**BOVINE VIRAL DIARRHOEA TESTING AND VACCINATION ON SMALLHOLDER DAIRY FARMS IN KENYA**

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## Thesis Abstract

Smallholder dairy (SHD) farming is a critical sector supporting many households’ income in rural Kenya. The SHD farmers are faced with numerous challenges that impair the optimal performance of their dairy cattle. Among these challenges are the poor state of infectious disease diagnosis and prevention, such as Bovine Viral Diarrhoea Virus (BVDV), and the low awareness of the potential benefits of vaccination commonly used in high-income countries. The aims of this research on SHD farms in Kenya were: 1) to evaluate the possible cross-reactivity between Classical Swine Fever Virus (CSFV) testing with BVDV testing using both antibody and antigen Enzyme-Linked Immunosorbent Assays (ELISA); 2) to determine associations between vaccination status for a multivalent BVDV vaccine and disease occurrence in a cohort study; 3) to determine associations between vaccination status for a multivalent BVDV vaccine and disease occurrence in a randomized controlled trial (RCT); and 4) to determine antibody titer response variability from BVDV vaccination when given to cows under different planes of nutrition, management, and body condition score (BCS).

For objective 1, a cross-sectional study was based on a single visit to farms to collect serum samples and other descriptive farm and animal information. IDEXX ELISA testing for BVDV antigen (Ag) and antibody (Ab) was conducted on 320 dairy cows’ and heifers’ serum samples, with CSFV Ag and Ab being tested on a subset of 133 and 74 serum samples, respectively. The CSFV testing was based on BVDV test results and the availability of enough sample volume from cattle on farms that kept pigs. For the 74 Ab tests, 40 (54.0%) were BVDV Ab positive, while 63 (85.1%) were CSFV Ab positive. Of the 40 BVDV Ab-positive samples, 36 cattle (90.0%) tested positive for CSFV Ab. However, of the 34 BVDV Ab negative samples, 27 (79.4%) were CSFV Ab test positive. For the 133 Ag tests, 125 (94.0%) were BVDV Ag positive, while 2 (1.5%) samples were CSFV Ag positive. None of the eight BVDV Ag negative samples was positive for CSFV Ag and only two (1.6%) of the 125 BVDV Ag positive samples were positive for CSFV Ag.

For objective 2, a retrospective cohort study compared 226 cows and 85 heifers vaccinated with a single multivalent modified live vaccine, including BVDV, against 215 cows and 60 heifers in the non-vaccinated cohort. One year after vaccination, during a follow-up visit, we recorded reported disease outcomes, and farm and animal factors during the previous year. Mixed multivariable logistic and Poisson regression modeling was used for the heifers and cows, respectively, to determine factors associated with the reported disease, allowing the control for other factors when exploring vaccine associations. There was significantly lower reported pneumonia, diarrhea, and overall disease for both cows and heifers in the vaccinated cohort. For the cows, poor appetite, tick-borne diseases, and uterine diseases were significantly lower in the vaccinated versus unvaccinated cohort. For the final cow model, factors associated with the disease count included: feeding grass weeds (Incidence risk ratio (IRR)=1.294); having more than two diseases in the farm (IRR=1.415); above 3rd parity (IRR =1.134); milking mastitis cow last (IRR=0.669); herd size (IRR=1.796); only one breeding to conception (IRR =0.778); Body Condition Score (BCS) above 2.25 (IRR=0.813); and an interaction between vaccination status and pregnancy checking practice. Cows in farms that did not do pregnancy checking had more reported disease counts for the unvaccinated cohorts than cows in the vaccinated cohort while there was no difference for farms that did pregnancy checking. In the final heifer mixed effects logistic model, factors associated with disease likelihood in the last year included: age of female farmer (Odds Ratio (OR) = 0.23); farm size over 2 acres (OR=0.13); Napier grass height fed in the dry season (OR=6.51); loss of a vaccinated cow in the farm (OR=8.91); concrete floor in the stall (OR=0.02); buying replacement heifers (OR=3.26); vaccination status (OR=0.11) and heifers over 30 months (OR=6.11). Higher categories of BCS, 2.25 – 2.75 (OR=4.00) and above 3 (OR=10.77) had higher OR compared to BCS of 2 and lower.

For objective 3, a randomized controlled trial recruited a total of 384 cows on 292 SHFs and 352 heifers on 290 SHFs, all randomly selected.  On the first farm visit, using a questionnaire and animal examinations, baseline level information of current disease and reported diseases in the last 12 months were collected, along with other cow- and farm-level management and production data. With random block allocation, 185 cows and 172 heifers were injected with a single dose of multivalent modified live vaccine, including BVDV (Pyramid® FP 5; Boehringer Ltd.), while 199 cows and 180 heifers were injected with a placebo. After one year, a return visit to the farms recorded the same data for time-varying variables. The vaccine is against four pathogens: bovine viral diarrhea virus, bovine herpesvirus type 1, bovine respiratory syncytial virus, and parainfluenza virus type 3. Mixed effects multivariable logistic and Poisson regression modeling were used for the heifers and cows, respectively, to determine factors associated with the reported disease in the last 12 months. There was an overall reduction in reported disease occurrence in the vaccinated group during the second visit compared to the first visit, in both cows and heifers. The specific reported disease conditions that were reduced in the vaccinated group included: pneumonia, diarrhoea, anorexia, and uterine disease. For both the cows and heifers, there was a significant reduction in reported disease count and likelihood, respectively, in the last 12 monthsfor the vaccinated group versus the placebo group on the second visit, while adjusting for the reported disease counts and likelihood on the first visit. In the final cow model, the following factors were associated with reported disease counts in the last 12 months: farms that bought in-calf replacement heifers (IRR= 0.80); farms having more than three acres of land (IRR= 0.81); farms which had more diseases in calves (IRR= 1.32); cows that had been bred within the last 12 months (IRR= 1.43) and cow’s weight in kilograms (IRR=1.001). For categorical variables, there were higher disease counts for cows on farms which had reported one case of mastitis prior to the study (IRR=1.27) and those that reported two or more cases of mastitis (IRR=1.50) compared with cows on farms that did not report any case of mastitis. There were higher counts of reported diseases in cows on farms using different towels for udder cleaning (IRR=1.00) and farms that did not use udder cleaning towels (IRR=1.31) than in farms using a single udder cleaning towel. The cow mixed multivariable model also had four significant interactions between the following variables: 1) household income dependency on dairy above 50% and number of cows; 2) farms feeding maize silage and cow pregnancy status; 3) number of farms feeding extra supplement post-calving and cow body condition score (BCS) and 4) visit number with intervention group.

In the final heifer model, higher likelihood of disease within the last 12 months was associated with: farms feeding grass silage (OR=3.27); buying replacement heifers (OR=2.45); heifers over two years during the visit (OR=2.16); heifers on farms that fed at least 4 kg colostrum to calves within the first 12 hours of life (OR=0.30) and heifers that had been bred within the last 12 months (OR=0.31). Ayrshire and other breeds had OR=1.88 and 0.45, respectively, compared to Holstein heifers. There were two significant interactions in the heifer model: 1) number of diseases in cows for the last one year, and season during the visit; and 2) visit number and intervention group.

For objective 4, we evaluated the variability in antibody response in 128 cows and 109 heifers, all non-pregnant, after a single modified live vaccine injection, including BVDV (Pyramid® FP 5; Boehringer Ltd.). The ELISA tests before and four weeks after vaccination determined pre- and post-vaccine sample-to-positive (S/P) ratios for BVDV antibody levels. A questionnaire and animal examination were used to collect information on animal health status, management practices, and reported disease outcomes for the last 12 months. Multivariable linear regression analysis modeling was used to determine factors associated with the change in antibody levels. Before vaccination, 40.6% and 7.3% of cows and heifers tested positive for BVDV antibodies (S/P ratio > 0.3), respectively. The mean increase in S/P ratio post-vaccination was 0.476 and 0.804 for cows and heifers, respectively. In the final cow model, the factors associated with the change in BVDV antibody S/P ratios included: body condition score (BCS) (Coefficient = 0.132); feeding protein supplements (Coefficient = -0.170); post-partum disease (Coefficient = -0.137); abnormal physical exam findings (Coefficient = -0.160) and BVDV antibody test status on visit one (Coefficient = -0.507). There was a significant three-way interaction between days in milk (DIM), age, and post-calving supplementation.

For heifers, factors associated with the change in BVDV antibody S/P ratios included: heifer numbers (Coefficient= -0.059); acaricide application (Coefficient=0.13); number of people living on farm (Coefficient=-0.053); and BVDV antibody test status on visit one (Coefficient=-0.574). There was a significant interaction between BCS and raising one’s replacement heifers for the heifer model. Management type was also a significant categorical factor for both the heifers and cows. Zero-grazed cows had better antibody response compared to mixed management farms and communal grazing farms (Coefficient=-0.137 and -0.124, respectively), and for the heifers, the coefficients were -0.33 and -0.34, respectively. We recommend the planning vaccination against BVDV when cattle have a BCS between 2 to 2.75.

Generally, the results indicated substantial cross-reactivity of the two Ab ELISA tests (BVDV and CSFV) or reactivity with some other protein in the samples that led to the positive Ab test results, and only limited evidence for cross-reactivity of the two Ag ELISA tests (BVDV and CSFV).The multivalent vaccine, including the ML BVDV component, was beneficial in reducing reported diseases in both cows and heifers after one year’s follow-up, while controlling for other farm-level and animal-level factors. The benefit of vaccination was evident in both the retrospective cohort and randomized controlled trial. The BVDV antibody response to vaccination varied with the current health status of animals, nutritional status, and BCS of both cows and heifers, as well as other farm management factors.

Our research supports the recommendation of a multivalent vaccination that includes a BVDV component to cattle within the SHD sector of Kenya. We also recommend planning multivalent ML vaccination, including BVDV, to non-pregnant cows and heifers who are not sick and have a BCS of 2 to 2.75 for best immune response.

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## Dedication

***To my Dad, Samuel Muasya Wambua (Deceased), my Mum, Mary Muasya, my wife, Rachael Ndune, my daughter, Daniella Nthenya, and my sons, Nathaniel Muasya and Joseph Nzilli***

## List if Abbreviations

Ab - Antibody

ACE - Antigen Capture ELISA

Ag - Antigen

AI - Artificial Insemination

APP - Atypical Porcine Pestivirus

BCS - Body Condition Score

BDV - Border Disease Virus

BVDV - Bovine Viral Diarrhoea Virus

CI - Confidence interval

Cm - Centimeters

CMT - California mastitis test

CP - Cytopathic

CSFV - Classical Swine Fever virus

DIM - Days in Milk

ECF - East Coast Fever

ELISA – Enzyme-Linked Immunosorbent Assay

FMD - Foot and Mouth Disease

GDP - Gross Domestic Product

IBR - Infectious Bovine Rhinotracheitis

IFNs - interferons

IHC - Immunohistochemistry

IRR – Incidence Risk Ratio

Kg – Kilograms

KV - Killed/Inactivated vaccine

LSD - Lumpy Skin Disease

MLV - Modified live vaccine

NCP - Non-Cytopathic

NGO - Non-Governmental Organization

PI – Persistent Infection

RT-PCR - Reverse Transcriptase Polymerase Chain Reaction

S/P – Sample to Positive

Se - Sensitivity

SHD - Small Holder Dairy

SDF - Smallholder Dairy Farm

Sp - Specificity

TI - Transient Infection

UoN - University of Nairobi

UPEI - University of Prince Edward Island

VNT - Virus Neutralization Test

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# Chapter 1: Introduction

## 1.1 Smallholder dairy (SHD) sector overview in Kenya

Smallholder dairies comprise of farms keeping few dairy cows and they are a very important sector of the economy in Kenya. The smallholder dairy (SHD) sector makes up about 80% of the dairy sector in Kenya and supports many households (Ettema, 2012; FAO, 2019; FAO et al., 2017; FAO, 2015; ). Kenya faces challenges of hunger and poor nutrition in low-income households (Bonilla et al., 2018; FAO et al., 2017). Rural communities in Kenya are mostly subsistence farmers keeping small numbers of livestock and growing crops in an interdependent form of an enterprise (Peters et al., 2012; Recha, 2018). Like other third-world countries, Kenya faces similar problems for its low-income households (FAO et al., 2017; Recha, 2018). One of the economic activities that has been of great contribution to mitigating the problems of hunger and nutrition is smallholder dairy (SHD) farming (Chen et al., 2021; Miller et al., 2021). Farming is a key income generator for SHD farmers and communities through the sale of milk and livestock (Bonilla et al., 2018). Dairy farming communities are less likely to have nutritional deficiencies in their children due to higher consumption of milk compared to non-dairy farming communities (Miller et al., 2021; Ruel et al., 2018). The SHD farms are also likely to produce better crops due to the income supplementation from milk sales, as well as getting cheap, good quality manure from cattle to use as fertilizer. (Ndambi et al., 2019; Odhiambo et al., 2019; Paul et al., 2009)

The SHD sector is varied in performance from region to region, with major factors being the climatic zone and the farmers' knowledge of dairy cow management (Recha, 2018). Feed availability and knowledge on preservation of fodder are important in SDFs, with fluctuations in supply being common (Duguma & Janssens, 2016; Njarui et al., 2011). The latter results in challenges to health due to poor nutrition and stress among cattle (Carroll & Forsberg, 2007). Poor cow reproductive performance is also common due to nutrition insufficiency and lack of knowledge on good reproductive management (Bisinotto et al., 2012; Lanyasunya et al., 2005; Mungube et al., 2014). The lack of preparedness and resources to mitigate shortages during droughts makes farmers vulnerable to preventable losses (Duguma & Janssens, 2016; Omolo et al., 2020).

### 1.1.1 **SHD's importance to the Kenyan economy and the dairy cooperatives**

Dairy cattle farming is a very important economic activity in Kenya, as it contributes 4% of the total gross domestic product (GDP), 44% of livestock GDP, and 12% of the agricultural GDP (FAO, 2019; MOALF, 2021). The dairy subsector is estimated to support 1.8 million people by employment and produces about 4.75 billion liters of milk annually (USAID-KAVES, 2014; USAID, 2018). Dairy cattle contribute about 75% (3.6 billion liters) of the milk produced in Kenya, with 25% coming from other animals such as camels and dairy goats (USAID-KAVES, 2014; USAID, 2018).

The anticipated milk production in Kenya in 2022 is 7.8 billion liters, with an estimated annual production of 1600 liters per cow (USAID-KAVES, 2014). The SHD farming sector is dynamic, with farmers being members of cooperative societies that help in marketing milk and offer supportive financial and technical services (USAID-KAVES, 2014; Waitituh, 2017). There is an observed positive correlation between well-performing dairy cooperatives and better performing farms with regard to profitability, knowledge, and training (Mwebia et al., 2019). The SHD industry is therefore key to food security and financial support for many rural livelihoods in Kenya (FAO, 2011).

### 1.1.2 Herd management and characteristics

The most common method of management in the smallholder dairy system is referred to as zero-grazing. Animals are kept in stalls and are fully dependent on humans for feed and water, which are delivered to them. Dairy cow housing typically depends on the environment, climatic conditions, and available construction material (Bebe, 2003). Alternate management systems include semi-zero-grazing and grazing, which can be on private paddocks or communal grounds during the day and within the homestead at night. Grazing and semi-zero-grazing represent less intensive management systems and are used where fodder resources are limited (Njarui et al., 2011; Omolo et al., 2020). Communal grazing management leaves cattle vulnerable to disease transmission between farms (Ogola, 2018).

The most common dairy breeds in Kenya are Holstein-Friesian, Ayrshire, Guernsey, Jersey, Zebu breeds, and crosses (Waitituh, 2017). The SDFs are typically on a small plot of land ranging from less than one acre to several acres of land, with renting of additional land to supplement fodder production being a common practice (Bebe, 2003; Duguma & Janssens, 2016; Njarui et al., 2011). Production is mostly based on the close integration of dairy cattle and crop farming, with the majority of farmers keeping one to three milking cows (USAID, 2018). Interdependence between SHD farming and crop farming mainly involves the utilization of manure to fertilize fodder and other crops, and cattle being fed the byproducts of crops such as maize stover and legume stover, where the maize kernels or beans are farmed for human consumption (Mugambi et al., 2015; Muia et al., 2011).

Cattle nutrition is varied across SDFs, with some providing good quality fodder throughout the year, while others are fluctuating between available fodder options and crop residues across the seasons (Maleko et al., 2018; Njarui et al., 2011). Of importance to feed supply and cattle nutrition are the amounts of rains, land size, resource capacity to preserve feed, and knowledge level of the farmers (Duguma & Janssens, 2016; Njarui et al., 2011). The seasonal variation in the quality and quantity of fodder results in variation in body condition score (BCS), productivity, and health performance (Duguma & Janssens, 2016). Supplementation with grains, dairy meals, and minerals licks is very common; however, the quality and quantity of these purchased feeds are varied (Gachuiri et al., 2012; Möller, 2018; Waitituh, 2017).

Mixed livestock farming is a common feature of some SDFs, with the most common species being poultry, pigs, sheep, and goats. Mixed livestock keeping, just like crop farming, offers the farmers complementary and supplementary income (Mugambi et al., 2015; Odhiambo et al., 2019; Peters et al., 2012). Herd replacements on SDFs are mainly by home raised, although the purchase and sale of both heifers and milking cows, driven by financial needs in the households, is common (Bebe, 2003, 2008).

### 1.1.3 Milk production, breeding, and farm income

Milk production on SHD farms in Kenya is variable due to various farming limitations and challenging marketing structures (Migose et al., 2018). Milk produced on SHD farms is either sold to private buyers or cooperative societies for processing or sold raw, locally at milk bars, to consumers and restaurants. Some better-organized dairy cooperative societies process the milk collected and make several value-added products such as yorghut for sale, and this results in increased income to their members (Wambugu et al., 2011; Wangu et al., 2021). The main constraints to efficient milk production in Kenya’s SDFs include the seasonality of fodder, limited use of supplements, poor farming practices, poor breeding plans, and diseases (Lukuyu et al., 2011; VanLeeuwen et al., 2012; Waitituh, 2017). Limitations in production and suboptimal efficiency have led many farms to be subsistence enterprises (FAO, 2015; Gachuiri et al., 2012; Muraguri et al., 2004).

Farmer knowledge and awareness of best practices for milk production management are lacking due to limited short training sessions, seminars, and field demonstrations that are primarily provided by the dairy cooperatives (Nyokabia et al., 2021; USAID, 2018); government extension services are poorly resourced. Farm management and characteristics, such as type of feed, routine practices, size of household, and income dependency, are very important determinants of milk sales profitability (Richards et al., 2015). The farmer usually gets additional income from selling bull calves, heifers, or adult culled cows to other farmers for keeping or slaughter (Mburu et al., 2007). Farmers utilizing zero grazing management usually keep exotic breeds such as Holstein, Ayshire, Guernsey and Jersey) or their crosses while the farmers in less intensive production systems prefer local crosses for better disease and drought tolerance (Ajak et al., 2020; Waitituh, 2017). Artificial insemination (AI) is common in order to control infectious diseases from bull breeding, and to enhance genetic improvement, using both local and imported semen, with European breeds being the most preferred. The use artificially inseminated semen is a potential source of BVDV transmission if semen used is not from an institution that is not routinely testing for BVDV (Meyling and Mikél 1988).

### 1.1.4 Infectious disease biosecurity and vaccinations in the SDFs

Infectious diseases cause major losses to production on SHDs through morbidity, mortality, and poor reproduction (Odhiambo et al., 2019; Waitituh, 2017). Farmer knowledge on common diseases that affect dairy farming is limited to a few diseases that are most common and with high morbidities and mortalities, such as Foot and Mouth Disease (FMD), East Coast Fever (ECF), and Lumpy Skin Disease (LSD) (Gizaw et al., 2020; Peters et al., 2012). Other diseases, such as Bovine Viral Diarrhea Virus (BVDV), Brucellosis, and Infectious Bovine Rhinotracheitis (IBR), despite having a potential for causing large losses, are less understood by dairy farmers, because they can persist within farms in subclinical forms (Kainga et al., 2022; Nyangau et al., 2021).

There is little awareness or adherence to biosecurity practices as preventive measures for infectious diseases, therefore high disease burdens exist (Mutua et al., 2022). There is also the challenge of limited resources that minimize the employment of basic biosecurity practices (Mekonnen et al., 2006; Mutua et al., 2022). The lack of enough fodder results in practices that contravene good biosecurity, such as grazing cows along the roads and on communal grazing grounds. Other practices, such as the use of shared bulls for breeding, purchase of cattle, sharing of equipment, and the unrestricted movement between farms, serve to increase the transmission of disease ( Bebe et al., 2003; Ogola et al., 2018).

Other disease prevention practices, such as deworming and vector control within the SHD farms, are not consistently practiced, leading to higher disease risk as well as parasitic infestation stress (Maingi & Njoroge, 2010; Ohaga et al., 2007). Furthermore, identification and diagnostic confirmation of diseases are very important in informing herd prevention strategies and surveillance policies in cattle. These components are not always possible for the SDFs since there is either very little awareness of many diseases, or there is limited veterinary services, laboratory facilities, and/or resources to some farmers (Kidali et al., 2021; Singh et al., 2020).

Vaccination of cattle within the SDFs is limited to a few diseases deemed important by government livestock services (e.g. anthrax, foot-and-mouth disease) due to a lack of awareness of other vaccines available, lack of resources to buy these vaccines for the few cows on a farm, and some logistic problems such as some vaccines that are only commercially available in large-pack doses for these small farms (Peters et al., 2012). Vaccinations on SHD farms are mainly done by government or Non-Governmental Organizations (NGOs) funded by charity campaigns (Singh et al., 2020).

## 1.2 Bovine Viral Diarrhea Virus (BVDV) overview

Bovine Viral Diarrhea (BVD) is an important pestivirus disease of cattle caused by the Bovine Viral Diarrhea Virus (BVDV). It is distributed globally in most cattle herds and some wild animals, causing clinical or subclinical disease (Pinior et al., 2017; Scharnböck et al., 2018). The BVD virus consists of two main genotypes, BVDV 1 and 2, and two biotypes, cytopathic (CP) and noncytopathic (NCP). BVDV was first identified in the USA in a herd of cattle that reported acute gastroenteritis and high mortalities (Olafson et al., 1946; Walz et al., 2020). Within susceptible cattle herds, BVDV may cause devastating losses through clinical disease and reproductive inefficiency (Richter et al., 2017).

