Do you usually feed dairy meal or grain to cows for the month before calving? __YES __NO

13a. Do you feed vitamins/minerals to cows during the month before calving? ___YES ___NO
13b. If yes, what brand?
Brand: _______________ (*from bag: Ca:P ratio:* ____ *Selenium amount & unit:* ______*)*
13c. If yes, how much is given to the cow? Amount (in spoons or grams per day): ___________

14. How much dairy meal and/or grain (e.g. maize "jam") do you give cows on the day they calve?
dairy meal ______ kg in morning ______ kg in evening
other grain ______ kg in morning ______ kg in evening

15a. In general, during the first 5 months after calving, do you normally change the amount of dairy meal or grain you feed per day to your cows? _____YES _____NO
b. If yes, what factors affect how much dairy meal or grain you feed per day?
____

16. At what height do you normally cut and feed your Napier grass for milking cows?
a. Cows (rainy season) b. Cows (dry season)

1. mostly < 1.0 meter _______	1. mostly < 1.0 meter _______
2. mostly < 1.5 meters _______	2. mostly < 1.5 meters _______
3. mostly < 2.0 meters _______	3. mostly < 2.0 meters _______
4. mostly > 2.0 meters _______	4. mostly > 2.0 meters _______

c. Are these the same heights for Napier grass fed to calves and heifers too? Yes_____ No_____
If no, what is different? ____

17a. For your cows, did you experience a shortage of feeds over the last year? Yes_____ No_____
17b. If yes, which feeds were inadequate (check all that apply)?
___Forages ___Grain or meals ___Vitamin-minerals ___Water ___Other(specify)___________

18a. Did you store forage for the dry season over the last year? Yes_____ No_______
18b. If yes, what is the method of storage (circle all that apply)?
___Grass hay ___Silage ___Maize stover ___Other (specify)_____________________

19a. How frequently do you normally deworm your cows?
Every ___ months ____when suspect it is a problem ___when not pregnant

19b. How frequently do you normally deworm your calves/heifers?
Every ___ months ____when suspect it is a problem ___other (specify: ___________________)

20. How many calves died in the last year? ____
If yes, from what causes____

21. How many heifers died in the last year? _____
If yes, from what causes ____

22. How many heifers died in the last year? _____
If yes, from what causes ____

III. Mastitis Prevention Management Ask AFTER initial accelerometer readings

23. a) Do you wash the udder pre-milking?	Yes __No __
b) Do you use soap when washing the udder?	Yes__ No__
c) Is a different wash cloth used for each milking cow?	Yes __No __
d) Is the udder dried before milking with a clean cloth or paper?	Yes __No __

e) Is a different drying cloth used for each milking cow? Yes __No __
f) If you have > 1 milking cow, do you wash your hands between milking cows? Yes __No __
g) Do you wash and rinse out your wash cloth(s) & hang them to dry out between milkings? Yes __No __
h) Do you use a teat dip post milking? Yes __No __
i) Do you give fresh feed after milking? Yes __No __
j) Do you use dry cow treatment when drying cows off prior to calving? Yes __No __
k) If you have more than one cow, and are treating a cow for mastitis, do you milk her last? Yes __No __

24. a) How many cases of mastitis did you have in the last year? _____
b) How many cases of mastitis did you treat in the last year? _____
c) How many cows leaked milk in the last year? _____
d) How many times did you have milk rejected in the last year? _____

IV. Cow Stall Design and Management

25. How often do you remove manure from where the milking cows lie down?
a) more than once a day
b) once a day
c) every other day
d) twice a week
e) once a week
f) less than once a week

26. What kind of bedding is used where the milking cows lie down?
a) grass/hay b) straw c) sawdust d) pea/bean waste e) none f) other (please specify _________)

27. How often do you add new bedding to where milking cows lie down?
a) every day
b) every other day
c) twice a week
d) once a week
e) less than once a week

28. How often do you add new bedding to where dry cows lie down?
a) every day
b) every other day

c) twice a week
d) once a week
e) less than once a week

29. How often do you trim your cows' feet?
a) every 3 months
b) every 6 months
c) every 12 months
d) less often or never

30. Do your cows do any of the following behaviours (circle all that apply – observe to confirm)?
a) perching (standing partly in the stall and partly out of the stall for more than a few minutes)
b) standing backwards in stall
c) idle standing in the stall (standing fully in the stall for more than a few minutes)
d) dog-sitting in the stall (sitting on hind legs but standing on front legs)
e) kneeling in the stall (sitting on hind legs but kneeling on front legs)
f) lying restless in the stall (shifting position in the stall every 15 minutes or less)
g) nose-pressing against a post or board while standing or sitting in the stall
h) lying somewhere other than in the stall
i) Other behaviours you wonder about (please specify _________)

31. Do cows hit any of the following body parts when lying down or getting up
(circle all that apply – observe to confirm)?
a) chin
b) skull
c) withers / shoulders
d) feet
e) knees
f) other body parts (please specify _________)

For observation:

32. Is the roof appropriate (observe – no holes, extends to cover udder area)? Yes __No __

33. What is the type of the floor where the milking cows lie down?
1) concrete 2) dirt 3) other (please specify: ________________)