Infection with BVDV can result in the birth of persistently infected (PI) calves when the dam is exposed to a NCP biotype of BVDV between 40 to 125 days of pregnancy. Outside this time frame, and with CP infection, fetal BVDV infection can result in early embryonic deaths (up to 40 days), abortion, or congenital defects (Burgstaller et al., 2016; Walz et al., 2020). Youngstock or mature cattle that become infected may develop a transient infection (TI) and can develop signs of acute pneumonia or diarrhea which may result in death (Burgstaller et al., 2016; Walz et al., 2020).

Immunosuppression is an important feature of BVDV infection in both the TI and PI forms of the disease, making animals more susceptible to other opportunistic infections (Walz et al., 2020; Wathes et al., 2020). Infection with BVDV causes immunosuppression in cattle through the ability to suppress interferon (IFN) production, which delays immune responsiveness and therefore enhances pathogen establishment and virus replication (Baigent et al., 2004; Charleston et al., 2001). A BVDV-induced immunosuppression has been related to infertility that is manifested as high age at first calving and low conception rates. Infertility is caused by impaired ovulation and ovarian steroidogenesis and oophoritis (Fray et al., 2000; Grooms et al., 1998; Wathes et al., 2020).

The primary method of transmission is through contact with body secretions and aborted fetal materials, either directly (e.g. natural breeding or cattle in close proximity to each other) or indirectly via shared cattle equipment, workers’ clothing, and in some cases, through flies (Chamorro et al., 2011; Khodakaram-Tafti & Farjanikish, 2017; Meyling & Mikél, 1988; Tinsley et al., 2012). Cattle of all countries, breeds, and age groups are susceptible to BVDV infection, and PI cattle are the main source exposure because of the large volumes of viral secretions emitted during their entire lives versus the 1-2 weeks of transmissibility coming from TI animals (Grooms, 2004).

### 1.2.1 Genetic Diversity and diagnosis of BVDV

There are two BVDV genotypes, BVDV1 and BVDV2, and a third Hobi-like serotype was recently described (Decaro et al., 2011, 2013; Huang et al., 2021). Advances in molecular diagnosis and BVDV phylogenetic research have identified 21 BVDV1 strains (BVDV1a-u), 4 BVDV2 strains (BVDV2a-2d), and 4 Hobi-like strains (HoBi a-d) (Yeşilbağ et al., 2017). The molecular similarity in the glycoproteins of Pestiviruses provides an advantage for consistent diagnosis but remain a challenge in differentiation between variants (Arce et al., 2009; Podgórska et al., 2012; Postel et al., 2015), especially the Hobi-like variants that are not well identified (Walz et al., 2020).

There is a similarity in the way Pestiviruses trigger immune responses, thereby having the potential for cross-reaction on various tests, including Enzyme-Linked Immunosorbent Assays (ELISA) (de Oliveira et al., 2020; Loeffen et al., 2009). Other members of the Pestivirus genus affecting pigs and sheep are Classical Swine Fever Virus (CSFV) and Border Disease Virus (BDV), respectively, and have the potential for diagnostic cross-reactivity and cross-species infection (Huang et al., 2021; Kaiser et al., 2017). Other species in the genus Pestivirus include Pronghorn Pestivirus, Bungowannah virus, Giraffe Pestivirus, Aydin-like Pestivirus, Rat Pestivirus, and Atypical Porcine Pestivirus (Ridpath, 2013; Smith et al., 2017). Possible cross-reactivity implications in the diagnosis of BVDV have been poorly researched among some of these species.

Infection with BVDV causes disease primarily in cattle, however, it has been shown to infect other mammalian hosts such as pigs, camelids, and other domestic and wild ruminants (Simmonds et al., 2015). Infections in these other host are thought to have analogous clinical and pathological syndromes to those in cattle (Passler & Walz, 2010). The multiple species infections make these other hosts potential reservoirs or maintenance carriers for BVDV transmission to cattle, especially where mixed species farming is done in close contact (Rodríguez-Prieto et al., 2016; Walz et al., 2020).

The main diagnostic tests that are used to screen for BVDV infections in cattle are Antigen Capture ELISA (ACE), and competitive and indirect antibody ELISA assays in ear-notch, milk, and serum samples. Commercially available antibody indirect ELISA kits have been shown to have good sensitivities (Se) and specificities (Sp) in the range of 85% to 100% and 94% to 100%, respectively. With the use of competitive ELISA diagnostic kits and utilizing milk samples for antibody testing have lower Se and Sp (Hanon et al., 2017; Jenvey et al., 2015). Infections with BVDV can be confirmed using a reverse transcriptase polymerase chain reaction (RT-PCR), which has been shown to have a 100% agreement with antigen capture ELISA (ACE).

The use of RT-PCR is considered the gold standard in the diagnosis of BVDV as well as other Pestiviruses and serves as also for identification of circulating variants. (Barlič-Maganja & Grom, 2001; Baule et al., 1997; Deng et al., 2015). An ACE diagnostic test is a very good economically viable method of PI identification and a key tool in the actualization of control programs (Lanyon et al., 2014; Walz et al., 2020). Virus Neutralization Tests (VNT), virus isolation, and RT-PCR, are mostly performed and limited to research or confirmation of homologous BVDV strain (Khodakaram & Farjanikish, 2017; Sozzi et al., 2020). An improved indirect ELISA utilizing truncated E2 and recombinant Erns glycoproteins have been proposed to separate anti-CSFV from anti-BVDV antibodies (Ji et al., 2018; Yi et al., 2022).

The immunohistochemistry (IHC) and tissue ACE tests, both utilizing ear notch samples, are cheaper tests for identifying only PIs, with very good sensitivities and specificities, with TI cattle testing negative (Khodakaram & Farjanikish, 2017; Lee et al., 2018). These tests are cheaper because only one sample is needed to confirm PI status, whereas with blood or serum samples, confirmation of PI status requires two samples testing positive 3-4 weeks apart to rule out transient infection (Fulton, 2013).

### 1.2.2 BVDV status in Kenya and elsewhere

Herd-level prevalence of BVD virus antibodies globally is varied, ranging from very high (e.g. up to 53% in China and Botswana) to very low (e.g. less than 1% in Belgium and Germany (Deng et al., 2015; Ran et al., 2019; Scharnböck et al., 2018; Simon et al., 2017)). In some western European countries, such as Norway and Spain, there has been no evidence of recent cases according to surveillance reports (Simon et al., 2017). Previous reports of BVDV infection in Kenya have shown varying prevalence based on antibodies in cattle. An antibody prevalence in exotic breed dairy cows in the Kenyan Rift Valley was 79.1% (Okumu et al., 2019), 45.8% in Zebu cows in the Coastal area (Kenyanjui et al., 1994), and 19.8% in Zebu cows in the western region of Kenya (Callaby et al., 2016). Another study conducted in Kakamega, Makueni, and Nandi Counties of Kenya reported an antibody prevalence of 52.3% (Olum et al., 2020). A recent study in Meru County of Kenya utilizing antibody and antigen ELISA reported a high antigen prevalence of 36.2% in cattle and an antibody prevalence of 47.1.% BVDV ( VanLeeuwen et al., 2021).

Recently in Meru County, there has been an identification of possible diagnostic cross-reactivity between BVDV and Classical Swine Fever virus (CSFV) in SDFs practicing mixed livestock farming (Muasya, et al., 2022). Therefore, where these prevalence estimates included farms with swine, it is possible that the estimates may not actually reflect the prevalence of BVDV infections. In 1967, there was a diagnosis of a Pestivirus causing mucosal disease-like syndrome in giraffes in Nanyuki, Kenya (Harasawa et al., 2000). There has not been any evidence of a further investigation into the relationship between BVDV and the giraffe virus or other Pestiviruses in livestock since then. Elsewhere in Africa, there are varied prevalence reports, an absence of control programs and a paucity of data on circulating variants of BVDV (Bahgy et al., 2018; Handel et al., 2011; Kabongo & Van Vuuren, 2004; Lysholm et al., 2019; Tadesse et al., 2019; Tesfaye et al., 2021).

### 1.2.3 Control and prevention

The control of BVDV involves several options that are often more successful when combined due to the complexity of the infection and disease persistence in herds. These control strategy options include periodic BVDV testing and culling of identified PI animals, biosecurity that minimizes or eliminates contact with infected cattle, and vaccination of animals (Mishra & Kalaiyarasu, 2019). Biosecurity practices can be effective methods of disease control; however, in the SDFs, there are limited biosecurity practice (Mutua et al., 2022). People operating within the SDFs system often lack knowledge and awareness of good preventive practices, with many farmers also lacking capacity to practice some biosecurity aspects such as fencing and disinfection of facilities (Mutua et al., 2022).

Entry onto SHD farms remains very much uncontrolled and without fomite or personnel disinfection. The livestock health authority’s enforcement of movement restrictions especially during disease outbreaks is limited, and there is constant contact of animals in places such as common grazing grounds and markets (Aklilu, 2008; Yusuf, 2008). Sharing of equipment for routine practices such as hoof trimming, ear tag application, vaccination, and dewormer drenching is a common practice in the SHFs. Communal pastures and roadside grazing are common within SHD farms, which are potential ways in which diseases can be transmitted between farms. (Rotich, 2018; Wesonga, 2013). Many SHD farms frequently get new cattle introductions through purchase, lending out cattle when there are feed shortages, and sometimes the use of cattle as a dowry or gift. With these challenges and limitations, the SHD system farms rarely employ quarantine and isolation of cattle for observation before introduction of the new stock (Rotich, 2018; SPARD Africa, 2022; Wesonga, 2013).

The identification of PI cattle is very important in control and prevention programs (Walz et al., 2020), due to its central strategic role in preventing within farm infection spread. Countries with control programs focused on identification and removal of PIs especially in Europe have seen great success in the reduction of BVDV prevalence (Moennig et al., 2005; Scharnböck et al., 2018). Consistent surveillance and testing availability coupled with the disease knowledge by cattle farmers are all important to the success of this strategy (Bitsch et al., 2000; Greiser-Wilke et al., 2003). The situation for SHFs in Kenya is different with no evidence of an existing program of surveillance, PIs identification, or even routing herd screening. Despite BVDV being reported in several prevalence studies in Kenya (Muasya et al., 2022; Okumu et al., 2019; VanLeeuwen et al., 2021), the status of PIs prevalence and consequent economic impact remain unknown.

Improved herd immunity through vaccination is critical in reducing losses from sickness and deaths associated with BVDV infection in cattle where there is a risk of exposure to the virus. Bovine viral diarrhoea vaccines are commercially available in killed vaccine (KV) or modified live vaccine (MLV) forms and can be used in both calves and adult cows (Chamorro et al., 2015; Newcomer et al., 2017). Research reports indicate that the use of MLV or KV successfully prevents the presentation of clinical symptoms such as pneumonia, fever, and diarrhoea in addition to reduced viremia and shedding (Chamorro et al., 2015; Makoschey et al., 2001). In the US, BVDV vaccination has been shown to have general health and productivity benefits through recorded lower morbidities and mortalities, as well as better weight gain in feedlot calves (Grooms et al., 2014). BVDV vaccination has also been shown to provide reproductive benefits by reducing the incidences of abortions, PI generation and early embryonic deaths (Fulton, 2013; Gates et al., 2019).

Maternal BVDV antibodies are a key protection factor among calves less than six months of age and are thought to interfere with vaccine response (Chamorro et al., 2015), leading to the recommendation to initiate BVDV vaccination after 6 months of age (Chase et al., 2004; Chase et al., 2008; Walz et al., 2020 ). If BVDV vaccination is needed to prevent BVDV infections in calves less than 6 months of age, those youngstock should be revaccinated again after 6 months of age as recommended by the manufacturer for the vaccine we used in the study.

Modified live vaccines trigger longer-lasting humoral and cell-mediated immune responses while KV causes weaker humoral protection (Newcomer et al., 2017). Therefore, the MLV is better in protecting against abortions and fetal infections than KV, thereby explaining difference in the prevention of PIs observed for the vaccine types (Walz et al., 2018). Due to the weaker response triggered by the KV, it has been recommended that this vaccine type be used for boosting after the use of a MLV (Walz et al., 2020). A NCP virus strain challenge generates a higher serum antibody titer than exposure to the CP virus strain; however, the CP virus challenge produces higher lymphocyte responses than the NCP virus strain challenge (Lambot et al., 1997). Most vaccines contain CP strains, while NCP strains are more common in natural infections (Deregt & Loewen, 1995).

There are three important capsule glycoproteins (E1, E2, and Erns) in the BVDV virus that are responsible for immune response (Wang et al., 2015). The E2 envelope glycoprotein is the most important due to its immunodominance and having two very conserved domains (I and II) (Chernick et al., 2018). The E2 glycoprotein is very important in vaccine development due to the possibility of triggering cross protection while Erns has been utilized in ELISA diagnostic tests for better identification of different Pestiviruses (Kampa et al., 2007; Wang et al., 2015). The mismatches between vaccine variants and naturally circulating variants can limit the effectiveness of available vaccines. Unfortunately, the exact antigenic diversity of BVDV field viruses in circulation within specific farms or areas remains largely unknown, making the development of widely protective vaccines in every scenario difficult (Neill et al., 2019; Ridpath, 2005).

As a result of the antigenic diversity of BVDV strains on farms, most of the vaccines available for use against BVDV are multivalent, containing both BVDV 1 and 2 serotypes, along with other viral and bacterial antigens, providing broad cover against reproductive, gastrointestinal, and respiratory disease (Walz et al., 2018). These multivalent vaccines make it difficult to separate the effect of the BVDV components from the effect of other antigens when assessing the benefits of the vaccine protection (Walz et al., 2020). Multivalent vaccines with BVDV 1 and 2 components have been shown to be beneficial in reducing herd infertility diseases, abortions, BVDV persistently infected (PI) animals, respiratory diseases, and diarrheal diseases (Chamorro et al., 2015; Newcomer et al., 2015; Walz et al., 2010). Feedlot calves vaccinated with multivalent vaccines versus univalent BVDV vaccines showed better prevention of Bovine Respiratory Disease (BRD) and overall performance (Schunicht et al., 2003; Wildman et al., 2008).

There have been varied adoption of the various control programs in different countries with varying success and cost (Thomann et al., 2017). In North America, there has been success in the prevention of BVDV mainly by use of the three-dimensional approach of vaccination, identification and elimination of PIs, and biosecurity (Walz et al., 2020). Some countries in Europe, such as Austria, Denmark, Finland, Sweden, and Norway, do not permit the use of BVDV vaccination (Scharnböck et al., 2018), and rely heavily on biosecurity and active monitoring for infections. In South America, Asia, Australia, and Africa, there is little or no evidence of implementation of control programs at the national level with the responsibility is left to farmers (Scharnböck et al., 2018).

In Kenya and other African countries, there is very limited information about the benefits of BVDV vaccination, and no formal control programs, with the exception of Egypt (Mutua et al., 2022; Scharnböck et al., 2018; Soltan et al., 2015). The awareness and vaccination for BVDV are very limited within the SDFs of Kenya, despite the disease being prevalent (MOHK, 2017; Muasya, et al., 2022; Okumu et al., 2019). Since BVD is a very important disease limiting productivity and reproduction on SDF in Kenya and other developing countries, the benefits of BVDV prevention in this context need to be studied.

## 1.3 Research rationale and objectives

The SDFs in Kenya face substantial disease challenges, among other limitations, leading to poor productivity. Several researchers have highlighted the major health challenges in dairy cattle (Bebe et al., 2003; Bonilla et al., 2018; Maingi & Njoroge, 2010; Odhiambo et al., 2019). Disease prevention strategies are poorly adopted by the SHD farms due to the lack of knowledge, little awareness, or limitation of resources (FAO, 2011; Waitituh, 2017; Wanyoike et al., 2019).

In Kenya BVDV is one of the prevalent and endemic cattle diseases, yet there is little awareness among the SHD farmers of this disease, as well as lack of a formal control policy. A recent study in Meru County, Kenya, reported a high prevalence of BVDV infections (47% of unvaccinated tested cattle had antibodies) and its potential negative impact on cattle reproduction, among other diseases (VanLeeuwen et al., 2021). For this reason and the fact that vaccination can be a cheap method of BVDV control, assessing the potential benefits of BVDV vaccination in the SDF context is critical to convincing SHD farmers to vaccinate. Understanding the factors that affect efficiency and effectiveness of vaccination in cattle will help farmers to strategically ensure proper immune response is likely (e.g., good body condition) would also be very beneficial. Investigating the possibility of cross-reactivity of BVDV test results from infection by other Pestiviruses would also help to clarify the interpretation of BVDV test result in prevalence estimates and control programs.

The overall objective of this thesis was to understand how to control BVDV infections better within the SHD farming sector in Kenya with the following specific study objectives:

1. To evaluate the possible cross-reactivity between Classical Swine Fever Virus (CSFV) testing with BVDV testing using both antibody and antigen ELISA,
2. To determine associations between vaccination status and disease occurrence among cattle on SDFs in Kenya in a cohort study,
3. To determine associations between vaccination status and disease occurrence among BVDV vaccinates and placebo-treated cattle in a randomized controlled trial (RCT),
4. To determine antibody titer response variability from vaccination when given to cows under different planes of nutrition, management, and BCS on smallholder dairy farms in Kenya.

## 1.4 Thesis hypotheses and structure

The rest of the thesis contains 4 substantive chapters, one for each of the four objectives, along with a concluding summary chapter that can be read by those who do not have time to read the four substantive chapters but want more information than the abstracts. The concluding chapter also provided a section that interprets and integrates the chapter results together, along with some overall recommendations from the thesis.

The hypotheses for each objective are listed below.

1. Chapter 2: The diagnosis of BVDV using both antibody and antigen ELISA in cattle is not affected or complicated by potential cross-reactivity with CSFV among cattle on SDFs in Kenya.
2. Chapter 3: There are health benefits and lower disease occurrence in the vaccinated cohort compared to the unvaccinated cohort, with good management practices complementing the benefits of vaccination among cattle on SDFs in Kenya.
3. Chapter 4: There are health benefits and lower disease occurrence in the vaccinated group compared to the placebo group after one year of follow-up, with other preventive management practices complementing the benefits of vaccination among cattle on SDFs in Kenya.
4. Chapter 5: The BVDV vaccination antibody response will be different under different planes of nutrition, management, and BCS on smallholder dairy farms in Kenya, with the BVDV vaccination antibody response being better at a higher body condition score.

It is expected that good SHD performance and efficiency will lead to better household incomes and nutrition, thus empowering rural communities. The improvement of dairy herds through better knowledge and disease prevention, and better financial capacity from better performance offers a sustainable solution for better quality of life for the SHD farmers of Kenya. The BVDV vaccination could be a key disease prevention intervention, in addition to other important and complementary methods, such as biosecurity and correct diagnosis of BVDV.

## 1.5 Study location and context

The study was done in the Buuri and Naari areas of the Buuri sub-county in Meru County, Kenya. Meru County is in central Kenya to the East of Mount Kenya and lies on the equator and within longitudes 37° and 38° East, about 270 kilometers north of Nairobi, the capital city of Kenya. The study area is in a high potential agricultural region at an altitude of approximately 2,000 meters above sea level. The County has a population of 1.55 million people and is about 7,000 square kilometers, with animal and crop agriculture being the main economic activity.

Farms that were members of the Buuri and Naari Dairy Farmers Cooperative Societies from May of 2019 formed the sampling frame for the research studies. The study was done from May 2019 to January 2021. These cooperatives have an active partnership with Farmers Helping Farmers, the University of Prince Edward Island (UPEI), and the University of Nairobi (UoN). In the Buuri area, there were no smallholder dairy farms that had ever vaccinated with a BVDV vaccine in the past. In the Naari area, some farms had their cattle vaccinated in 2018 and early 2019 in a related project and formed the sampling frame for objective 2.

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Figure 1.1 Map of Kenya showing Meru County

# Chapter 2: Evaluation of antibody and antigen cross-reaction in Kenyan dairy cattle naturally infected with two pestiviruses: Bovine viral diarrhea virus and classical swine fever virus

## 2.1 Abstract

**Background:** Bovine viral diarrhea virus (BVDV) and classical swine fever virus (CSFV) are important pathogens of cattle and pigs, respectively, and belong to the genus Pestivirus. As CSFV has been shown to infect cattle, it can create diagnostic challenges for BVDV results through possible cross-reactivity where cattle could be exposed to pigs and CSFV. This study aimed to determine possible cross-reactivity of BVDV and CSFV enzyme-linked immunosorbent assay (ELISA) results for antigen (Ag) and antibody (Ab) among smallholder dairy cattle in Kenya.

**Methods:** This was a cross-sectional study based on a single visit to farms to collect serum samples and other descriptive farm-level and animal-level information. Testing for BVDV Ag and Ab was conducted on serum samples from 320 dairy cows and heifers, with CSFV Ag and Ab testing conducted on a subset of 133 and 74 serum samples, respectively. Testing of CSFV was based on BVDV test results and availability of enough sample volume from farms that kept pigs. The IDEXX ELISA kits (IDEXX Laboratories, Switzerland) were used to detect Ag and Ab for both BVDV and CSFV.

**Results:** For the 74 samples with tested for Ab to both viruses, 40 (54.0%) were BVDV Ab positive, while 63 (85.1%) were CSFV Ab positive. Of the 40 BVDV Ab positive samples, 36 cattle (90.0%) tested positive for CSFV Ab. However, of the 34 BVDV Ab negative samples, 27 (79.4%) were CSFV Ab test-positive. For the 133 samples tested for Ag of both viruses, 125 (94.0%) were BVDV Ag positive, while 2 (1.5%) samples were CSFV Ag positive. None of the eight BVDV Ag negative samples was positive for CSFV Ag and only two (1.6%) of the 125 BVDV Ag positive samples were positive for CSFV Ag.

**Conclusions:** The results indicate either substantial cross-reactivity of the two Ab ELISA tests, or reactivity with some other protein in the samples that led to the positive Ab test results. There was only limited evidence for cross-reactivity of the two Ag ELISA tests. We recommend that Pestivirus genus cross-reactivity be considered when interpreting BVDV ELISA results in cattle, more for Ab than Ag tests. Further research is needed to clarify the levels of cross-reactivity for other Pestivirus Ag and Ab tests from animals on mixed-species farms.

**Keywords:** antibody, antigen, bovine viral diarrhea virus, classical swine fever virus, cross-reactivity, smallholder dairy.

Muasya, D., Van Leeuwen, J., Gitau, G., McKenna, S., Heider, L., & Muraya, J. (2022). Evaluation of antibody and antigen cross-reaction in Kenyan dairy cattle naturally infected with two pestiviruses: Bovine viral diarrhea virus and classical swine fever virus. *Veterinary World*, *15*(5), 1290–1296. <https://doi.org/10.14202/VETWORLD.2022.1290-1296>

## 2.2 Introduction

Bovine viral diarrhea virus (BVDV) is an economically important and genetically diverse member of the genus Pestivirusin the *Flaviviridae* family (Waltz, 2008). The virus has two main genotypes, BVDV1 and BVDV2, with multiple sub-strains, and a third Hobi-like strain has been described recently (Decaro et al., 2011, 2013). There are two other Pestivirus species, classical swine fever virus (CSFV) and border disease virus (BDV) of importance to domestic animals (Simmonds et al., 2015). Other species in the genus Pestivirus include: Pronghorn pestivirus, Bungowannah virus, giraffe pestivirus, aydin-like pestivirus, rat pestivirus, and atypical porcine pestivirus (APP) (Ridpath, 2013; Simmonds et al., 2015; Smith et al., 2017). The classification of Pestivirus genus was updated in the year 2017, and there is a new proposal to add eight new species recently described (Postel et al., 2021). It has been demonstrated that the viruses in the Pestivirus genus have some level of genetic similarity with respect to diagnostic target molecules (Arce et al., 2009; Podgórska et al., 2012; Postel et al., 2015). This similarity makes the molecular aspects for diagnosis complex, with research attention being focused on more specific diagnosis (Riitho et al., 2020).

There is a very close similarity in the way Pestiviruses trigger immune-responses, thereby having the potential for cross-reaction on various tests, including enzyme-linked immunosorbent assays (ELISA) (de Oliveira et al., 2020; Loeffen et al., 2009). Infections with BVDV causes disease primarily in cattle; however, it has been shown to infect other mammalian hosts, such as pigs, camelids, and other domestic and wild ruminants across the globe (Simmonds et al., 2015). The heterologous host infections may have analogous clinical and pathological syndromes to those in cattle (Passler & Walz, 2010). Infections in multiple species could mean that some of these animals may be potential reservoirs or maintenance hosts, transmitting BVDV to cattle where there may be in contact, thereby hindering successful control (Rodríguez-Prieto et al., 2016; Walz et al., 2020).

Antibody (Ab) cross-reaction between CSFV and BVDV has been demonstrated as a challenge in the diagnosis and surveillance of BVDV in cattle herds (Giangaspero et al., 2019; Ji et al., 2018). Pestivirus Ab response for BVDV and CSFV infections in cattle has been shown to target glycoproteins Erns, E1, and E2, with the latter being more dominant (Chen et al., 2020; Wang et al., 2015). The epitope mapping has been used to understand the cross-reactivity between the BVDV E2, CSFV E2, and BDV E2 glycoproteins as a means to identify monoclonal Ab domains (Burton, 2016; Huang et al., 2021). The importance of BVDV in cattle populations is well documented, leading to direct and indirect losses to productivity and reproduction (Newcomer et al., 2015). The disease has equally presented challenges with regards to a successful and sustainable control program (Sandvik, 2004). Control programs have utilized diagnosis, vaccination or both as key components, in addition to biosecurity (Walz et al., 2020).

For successful control, vaccination and serological diagnosis require more research on the genetic variation and cross-reactivity dynamics among pestiviruses (de Oliveira et al., 2020; Walz et al., 2020), particularly in places practicing mixed livestock keeping of cattle, pigs, and small ruminants in close contact, as is the often the case in Kenya. In a related primary research study preceding this work, there were 158 randomly selected farms in Meru County, Kenya. Among the 467 and 323 serum samples tested for BVDV antigen (Ag) and Ab using ELISA tests, respectively, the seroprevalences of BVDV Ab and Ag were 47.1% (152/323) and 36.2% (169/467), respectively (VanLeeuwen et al., 2021).

The previous reports of BVDV in other parts of Kenya have shown varying prevalence in cattle (Callaby et al., 2016; Okumu et al., 2019; VanLeeuwen et al., 2021); however, none has tested for the presence of cross-infection with CSFV between cattle and pig populations. In large-scale farms in the Rift Valley, Kenya, an Ab prevalence in dairy cows of 79.1% was recorded (Okumu et al., 2019). 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). There is recent evidence for possible cattle infection with CSFV in China and India, which could further complicate the interpretation of the BVDV test results (Giangaspero et al., 2017), if this means that the long-held belief that CSFV only infects swine is confirmed to be untrue. This study aimed to determine the possible cross-reactivity of BVDV and CSFV ELISA results for Ag and Ab among dairy cattle.

## 2.3 Materials and Methods

### 2.3.1 Ethical approval

The research was approved on March 14th, 2019, by the University of Prince Edward Island (UPEI) Research Ethics Board (REB Ref # 6008082). Serum sampling was carried out in accordance with UPEI animal use approval, and laboratory testing was done in accordance with standard laboratory operating procedures.

### 2.3.2 Study period, area, and population

The study was conducted from May 2019 to April 2020 using unpublished data from a previous study (VanLeeuwen et al., 2021). The study location was the Naari area of Meru County, Kenya. All the farms recruited were SDFs and members of the Naari Dairy Farmers Cooperative Society. The cattle recruited were above 6 months of age, and the population consisted of mainly exotic breeds with some local breeds and their crosses. The farms practiced zero-grazing, communal grazing, or a combination of both management practices.

### 2.3.3 Experimental design

This was a cross-sectional study based on a single visit to the farm to collect serum samples and other descriptive farm-level and animal-level information. The sampling frame for the study was the 470 serum samples from the primary study conducted by VanLeeuwen and colleagues (2021). From that sampling frame, there were 320 samples that underwent testing for both BVDV Ag and Ab. Testing for CSFV Ag (n=133) and Ab (n=74) was conducted on a subset of the 320 samples, purposively selected, based on the following factors.

1. They came from animals on farms with at least one BVDV test positive sample in the primary study.
2. They came from farms rearing multiple animal species, especially pigs, in addition to cattle.
3. To identify CSFV exposure in cattle samples that were negative for BVDV Ab and Ag, a limited number of samples (~30%) that were negative for BVDV Ab and Ag were tested.
4. They were available and had enough volumes of sera remaining in the frozen sample vials for follow-up testing.

Figure 2.1 shows a flow diagram of the sample numbers tested in relation to the above criteria.

### 2.3.4 Laboratory testing

The serum samples were tested for Ab and Ag for CSFV and BVDV using commercial ELISAs (IDEXX Laboratories, Switzerland) conducted at the Department of Clinical Studies, University of Nairobi. The University of Nairobi laboratory staff member was blinded to the identification of the tested animals and other test results. The ELISA results produced an optical density, and then a sample-to-positive (S/P) ratio for each sample was calculated, indicating the reading was adjusted for the positive control on the plate, after confirming that the negative control was in the correct range.

The presence of BVDV Ag was tested with the BVDV Ag/Serum Plus Test® kit (IDEXX Laboratories, Switzerland) according to the manufacturer’s instructions. Samples were considered positive when the S/P ratio was equal or above 0.3. This test is reported to have a sensitivity of 98.7% and specificity of 95% and can detect the majority of BVDV 1 and 2 Ag. For BVDV Ab testing, the BVDV Total Ab Test® kit (IDEXX Laboratories, Switzerland) was used according to the manufacturer’s instructions, and an S/P ratio equal to or above 0.3 was considered positive. According to the manufacturer, this test is reported to have a sensitivity of 100% and specificity of 95% and can detect BVDV 1 and 2 Ab. Samples with an S/P ratio of between 0.2 and 0.3 for both Ab and Ag were considered suspect according to the manufacture’s guidance.

The CSFV Ag detection was conducted with CSFV Ag Serum Plus Test® kits (IDEXX Laboratories, Switzerland) following the manufacturer’s instructions. Samples were considered positive when the S/P ratio was equal or above 0.3. This test is reported to have a sensitivity of 90% and specificity of 100%. For CSFV Ab testing, CSFV Ab Test® kits (IDEXX Laboratories, Switzerland) were used, according to the manufacturer’s instructions. Samples were considered positive when the S/P ratio was equal or above 0.3. This kit is reported to have a sensitivity of 97.8% and specificity of 99.7%.

### 2.3.5 Statistical analysis

Data were entered and organized into an Excel spreadsheet (Microsoft, Sacramento, California, USA). The CSFV data were merged with the BVDV data by farm name and animal name. Descriptive statistics of cross-tabulations were carried out using STATA/IC 16.0 (StataCorp LLC, College station, Texas, USA).

## 2.4 Results

The testing of BVDV and CSFV was compared between the four tests to assess the possibility of cross-reactivity and reliability of interpretation. The comparison combinations shown below have different possible meaning in this SHF study. Of the 320 cattle that were tested for both BVDV Ab and Ag, 79 (24.3%) tested positive for both BVDV Ab and Ag, and 87 (27.2%) tested negative for both BVDV Ab and Ag (Table 2.1). There were 71 (22.2%) samples that were positive for BVDV Ab but negative for BVDV Ag and 81 (25.3%) samples that were negative for BVDV Ab but positive for BVDV Ag. Three samples were suspect on the BVDV Ab test and were considered positive. Half (81) of the 162 BVDV Ag positive cattle were also positive for BVDV Ab, and 71 (45%) of the 158 BVDV Ag negative were BVDV Ab positive. The BVDV Ag positives that were Ab negative were most likely acute infections yet to seroconvert, while the BVDV Ab positives that were Ag negative were possible cleared infections.

Of the 133 CSFV Ag tests, only 2 (1.5%) samples were CSFV Ag positive (Table 2.2). None of the eight BVDV Ag negative samples were positive for CSFV Ag. Only two (1.6%) of the 125 BVDV Ag positive samples were positive for CSFV Ag. This would possibly mean that both Ag tests are cross reactive or there was a co-infection in the two samples. Table 2.3 compares results from cattle that were tested for BVDV Ab and CSFV Ag, with 68 (51.9%) being BVDV Ab positive. The two cattle that were CSFV Ag positive were negative for BVDV Ab. None of the 68 BVDV Ab positive samples was positive for CSFV Ag. The two CSFV Ag positive samples could have possibly been acute infections that had not seroconverted or were not detectable by the BVDV Ab ELISA test.

For the 74 CSFV Ab tests, 63 (85.1%) were CSFV Ab positive, and 40 (54.0%) were BVDV Ab positive (Table-2.4). Of the 40 BVDV Ab positive samples tested for CSFV Ab, 36 cattle (90.0%) tested positive for CSFV Ab. Of these 36 samples testing positive for both BVDV and CSFV Ab, none tested positive for BVDV Ag or CSFV Ag. A majority of BVDV Ab positives being CSFV Ab positive could possibly mean that there was a cross reactivity of the two tests with a past exposure of either virus or that the cattle could have been exposed to both in the past.

Of the 34 BVDV Ab negative samples tested for CSFV Ab, 27 (79.4%) were CSFV Ab test positive (Table 2.4). Of the 63 CSFV Ab positive samples tested for BVDV Ab, 36 cattle (57.1%) tested positive for BVDV Ab. However, of the 11 CSFV Ab negative samples tested for BVDV Ab, 4 (36.4%) were BVDV Ab test positive. Comparing the two Ab tests, there were more CSFV positives only than there were BVDV Ab positives only. For the two samples that were positive for both CSFV Ag and BVDV Ag, an attempt was made to extract RNA from the samples to determine if they were truly CSFV or BVDV. Unfortunately, due to freezer storage issues, the sample quality was compromised and therefore it was not feasible to do RNA extraction.

## 2.5 Discussion

This investigation of CSFV and BVDV Ab and Ag test results in dairy cattle in Kenya provides data on possible diagnostic cross-reactions . As reported previously, the identification of Pestviruses by Ag or Ab ELISA has the potential for cross-reactivity due to the similarity in Pestivirus Ag and response Ab used in various diagnostic tests (Ji et al., 2018; Lihua et al., 2020; Postel et al., 2015). There were 40 BVDV Ab positive cattle, with 36 of these (90.0%) cattle also testing positive for CSFV Ab (Table 2.4). The 36 samples testing positive for both BVDV and CSFV Ab likely have been previously infected by either or both of the two viruses. These results could suggest that there is substantial cross-reactivity of the two Ab ELISA tests, or the animals had antibodies to both CSFV and BVDV. Evidence of cross-reactivity between CSFV and BVDV complicating serological diagnosis has been demonstrated (Burton, 2016; van Rijn, 2007). The antigenic epitope relied on for diagnosis is similar for BVDV and CSFV; thus, there is great potential for cross-reactivity (van Rijn, 2007).

It may also be possible that there is some other Pestivirus Ab in these samples that is leading to positive CSFV Ab test results, positive BVDV Ab test results, or both. A study in Turkey reported that sheep and goats infected with BDV also tested positive for CSFV. Genetic sequencing of the DNA in the samples demonstrated the presence of Pestiviruses *Aydin/04* and *Burdur/05,* which were new variants of BDV (Postel et al., 2015). There has been recorded evidence of cross-reactivity between other Pestiviruses of importance to livestock, including BDV and APP, rendering diagnosis a challenge (Ridpath, 2015; Bingham et al., 2010; Riedel et al., 2021). Cross-reactivity between BDV and BVDV has been reported to be a potential impediment in surveillance and diagnosis (Kaiser et al., 2017). Information on BDV prevalence has not been documented in Kenya, but it is thought to be present and may have impacted this study

Of the 34 BVDV Ab negative samples, 27 (79.4%) were CSFV Ab positive. This result could mean that there were CSFV infections among the cattle population. Infections with CSFV have been demonstrated in cattle populations, as well as BVDV infections in pig populations, in other studies in different places globally (Chakraborty et al., 2018; de Oliveira et al., 2020; Giangaspero et al., 2017; Tao et al., 2013; van Rijn, 2007). It has been shown that BVDV infection in pigs is a challenge to the diagnosis of CSFV in pig herds where pigs and cattle are kept in close proximity (Almeida et al., 2017; Loeffen et al., 2009). This result means that cattle and pigs reared together can compound the maintenance of both pathogens and thus possible cross-reaction or co-infection.

Only two (1.6%) of the 125 BVDV Ag positive samples were positive for CSFV Ag (Table 2.2). Therefore, it would seem that there is not much cross-reactivity of the two Ag ELISA tests. Other studies have reported CSFV Ag in cattle serum using Ag capture ELISA (Chakraborty et al., 2018). Given that cattle may become infected with CSFV, it is also possible that these two samples were from cattle that were infected with BVDV and CSFV at the time of sampling. A study of Pestiviruses in Asia showed that there was about 30% genetic divergence between BVDV and CSFV (Giangaspero et al., 2019: 2017). However, the genetic variation between BVDV 1 and 2, which are also classified as distinct species, has been shown to have similar capsule antigens, leading to some level of diagnostic cross-reactivity (Couvreur et al., 2002; Walz et al., 2020). Unfortunately, due to freezer issues, the sample quality was compromised; therefore, it was not feasible to do RNA extraction.

The high seroprevalences of BVDV Ab and Ag reported in the preceding primary study by Van Leeuwen *et al*. (2021) approached or exceeded those of other reports (Tesfaye et al., 2021; Velasova et al., 2017). Having a third of tested cattle test positive for BVDV Ag (VanLeeuwen et al., 2021) 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. The BVDV test results may be partly a function of test cross-reaction with CSFV since this has been demonstrated in other studies (Burton, 2016; Huang et al., 2021; Loeffen et al., 2009).

Infections with BVDV can easily be transmitted between cattle through body secretions, and BVDV antibodies can remain in circulation for long periods of time (Collins et al., 2009; Givens & Marley, 2013; Lindberg & Houe, 2005). A study looking at *Neospora caninum* and BVDV in export cattle from Rio Grande Brazil found 75.4% being positive for BVDV Ag (Alves et al., 2020). The prevalence of BVDV had been reported to be high in situations where mixed livestock are kept together, and where wildlife and domestic cattle co-mingle (Casaubon et al., 2012; Loeffen et al., 2009; Sandvik, 2004). Many SDFs in Kenya have these conditions; therefore, there is the possibility of other Pestiviruses along with the presence of BVDV and among other pathogens on Kenyan SDFs (Callaby et al., 2016; Okumu, 2014). In the primary study by VanLeeuwen *et al*. (2021), pigs were identified to be an important associated exposure with the odds of samples testing positive for BVDV Ag being 6.1 times higher on farms with pigs than farms without pigs (p=0.02).

## 2.6 Conclusion

Our findings clearly demonstrate the challenges of interpreting Ab test results for BVDV and CSFV on farms where livestock species (especially cattle and pigs) mingle on the same farm. The results indicate either substantial cross-reactivity of the two Ab ELISA tests, possible combined infections, or reactivity with some other Pestivirus in the samples, such as BDV, that led to positive CSFV Ab test results, positive BVDV Ab test results, or both. There was only limited evidence for cross-reactivity of the two Ag ELISA tests which could have been also a result of few samples tested.

This study was limited by using a subset of samples tested for CSFV that were originally tested for BVDV Ag and Ab. Having a larger proportion of the original samples tested would likely have provided clearer results, but logistical challenges precluded more samples from being tested. It is unlikely that the reasons for samples not being tested by CSFV are related to the cross-reactivity’s being investigated, so there is unlikely to be a selection bias in the results. Further research is needed to quantify the proportion of BVDV Ag false positives due to other Pestviruses. We recommend a study comparing the serological test to other more specific tests, such as RT-PCR, sequencing, virus neutralization, or a combination thereof. It could be good to explore and utilize improved diagnostic ELISA kits for cattle populations in Kenya, which could lead to more accurate establishment of seroprevalence for CSFV and BVDV infection.

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Table 2.1: BVDV antibody and antigen ELISA results of 320 dairy cattle over 6 months of age from 134 randomly selected farms in Meru County, Kenya.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **BVDV antigen** | **BVDV antibody** | | | **Total** |
| **Negative** | **Positive** | **Suspect positive** |
| Negative | 87 | 70 | 1 | 158 |
| Positive | 81 | 79 | 2 | 162 |
| Total | 168 | 149 | 3 | 320 |
| BVDV=Bovine viral diarrhea virus, ELISA=Enzyme-linked immunosorbent assays | | | | |

Table 2.2: CSFV antigen and BVDV antigen ELISA results of 133 purposively selected dairy cattle over 6 months of age in Meru County, Kenya.

|  |  |  |  |
| --- | --- | --- | --- |
| **CSFV antigen** | **BVDV antigen** | | **Total** |
| **Negative** | **Positive** |
| Negative | 8 | 123 | 131 |
| Positive | 0 | 2 | 2 |
| Total | 8 | 125 | 133 |
| CSFV=Classical swine fever virus, BVDV=Bovine viral diarrhea virus, ELISA=Enzyme-linked immunosorbent assays | | | |

Table 2.3: CSFV antigen and BVDV antibody ELISA results of 133 purposively selected dairy cattle over 6 months of age in Meru County, Kenya.

|  |  |  |  |
| --- | --- | --- | --- |
| **CSFV antigen** | **BVDV antibody** | | **Total** |
| **Negative** | **Positive** |
| Negative | 63 | 68 | 131 |
| Positive | 2 | 0 | 2 |
| Total | 65 | 68 | 133 |
| CSFV=Classical swine fever virus, BVDV=Bovine viral diarrhea virus, ELISA=Enzyme-linked immunosorbent assays | | | |

Table 2.4: CSFV antibody and BVDV antibody ELISA results of 74 purposively selected dairy cattle over 6 months of age in Meru County, Kenya.

|  |  |  |  |
| --- | --- | --- | --- |
| **CSFV antibody** | **BVDV antibody** | | **Total** |
| **Negative** | **Positive** |
| Negative | 7 | 4 | 11 |
| Positive | 27 | 36 | 63 |
| Total | 34 | 40 | 74 |
| CSFV=Classical swine fever virus, BVDV=Bovine viral diarrhea virus, ELISA=Enzyme-linked immunosorbent assays | | | |

Diagram

Description automatically generated

Figure 2. 1: Flow diagram of samples used for testing for CSFV and BVDV Ag and Ab among purposively selected dairy cattle over 6 months of age in Kenya

CSFV=Classical swine fever virus, BVDV=Bovine viral diarrhea virus, Ag=Antigen, Ab=Antibody

# Chapter 3: A retrospective cohort study of disease among vaccinates and non-vaccinates after a single injection of multivalent vaccine including modified live bovine viral diarrhea virus on smallholder dairy farms in Kenya

## 3.1 Abstract

**Background:** Vaccination can be an important component in the control and prevention of bovine viral diarrhoea virus (BVDV). In Kenya, BVDV is prevalent but there are limited control and prevention practices. The research objective was to determine associations between vaccination status and disease occurrence among cattle on smallholder dairy farms in Kenya in a retrospective cohort study.

**Methods:** This study compared 226 cows and 85 heifers injected with a single multivalent modified live vaccine including BVDV with 215 cows and 60 heifers in control cohorts. One year after vaccination, a follow-up visit recorded reported disease outcomes, and farm and animal factors. Mixed multivariable logistic and Poisson regression modeling was used for the heifers and cows, respectively, to determine factors associated with the disease outcomes.

**Results:** There was significantly lesser pneumonia, diarrhoea, and other diseases reported in the vaccinated versus unvaccinated heifers. For the cows, there was significantly lower pneumonia, diarrhoea, poor appetite, tick-borne diseases, and uterine diseases reported in the vaccinated versus unvaccinated cow. For the final cow model, factors associated with the disease count included: feeding grass weeds (IRR=1.294), having more than two diseases in the farm (IRR=1.415), parity (IRR=1.340), milking mastitic cow last (IRR=0.669), herd size above 6 (IRR=0.769), needing only one breeding (IRR=0.778), Body Condition Score (BCS) above 2.25 (IRR=0.813), and an interaction between vaccination status and pregnancy checking practice. In the final heifer model, factors associated with dichotomized disease in the last year included: age of female farmer above 55 years (OR=0.23), farm size over 2 acres (OR=0.13), short Napier grass fed in the dry season (OR=6.51), loss of a vaccinated cow in the farm (OR=8.91), concrete floor in the stall (OR=0.02), buying replacement heifers (OR=3.26), vaccination status (OR=0.11), and age of heifers above 30 months (OR=6.11). Higher categories of BCS, 2.25 to 2.75 (OR=4.00) and above 3 (OR=10.77) were compared to BCS of 2 and lower.

**Conclusions:** The study established that multivalent modified live vaccination that included BVDV was beneficial in reducing reported diseases in both cows and heifers one year after vaccination, while controlling for other farm-level and animal-level factors.