34. Is the floor (observe - check all that apply):
1) flat (no pooling water)
2) lumpy
3) wet in the udder area

35. Is water/urine/feces able to flow (by gravity) under the udder where the milking cows lie down (observe)?

36. Results of the stall knee tests?
a) Impact force Pass______ Fail_______
b) Level of Wetness Pass______ Fail_______

Dimensions of stalls Preintervention:

Stall	a. Width (cm)	b. Body Resting Length (cm)	c. Total Stall Length (cm)	d. End Board Height (cm)	e. Neck Rail Height (cm)	f. Brisket Board Height (cm)	g. Side Rail Height Lowest Board (cm)	h. Side Rail Mid Board (cm)	i. Side Rail High Board (cm)
#1 (Q37)									
#2 (Q38)									
#2 (Q39)									
#2 (Q40)									

VI. Health and Productivity of Cows

Examination of Cows:	Cow1 (Q41) ID_________	Cow2 (Q42) ID________	Cow3 (Q43) ID_________	Cow4 (Q44) ID_________
a. "Age (years)"				
b. Breed				

c. "Number of calvings"				
d. "Last calving date"				
e. "Last breeding date"				
f. "Number of breedings for last calving"				
g. "Number of times used sexed semen"				
h. "Number of times used Canadian semen"				
i. "# of times used hormones for breeding"				
j." Current daily milk yield (kg/day)"				
k. "Is this expected yield, based on feeding?"	Y/N	Y/N	Y/N	Y/N
l. "Mastitis in last 12 months"	Y/N	Y/N	Y/N	Y/N
m. "Abortion in last 12 months"	Y/N	Y/N	Y/N	Y/N
n. "Other disease (RP) in last 12 months" ____	Y/N	Y/N	Y/N	Y/N
o. Weight				
p. Height				
q. Body condition score				
r. TPR/physical exam Normal / Abnormal? (manure, feet, skin, lymph nodes, eyes, rumen)	N / A	N / A	N / A	N / A
s. CMT (circle CMT result if milk looks abnormal as well)	LF LH RF RH __ __ __ __	LF LH RF RH __ __ __ __	LF LH RF RH __ __ __ __	LF LH RF RH __ __ __ __
t. Reproductive status (*preg confirmed?)*	Preg Y/N	Preg Y/N	Preg Y/N	Preg Y/N
u. Ovaries cycling	Y/N	Y/N	Y/N	Y/N

v. Month of last deworming				
w. Hock lesion score (1-3)				
x. Neck lesion present (Yes/No)				
y. Knee lesion score (1-3)				
z. Lameness score (1-3)				
aa. Udder hygiene score (1-4)				
ab. Leg hygiene score (1-4)				
ac. Cow photo digital file name				

<u>Youngstock health and productivity</u> (calves and heifers that have never calved yet):

	Calf/Heifer #1 (Q45) ID_______	Calf/Heifer #2 (Q46) ID________	Calf/Heifer #3 (Q47) ID_________	Calf/Heifer #4 (Q48) ID_________
a. "Birthdate or Age (months)"				
b. Sex				
c. Breed				
d. "Last breeding" (month and year or n/a)				
e." Number of breedings to date"				
f. "Had diarrhea"	Y/N	Y/N	Y/N	Y/N
g. "Had pneumonia"	Y/N	Y/N	Y/N	Y/N
h. "Had navel-ill"	Y/N	Y/N	Y/N	Y/N
i. Weight				
j. Height				

k. Body condition score				
l. TPR/physical exam Normal / Abnormal? (manure, feet, skin, lymph nodes, eyes, rumen)	N / A	N / A	N / A	N / A
m. Udder hygiene score (1-4)				
n. Reproductive status (*preg. confirmed?)*	Preg Y/N	Preg Y/N	Preg Y/N	Preg Y/N
o. Ovaries cycling	Y/N	Y/N	Y/N	Y/N
p. Month of last deworming				

49. How do your calves usually receive their first colostrum?
Choose only ONE of the options that is MOST commonly used
___ free choice suckles ____assisted suckle ____nursing bottle _____bucket
____other -specify:__________

50. How soon would most of your calves receive 4L of colostrum? Choose ONE answer only
___ < 6 hours ___ 6 - 12 hours ___ 12 - 24 hours ___ > 24 hours ___ unknown

51. What do you usually do if a calf is weak and unable to drink colostrum during the first day of life?
_____Bottle feed ___Tube feed _____Other (specify)______________________

52a. In the last year, how frequently did your cows have an abrupt feed change? (for example, you completely run out of one type of feed one day, such as napier grass, so you switch to a different type of feed the next day, such as maize stover) Choose ONE
_____Never _____Occasionally in the dry season _____ 1 time/month _____more than 1 time/month

52b. In the last year, how frequently did your calves have an abrupt feed change? Choose ONE
_____Never _____Occasionally in the dry season _____ 1 time/month _____more than 1 time/month

6.4 Experimental Study Survey for Cost-Benefit Nutritional and Reproductive Study of Smallholder Kenyan Dairy Farmers: for subsequent farm visits after the study calf is born