**Key words:** BVDV, vaccine, retrospective cohort study, smallholder dairy

## 3.2 Introduction

Bovine viral diarrhoea virus (BVDV) is an important pathogen responsible for significant losses through poor health and reproductive performance in the cattle industry worldwide (Waltz, 2008). Vaccination can be an important control strategy in the prevention of BVDV and has been shown to offer significant benefits in herds (Newcomer et al., 2015, 2017b; Walz et al., 2020). BVDV vaccines mostly come in multivalent combinations with other pathogens including Herpesvirus type 1, bovine respiratory syncytial virus, and parainfluenza virus type 3. The use of multivalent Modified Live Virus (MLV) BVDV vaccination is a key disease prevention practice in dairy production and has been shown to offer broader protective benefits than killed BVDV vaccines (Chowdhury et al., 2021; Cusack et al., 2020; Marschika et al., 2018; Walz et al., 2018). The benefits of multivalent MLV BVDV vaccine have also been shown to be critical in the health performance of calves (Chamorro et al., 2015). The multivalent MLV vaccines provide sufficient genotype specific responses in cattle compared to killed vaccine (Dubovi et al., 2000; Fulton et al., 2020; Smith et al., 2021; Sozzi et al., 2020).

Multivalent vaccination to prevent pathogens associated with bovine respiratory disease including BVDV have been used with good success in different places (Chowdhury et al., 2021; Cusack et al., 2020; Smith et al., 2021). Vaccination has also been shown to have reproductive benefits (Bolin, 1995; Fulton, 2013; Gates et al., 2019) and reduced diseases associated with stress and immunosuppression (Barrett et al., 2018; Grooms et al., 2014; Lemon & McMenamy, 2021), especially with auction handling and introductions to feedlots (Barrett et al., 2018; Newcomer et al., 2017). Vaccination against BVDV has also been found to protect against clinical presentation of respiratory disease and diarrhoea upon experimental infection with BVDV (Makoschey et al., 2001). Calf BVDV vaccination remains a major component of control programs either as a primary method of control or complementary to other methods (Volker Moennig & Becher, 2018; Pinior et al., 2017). The application of BVDV vaccination as part of BVDV control is more adopted in North America than in European countries (Bitsch et al., 2000; Kelling, 2004; V. Moennig et al., 2005; Rat-Aspert & Fourichon, 2010).

In Africa, there is very limited information about the benefits of BVDV vaccination as a component of a control program, with exception of Egypt and Botswana (Lysholm et al., 2019; Rasha & Mostafa, 2017; Soltan et al., 2015). Recent studies in Kenya utilizing antibody and antigen ELISA have shown a high cattle and farm prevalence of BVDV and Infectious Bovine Rhinotracheitis (IBR) (Kipyego et al., 2020; Okumu et al., 2019; J. VanLeeuwen et al., 2021). However, there is limited documented use of BVDV vaccine in the different dairy cattle management systems in Kenya. Therefore, the assessment of BVDV vaccination in Kenyan dairy herds is relevant. Our project objective was to determine associations between vaccination status and disease occurrence among cattle on smallholder dairy farms in Kenya in a retrospective cohort study.

## 3.3 Materials and Methods

### 3.3.1 Study Area and Population

The study was done in the Naari area of Buuri sub-county in Meru County, Kenya. Meru County is located in central Kenya to the east of Mount Kenya and lies on the equator, within longitudes 37° and 38° East. The county has a population of 1.55 million people and is about 7,000 square kilometers, with animal and crop agriculture being the main economic activity. The dairy farms are mainly small and members of the Naari Dairy Farmers Cooperative Society which helps in collecting and marketing milk.

In June and July 2018, 909 non-pregnant cattle on 497 smallholder dairy farms in the Naari area of Kenya were vaccinated with a modified live vaccine (Pyramid® FP 5; Boehringer Ltd.) against four pathogens: bovine viral diarrhea virus, bovine herpesvirus type 1 (IBR virus), bovine respiratory syncytial virus, and parainfluenza virus type 3. The BVDV components in the vaccine included BVDV type 1 (Singer 1a cytopathic) and BVDV type 2 (296 cytopathic). The vaccine was reconstituted and kept refrigerated as recommended by the manufacturer and used within 12 hours of reconstitution. Cattle on farms belonging to active members of the Naari Dairy Farmers Cooperative Society were eligible to receive the vaccine. This cooperative has an active partnership with Farmers Helping Farmers and the University of Prince Edward Island. No smallholder dairy farms in this region were ever vaccinated with a BVDV vaccine in the past.

Farm eligibility criteria for our study included: 1) being a member of the Naari Dairy Farmers Cooperative Society; 2) having cattle vaccinated with a multivalent vaccine including BVDV in 2018; 3) the vaccinated cattle were above 6 months at the time of vaccination; and 4) the farm had available animal records. Cattle eligibility criteria included: 1) cows and heifers present in the eligible farms during the visit in the year 2018, and 2) cattle above six months of age and not pregnant in the vaccinate group during the vaccination time in the year 2018. These cattle inclusion criteria were to avoid maternal antibody interference of vaccine efficacy and to avoid inducing abortions. It was expected that vaccination should reduce disease incidence and prevalence among the vaccinated animals for diseases related to the vaccine pathogens (e.g. pneumonia, diarrhoea, abortion), and perhaps other diseases occurring due to immunosuppression from BVDV infection, such as mastitis and skin infections.

### 3.3.2 Study Design, and Data collection

This was a cohort study following cohorts of vaccinated and unvaccinated cattle for information on the eligible cows and heifers present in the eligible farms. Starting in late May 2019, farms that received a multivalent modified live vaccine that included BVDV in 2018, called BVDV-vaccinated cattle, received follow-up visits by the researchers for the following four purposes: 1) to record information from the farms on reported disease occurrences that could be remembered in the vaccinated cattle since the vaccine was given (vaccinated cohort); 2) to record information from the farms on disease occurrences that could be remembered in unvaccinated cattle on the farm since the time that the vaccine was administered (i.e. pregnant cows and heifers during the 2018 vaccination visits); 3) to conduct physical exams of both sets of animals on the farm for current diseases (e.g. California mastitis test (CMT), visible signs of skin infections, signs of lung pathology); and 4) to record management activities that could be related to the incidences of various diseases in the cattle.

During the study visits to accomplish the above purposes, a questionnaire was utilized to capture information for the period of the previous one year. Various health outcomes for the last 12 months were recorded, including: deaths of different cattle groups, diseases treated, cases of mastitis, milk spoilage, calving difficulties, and peri-calving conditions. Lists of diseases and brief descriptions were used to prompt the farmers to remember the diseases that occurred, especially for common diseases, such as mastitis, and diseases expected to be related to the vaccine pathogens, such as pneumonia, diarrhoea, and abortion. For the heifers, the diseases reported that were recorded were pneumonia, diarrhoea, navel ill, and all other diseases that included among others, tick borne diseases and skin diseases in the last one year. The disease outcomes recorded for the cows were mastitis, abortion, pneumonia, diarrhoea, skin diseases, tick-borne diseases, uterine infection, and other disease. These reported disease outcomes were either attended by veterinary professionals, self-attended by the farmers, or in some cases left to heal without any intervention. The time limit for the disease outcomes was the last one year since the last visit.

For farm-level predictor variables, farm demographics and statistics were recorded, such as: age and education level of farmers; the size of the farm and proportion of land in fodder: attendance of farmer training sessions; and the proportion of income that the dairy enterprise contributed to the household income. We also recorded management practices of importance, such as disease prevention and welfare factors used: feed types and feeding methods used, especially during the cow transition period, and during different seasons; deworming; seeking of veterinary services; pregnancy checking; mastitis prevention; status of stalls; and the sources of animal replacements. The animal-level factors recorded included: identification; age; breed; parity; breeding status; breeding history; BCS; body weight; shoulder height; physical examination information; cow’s subclinical mastitis status (based on CMT results), cow’s current milk production status; and vaccination status. The definition of heifer was nulliparous cattle (never calved), and cow was defined as those with at least one calving.

### 3.3.4 Statistical analysis

Descriptive statistics were used to determine the self-reported frequency of diseases among the two cohorts of cattle during the last year after vaccination, along with prevalence of disease at the time of the visit based on physical exams. Chi square testing was done to compare the significance of reported disease between the vaccinated and cohort groups. The planned outcome variables for the modeling were the number of reported diseases at the individual animal level within the last 12 months. For heifers, the frequency of reported disease was low, and therefore the outcome was dichotomized into no disease versus at least one disease event reported for the last 12 months. With more disease events, the cow outcome remained the number of disease event reported for the last 12 months. The physical examination findings during the study visit were not included in the outcome variable because there was very little clinical disease at the study visit.

With these outcome variables, mixed effects logistic regression models for heifers were developed to determine the risk factors associated with the dichotomized disease outcome. Conversely, mixed effects Poisson regression models for cows were developed to determine the risk factors associated with the disease count outcome. For both modeling processes, univariable mixed logistic and Poisson regression models were initially used to determine associations with the disease outcome for heifers and cows, respectively, to determine the predictors eligible for multivariable modeling. Linearity of continuous predictors was assessed by plotting them against the log odds of the outcome and through "best" fitting fractional polynomial calculations. For some continuous variables, exploration to categorize or divide into binary variables was done at points of meaningful interpretation or at the median point.

Univariable associations with p<0.15 were eligible for multivariable analysis. Candidate variables for multivariable models were checked for collinearity by means of variance-covariance matrix of the estimators (VCE). Multicollinearity was considered to be present if VCE> 5 for any pair (Dohoo et al., 2009). Among the collinear variables, the one that had more biological plausibility was left in the model. Mixed multivariable regression was conducted to determine factors associated with the diseases outcome, while controlling for possible confounding among model variables (based on model coefficients changing by more than 20% with the addition of a variable). The final models were built using backward stepwise elimination to retain those variables which had a P-value ≤0.05. Biologically plausible pair-wise interactions between significant variables from the final models were assessed.

The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was used to evaluate logistic model performance for the heifers. Hosmer-Lemeshow test was used as a test for the cow model goodness-of-fit. Residual and influential analyses were also used to assess the reliability of the models, as described by Dohoo (2009). The clustering effect within farm for the Poisson final model was determined using inter-cluster coefficient (ICC) exact calculation (Austin et al., 2018).

### 3.3.5 Sample size calculation

Sample size calculations were determined assuming 5% and 20% type 1 and 2 errors, respectively. It was assumed that there would be a beneficial vaccination effect lowering the reported disease occurrence for the cows and heifers during the year of follow-up. The unvaccinated group was assumed to have 47% reported disease, which was the reported prevalence of BVDV infection recorded in a related study two years earlier (VanLeeuwen et al. 2021). The proportions are drawn from a seroprevalence study due to limitation in the availability of disease prevalence or incidence research in a similar set-up of SHD farms. The vaccinated cohort of heifers were predicted to have the larger reduction in reported disease occurrence at 50% due to a higher proportion of vaccination in the heifers than the cows. For the cows, the reduction of reported disease occurrence in the vaccinated cohort was assumed to be 30% since many were left out for being pregnant or being recently served. The required two-tailed sample size was calculated using Fleiss’ formula for cows and heifers (Fleiss et al., 1980). The total calculated minimum sample size for heifer and cow cohorts was 134 and 290 respectively.

**n** = {*z***α***0.05* /2√(m+1) p(1-p) + *z***β**√(mp1(1-p1)+[p2(1-p2)]}2/m(p1-p2)[2](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7190503/#bib0002)

**p** = (p1+p2)/m+1

**n** = Sample sizeforheifers or cow data

**Zα** *=* 1.96 Z score for an error of 5% and CI of 95%

**Zβ** *=* 0.84 Z score for power of 80%

**m =** Number of cohort-negative animals per cohort-positive animal (assumed 1:1)

**p1 =** The probability of event in non-vaccinated animals (0.47)

**p2=** Probability of event in vaccinated animals (0.31 for cows and 0.24 for heifers)

## 3.4 Results

### 3.4.1 Descriptive results

The final dataset included 441 cows from 194 smallholder dairy farms, with the two largest sampled farms having 11 and 16 cows. Most of the farms (70%) had only 1 or 2 cows participating in this study, with the mean and median number of study cows per farm being 2.3 and 2, respectively. The age of cows sampled ranged between 2.5 and 17 years, with the mean age being 6.9 years. The most common breed was Holstein-Friesian at 58.3%, followed by Ayrshires at 20.2%, with the rest being other breeds, such as Jersey and Zebu. The study cows had a weight range of 166 to 643 kg, with a mean of 350.5 kg. Cows’ shoulder height was between 100 centimeters and 146 cm, with a mean of 121.5 centimeters. There were 204 (46.3%) reportedly pregnant cows in the study at the time of our visit 12 months after vaccination, with 44.2% and 46.5% being among the unvaccinated and vaccinated groups, respectively. The remaining 237 cows were either not bred or the owner did not have the breeding record available to confirm a breeding date. Pregnancy status was confirmed by local animal health personnel in some cows that were over 3 months since their service, but other cows had only failed to show subsequent heats after service.

There were 145 nulliparous heifers within 100 smallholder dairy farms in the final dataset, with the largest sample being from two farms having 5 and 6 heifers. A majority (70%) of farms had one heifer sampled, while 20% had 2 sampled heifers. The age of heifers sampled ranged between 16 months and 60 months, with the mean age being 27.4 months. The most common breed in the farms was Holstein-Friesian at 71.7%, followed by Ayrshires at 15.2%, with the rest being other breeds, such as Jersey and Zebu. The weight of the heifers ranged between 87 and 473 kg, with a mean of 263.5 kg. Heifers’ shoulder height was between 87 and 138 cm, with a mean of 113.6 cm.

For heifer reproductive performance, 54 (37.2%) of the heifers had been bred, with 41 (75.9%) of them being in the vaccinated cohort and 36 (66.6%) being over 2.5 years old. During the study visit, 44 (31.0%) of the heifers were reported as pregnant, using a similar pregnancy status confirmation process. There were more reportedly pregnant heifers among the vaccinated cohort at 38 (45.8%) than in the unvaccinated cohort at 14 (23.7%). The vaccinated cohort had 85 (58.6%) heifers on 60 farms while the unvaccinated cohort had 60 (41.4%) heifers on 40 farms (Table 3.1). There were 43 heifers (29.7%) with at least 1 disease reported during the last 12 months. Among the 43 heifers with at least one reported disease, 27, 12, 3 and 1 heifers had 1, 2, 3 and 4 reported diseases, respectively. The most reported disease condition among the heifers were pneumonia and diarrhoea at 13.8% and 11.7%, respectively.

Among the heifers, 21.2% of the vaccinated cohort had a reported disease, while 41.7% of the unvaccinated cohort had a reported disease in the last 12 months. On a Chi-squared test, there was a significant difference in reported disease for pneumonia, diarrhoea, other diseases, and the overall disease outcome for the last 12 months in heifers. For the heifers, the individual diseases and overall disease binary outcome proportion showed a reduction in the vaccinated cohort compared to the unvaccinated cohort, as expected (Table 3.2. Other diseases, which included tick-borne infections, was most reported, followed by pneumonia. During the data collection visit, the vaccinated cohort had a mean BCS of 2.63 while the unvaccinated cohort had a mean BCS of 2.46.

For the cows, the vaccinated cohort had 226 (51.2%) on 97 farms, while the unvaccinated cohort had 215 (48.8%) on 97 farms (Table 3.2). During the study visit, 81.9% (361) of the cows were lactating, with the rest being dried off due to late pregnancy. The mean milk production for the 80% (181) of the vaccinated cohort that was being milked was 7.3 kilograms of milk per day, while the unvaccinated cohort had 83.7% (180) being milked, with a mean production of 8.7 kilograms of milk per day. The days in milk for the cows in this study had a mean of 317 days and a range of 1 to 1293 days among the 335 cows with available records of calving date. The vaccinated cohort had a mean of 339 days while the unvaccinated cohort had a mean of 294 days. The cow vaccinated and unvaccinated cohorts had a mean BCS of 2.02 and 2.20, respectively.

The most reported disease condition among the cows was pneumonia at 27.4%, followed by tick-borne diseases at 25.4%, with the least reported diseases being abnormalities on physical examination findings at 4.1% and abortion at 4.8%. Among the 292 cows (66.2%) that had at least one reported disease within the last 12 months, 53.5% were in the vaccinated cohort, while 79.5% were in the unvaccinated cohort. For the cows, most of the reported disease conditions were proportionally less frequent in the vaccinated than unvaccinated cohort even when not significantly different. However, for abortions, skin and other diseases and abnormal physical examination conditions, including CMT results, there was no difference between cohorts.

### 3.4.2 Factors associated with presence of reported disease in the last 12 months among heifers

Table 3.3 shows the eleven out of twelve variables univariably associated with reported disease presence during the last 12 months for the heifers (P-value<0.15). Eleven variables were farm-level, while vaccination status and BCS were animal-level factors. Low BCS of less than 2.25 had a protective association with disease compared to scores above 2.25 categories. Five variables had a protective association, while 7 variables had a positive association to presence of reported disease.

The final multivariable logistic regression model (Table 3.4) had nine variables significantly associated with reported disease presence in the last 12 months at a P-value<0.05. The model included four protective factors and five positively related to disease likelihood. Vaccinated heifers were 0.11 times as likely to have a reported disease within the last 12 months compared to the unvaccinated heifers. Heifers on farms managed by a woman older than 55 years of age were 0.23 as likely to have had a reported disease in the last 12 months than heifers on farms managed by younger women. Heifers on farms with over 2 acres of land were 0.13 as likely to record a disease within the last 12 months than heifers on smaller farms. Heifers on farms feeding dry season Napier grass that was less than 1 meter in height for the last 12 months during the dry season were 6.5 times more likely to have a reported disease within the last 12 months compared to heifers on farms feeding taller Napier grass, among those using Napier grass. Heifers on farms whose dairy cattle stalls had concrete floors were 0.02 times as likely to have a reported disease within the last 12 months compared to heifers on farms with other types of floors.

Heifers on farms that had lost (by death) a cow vaccinated with a multivalent vaccine including BVDV within the last 12 months were 8.9 times more likely to have a reported disease within the last 12 months, compared to the heifers on farms that did not have deaths of vaccinated cows. Heifers on farms that buy replacement heifers were 3.3 times more likely to have a reported disease within the last 12 months compared to heifers on farms that raised their replacements. Heifers above 30 months of age had 6.1 times more likely to have reported disease in the last 12 months compared to heifers below 30 months of age. Body condition score was categorized into 3 categories based on the linear trend function and showed an increase in the likelihood of reported disease with higher BCS. Compared to the baseline BCS of 2.25 and below, heifers with a moderate BCS of 2.25 to 2.75 were 3.9 times more likely to have a reported disease in the last 12 months, while heifers with a BCS of 3 and above were 10.7 times more likely to have had a reported disease within the last 12 months. The farm random effect was very low, with the the model having no difference with multivariate model without farm random efffect, therefore it was not used.

### 3.4.3 Factors associated with counts of reported disease in the last 12 months among cows

There was a total of twenty-three variables univariably associated with the count of reported disease in the 441 cows, 19 being binary, 2 being categorical, and 2 being continuous variables (Table 3.5, 3.6, and 3.7). Among these variables, 12 were protective while the rest were associated with higher reported disease counts for the last year. The final multivariable mixed effects Poisson model (Table 3.8) had nine binary variables and an interaction among two of the variables significantly associated with reported disease count in cows in the last year. Six of the variables were protective while three variables were associated with more risk of reported disease count. The model assessed random effects at the farm level, with a random coefficient of 0.019.

Feeding grass weeds was associated with higher reported disease counts in the last year with an incidence risk ratio (IRR) of 1.29 (i.e. 29% higher rate compared to not feeding grass weeds). Farms that had more than two reported diseases at the farm level had higher reported disease counts for their cows within the last year, at an IRR of 1.41, compared to cows on farms with 2 or fewer reported diseases. Cows that had over three calvings had higher reported disease counts for the last one year, at an IRR of 1.34, compared to cows with 3 or fewer calvings. Farms that practiced milking of mastitis cows last had lower reported disease counts in that last year, at an IRR of 0.67, compared to farms not milking mastitis cows last.

Farms with more than six cows during the time of the visit had reported lower disease counts in their cows in the last year, at an IRR of 0.80, compared to cows on farms with five or fewer cows. The cows that only needed a single breeding in the last year had lower reported disease counts in the last year, at an IRR of 0.78, compared to cows requiring multiple breedings to get pregnant. Cows with a BCS above 2.25 had lower reported disease counts in the last year, at an IRR of 0.81, compared to cows with a lower BCS.

Pregnancy checking status for the last 12 months as a cow-level factor and vaccination status had a significant interaction, with both factors having a protective effect at the univariable association. The effect modification between the two variables is presented in Figure 3.1. The reported disease counts for the last year for vaccinated cows that were pregnancy checked and those not pregnancy checked were not different graphically. The non-vaccinated cows that were not pregnancy checked had higher reported disease counts during the last year than non-vaccinated cows that were pregnancy checked. The random effect within farm for the Poisson final model determined using ICC exact calculation was 0.019, suggesting that the correlation between disease counts of two cows in the same farm is quite low.

### 3.4.4 Model evaluation

For the final cow Poisson regression model, the Pearson’s goodness-of-fit test showed no lack of fit at (p=0.146). There was no evidence of multicollinearity between the explanatory variables, with all correlation matrix coefficients of the Poisson model being less than 0.5. There were only twenty-six Anscombe residuals outside the -2 and 2 limits with 13 outside -3 and 3. The twenty-six larger Anscombe residuals represented about 5% of the 441 total observations; therefore, there was no concern for extreme observations.

The heifer multivariable model evaluation was done without controlling for clustering due to the very low random effect and the similarity of odds ratios with and without random farm effects. The final heifer logistic regression model’s Hosmer–Lemeshow goodness-of-fit test showed no lack of fit at (p= 0.13). The final heifer logistic regression model had only five standardized residuals outside the -2 and +2 limits, with 2 outside -3 and 3. The five larger standardized residuals represented less than 5% of the 145 total observations; therefore, there was no concern for extreme observations. There was no evidence of multicollinearity between the explanatory variables, with all correlation matrix coefficients of the logistic model being less than 0.5. The overall model predictive ability was very good with a sensitivity of 80%, specificity of 84% and the area under the curve (AUC) for the receiver operating characteristic (ROC) curve being 90.3%.

## 3.5 **Discussion**

### 3.5.1 Associations between vaccination and the disease outcome in cows and heifers

The health benefits of BVDV vaccination have not been recorded adequately in scientific literature in the SHD context (Campbell, 2004; Perino & Hunsaker, 1997). The benefits of immunization against BVDV and the other vaccine components in the SHD sector in Kenya are evident in our retrospective cohort study by the associated reduction of reported disease in the last year among the vaccinated cohorts compared to non-vaccinates. This prevention and control of diseases by vaccination can lead to improvements in animal welfare and productivity, and therefore can be economically beneficial to the dairy cattle and farmers as seen in other vaccines (Mutua et al., 2019). In our study we had a total of 306 both heifers and cows out of the 909 cows and heifers vaccinated the previous year. In this study it was only possible to recruit a third of the cattle that had been vaccinated, however, this proportion was more than the minimum calculated sample size. The other reasons for not reaching all the previously vaccinated cattle were due to losses through sales, deaths, and some farms being outside of the study area. The low proportion of previously vaccinated recruited could be a source of selection bias; however, the results are still a valuable addition of information for future research.

Other studies have shown better health performance with respect to lower incidences of Bovine Respiratory Disease (BRD) when combined vaccines were used (Cusack et al., 2020). Similarly, vaccination of calves and heifers using a multivalent vaccine, which included BVDV, showed that there was lower cost of treatment in the vaccinated group (Kirkpatrick et al., 2008). While there were a number of components in the vaccine used in our study, this discussion will focus primarily on the BVDV component because there is ample evidence of BVDV infection in Kenyan smallholder dairy cattle (Okumu et al., 2019; VanLeeuwen et al., 2021). Infectious bovine rhinotracheitis (IBR) having been reported in same area of Meru, recently (Kipyego et al., 2020); there could, therefore, be some benefits of the vaccination related to the prevention and control of IBR.

In heifers, our study established that in the SHD context, vaccination was significantly associated with reduction of pneumonia, diarrhoea, and other diseases reported in the last year in our univariable analyses, and overall reported disease in our multivariable modeling, accounting for the effects of other possible confounders. In cows from this study, vaccination was significantly associated with reduced pneumonia, diarrhoea, poor appetite, tick-borne disease, and uterine disease reported in the past year in our univariable analyses, and overall reported disease counts in our multivariable modeling, again accounting for the effects of other possible confounders.

A challenge study to assess the protection against BVDV subtype 2 by vaccination with an inactivated BVDV type 1 vaccine found that there was protection against clinical presentation of respiratory disease and diarrhoea upon experimental infection with BVDV subtype 2 (Makoschey et al., 2001). Another similar challenge study demonstrated cross-protection as well as reduced clinical manifestation in the vaccinated calves (Chen et al., 2020). Although these two challenge studies involved cattle younger than in our study, they demonstrate similar results to our findings, which showed less reported clinical syndromes of pneumonia and diarrhoea.

Multivalent vaccination, including BVDV, has been shown to have respiratory disease benefits in prevention of associated disease outcomes by virtue of the infection being immunosuppressive (Lemon & McMenamy, 2021). A study assessing the benefits of BVDV vaccination in cattle upon introduction to a feedlot found that mortality and morbidity of vaccinated calves was reduced significantly, and average daily weight gain improved significantly in calves where there was constant exposure to PI animals (Grooms et al., 2014). The vaccination of BVDV has been shown to reduce clinical diseases associated with BVDV infection’s immunosuppression (Newcomer et al., 2017), and feedlot calves are often stressed from transportation, auction handling, and the new environment at the feedlot. This was similar to the findings of a study assessing the prevalence of BVDV, Bovine herpes-1 (BHV1), neosporosis and leptospirosis, which showed that vaccination status at the herd level was protective to BHV1 (Barrett et al., 2018).

Our study showed no significant difference for reported abortion between the vaccinated and the unvaccinated group; however, the overall number of reported abortions was very low. There was also a significant difference in the number of reported uterine infections among the vaccine cohorts. Assessing the efficiency of BVDV vaccination in preventing reproductive disease in cattle elsewhere showed overall better reproductive health performance in vaccinated cattle (Newcomer et al., 2015). The improvement of reproductive performance from BVDV vaccination can be considered an indicator for good health performance, as demonstrated by less reproductive diseases in a Portugal study (da Silva et al., 2019).

### 3.5.2 Other factors associated with the disease outcome in heifers

In the final multivariable logistic model, the heifers on farms where the woman’s age was over 55 years had lower odds of reported disease than heifers where the woman was younger. Older female farmers may have more experience in running a dairy enterprise, and a majority of smallholder dairy farmers in Kenya are female (Gitau, 2013; J. A. VanLeeuwen et al., 2012). A study of Swedish herds showed that the age of farmers was associated with cow longevity; however, the study did not directly link the longevity to health performance (Alvåsen et al., 2018). A study in Ethiopia reported that less experienced workers and owners had farms with higher mortalities and morbidities (Asmare & Wubshet, 2016).

Heifers on farms that owned more than two acres of land had lower odds of reported diseases than heifers on farms that had a smaller land size. Most farmers depend on farmed fodder, and less land potentially means fodder shortages, especially in the dry seasons, likely leading to poorer nutrition and therefore more disease. Limited land size has been shown to be related to more incidences of diseases in SDH in Ethiopia (Mekonnen et al., 2006). A study assessing the performance of SDFs in an econometric approach found that land size was a very important factor in overall milk productivity and farm efficiency (Mugambi et al., 2015).

The heifers on farms that fed Napier grass less than one meter in height during the dry season had a higher odds of reported disease for the last twelve months. Napier grass is a very important fodder for dairy cattle in the SHD farming system, especially under intensive and semi-intensive management, and is grown by over 70% of SHD dairy farmers in Kenya (Kabirizi et al., 2015). The optimum height for best quantity and quality of nutrition of Napier grass is 130 to 140 centimeters (Muia et al., 1999). It has been shown that the nutritive values of Napier grass reduce beyond the optimum height (Bayble et al., 2007; Haryani et al., 2018). We can attribute the cutting and feeding of short Napier grass in the dry season to the likelihood that the Napier grass took longer to grow with the drought, requiring harvesting at less than one meter, due to dry season scarcity of fodder biomass (Maleko et al., 2019; Wambugu et al., 2011).

There was a higher odds of reported disease in heifers from farms that had lost (by death) a vaccinated cow or heifer by death in the last year. For clarity, the vaccinated animal that died was not part of this statistical analysis because only vaccinated cattle that were still alive on the farm at the time of the study were in our final dataset. Therefore, this variable is referring to other cattle on the farm that were vaccinated but died since the vaccination, which may or may not be related to the vaccination (there was no record of the cause of death on most farms). This farm-level variable may be an indicator of disease pressure on the farm, which appears to be higher on farms with heifers with higher odds of disease. Farms that reported a loss to death of a BVDV vaccinated cow had a similar mean number of reported diseases for both the vaccinated and non-vaccinated groups for the last year. Further research should explore this finding.

There were lower odds of reported disease in heifers kept on farms rearing cattle on concrete floors than farms where heifers were on other forms of flooring, such as earth or wood for the last year. This finding can be attributed to better hygiene from possible better drainage and ease of cleaning on concrete. Similar outcomes have been reported by other studies showing associations between diseases and poor hygiene related to type of floors (Dutta et al., 2020; Islam et al., 2020; Kimeli et al., 2019; Mostafa & Mahran, 2016). Conversely, a recent study assessing the welfare of dairy calves in Meru, Kenya, found that concrete floors were associated with dirtier stalls and poor health status for calves (Kathambi et al., 2018). Additional research is needed in this area to clarify these conflicting results.

The heifers on the farms that bought replacements had higher reported disease odds for the last year than farms that did not buy replacement heifers. It has been shown in Kenya that many zero-grazing farms depend on the free-grazing farms to acquire replacement heifers (Bebe, 2008). Replacements are a potential source of disease entry into farms, especially more likely in farms that may not be adhering to strict biosecurity practices of disease prevention (Dutta, 2016). A recent study in Nakuru, Kenya, showed that the introduction of cattle in dairy farms was associated with higher Lumpy Skin Disease (LSD) incidence during outbreaks (Kiplagat et al., 2020).

In our study, the heifers which were over 30 months in age had higher odds of reported disease within the last year than the younger heifers. This finding can likely be attributed to both length of exposure to diseases by virtue of higher age as well as the older heifers being kept together with the cows. It has been reported elsewhere that at advanced ages cattle have higher risk of disease than at a younger age (Lasser et al., 2021; Ruprechter et al., 2018). A study investigating integrated leptospirosis outbreak management in dairy herds of Padua, Italy reported better immunocompetence with age in calves less than one year of age with respect to protective antibody titers (Mughini-Gras et al., 2014).

For the heifers in our study, there was an unexpected finding of higher categories of BCS being associated with higher odds of reported disease. In heifers, BCS can vary with age, seasonal feed availability, prioritization of feeding available high-quality feeds to animals with higher nutritional requirements (e.g. better feed allocated to cows giving milk versus nulliparous heifers), and diseases in the past (Chebel et al., 2018; Gallo et al., 1996; Gearhart et al., 1990; Roche et al., 2009). The reasons for the heifers with higher BCS in our study being associated with higher odds of reported disease must be related to the other BCS factors other than disease. Further research is needed to clarify this finding.

### 3.5.3 Other factors associated with the disease outcome in cows

Feeding grass weeds was associated with more reported disease counts in the last 12 months, which can be attributed either to farms having less feed supply or poor feed quality from the alternative source of feed. Feeding weeds and other grasses harvested from the gardens and roadsides has been reported as a common practice among farms facing feed resource constraints among SDFs in most areas of Kenya and Ethiopia (Duguma & Janssens, 2016; Kiptot et al., 2015; Lukuyu et al., 2011; Njarui et al., 2016). However, those studies did not relate the feeding of weeds to any health outcome. Feed challenges have been associated with more health challenges in the SHD system (Ajak et al., 2020; Lanyasunya et al., 2005).

Farms that had more than two reported diseases also had more reported disease counts per individual cow in our study. This farm-level variable association can be attributed to disease pressure in the herd, where diseases can easily be transmitted between individuals. A study in the Hawassa region of Ethiopia found that there were more diseases reported in animals which had more diseases at the farm level (Debebe & Haben, 2020). A study in Irish beef herds showed that herd-level pathogen status was an important determinant for exposure to more and other different pathogens at the cow level (Barrett et al., 2018).

There were higher reported disease counts for the last year in cows that were beyond third parity versus younger cows. This finding may be related to higher milk production and associated reproductive stress, making the cows more susceptible to more disease. Many studies elsewhere have reported higher disease prevalence or incidence in higher parity or aged cattle for both infectious and non-infectious conditions in dairy cattle (Abera et al., 2019; Lasser et al., 2021; Ruprechter et al., 2018). A study assessing the reasons for culling dairy cattle in Tigrey, Ethiopia, also showed that there was a close relationship between age, diseases, and parity above second calving cows, making farmers voluntarily cull their older cows (Idesa & Aman, 2021).

In our study, there was a lower count of diseases reported in cows when the farms practiced milking mastitis cows last. A study in Kajiado and Embu counties of Kenya also showed that milking a cow last was an important factor for lower prevalence of mastitis reported (Mbindyo et al., 2020). Good milking practices have been shown to lower the presence of mastitis and thus better health performance in dairy cows in Malawi (Tebug et al., 2012). Cows in herd sizes larger than six cattle were associated with lower reported disease counts in the last year than cows in smaller herds in our study. Other studies have reported the opposite, of more health challenges with an increase in herd size (Barrett et al., 2018; Debebe & Haben, 2020; Ghebremariam et al., 2018; Kiplagat et al., 2020). This finding in our study may be due to the possibility that farmers with more animals have had more experience in keeping cattle and thereby, better disease preventive practices. This finding requires further investigation.

The cows that were bred once within the previous year had lower reported disease counts for the last year than cows requiring more than one breeding. Cows with infectious diseases are likely to have reduced conception and therefore be repeat breeders (Wathes et al., 2020). Therefore, this variable is a good example of reverse causation where it is an indicator of less disease stress for the last year. In contrast to the heifer results, body condition score above 2.25 was associated with lower diseases reported for the last year in cows. Having a better body score during the visit can be attributed to better nutritional management of the cows (Tienken et al., 2015) and better health performance. Other studies have shown that good body condition score is positively related to good health outcomes (Roche et al., 2009). A study assessing the relationship between BCS and health trait heritability in Canadian Holstein dairy herds found that there was a 0.73 correlation between mastitis and low body condition score (Loker et al., 2012). It has also been reported that there is a negative genetic correlation between BCS and other health disorders in first lactation cows (Koeck et al., 2012).

Vaccination status and cows that had been pregnancy checked within the last one year interacted significantly in the final reported disease count model, and both variables had a protective relationship with disease counts (Fig. 3). Whether pregnancy checking was associated with reported disease counts appeared to depend on the vaccination status. Not practicing pregnancy checking in cows not being vaccinated had the highest disease counts reported within the last year. In vaccinated cows, there was no difference in disease counts with pregnancy checking. Pregnancy diagnosis can detect breeding failure from a disease origin, and therefore this practice may be an indicator of better management (Abdullah et al., 2015; Mohanty et al., 2014; Waldner, 2005).

### 3.5.4 Study limitations

Because of the infrequent occurrence of disease on the clinical examinations at the follow-up visits, this study was limited to observational reporting of management and diseases during the last 12 months by the farmers, which may be susceptible to recall bias with regards to the animal health history. This bias was complicated by the challenges of poor record-keeping, especially on the age, parity, and reproduction data. To reduce recall bias, the study could have had multiple follow-up visits; however, logistically this was not possible. Nevertheless, recall bias is more likely for potential exposures in a case-control study of a rare disease than for common diseases that may have occurred within a retrospective cohort study (Dohoo et al., 2009). Also, normally in a cohort study, the researchers would confirm lack of disease in the cohorts; however, this was not possible within our study because it was retrospective. However, when the vaccines were given, the administrators of the vaccines did confirm with the owners that they considered the cattle were healthy.

Another possible limitation is that we did not have serological antibody titer assessment for determining BVDV exposure prior to vaccination or proof of vaccine efficacy after vaccination during the follow-up visit. Determining the BVDV status in the beginning and following up only the BVDV-negative cattle could have added more control in the statistical analyses in separating the effects of previous BVDV disease and the effects of exposure after vaccination. However, even without those data, which would have been useful predictors in the final models, the study recorded the benefits of vaccination in the SHFs situation.

This study used a multivalent MLV vaccine, and it was not possible to separate the effects of each of the five pathogens on the disease outcomes during the 12 months of follow-up. However, because the vaccination program was conducted as part of a development project by a research partner, Farmers Helping Farmers, the multivalent vaccine was the logical commercial option utilized. Therefore, the disease benefits could be attributed not only to BVDV vaccination but any of the other pathogens in the vaccine, and possible exposures to these pathogens prior to vaccination.

### 3.5.5 Future Research

There is a need to study further the relationship between vaccine benefits and other methods of disease prevention to determine the success of integration of vaccinations with other disease prevention programs in the smallholder dairy system, such as enhanced biosecurity. There is also a need to investigate BVDV vaccine benefits in seronegative cattle cohorts to determine the true protective capacity for vaccination in the SDFs system. Future investigations could also integrate antibody titer trend checks at multiple times during the follow-up to assess the immunocompetence period and its associated factors in the SDFs system in Kenya. It would also be good to investigate the benefits of vaccination on the key dairy farming productivity outcomes of reproduction and milk production controlling for other factors affecting those outcomes, such as nutritional management and BCS.

## 3.6 Conclusions

The study established that vaccination was significantly associated with reduction of overall disease, as well as specifically pneumonia and diarrhoea in both the cows and heifers reported in the last year. In the heifers, there was a significant association with other reported diseases, which included any other conditions such as tick-borne diseases and skin diseases reported for the last year. Additionally, vaccination in cows was significantly associated with reduced poor appetite, tick-borne disease, and uterine disease reported for the past year.

In cows, the study also determined other factors associated with lower reported disease counts in the last year were: farms milking mastitis cows last; farms with more than six cows; cows needing only one breeding, and with BCS above a score of 2.25. Higher reported disease counts for the last year in cows was associated with: farms reporting more than two disease conditions; farms feeding grass weeds; and cows being third parity or higher. In an interaction variable, practicing pregnancy checking was associated with lower reported disease counts, but only among vaccinated cows.

For heifers, the other factors associated with reduced reported disease odds in the last year were: farms where a women farmer was over 55 years of age; farms with over 2 acres of land; and heifers kept on concrete floor structures. Increased reported disease odds in heifers over the last year was associated with: farms cutting and feeding Napier grass less than 1 meter tall in the dry season; farms that lost a BVDV-vaccinated animal within the last year; farms that bought replacements from other farms; and heifers that were over 30 months of age and having a BCS above 2.25.

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Table 3. 1: Reported disease proportions in the last 12 months and physical exam findings for the vaccinated and unvaccinated cohorts in heifers on smallholder dairy farms in Kenya

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Disease variable** | **Unvaccinated n=60** | | **Vaccinates n=85** | | **Chi 95%** | **Overall n=145** | |
| **Number** | ***%*** | **Number** | ***%*** | **P-value** | **Number** | ***%*** |
| Reported Pneumonia12\* | 15 | *25.0* | 5 | *6.0* | **0.001** | 20 | *14.0* |
| Reported Diarrhoea12\* | 11 | *18.0* | 6 | *7.0* | **0.037** | 17 | *12.0* |
| Reported Other Diseases 12\* | 16 | *26.7* | 11 | *13.0* | **0.037** | 25 | *17.0* |
| Physical exam 1 | 3 | *5.0* | 4 | *5.0* | 0.935 | 7 | *5.0* |
| Overall Reported Disease 12\* | 25 | *42.0* | 18 | *21.0* | **0.008** | 43 | *29.7* |

**1**Status during the study visit, 12 months after vaccination

**12**Status for the 12 months prior to the study visit

\* Significant difference in disease outcome between vaccinated and unvaccinated cohorts

Table 3.2: Reported disease proportions in the last 12 months and physical exam findings for the vaccinated and unvaccinated cohorts in cows on smallholder dairy farms in Kenya

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Disease variable** | **Unvaccinates n=215** | | **Vaccinates n=226** | | **Chi 95%** | **Overall n=441** | |
| **Number** | ***%*** | **Number** | ***%*** | **P-value** | **Number** | ***%*** |
| Reported Mastitis 12 | 51 | 23.7 | 41 | 18.1 | 0.149 | 92 | 20.9 |
| Reported Abortion 12 | 9 | 4.2 | 12 | 5.3 | 0.580 | 21 | 4.8 |
| Reported Pneumonia 12\* | 80 | 37.2 | 41 | 18.1 | <0.001 | 121 | 27.4 |
| Reported Diarrhoea 12\* | 45 | 20.9 | 6 | 2.7 | <0.001 | 51 | 11.6 |
| Reported Poor Appetite 12\* | 42 | 19.5 | 21 | 9.3 | 0.002 | 63 | 14.3 |
| Reported Tick-borne disease 12\* | 70 | 32.6 | 42 | 18.6 | <0.001 | 112 | 25.4 |
| Reported Skin Disease 12 | 12 | 5.6 | 13 | 5.8 | 0.900 | 25 | 5.7 |
| Reported Uterine Disease 12\* | 35 | 16.3 | 16 | 7.? | 0.002 | 51 | 11.6 |
| Reported Other diseases 12 | 18 | 8.4 | 12 | 5.3 | 0.202 | 30 | 6.8 |
| Physical exam 1 | 6 | 2.8 | 12 | 5.3 | 0.182 | 18 | 4.1 |
| California Mastitis Test (CMT) 1 | 49 | 22.8 | 54 | 23.9 | 0.784 | 103 | 23.4 |
| Overall Reported Disease 12\* | 171 | 79.5 | 121 | 53.5 | <0.001 | 292 | 66.2 |

**1**Status during the study visit, 12 months after vaccination

**12**Status for the period of 12 months prior to the study visit

\* Significant difference in disease outcome between vaccinated and unvaccinated cohorts

Table 3.3: Descriptive statistics for predictor variables with a P-value less than 0.15 for associations with overall dichotomized reported disease for the last 12 months among 145 heifers on 100 smallholder dairy farms in Kenya

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Predictor variable** | **Proportion of the Variable that is Yes % (#/#)** | **Reported disease in heifers when Variable is Yes (%) (#/#)** | **Reported disease in heifers when Variable is No (%) (#/#)** | **Odds Ratio** | **Odds Ratio 95% CI** | **P-value** |
| Woman age over 55 years 1 | 42.3 (41/97) | 15.3 (9/59) | 38.3 (31/81) | 0.179 | 0.041, 0.784 | 0.022 |
| Owned over 2 acres land 1 | 29 (29/100) | 11.4 (5/44) | 37.6 (38/101) | 0.106 | 0.018, 0.634 | 0.014 |
| Own over 1 acre land in fodder 1 | 37 (37/100) | 21.4 (12/56) | 34.8 (31/89) | 0.280 | 0.063, 1.240 | 0.094 |
| Dairy training or seminar 12 | 75 (75/100) | 34.2 (38/111) | 14.7 (5/34) | 3.636 | 0.846, 15.620 | 0.083 |
| Fed dry season Napier <1 Meter 12 | 59 (59/100) | 38.2 (34/89) | 16.1 (9/56) | 4.301 | 1.199, 15.428 | 0.025 |
| Cow died on the farm 12 | 15 (15/100) | 45.8 (11/24) | 26.5 (32/121) | 3.235 | 0.706, 14.832 | 0.131 |
| Lost a vaccinated animal 12 | 13 (13/100) | 57.1 (12/21) | 25.0 (31/124) | 7.467 | 1.304, 42.741 | 0.024 |
| Zero-grazing 1 | 38 (38/100) | 42.7 (21/49) | 22.9 (22/96) | 3.402 | 0.993, 11.649 | 0.051 |
| Buys replacement heifers 1 | 40 (40/100) | 19.8 (17/86) | 44.1 (26/59) | 6.491 | 1.373, 30.691 | 0.018 |
| Concrete floor type 1 | 10 (10/100) | 7.1 (1/14) | 32.1 (42/131) | 0.073 | 0.003, 1.458 | 0.087 |
| BVDV Vaccinated in 2018 1 | 58.6 (85/145) | 21.2 (18/85) | 41.7 (25/60) | 0.229 | 0.064, 0.824 | 0.024 |

**1** - Statusduring the study visit, 12 months after vaccination

**12** - Status for the 12 months prior to the study visit

**gp** - Global P-value for entire categorical variable association

**Y/N** - Yes /No Binary variable

**CI** - Confidence interval

BVDV – Bovine Viral Diarrhoea Virus

Table 3. 4: Multivariable logistic regression model results showing associations with overall dichotomized reported disease for the last 12 months among the 145 heifers on 100 smallholder dairy farms of Meru Kenya