Farmer Name: Farm Number:

Temp: Time Visit Date: Group:

Visit Number: 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 Interviewer Initials:

<u>Part A. Post-birth visit question (ask these only at the first visit after a birth)</u>

i. Was the birth observed (was someone around)? Y / N

ii. What time of day was the birth? _________________________

iii. Did you need to give assistance to deliver the calf? Y / N

iv. Sex of calf M / F

v. Was the calf weak during the first 6 hours (not drink on its own)? Y / N

vi. Were any treatments given to the calf? Y / N (specify _____________________

<u>Part B. For every post-natal visit</u>

1. If the owners did not fill in the weekly log of what was fed to the calf and cow:
Please indicate the brand/product (if applicable) and average amounts (in kg, or kasuku container levels, or cups or spoons/day) of the feeds listed that were given to the study calf and cow <u>daily since the last visit</u>. Indicate if always available free choice (FC).

	Brand	Calf yes/no	Calf amount	Cow yes/no	Cow amount
Milk					
Calf pellets					
Calf pencils					
Mineral block lick					

Vitamin/mineral powder					
Dairy meal					
Maize “jam” germ					
Wheat bran					
Other purchased feed (specify)					
Calliandra or Sesbania					
Napier grass					
Other grasses					
Grass silage					
Maize silage					
High-protein plants (like Desmodium, Lucerne)					
Other forage (weeds, banana leaves, other -specify)					
Water availability?		Always / Sometimes		Always / Sometimes	
Milk Produced (kg/d)					

3a. At what height did you cut and feed your Napier grass for cows since the last visit?
_____ most < 1.0 meter _____ most < 1.5 meters
_____ most < 2.0 meters _____ most > 2.0 meters

3b. At what height did you cut and feed your Napier grass for calves since the last visit (answer only if different from above)?
_____ most < 1.0 meter _____ most < 1.5 meters
_____ most < 2.0 meters _____ most > 2.0 meters

4. a) Did you make any sudden / unusual changes in feed (amount or type) to your cows or calves since the last visit? _____YES _____NO
b. If yes, describe: ___________________________________

5. a) Did you change the amount of dairy ration or grain you fed according to the cow’s milk yield since the last visit? _____YES _____NO
b) If yes, describe: ___________________________________

6. a) Do you still own the study cow? Yes_______ No______
b) If no why____

7. a) Do you still own the study calf? Yes_______ No_______
b) If no why____

8. a) Are you spending more on feed since the last visit (Y/N)
b) By how much more (give range) ____________

9. a) Has your revenue from dairy cows increased since the last visit (Y/N)
b) Describe how ____

10. Health Examination of Cattle. Study Calf and Cow

Italics are answered by owner	Calf ID_______	Calf ID_______	Calf ID_______	Cow ID________	Cow ID________	Cow ID________
a. Navel status	N / A	N / A	N / A			
b. Weight (calf) or BCS (cow)						
c. Height						
d. "Any disease since last visit?"	Y/N ________	Y/N ________	Y/N ________	Y/N ______	Y/N ________	Y/N ________
e. "Appetite normal?"	N / A	N / A	N / A	N / A	N / A	N / A
f. TPR normal?	N / A	N / A	N / A	N / A	N / A	N / A
g. Cardio/pulmonary system normal?	N / A	N / A	N / A	N / A	N / A	N / A
h. Gastrointestinal system normal?	N / A	N / A	N / A	N / A	N / A	N / A
i. Feet condition normal?	N / A	N / A	N / A	N / A	N / A	N / A
j. Skin parasite/condition normal?	N / A	N / A	N / A	N / A	N / A	N / A

k. Uterus normal? (preg?)	[redacted]			N / A Y/N	N / A Y/N	N / A Y/N
l. Ovaries (CL, follicle, in heat, anestrus):				L R	L R	L R
m. Given repro medicine?				Y /N (specify: ____________)	Y /N (specify: ____________)	Y /N (specify: ____________)
n. Eligible for breeding? (Uterus OK, BCS 2.5 or 2.0 but up, cycling?)				Y/N	Y/N	Y/N
o. Eligible for PG shots to set up for sexed semen				Y/N	Y/N	Y/N
p. Any other current ailment	Y /N_________	Y /N_________	Y /N_________	Y /N________	Y /N________	Y /N________
q. Pen comfort / hygiene (out of 6) if changed from last visit						
r. CMT status	[redacted]			LF LH RF RH __ __ __ __	LF LH RF RH __ __ __ __	LF LH RF RH __ __ __ __

Note: Comfort Score (/6) is a function of:

For cows: 1) Stall Length 2) Stall Width 3) Stall Lunge Space 4) Stall Softness 5) Stall Shade 6) Stall hygiene.

So, an index from 1 to 6, getting a 1, ½ or 0 for each of these for good (equals or surpasses minimum requirement), fair (approaches the minimum requirement) or poor (nowhere near the minimum requirement). If the cow uses the stall appropriately, then it is likely not too bad. (Stall wetness will affect mastitis - but we will assess udder CMT).

For calves: put emphasis on stall space (1/6), shade (1/6), and hygiene (4/6).