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Predictor variable** | **Description** | **Odds Ratio** | **Odds Ratio 95% CI** | **P-value** |
| Woman age over 55 years 1 | Y/N | 0.23 | 0.07, 0.76 | 0.016 |
| Owned over 2 acres land 1 | Y/N | 0.13 | 0.03, 0.62 | 0.010 |
| Fed dry season Napier <1 Meter 12 | Y/N | 6.51 | 1.74, 24.35 | 0.005 |
| Lost BVDV vaccinated animal 12 | Y/N | 8.91 | 1.81, 43.79 | 0.007 |
| Concrete floor type 1 | Y/N | 0.02 | 0.002, 0.23 | 0.002 |
| Buys replacement heifers 1 | Y/N | 3.26 | 1.08, 9.84 | 0.036 |
| BVDV Vaccinated in 2018 1 | Y/N | 0.11 | 0.03, 0.44 | 0.002 |
| Heifer age over 30 months1 | Y/N | 6.11 | 1.47, 25.43 | 0.013 |
| Body condition Score 1 | 2 and below | Baseline | Baseline | 0.007gp |
| 2.25-2.75 | 4.00 | 1.05, 15.30 | 0.043 |
| 3 and above | 10.77 | 2.45, 47.33 | 0.002 |

**1**Statusduring the study visit, 12 months after vaccination

**12**Status for the 12 months prior to the study visit

**gp** Global P-value for entire categorical variable association

**CI** - Confidence interval

**Y/N** – Yes /No binary variable

BVDV – Bovine Viral Diarrhoea Virus

Table 3. 5: Descriptive results and mixed effects Poisson incidence risk ratio for reported disease in heifers when Variable is Yes ratio statistics for binary predictor variables with a P-value less than 0.15 for associations with overall reported disease count for the last 12 months

| **Predictor Variable** | **Variable Yes (%) (#/#)** | **Mean Count of Reported disease in cows when Variable is Yes** | **Mean Count of Reported disease in cows when Variable is No** | **IRR** | **IRR 95% CI** | **P-Value** |
| --- | --- | --- | --- | --- | --- | --- |
| Owned over 2 acres land (Y/N) 1 | 16.5(32/194) | 1.03 | 1.35 | 0.758 | 0.583, 0.986 | 0.039 |
| Dairy training (Y/N) 12 | 74.2(144/194) | 1.38 | 1.02 | 1.402 | 1.106, 1.777 | 0.005 |
| Fed high protein fodder (Y/N) 12 | 25.8(50/194) | 1.31 | 0.76 | 1.735 | **0.968, 3.109** | 0.064 |
| Fed grass weeds (Y/N) 12 | 20.1(39/194) | 1.54 | 1.21 | 1.308 | 1.038, 1.649 | 0.023 |
| Transition meal constant (Y/N) 12 | 37.5(61/160) | 1.44 | 1.19 | 1.239 | 1.001, 1.535 | 0.049 |
| Deworm pregnant cows (Y/N) 12 | 49.5(96/194) | 1.18 | 1.38 | 0.815 | 0.667, 0.996 | 0.046 |
| Veterinarians visit (Y/N) 12 | 90.2(175/194) | 1.35 | 0.29 | 4.819 | 2.357, 9.850 | <0.001 |
| Over 2 diseases in farm (Y/N) 12 | 49.0(95/194) | 1.49 | 1.02 | 1.514 | 1.239, 1.849 | <0.001 |
| Post-calving disease (Y/N)12 | 40.6(78/192) | 1.39 | 1.17 | 1.212 | **0.992, 1.482** | 0.060 |
| Mastitis cow milked last (Y/N) 12 | 85.6(166/194) | 1.23 | 1.54 | 0.748 | 0.580, 0.965 | 0.026 |
| Milk rejected (Y/N) 12 | 16.5(32/194) | 1.52 | 1.23 | 1.307 | 1.007, 1.697 | 0.045 |
| Breed AI only (Y/N) 12 | 69.1(134/194) | 1.20 | 1.47 | 0.807 | 0.655, 0.992 | 0.043 |
| Parity over 3rd calving (Y/N) 1 | 30.2(133/441) | 1.53 | 1.17 | 1.337 | 1.110, 1.610 | 0.002 |
| Pregnant (Y/N) 12 | 51.9(229/441) | 1.17 | 1.37 | 0.846 | **0.715, 1.023** | 0.087 |
| Last bred only once (Y/N) | 69.8(308/441) | 1.17 | 1.77 | 0.728 | 0.600, 0.882 | 0.001 |
| Constant milk last 7 days (Y/N) 1 | 79.3 (288/363) | 1.24 | 1.52 | 0.785 | **0.615, 1.002** | 0.052 |
| BCS over 2.25 (Y/N) 1 | 45.1(199/441) | 1.10 | 1.43 | 0.771 | 0.638, 0.932 | 0.007 |
| Does pregnancy check (Y/N) 12 | 33.3(147/441) | 1.06 | 1.39 | 0.752 | 0.608, 0.931 | 0.009 |
| BVDV Vaccinated in 2018 (Y/N)12 | 51.2(226/441) | 0.90 | 1.68 | 0.528 | 0.440, 0.633 | <0.001 |

**Y/N -** Binary variable code, Yes and No

**IRR -** Incidence risk ratio

**1 -** Statusduring the study visit, 12 months after vaccination

**12 -**Status for the 12 months prior to the study visit

BVDV – Bovine Viral Diarrhoea Virus

Table 3. 6: Descriptive results and mixed effects Poisson incidence risk ratio statistics for categorical predictor variables with a P-value less than 0.15 for associations with overall reported disease count for the last 12 months among the 441 cows on 194 smallholder dairy farms

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Predictor variable** | **Variable Category Proportion**  **% (#/#)** | **Mean Count of reported disease in cows for Variable category** | **IRR** | **IRR 95% CI** | **P-Value** |
| Lying posture in stalls 12\*\* | -- | -- | -- | -- | 0.009gp |
| *Lie down well* | 26.8 (52/194) | 1.62 | Baseline | Baseline | Baseline |
| *Lie down wrong* | 51.0 (99/194) | 1.14 | 0.721 | 0.580, 0 .897 | 0.003 |
| *No stalls* | 22.2 (43/194) | 1.21 | 0.747 | 0.566, 0 .986 | 0.040 |
| Other lying behavior 12\*\* | -- | -- | -- | -- | 0.011 gp |
| *Normal* | 26.3 (51/194) | 1.63 | Baseline | Baseline | Baseline |
| *Wrong* | 51.5 (100/194) | 1.15 | 0.722 | 0.580, 0.899 | 0.004 |
| *No stalls* | 22.2 (43/194) | 1.21 | 0.746 | 0.565, 0.986 | 0.039 |

**IRR –** Incidence risk ratio

**12**– Status for the 12 months prior to the study visit

**gp -** Global P-value for entire categorical variable association

\*\* - No stall category is not comparable to the other two categories because the behavior could not be recorded in the absence of housing structures needed for separated stalls for lying down.

Table 3.7: Descriptive results and mixed effects Poisson incidence risk ratio statistics for continuous predictor variables with a P-value less than 0.15 for associations with disease counts for the last 12 months among the 441 cows on 194 smallholder dairy farms in Kenya

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Predictor variable** | **Range** | **Number** | **Mean** | **IRR** | **IRR 95% CI** | **P-value** |
| Cow age in years 1 | 2.5-17 | 424 | 6.93 | 1.045 | 1.011, 1.080 | 0.008 |
| Farm mortality risk 12 | 0-0.67 | 194 | 0.07 | 1.967 | 0.888, 4.354 | 0.095 |

**IRR-** Incidence risk ratio

**1**Statusduring the study visit, 12 months after vaccination

**12**Status for the 12 months prior to the study visit

Table 3. 8: Multivariable Poisson regression model results showing associations with overall reported disease counts for the last 12 months among the 441 cows on 194 smallholder dairy farms in Kenya

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Predictor variable** | **Description** | **IRR** | **IRR 95% CI** | **P-value** |
| Fed grass weeds ***co*** | Y/N | 1.294 | 1.05, 1.59 | 0.015 |
| Over 2 diseases in the farm 12 | Y/N | 1.415 | 1.17, 1.71 | <0.001 |
| Parity over 3rd calving 1 | Y/N | 1.340 | 1.12, 1.60 | 0.001 |
| Mastitic cow milked last 12 | Y/N | 0.669 | 0.54, 0.84 | <0.001 |
| Herd size over 6 1 | Y/N | 0.796 | 0.66, 0.96 | 0.017 |
| Last bred only once 12 | Y/N | 0.778 | 0.65, 0.94 | 0.008 |
| BCS over 2.25 1 | Y/N | 0.813 | 0.67, 0.98 | 0.03 |
| Does pregnancy check ***i*** 12 | Y/N | 0.674***i*** | 0.52, 0.88***i*** | 0.003 ***i*** |
| BVDV Vaccinated ***i*** 12 | Y/N | 0.478 ***i*** | 0.38, -0.60***i*** | <0.001 ***i*** |
| 1Does pregnancy check & BVDV Vaccinated | Interaction | \*\* | \*\* | 0.021\*\* |

**Y/N -** Binary variable code, Yes and No

**IRR-** Incidence risk ratio

***1*** Statusduring the study visit, 12 months after vaccination

***12***Status for the 12 months prior to the study visit

***co*** Common practice not limited to the 12 months’ time prior to the study visit

***i*** Main effects of interaction variable output - should not be interpreted without considering the interaction

\*\*Interaction outputs - cannot be interpreted without considering the main effects

BVDV – Bovine Viral Diarrhoea Virus

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Figure 3. 1: Final multivariable model interaction between BVDV vaccination and pregnancy check and how they are associated with overall reported disease counts for the last 12 months among the 441 cows on 194 smallholder dairy farms in Kenya

BVDV – Bovine Viral Diarrhoea Virus

## Chapter 4: A randomized controlled trial of a multivalent vaccine including modified live Bovine viral diarrhoea virus on smallholder dairy farms in Kenya

## 4.1 Abstract

**Background:** Use of a multivalent bovine viral diarrhea virus (BVDV) modified live vaccine (MLV) is considered a beneficial prevention intervention to mitigate substantial cattle losses from infection. On Kenyan smallholder dairy farms (SDFs), there are limited BVDV control and prevention practices and lack of awareness on the benefits of vaccination for BVDV. The research objective was to determine the benefits of a multivalent vaccination including BVDV component in a randomized controlled trial on SDFs in Kenya.

**Methods:** The study recruited a total of 384 and 352 randomly selectedcows andheifers on 292 and 260 SDFs, respectively. On the first and second (12 months later) farm visits, through a questionnaire and animal examinations, we recorded the diseases reported in the last 12 months, along with other cow- and farm-level information. With random block allocation, 185 cows and 172 heifers were injected with a single dose of multivalent modified live vaccine including BVDV component (Pyramid® FP 5; Boehringer Ltd.), while 199 cows and 180 heifers were injected with a placebo. Mixed multivariable logistic and Poisson regression modeling were used for the heifers and cows, respectively, to determine factors associated with the reported disease in the last 12 months.

**Results:** The overall count of reporteddiseases in the last 12 months was the cow outcome of interest for the modeling, with a range between 0 and 6 diseases, with a mean of 1.4 diseases/cow. For the heifers overall, 34.1% (240/704) had at least one disease in the last 12 months, the heifer outcome of interest. There was also a significant reduction in overall reported disease in the last 12 monthsin the vaccinated group versus the placebo group on the second visit in both cows and heifers, adjusting for the reported disease on the first visit.

For the cows, the specific reported disease conditions that were reduced in the vaccinated group included: pneumonia, diarrhoea, anorexia, and uterine disease.

In the final cow model, there were lower reported disease counts in cows in the last 12 months associated with two variables, farms that bought in-calf replacement heifers (IRR= 0.80) and those with more than three acres of land (IRR = 0.81). There were higher reported disease counts in cows in the last 12 months associated with the following variables: farms which had more diseases in calves (IRR= 1.32), cows that had been bred within the last 12 months (IRR = 1.43) and weight in kilograms (IRR = 1.001). For categorical variables, there were higher disease counts for cows on farms which had reported one case of mastitis prior to the study (IRR=1.27) and those that reported two or more cases of mastitis (IRR=1.50) compared with cows on farms that did not report any case of mastitis. There were higher counts of reported diseases in cows on farms using different towels for udder cleaning (IRR = 1.00) and farms that did not use udder cleaning towels (IRR = 1.31) compared to farms using a single udder cleaning towel. The cow multivariable model also had four significant interactions between the following variables: 1) household income dependency on dairy above 50% and number of cows; 2) farms feeding maize silage and cow pregnancy status; 3) number of farms feeding extra supplement post-calving and cow body condition score (BCS); and 4) visit number with intervention group

For the heifers, the specific reported disease conditions that were reduced in the vaccinated group included pneumonia and other disease. In the final heifer model, higher likelihood of disease within the last 12 months was associated with: farms feeding grass silage (OR = 3.27), buying replacement heifers (OR = 2.45), heifers over two years of age during the visit (OR = 2.16), and Ayrshire and other breeds were (OR = 1.88 and 0.45), respectively, compared to Holstein heifers. Also, there was lower likelihood of disease in heifers on farms that fed at least 4 kg colostrum to calves within the first 12 hours of life (OR = 0.30) and heifers that had been bred within the last 12 months (OR = 0.31). There were two significant interactions in the heifer model: 1) number of diseases in cows for the last one year, and season during the visit; and 2) visit number and intervention group.

**Conclusions:** In both the cows and heifers, the vaccinated animals had lower reported disease than unvaccinated during the 1-year follow-up period than during the 12 months prior to the first visit. Furthermore, for the cows and heifers, there was less reported diseasesin the last 12 monthsin the vaccinated group versus the placebo group on the second visit, adjusting for the reported disease on the first visit. The multivalent MLV including BVDV component was shown to be highly beneficial in SDF herds.

**Key words:** infectious disease, prevention, vaccine benefits, smallholder dairy.

## 4.2 Introduction

Bovine Viral Diarrhoea (BVD) is an important disease responsible for substantial losses through morbidity, mortality, culling, and decreased health, welfare, productivity and reproductive performance in cattle populations worldwide (Richter et al. 2017; Scharnböck et al. 2018). Bovine Viral Diarrhea Virus infection is caused by a *Pestivirus* in the family *Flaviviridae* (Waltz 2008). Infection with BVDV can affect multiple body systems, including the respiratory, reproductive, and gastrointestinal systems, producing a wide range of clinical signs, but can also be asymptomatic in some cattle, depending on the virus strain and animal immunity (Flores et al., 2000; Passler et al., 2007). Immunosuppression from BVDV is also reported, making infected cattle more susceptible to another pathogen’s opportunistic establishment (Waltz 2008; Walz et al. 2020).

Vaccination for BVDV can be an important control strategy in the prevention of both its vertical and horizontal transmission, leading to significant health and reproductive benefits in herds (Newcomer et al. 2015; Newcomer, Chamorro, and Walz 2017; Walz et al. 2020). Experimental studies have additionally shown that multivalent modified live vaccines (MLV) including BVDV triggered higher protection compared to multivalent killed vaccine (KV) including BVDV (Downey-Slinker et al. 2016; Walz et al. 2020). The use of MLV vaccines have been shown to be safe and effective in the prevention of fetal infection and the consequent reduction of Persistently infected (PI) animals (Dubovi et al., 2000; Kovács et al., 2003; Walz et al., 2020). However, the losses caused by BVDV and prevented by vaccination have not been studied well in smallholder dairy farms (SDFs) in developing countries; therefore, there is a need for more studies to quantify the impact of the disease and benefits of disease mitigation through vaccination in this context (Pinior et al., 2017).

Most of the BVDV vaccines commercially available are multivalent combinations with other pathogens, typically including parainfluenza virus type 3, bovine respiratory syncytial virus (BRSV), and bovine herpes virus type 1. They therefore offer broader health benefits than BVDV only vaccines (Chowdhury et al., 2021; Cusack et al., 2020; Walz et al., 2018), but this makes it difficult to separate the benefits of the different antigen components (Walz et al. 2020).

Recent studies in Kenya utilizing antibody and antigen ELISA have shown high cattle- and farm-level prevalence of infection of BVDV and parainfluenza virus type 3 (Kipyego et al., 2020; Okumu et al., 2019; VanLeeuwen et al., 2021). However, there is very limited documented use of BVDV vaccine in the different systems of cattle populations in Kenya, particularly SDFs which make up 80% of the milk production by dairy farms in Kenya (Ettema, 2012; FAO, 2015). Therefore, the assessment of BVDV vaccination benefits in a study on Kenyan SDFs is needed. Our project objective was to determine associations between multivalent vaccination including BVDV and disease occurrence controlling for farm and animal factors among cattle on SDFs in Kenya in a randomized controlled double-blinded trial.

## 4.3 Materials and Methods

### 4.3.1 Study area and population

The study was done at Buuri and Naari areas of Buuri sub-county in Meru County, Kenya. Meru County is in central Kenya to the east of Mount. Kenya and lies on the equator and within longitudes 37° and 38° East. The county has a population of 1.55 million people and is about 7,000 square kilometers, with animal and crop agriculture being the main economic activity.

Farms that were members of the Buuri and Naari Dairy Farmers Cooperative Societies in May of 2019 formed the sampling frame for the trial. These cooperatives have an active partnership with Farmers Helping Farmers, the University of Prince Edward Island, and the University of Nairobi. In the Buuri area, there were no smallholder dairy farms that had ever been vaccinated with a BVDV vaccine in the past. In the Naari area, some farms had their cattle vaccinated in 2018 and in early 2019 in a related project, and the list of farms and animals that had been vaccinated was available for exclusion.

### 4.3.2 Farm and animal eligibility

Farm eligibility criteria for our study included: 1) being an active member of the Buuri or Naari Dairy Farmers Cooperative Societies; and 2) having cattle that had not been vaccinated against BVDV in the year 2018 and 2019. Animal-level eligibility criteria included: 1) female cattle being above 6 months at the time of vaccination; and 2) cows and heifers being not pregnant. These animal inclusion criteria were to avoid maternal antibody interference of vaccine efficacy in the youngstock, and to avoid abortions in the pregnant cattle. It was expected that vaccination should reduce disease occurrence among the vaccinated animals for diseases related to the vaccine pathogens (e.g. pneumonia, diarrhoea, abortion), and perhaps other diseases occurring due to immunosuppression from BVDV infection, such as mastitis and skin infections.

### 4.3.3 First visit data collection

The first farm visits occurred in May to December 2019. During the first visit, data were collected using the following methods (details provided below). First, using a questionnaire, the lead author collected information from the farms on reported disease occurrences that were recorded or could be remembered in the recruited cattle (cow-level) or all cattle (farm-level) for the last one year. Second, also using a questionnaire, farm management activities that could be related to the diseases of interest in the cattle were collected. Finally, physical exams were conducted by the lead author or an assistant on recruited animals for current diseases (e.g. California mastitis test (CMT), clinical signs of skin infections, pneumonia, uterine infection, etc.). Pregnancy status was determined by farmers’ records and rectal exams for cows bred more than 30 days before the visit to determine eligibility if animal were not pregnant.

For the reported disease data, lists of diseases and brief descriptions were used to prompt the farmers to remember the diseases that occurred, especially for common diseases, such as mastitis, and diseases expected to be related to the vaccine pathogens, such as pneumonia, diarrhoea, and abortion. For the heifers, the reported diseases in the last one year that we recorded were pneumonia, diarrhoea, and all other diseases, which included tick-borne diseases and skin diseases. For cows, the reported disease in the last one year included: mastitis, abortion, pneumonia, diarrhoea, skin diseases, tick-borne diseases, uterine infections, and other diseases. These reported disease outcomes were either diagnosed and treated by veterinary professionals, self-attended by the farmers, or in some cases, left to heal without any intervention.

For farm-level predictor variables, farm demographics were recorded, such as age and education level of farmers, the size of the farm, and proportion of land in fodder, attending farmer training sessions, and the proportion of income that the dairy enterprise contributed to the household income. We also recorded management practices of importance, such as: specific disease prevention and welfare management utilized (e.g., floor type, beddings and drainage): feed types and feeding methods used, especially during the cow transition period, and during different seasons; deworming frequency; seeking of veterinary services; pregnancy checking; mastitis prevention (e.g. teat dip use, single towel use for multiple cows); and sources of animal replacements. We also measured the cow comfort status of the stall bases (e.g. knee wetness test and knee impact test) (McFarland, 1991).

The animal-level factors recorded during physical exams included (where relevant): identification; age; breed; parity; breeding history; breeding status (based on rectal exam); body condition score (BCS); body weight (using a heart girth tape); withers height; subclinical mastitis status (based on CMT results); and cow’s current milk production status and udder hygiene score. Udder hygiene score followed the Cook and Reinemann assessment tool with a scale of 1 (clean) to 4 (>30% dirt covered) (Cook & Reinemann, 2007). Body condition score was done on a scale of 1 (lowest) to 5 (highest) using the Wildman scoring guideline (Wildman et al., 1982).

### 4.3.4 Intervention

Each trial animal received either the vaccine or a placebo (described below), which was randomly decided by picking a letter from a hat of either A (for vaccine) or B (for placebo). For each farm with multiple cows and/or heifers, there was an attempt to balance the number of cows and heifers getting either the vaccine or placebo by starting with either the vaccine group or placebo group on a farm, depending on which group had lower numbers of recruited animals at that point in time, as much as possible.

The vaccine group was vaccinated with a MLV (Pyramid® FP 5; Boehringer Ltd.) against four pathogens: BVDV, IBR virus, BRSV and parainfluenza virus type 3. The BVDV components in the vaccine included BVDV type 1 (Singer 1a cytopathic) and BVDV type 2 (296 cytopathic). The vaccine was stored in a refrigerator. It was removed from the refrigerator each day, reconstituted on the first farm of the day, kept in a cool box with ice packs and used that day, as recommended by the manufacturer. The vaccination was done through an injection of a 2 ml dose subcutaneously around the neck region. Approximately 10 vaccine doses were used on a normal day and about 5 vaccine doses on a day with less farm visits depending on logistics and distance covered.

The placebo group received a 2 ml injection subcutaneously of a liquid resembling the vaccine, formulated using 450 ml of normal saline, 45 ml of injectable multivitamin to give it color, and 5 ml of an antimicrobial (200 mg/ml sulfadoxineand 40 mg/ml trimethoprim) to suppress bacterial contamination.

### 4.3.5 Second visit data collection

After one year, we returned to the recruited farms to record the same three sets of data, for time-varying variables. For variables that do not change over time such as animal breed, these questions were omitted from the second visit information sheets. For logistics planning and consequences of COVID-19 movement restrictions, the flexibility to return to the farms for the second visit was maintained at plus (+) or minus (-) two months of the 12 months follow-up period. The delayed or early return only affected few (10%) farms at the end of data collection.

### 4.3.6 Data management and analysis

Information from the questionnaires and animal examination recordings was entered into Microsoft Excel - Office 2010 (Microsoft Corporation Washington, USA), error checked and cleaned, and then exported to Stata 17.0 (Stata Corporation TX, USA) for analysis. Descriptive statistics were utilized to determine the self-reported frequency of diseases among the two groups of cattle during the last year for each visit, along with prevalence of disease at the time of the visit, based on physical exams.

The planned outcome variables for the modeling were the number of reported diseases within the last 12 months. For heifers, since the frequency of reported disease was low, the outcome was dichotomized; no disease versus at least one disease event reported for the last 12 months. With more disease events, the cow outcome remained the number of disease event reported for the last 12 months. For both visits, the physical examination findings during the study visit were not included in the outcome variable because there was very little clinical disease at the time of the study visits.

With these outcome variables, mixed effects multivariable logistic regression models were developed for heifers to determine risk factors associated with the dichotomized disease outcome. Conversely, mixed effects multivariable Poisson regression models for cows were developed to determine the risk factors associated with the disease count outcome. The mixed effect modeling was done to control for random farm effect since a substantial number of farms had more than one cow or heifer recruited to the study. For both modeling processes, the clustering within visit for the cows and heifers were controlled by fixed effect of visit in the model.

For both modeling processes, univariable mixed logistic and Poisson regression models were initially used to determine associations with the disease outcome for heifers and cows, respectively, to identify the predictors eligible for multivariable modeling, using the proposed causal diagram in Figure 4.2 for guidance. Linearity of continuous predictors was assessed by plotting them against the log odds of the outcome, and through “best” fitting fractional polynomial calculations. Univariable associations with P-value<0.10 were eligible for multivariable analysis. Eligible variables for multivariable models were checked for collinearity by means of variance-covariance matrix of the estimators (VCE). Multicollinearity was considered present if VCE > 5 for any pair (Dohoo et al., 2009). For some continuous variables, exploration to categorize or dividing into binary variables was done at points of meaningful interpretation or at the median point. Among the collinear variables, the one that made more biological plausibility was left in the model. The clustering effect within farm for the Poisson final model was determined using inter-cluster coefficient (ICC) exact calculation (Austin et al., 2018).

The mixed multivariable regression modelling was conducted to allow for controlling of possible confounding among model variables (based on model coefficients changing by more than 20% with the addition of a variable). The final models were built manually and using backward stepwise elimination to retain those variables which had a P-value≤0.05. Biologically plausible pair-wise interactions between significant variables from the final models were assessed. The Poisson multivariable analysis was confirmed to be the most fitting count model through the Akaike's Information Criteria (AIC) and means Bayesian Information Criteria (BIC). The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was used to evaluate logistic model performance for the heifers. Hosmer-Lemeshow test was used as a test for the cow model goodness-of-fit. Residual and influential analyses were also used to assess the reliability of the models, as described by Dohoo et al. (2009).

### 4.3.7 Sample size calculation

Sample size calculations were determined assuming 5% and 20% type 1 and 2 errors, respectively. It was assumed that there would be a 50% beneficial effect of vaccination in lowering the disease occurrence during the year of follow-up, with the control group having reported disease in 47% of animals, which was the prevalence of BVDV infection recorded in a related study two years earlier (VanLeeuwen et al. 2021). The proportions are drawn from a seroprevalence study due to limitation in the availability of disease prevalence or incidence research in a similar set-up of SHD farms. With a 30% reduction in prevalence, the vaccinated group was assumed to be 31% prevalence. The required two-tailed sample size was calculated using Fleiss’ formula for cows and heifers (Fleiss et al., 1980). The calculated minimum sample size for each group was 145 cows or heifers and a total minimum of 290 per group.

**n** = {*z*α*0.05* /2√(m+1) p(1-p) + *z*β√(mp1(1-p1)+[p2(1-p2)]}2/m(p1-p2)[2](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7190503/#bib0002)

**n** = {1.96/2√ 2\*0.39(0.61) + 0.84√ (1\*0.47\*(1-0.47) + [0.31(1-0.31)]}2/ (0.0256)

**p** = (p1+p2)/m+1= 0.39

**n** = Sample sizeforheifers or cow data

Zα *=* 1.96 Z score for an error of 5% and CI of 95%

Zβ *=* 0.84 Z score for power of 80%

**m =** Number of cohort-negative animals per cohort-positive animal (assumed 1:1)

**p1 =** The probability of event in non-vaccinated animals (0.47)

**p2=** Probability of event in vaccinated animals (0.31)

## 4.4 Results

### 4.4.1 Animal population distribution by age group, herd, region, and trial group

On the first visit, 49.0% (237/484) and 50.6% (281/555) heifers and cows that were administered the placebo, respectively, with the remainder being vaccinated. The losses to follow-up from the first visit were 132 heifers (75 vaccinated and 57 placebo), 171 cows (89 vaccinated and 82 placebo) from a total of 95 farms. There were slightly higher losses to follow-up among the vaccinated than the placebo groups for both the cows and the heifers (Figure 4.1).

The study ultimately involved 736 animals, which included 384 (52.2%) cows and 352 (47.8%) heifers providing data for both the first and second visits. Excluded from the study were 22 animals that were heifers on the first visit and had calved, becoming cows by the time of the second visit. The reason for this exclusion was to avoid bias due to the transition and possible different management from the heifer to cow status. These animals would not fairly be compared to other heifers or to other cows in the study. In total, there were 405 farms, with cows coming from 292 farms and heifers coming from 260 farms. In total, 48.5% (357/736) animals were vaccinated with 48.2% (185/384) of the cows and 48.9% (172/352) of the heifers being vaccinated, and the remainder receiving the placebo treatment.

Comparing the two dairy cooperative societies, there were 49.7% (366/736) cattle from Buuri and 50.3% (370/736) from Naari in the trial. There were 55.2% (202/366) cows in Buuri and 49.2% (182/370) cows in Naari, with the rest being heifers. The vaccinated cows were 49.5% (181/366) from Buuri and 47.6% (176/370) from Naari, with the rest being injected with a placebo. These percentages show an almost equal/balanced distribution of vaccinates and placebo cattle in the two areas.

### 4.4.2 Farm and farmer characteristics

The female gender was more involved in running the day-to-day business of the farms. In the 405 farms, 48.6% of principal farmers were female (197/405), followed by farms where management was shared between the male and female at 35.6% (144/405), and male principal farmers constituting 15.8% (64/405) of farms. The female age ranged from 23 to 90 years, with a mean of 49.8, while for the male, the range was 25 to 96 years with a mean of 54.1. For the largest portion of the principal farmers, the highest level of education achieved by females and males was primary or basic education (elementary school) at 48.0% (189/394) and 44.3% (148/334), respectively. Only 14.4% (57/394) and 19.2% (64/334) of the females and males, respectively, had attained a tertiary education level, with the rest having attained secondary level.

The size of households varied from 1 person to 10 people living within the farm, with a mean of 3.5. More farmers had attended dairy training functions or seminars in the 12 months before the first visit, at 74.8% (303/405), than during the 12-month trial follow-up period, at 28.9% (117/405). Dairy farming contributed less than half of the household income for 45.9% (186/405) of farms, while 35.6% (144/405) of households relied on dairy farming for half to three-quarters of their income, and 18.5% (75/405) of households relied on dairy farming for more than three-quarters of their income.

For the farmer-owned land, sizes ranged from 0.125 to 26 acres, with a mean of 2.1 acres, while rented land sizes ranged from 0.125 to 40 acres, with a mean of 3.5 acres. The total land size available for use by the farmers, either owned or rented, ranged from 0.125 to 44 acres, with a mean of 3.0 acres. Most farms practiced a zero-grazing form of management at 51.4% (208/405), while mixed management farms (i.e., communal and zero-grazing) constituted 44.4% (180/405), and a handful utilized communal grazing all year round at 4.2% (17/405). The herds had a mean size of 5.6 cattle for both visits and a range of 1 to 34 animals (Table 4.1).

### 4.4.3 Disease conditions reported on the trial farms

The total number of reported disease conditions on the farms during the 12 months before the visits ranged between 0 and 8, with a mean of 2.4 disease conditions per farm at the first visit and 2.5 diseases per farm at the second visit (Table 4.1). The number of cattle deaths on the farms during the last 12 months for both visits ranged between 0 and 5, with a mean at first visit of 0.4 deaths per farm and mean at second visit of 0.5 deaths per farm.

Table 4.2 shows that the most common of the five specific diseases reported by the farms during the 12-month follow-up period was pneumonia at 47.7%, followed by diarrhea at 31.1%, with abortions and birth defects being the least reported diseases at the farm level. When the diseases were encountered during the follow-up period, we asked if they encountered those diseases on their farms more often during the follow-up period than during the 12 months prior to the study. Approximately 40-60% of farms said yes, and only abortions and birth defects were reported to be more often within the follow-up period, at 57.1% (24/42) and 60.0% (6/10), respectively. When the diseases were not encountered during the follow-up period, we asked if they encountered the diseases on their farms more often during the 12 months prior to the study than during the follow-up period. Approximately 20-50% of farms said yes, with a third or more of farms reporting diarrhea, pneumonia, and mastitis more often prior to the follow-up period thanduring the follow-up period. This information provides context for understanding the reported disease results at the animal level.

**4.4.4 Descriptive results for the cow portion of the study**

Regarding biosecurity, the proportion of farms that reported buying in-calf heifers for replacement was 6.5% (19/292) in the 12-month period before the first visit and 35.3% (103/292) during the 12-month follow-up period. The farms that reported buying cows were 43.2% (126/292) in the 12 months before the first visit and 46.9% (137/292) during the follow-up period.

Of note regarding nutritional management, most farms reported practicing supplementation of dairy meal post-calving, at 82.9% (242/292), with a mean of 2.1 kg/cow/day for the 12 months before the first visit, and 74.3% (217/292) with a mean of 1.9 kg/cow/day for the 12 months before the second visit. Due to the high price of dairy meal, there were some farms who reported providing grain supplementation in addition to, or instead of, dairy meal. There were 21.9% (64/292) of farms providing more than 1.5 kg grain post-calving on the first visit and 27.7% (81/292) at the second visit. The proportion for farms that fed the cows maize silage for the 12 months before the first visit was 32.5% (95/292) versus 26.0% (76/292) for the 12 months before the second visit.

Of note regarding mastitis and its prevention, at the first visit, 53.7% (157/292) of farmers did not report a mastitis case for the last 12 months, while 35.3% (103/292) reported one case of mastitis, and 10.9% (32/292) reported more than one case of mastitis. For milking hygiene reported on the first visit, 32.5% (95/292) of the farms used different towels to clean the udder of each cow before milking, while 48.0% (140/292) did not use different towels, and 19.5% (57/292) did not use a towel or had only one cow for the last 12 months.

The age of cows sampled ranged between 1.5 years, the youngest cow found at the first visit, and 16 years, the oldest cow found at the second visit, with the mean age being 6.3 years for the first visit. The most common breed was Holstein-Friesian at 53.4% (205/384), followed by Ayrshire at 24.4% (93/384), with the rest being other breeds, such as Jersey and Zebu. The study cows had a weight range of 140 kg, the smallest cow, measured on the first visit, to 659 kg, the largest cow, measured on the second visit, with a mean of 334.4 and 346 kg on the first and second visits, respectively. Cows’ shoulder height was between 97 centimeters (cm) and 145 cm, with a mean of 121 centimeters.

For the parity of cows, as reported by the farmers, they ranged between 1 and 8 with a mean of 2.5 and 2.8 at the first and second visits, respectively. There were 28.9% (110/381) and 23.6% (87/373) first-parity heifers for the first and second visits, respectively, with the rest being multiparous. During the first visit, 96.9% (372/384) of cows were being milked, with a production range of 0.5 to 26 L/cow/day and a mean of 8.3 L/cow/day, while at the second visit, 77.6% (298/384) of cows were in milk, with a similar range of 0.5 to 26 L/day but a mean of 5.8 L/day. The cows’ BCS on a scale of 1 to 5 ranged from 1.25 to 3.75, with a mean of 2.1 for both first and second visits.

Overall, the most reported disease during the follow-up period among the trial cows were tick-borne diseases at 25.5% (98/384), followed by pneumonia at 24.7% (95/384), while these diseases were reported to be even higher during the 12 months prior to the trial (Table 4.3). The overall count of reporteddiseases in the last 12 months had a range between 0 and 6 diseases per animal, with a mean of 1.4 diseases per animal. Clinical abnormalities on physical examination were infrequent during the first and second visits and were not significantly associated with disease reduction in the vaccinated cow group.

The vaccinated and non-vaccinated cows showed no significant difference in reported diseases during the 12 months prior to the first visit, except for abortions which had few numbers (Table 4.3). In the vaccinated group, on the second visit compared to the first visit, there was a significant (P < 0.05) reduction in the reported numbers of five disease conditions, namely: pneumonia, diarrhea, poor appetite, tick-borne diseases, and uterine diseases

### 4.4.5 Univariable Poisson model associations for cow disease count

There were 21 binary variables, 7 continuous variables, and 6 three-category variables significantly univariably associated (P < 0.1) with reported cow disease count during the previous 12 months. Sixteen of the 21 binary variables were at the farm level, with 13 being protective associations and 8 variables being associated with higher disease counts (Table 4.4 and 4.5). The 7 continuous variables were largely at the animal level (n=5), with 5 variables being associated with higher disease counts and 2 being protective (Table 4.6). All 6 categorical variables were measured at the farm level (Table 4.7 and 4.8).

### 4.4.6 Cow multivariable Poisson model

The final cow model had five binary variables, two categorical variables and eight variables involved in four interactions (Table 4.9 and 4.10). The cows that were on farms having more than three acres of land had lower reported counts of diseases for the last 12 months at an Incidence Risk Ratio (IRR) of 0.82 compared to farms with less land. Cows on farms that bought in-calf replacement heifers (pregnant heifers) had lower reported counts of disease for the last 12 months (IRR of 0.80) compared to farms not buying in-calf replacement heifers. Farms that reported calves having more than two diseases for the last 12 months had higher reported disease counts for their cows in the last 12 months (IRR of 1.32) compared to farms with two or fewer diseases. Cows that had been bred within the last 12 months had higher disease counts in the last 12 months (IRR of 1.43) than the cows that had not been bred. For each additional 10 kg of cow weight during the visit, there was a slightly higher count of reported diseases for the last 12 months (IRR=1.01).

The cows on farms that had not reported any case of mastitis during the 12 months before the visit had lower reported disease counts than the cows on farms that had reported one or more mastitis cases, with the IRR increasing numerically with more mastitis cases. The cows on farms that had only one cow or reported using no towel when cleaning the udder before milking had higher reported disease counts than cows on farms using the same towel when cleaning the udder for multiple cows. There was no difference between using a single towel or different towels between cows; however, most farmers with multiple cows did not use different towels for udder cleaning. The random effect within farm for the Poisson final model determined using ICC exact calculation was 0.03, suggesting that the correlation between disease count of two cows in the same farm is quite low.

Figure 4.3 demonstrates an interaction whereby both the vaccinated and non-vaccinated cows had similar counts of reported diseases in the last 12 months at the first visit to the farm but during the second visit, there were much lower reported disease counts in the last 12 months for the vaccinated cows than the placebo cows. Figure 4.4 demonstrates an interaction, where herd size was not associated with reported disease count when the household relied on dairy farming for less than 50% of the household income, but when the household relied on dairy farming for more than 50% of the income, there were lower reported disease counts for cows on farms with three or more cows compared to the small herd size farms. Feeding maize silage was not associated with reported disease count in the last 12 months when cows were not pregnant on the day of the farm visit, but when cows were pregnant on the day of the visit, the reported count of disease was lower in the last 12 months, but more so on the farms not feeding maize silage (Figure 4.5). Figure 4.6 shows an interaction where feeding extra grain post-calving was not associated with reported disease count in the last 12 months when cows had low BCS, but when cows with higher BCS (2.25 or higher) had lower disease count, especially on farms feeding extra grain post-calving.

### 4.4.8 Descriptive results for the heifer population in the trial

Regarding heifer level characteristics, the age of heifers sampled ranged between 6 to 54 months with a mean of 15.6 months at the first visit. The proportions of heifers that had reached 2 years old were 17.3% (61/352) at the first visit and 63.1% (222/352) at the second visit. The most common breed was Holstein-Friesian at 71.3% (251/352), followed by Ayrshires at 16.5% (58/352), with the rest being other breeds, such as Jersey and Zebu. The study heifers had a weight range of 45 kg to 345 kg with a mean of 163 kg on the first visit, and a range of 88 kg to 572 kg with a mean of 256 kg on the second visit. Heifers’ shoulder height was between 75 cm and 137 cm, with a mean of 101 cm and 113 cm on the first and second visits, respectively.

On reproductive status, during the first visit, only 1.4 % (5/352) of the heifers had a history of having been bred in the last 12 months; however, during the second visit, 37.8% (133/352) had been bred during the 12-month follow-up period. The proportion of confirmed pregnant heifers on the second visit was 33.6% (117/348), with 4 being recently serviced within the last month.

Of note on nutritional management of the calves and heifers, the most practiced method of feeding colostrum on the farms with heifers was suckling at 73.1% (190/260) compared to using a nipple bottle and bucket. The proportion of farms that were providing at least 4 kg of colostrum within the first 12 hours of calving was 22.7% (59/260). There were 51.2% (133/260) and 46.9% (122/260) of the farms in the first and second visits, respectively, feeding calf pencils (form of calf meal in long pellets). Of note regarding herd management, 33.5 % (87/260) of the farms with heifers had bought replacement heifers within the last 12 months.

Regarding seasonality, the proportions of farms that were visited during wet season months were 64.2% (167/260) and 27.3% (71/260) for the first and second visits, respectively. We noted that three months (July 2019 and both January 2020 and 2021) that are usually dry received substantial rains (at least half of the days of that month) during our study and therefore we included them as wet season months.

For the heifers, 34.1% (240/704) had at least one disease in the last 12 months. During the first visit, there was no significant difference in reported disease occurrence in the last 12 months between the placebo and vaccination groups (Table 4.11). However, on the second visit, there was significantly (P < 0.05) less frequent reported disease occurrence in the last 12 months for all disease outcomes combined, and for specifically pneumonia and other diseases (including skin diseases, tick borne diseases, or helminthiasis) in the vaccinates compared to the placebo group. The specific reported disease conditions that were reduced in the vaccinated group during the follow-up period compared to the 12 months prior to the trial included pneumonia, other diseases and overall, for the heifers.

**4.4.9 Heifer univariable results**

The factors significantly associated with the binary reported disease outcome over the last 12 months at P-value<0.1 included 18 farm-level, 5 animal-level binary variables, and 2 categorical variables (Table 4.12, 4.13, and 4.14, respectively). Among the binary variables, there were 10 protective associations and 13 variables positively associated with reported disease occurrence. One categorical variable (method of colostrum feeding) was a farm-level variable while the other (breed type) was an animal-level variable. Number of sick cows within the last 12 months was the only continuous variable significantly related to the disease outcome for the heifers with an odds ratio of 1.3 (P-value=0.07), range of 0 to 10 and a mean of 1.4.

### 4.4.10 Heifer multivariable results

The final heifer multivariable logistic model had five binary variables, one categorical variable, and four variables involved in two interactions (Table 4.15). Heifers on farms that were feeding heifers grass silage during the last year were 3.3 times more likely to have reported disease in the last year than heifers on farms not feeding grass silage. Heifers on farms that were buying replacement heifers during the last year were 2.5 times more likely to have reported disease in the last year than heifers on farms not buying replacement heifers in the last year. Heifers on farms that administered at least four kg of colostrum within the first 12 hours of a calf’s life had lower disease likelihood than heifers on farms where less colostrum was administered, with an odds ratio of 0.3. Heifers that had been bred within the last 12 months were less likely to have reported disease in the last year than those that had not been bred, with an odds ratio of 0.3. Heifers that were more than two years old were 2.2 times more likely to have reported disease within the last 12 months than those of lower age. Compared to Holstein-Friesians, other breed heifers (Jerseys and Zebu etc.) had marginally (p=0.064) lower odds of reported disease in the last year (OR=0.45), while Ayrshires had higher odds of reported disease in the last year (OR=1.9). The random effect within farm for the heifer logistic final model had an ICC of 0.38, suggesting that the correlation between disease occurrence likelihood within the last one year of two heifers on the same farm was high.

In the Figure 4.7 interaction, vaccinated and non-vaccinated heifers had similar likelihood of reported disease at the first visit to the farm. However, during the second visit, there was much lower odds of reported disease in the last year for the vaccinated heifers than the placebo heifers. In the Figure 4.8 interaction, at low numbers of reported sick cows on the farm, the odds of reporting disease in heifers did not differ by season of the visit, but at higher number of reported sick cows on the farm, the odds of reporting disease in heifers were significantly higher when the farms were visited during the wet season months versus during the dry season months.

### 4.4.11 Models’ evaluation

For the final cow Poisson regression model, the Pearson’s goodness-of-fit test showed no lack of fit at (p= 0.06.). There was no evidence of multicollinearity between the explanatory variables, with all correlation matrix coefficients of the Poisson model being less than 0.5. There were only twenty-five Anscombe residuals outside the -2 and 2 limits with none outside -3 and 3. The twenty-five largest Anscombe residuals represented less than 5% of the 768 total observations; therefore, there was no concern for extreme observations.

For the final heifer logistic regression model, there were only six Pearson residuals outside the -2 and +2 limits, with none outside -3 and 3. The six largest Anscombe residuals represented less than 5% of the 704 total observations; therefore, there was no concern for extreme observations. There was no evidence of multicollinearity between the explanatory variables, with all correlation matrix coefficients of the logistic model being less than 0.5.

## 4.5 Discussion

### 4.5.1 Vaccinations and the diseases in cows and heifers

The multivalent MLV vaccination including BVDV was beneficial for both the cows and heifers in this first trial of its kind on SHFs in Kenya. Other studies have recorded similar benefits in other farming systems (Campbell, 2004; Downey-Slinker et al., 2016; Perino & Hunsaker, 1997). The benefits of multivalent MLV vaccination including BVDV in the SDFs sector in Kenya are evident in our double-blinded randomized controlled trial through the reduction of reported disease in the 12-month follow-up period among the vaccinated group compared to the placebo group, while controlling for other factors associated with disease occurrence. This preventive health benefit can lead to improve productivity and reproduction, which in turn gives the farming households better livelihoods.

In our trial, the heifers and cows in the vaccinated group both showed significant reduction of reported pneumonia during the year-long follow-up period compared to the 12-month period prior to the first visit while the unvaccinated did not. It has been demonstrated that there is lower incidence of respiratory disease when these multivalent BVDV vaccines were used elsewhere (Cusack et al., 2020). Vaccination benefits have also been shown in calves and heifers using a multivalent BVDV vaccine, with lower health cost in the vaccinated animals (Kirkpatrick et al., 2008). Though the vaccine was multivalent in our study, the discussion focuses primarily on the BVDV component because there is substantial evidence of BVDV infection in Kenyan SDF cattle with little evidence on control strategies and awareness (Okumu et al., 2019; VanLeeuwen et al., 2021).

There is evidence of infectious bovine rhinotracheitis (IBR) on SDFs in Kenya (Kipyego et al., 2020); therefore, some of the benefits of the vaccination seen in our study may also be from prevention and control of IBR from the multivalent vaccine. The adoption of vaccination to prevent BVDV has been shown to be tied to other important factors, such as biosecurity, farm characteristics, and farm practices; therefore, vaccination decisions should be made with consideration of other risk and prevention factors (Arnoux et al., 2021; Han, 2020; Rat-Aspert & Fourichon, 2010).

### 4.5.2 Other factors associated with reported cow disease counts

Cows on farms that had more than three acres of land had lower counts of reported disease over the one-year follow-up period than those on farms that had a smaller land size, while controlling for herd size. Most farmers depend on home-grown fodder, with less land leading to fodder shortages, likely poorer nutrition, and therefore poor health for animals in those farms. Limited land size has been identified to be associated with higher incidence of diseases in SDFs in Ethiopia (Mekonnen et al., 2006). An assessment of feed resources for SHD farms in Ethiopia identified feed constraint due to limited land and diseases to be major limitations to the dairy enterprise (Duguma & Janssens, 2016). A study among smallholder livestock farmers in Congo reported shortage of crop residue in dry seasons and diseases as major constraints to livestock productivity (Maass et al., 2012).

Cows on farms that bought replacements in-calf heifers had lower reported disease counts for the last year than farms that did not buy replacement in-calf heifers. However, heifers on farms that were buying non-pregnant replacement heifers for the last one year had higher odds of reported disease than heifers on farms not buying non-pregnant replacement heifers in the last one year. In Kenya, many zero-grazing farms acquire replacement heifers from free-grazing farms, which can keep higher numbers of cattle (Bebe, 2008). Replacements are a potential source of disease entry into farms, especially more likely on farms that may not be adhering to strict biosecurity practices of disease prevention (Dutta, 2016). A study in Nakuru, Kenya, showed that the introduction of cattle in dairy farms was associated with higher Lumpy Skin Disease (LSD) incidence during outbreaks (Kiplagat et al., 2020). The reverse direction relationships for cows on farms buying in-calf heifers could be related to the introduction of better quality replacements that were pregnant. Pregnancy status at the time of acquiring heifers can be an indicator of fertility consequent to good health performance. There is evidence that pregnant replacement heifers are indicative of good farm efficiency (Stevenson 2002).

Cows on farms that had more than two reported diseases in their calves also had higher reported disease counts than farms with fewer reported calf diseases. This factor can likely be attributed to disease pressure across the herd, where diseases can easily be transmitted between age cohorts. Keeping calves and cows in close proximity is common in SDFs, with nose-to-nose contact due to adjacent pens, allowing co-mingling (Callan & Garry, 2002; Stokka, 2010). A study in the Hawassa region of Ethiopia found that there were similar reported diseases in both heifers and cows on farms with high disease burden at the farm level, especially in larger herd sizes (Debebe & Haben, 2020). A study in Irish beef herds showed that herd-level pathogen status was an important determinant for exposure to more pathogens at the cow level (Barrett et al., 2018).

Cows that had been bred within the last 12 months had higher disease counts than the cows that had not been bred. We would expect that the cows that have been bred to be performing better, and thus, having less health challenges. Cows that are having heats being detected, and subsequently being bred, are more likely to be in good health or showing less diseases. This variable does not consider the number of breedings per conception, which can differentiate disease outcome between difficult breeders and those conceiving with fewer inseminations. In our model, however, cows that were pregnant during the visit had lower reported disease counts. Cows with diseases are likely to have reduced conception and therefore be repeat breeders (Wathes et al. 2020; Heringstad, Wu, and Gianola 2009; Fourichon, Seegers, and Malher 2000), and therefore need additional breedings.

Heavier cows during the visit were more likely to have a higher count of recorded diseases for the last 12 months. Cows with higher weights have been associated with predisposition to health challenges such as lameness, mastitis, and ketosis (Morrow, 1976; Østergaard & Gröhn, 1999). Other studies did not find an association between weight of cows and health performance around the peri-calving period (Berry et al., 2007). Body weight and BCS changes, especially moderate to high loss of body weight or BCS in early lactation, leads to poor health performance (Poncheki et al., 2015; Roche et al., 2013). Considering the varying nutritional status and seasonal feed challenges in the SDFs, this may be a reason for more diseases with higher body weights (Njarui et al., 2016). We can compare high body weight to high BCS when interpreting the association, since there is evidence of correlation between cattle body weight and BCS (Berry et al., 2006, 2007, 2011; Roche et al., 2013).

Cows on farms that had not reported any case of mastitis 12 months before the first visit had lower disease counts during the follow-up period than cows on farms that had reported one or more mastitis cases. Mastitis is one of the most common diseases of cattle worldwide on both large farms as well as on SDFs (Chen et al., 2022; Getaneh & Gebremedhin, 2017; Hasan, 2021; Mbindyo et al., 2020), making it an obvious predictor of subsequent disease. Mastitis is related to many risk factors, including poor peri-calving management, lameness, poor hygiene and welfare, among other conditions (Abebe et al., 2016; Barker et al., 1998; Hertl et al., 2010; Kumar et al., 2017). In our study, cows on farms that reported using different towels to clean the udder before milking had lower disease counts than cows on farms not using towels and those using a single towel for multiple cows. Consistent with our findings, there is little and varied level of hygiene observation in the milking process on SDFs, contributing to poor health performance (Duguma, 2020). Good milking practices have been shown to lower the presence of mastitis and thus better health performance in dairy cows in Malawi and Kenya SDFs (Mbindyo et al., 2020; Tebug et al., 2012).

### 4.5.3 Cow multivariable interactions

There was an interaction between income reliance from dairy farming and categories of numbers of cows, whereby the cows on the farms that had high numbers of cows and relied on dairy farming heavily for their income had less disease counts compared to those that had fewer cows and did not rely on them as much for their income. This interaction is likely due to better disease prevention management on larger farms who focus on their dairy farming activities when they rely on the dairy farm for a high proportion of their household income. Other studies have reported the opposite, of more health challenges with an increase in herd size (Barrett et al., 2018; Debebe & Haben, 2020; Ghebremariam et al., 2018; Kiplagat et al., 2020). However, in Muranga, Kenya, it was found that SDFs not reliant on dairy farming income were not having efficient performance (Van Schaik et al., 1996). A study investigating profit efficiency of SDFs in Meru, Kenya, found that herd size and expenditure on drugs was contributing to more efficient output, while relying on non-dairy income was associated with inefficiency (Nganga et al., 2010).

There was an interaction between feeding maize silage and pregnancy status of the cows, whereby there were higher disease counts in cows that were non-pregnant and in cows on farms feeding on maize silage. Higher disease when feeding on maize silage is opposite of what would be expected since silage is a high value feed, if prepared well (Ntakyo et al., 2020). In SDFs, there are frequently challenges of feed availability, especially during the dry seasons, which could mean that farms that were feeding silage indeed were experiencing feed supply challenges (Kiptot et al., 2015; Maleko et al., 2018). When cows are pregnant, we can interpret that they have been having good health performance, which leads to good reproductive performance, whereas lower fertility in cows would have had more diseases (Bisinotto et al., 2012; Khan et al., 2016).

Finally, there was an interaction between farms feeding more than 1.5 kilograms of extra grain post-calving and body condition score, whereby the use of grain supplementation in cows on the SDFs was beneficial to body condition score and health performance. This interaction can be attributed to better nutritional reserves of cows during times of critical energy need, such as the peri-calving period. Similar findings of a study in Uganda had reported that the extra supplementation of cows with brewer’s waste was beneficial to their health and productivity (Okwee-Acai et al., 2021). The interplay between BCS and nutritional supplementation is critical to the health status of cows before calving and early lactation (Poncheki et al., 2015; Roche et al., 2013).

**4.5.4 Associated factors for heifers’ disease outcome**

In our study, heifers over 24 months in age had higher odds of reported disease within the last one year than the younger heifers. This finding can likely be attributed to both length of exposure to diseases by virtue of higher age, as well as the older heifers being kept together with the cows. Mature cattle have higher risk of disease than in heifers (Lasser et al., 2021; Ruprechter et al., 2018). A study, investigating integrated leptospirosis outbreak management in calves less than one year of age in dairy herds of Padua, Italy, reported better immunocompetence to leptospirosis with age, as demonstrated by protective antibody titers (Mughini-Gras et al., 2014). A study in Bangladesh investigating the prevalence and incidence of different disease conditions found the opposite with more diseases in the younger cattle than in the adults (Md Al-Noman et al., 2020).

The heifers that had been bred within the last 12 months had less occurrence of reported disease than those that had not been bred. Having been bred as a heifer is likely an indicator of good performance by showing heat and possibly achieving the minimum requirements of age and weight for first service (Heringstad, Wu, and Gianola 2009; Wathes et al. 2014; Ajak, Gachuiri, and Wanyoike 2020). On the other hand, heifers on farms that have higher disease conditions could have lower fertility and signs of heat and thus higher chance of being bred often (Córdova - Izquierdo et al., 2017; Khan et al., 2016; Walsh et al., 2011). First calving at 24 to 30 months has been shown to have optimum benefits on lifelong milk production performance (Stevenson, 2002; Wathes et al., 2014; Wathes et al., 2008).

Heifers that were on farms that were feeding heifers grass silage for the last one year had higher reported disease occurrence than heifers on farms not feeding grass silage. As with the cows, this association was opposite of what would be expected. Silage is a high value feed and if prepared well and fed to cows and heifers, there would likely be improved general performance (Ntakyo et al., 2020). In SDFs, there are frequently challenges of feed availability, especially during the dry seasons (Kiptot et al., 2015; Maleko et al., 2018; Njarui et al., 2011). Silage is a technical preservation method requiring some resources and may be challenging to prepare properly by farmers with limitations in knowledge or farm resources. Therefore, it is possible to have poor nutritional provision or incidence of spoilage with silage (Balehegn et al., 2022; Mahanna & Chase, 2015), potentially leading to more disease. Further research should investigate this possible association on SDFs.

Breed categories of the heifers showed significant association with disease outcome. Other breeds (Jerseys, Zebu, etc.) had the lowest occurrence of reported disease outcome followed by Holstein-Friesian and Ayrshire having the highest odds of disease. Cross-bred cattle and indigenous breeds on SDFs have reported a similar lower disease outcome compared to the exotic breeds in Kenya (Ogola, 2015). This disease resistance of local breeds can be attributed to the selection and adaptability for resistance against common diseases over long time periods in the tropical environment (Kim & Rothschild, 2014; Nyamushamba et al., 2017).

Heifers on farms that administered at least four kg of colostrum within the first 12 hours of a calf’s life had lower disease likelihood than where less colostrum was administered. A large volume of colostrum gives calves a good start in growth, and good immunity and survival in the early months (Abuelo et al., 2021; Godden et al., 2019; Zheng & Leal Yepes, 2021). It has been shown that the administration of colostrum in the right amounts early after birth is also beneficial to long-term productivity and health performance (Faber et al., 2005; Godden et al., 2019).

There was an interaction between season at the time of visit and having sick cows within the last 12 months, whereby there was a higher likelihood of heifers being reported sick as the number of sick cows in the farm was higher when the visit was done during a wet season, with no difference when the visit was done in the dry season. This association may be attributed to disease pressure across the herd and modified by the wet season. Cows and heifers are likely to be kept together on SDFs, and therefore the spread of diseases from cows to heifers on farms reporting more sick cows is likely a result of their co-mingling (Callan & Garry, 2002; Stokka, 2010). During the wet, rainy season, there is more likelihood of diseases being transmitted between cows and heifers due to the poor housing and welfare in SDFs (Rehman et al., 2010; Waruiru et al., 2000). During the wet season also, there is a higher chance of vector-borne disease spread within farms (Chepkwony et al., 2021; Marufu, 2008). Other studies have found the opposite of this association, where there were more disease during the dry season (Nalianya et al., 2020). The direction of this association may depend on the severity of the dry season, and the degree to which zero-grazing farmers rely on community pasture when they incur feed shortages, leading to more cattle exposure from other farms, and therefore more disease. Further research should investigate this possible association on SDFs.

### 4.5.5 Study limitations

Due to the infrequent occurrence of disease observed during the clinical examinations at the first and second visits, the study was limited to reported disease outcomes during the last 12 months by the farmers, which may be susceptible to recall bias. This bias was complicated by the challenges of poor record-keeping, especially production, health, and reproduction data. The second visit follow-up was expected to have reduced recall bias. Due to the double-blind nature of this randomized controlled trial, the impact of this bias on the study results is likely minimal. This study used a multivalent MLV vaccine, and it was not possible to separate the effects of each of the five pathogens on the disease outcomes during the 12 months of follow-up. We did not have serological antibody titer assessment for determining proof of vaccine efficacy after vaccination during the follow-up visit.

The disease prevention benefits between the vaccination and placebo groups provided a good measure of vaccine effect, but it remains unclear which portion of the vaccine is responsible for what proportion of disease reduction benefits in the trial. Finally, if the BVDV portion of the vaccine was indeed responsible for the disease reduction benefits in the trial, knowing BVDV exposure prior to the trial would have been useful in the trial to adjust for this past exposure in the analyses. For those cattle who were exposed to BVDV prior to vaccination, the benefit is likely a combined benefit of both the prior exposure and vaccination. Some animals having just vaccine effects and some having this combined benefit would represent what would be expected in animals in a commercial setting such as SHD farms, as opposed to some controlled research setting where there was no exposure to BVDV previously. The study was done in commercial herds on purpose to ensure that the results are applicable to commercial herds. Further trials should investigate the difference in benefits with and without previous BVDV exposure on SDFs.

## 4. 6 Conclusions

The trial established that multivalent MLV vaccination including BVDV on smallholder dairy farms in Kenya was significantly associated with reduction of overall disease, pneumonia, and diarrhoea in both the cows and heifers reported in the follow-up period compared to the 12 months prior to the trial. In the heifers, there was a significant association between vaccination and other reported diseases, including tick-borne diseases and skin diseases reported for the last year. Additionally, vaccination in cows was significantly associated with reduced poor appetite, tick-borne disease, and other disease reported in the follow-up period. Furthermore, for the cows and heifers, there was a significant interaction variable between visit number and intervention group, confirming less diseasesreported in the last 12 monthsin the vaccinated group versus the placebo group on the second visit, adjusting for the reported disease counts on the first visit. A multivalent MLV vaccination including BVDV is recommended on smallholder dairy farms similar to those in Kenya.

Management factors such as biosecurity and nutrition are important components in complementing the benefits of vaccines. In this study we had the evidence of milking hygiene, colostrum administration, post calving supplementation all being associated with the disease outcome. Other farm factors, such as herd size, level of dairy farming reliance and management of replacements, are also critical factors to supplement and complement the beneficial effects of vaccination. Animals that were crossbreeds and local breeds had lower association with disease outcomes; therefore it is good for farmers to consider the use of crosses that produce more milk than local breeds.

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Figure 4.1:Flow chart for selection and allocation of the 736 cows and heifers recruited into the study.

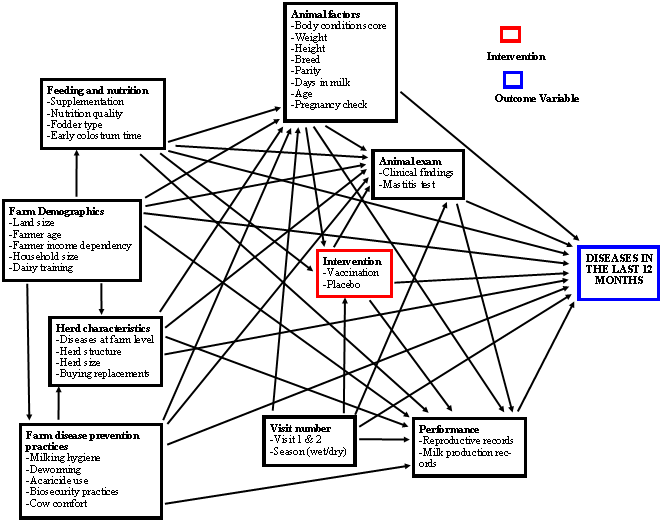
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Figure 4.2: Theoretical causal relationship model for the possible factors’ association with disease outcome in the 736 cows and heifers recruited

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Table 4.1: Herd structure during the time of each visit and reported number of deaths and diseases for the previous 12 months prior to both the first visit and second visit on the 405 smallholder dairy farms in Kenya.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Herd variable** | **Overall range** | **Overall mean** | **First visit means and range** | **Second visit mean and range** |
| Number of calves | 0 - 10 | 1.8 | 1.9 (0 - 9) | 1.7 (0 - 10) |
| Number of heifers\* | 0 - 11 | 1.3 | 1.2 (0 - 8) | 1.4 (0 - 11) |
| Number of cows | 1 - 23 | 2.5 | 2.6 (0 - 23) | 2.5 (1 - 13) |
| Total herd size | 1 - 34 | 5.6 | 5.6 (1 - 34) | 5.6 (1 - 32) |
| Number of deaths | 0 - 5 | 0.4 | 0.4 (0 - 5) | 0.5 (0 - 5) |
| Number of diseases | 0 - 8 | 2.5 | 2.4 (0 - 8) | 2.5 (0 - 8) |

\* Nulliparous cattle above 6 months of age

Table 4.2: Proportion of farms with specific reported diseases in the 12 month follow up period in comparison with the 12 months before the study for both the heifers and cows in the 405 smallholder dairy farms in Kenya

|  |  |  |  |
| --- | --- | --- | --- |
| **Diseases at farm level** | **Reported during follow-up period (#) (%)** | **If seen during the follow-up period, was it seen more in follow-up period than the previous 12 months (#) (%)** | **If not seen during the follow-up period, was it seen more in the 12 months before the follow-up period than during the follow up period (#) (%)** |
| Abortion | 42/405n (10.4) | 24/42i (57.1) | 89/363 ii (24.5) |
| Diarrhoea | 126/405 (31.1) | 56/126 (44.4) | 89/279 (31.9) |
| Pneumonia | 193/405 (47.7) | 80/193 (41.5) | 100/212 (47.2) |
| Birth defects | 10/405n (2.5) | 6/10 (60.0) | 70/395 (17.7) |
| Mastitis | 138/405\* (34.1) | 75/138 (54.4) | 124/267 (46.4) |

n Variable only relevant for farms where there were pregnant cows and calvings within the 12 month period. Information on periods without pregnant cows and calving not available

\* Relevant for farms with lactating cows. The periods with no lactating cows in the farm were not recorded but would be infrequent.

i  The column denominator is the numerator for the 1st column (farms reporting the disease).

ii  The column denominator is 405 minus the numerator for the 1st column (farms not reporting the disease).

Table 4.3: Reported disease in the last 12 months and physical exam findings by vaccinated and placebo groups and overall, in 384 cows on 292 smallholder dairy farms in Kenya

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Visit One** | | | | | | |  | **Visit Two** | | | | | |
| Disease variable | Chi 95% | Placebo  (n=199) | | Vaccinates  (n=185) | | Overall (n=384) | | Chi 95% | Placebo  (n=199) | | Vaccinates  (n=185) | | Overall (n=384) | |
| **P-value** | **#** | ***%*** | **#** | ***%*** | **#** | ***%*** | **P-value** | **#** | ***%*** | **#** | ***%*** | **#** | ***%*** |
| Mastitis12 | 0.692 | 45 | 22.6 | 45 | 22.3 | 90 | 23.4 | 0.097 | 42 | 21.1 | 27 | 14.6 | 69 | 18.0 |
| Abortion12 | **0.040** | 8 | 4.0 | 17 | 9.2 | 25 | 6.5 | 0.476 | 8 | 4.0 | 5 | 2.7 | 13 | 3.4 |
| Pneumonia12 | 0.592 | 62 | 31.2 | 53 | 28.7 | 115 | 30.0 | **0.001** | 63 | 31.7 | 32 | 17.3 | 95 | 24.7 |
| Diarrhoea12 | 0.383 | 27 | 13.6 | 31 | 16.8 | 58 | 15.1 | 0.008 | 25 | 12.6 | 9 | 4.9 | 34 | 8.9 |
| Poor Appetite12 | 0.212 | 36 | 18.1 | 43 | 23.2 | 79 | 20.6 | **<0.001** | 64 | 32.2 | 12 | 6.5 | 76 | 19.8 |
| Tick-borne disease 12 | 0.962 | 65 | 32.7 | 60 | 32.4 | 125 | 32.6 | **0.009** | 62 | 31.2 | 36 | 19.5 | 98 | 25.5 |
| Skin Disease 12 | 0.111 | 10 | 5.0 | 17 | 9.2 | 27 | 7.0 | 0.342 | 13 | 6.5 | 8 | 4.3 | 21 | 5.5 |
| Uterine Disease12 | 0.763 | 29 | 14.6 | 29 | 15.7 | 58 | 15.1 | **0.058** | 12 | 6.0 | 4 | 2.2 | 16 | 4.2 |
| Other diseases12 | 0.970 | 17 | 8.5 | 16 | 8.7 | 33 | 8.6 | **0.039** | 35 | 17.6 | 19 | 10.3 | 54 | 14.1 |
| Physical exam1 | 0.605 | 2 | 1.0 | 1 | 0.5 | 3 | 0.8 | 0.261 | 7 | 3.5 | 11 | 6.0 | 18 | 4.7 |
| CMT test1 | 0.923 | 46 | 23.3 | 42 | 22.7 | 88 | 22.9 | 0.637 | 57 | 28.6 | 49 | 26.5 | 106 | 27.6 |
| Overall Disease12 | 0.692 | 146 | 73.4 | 139 | 75.1 | 285 | 74.2 | **<0.001** | 137 | 68.8 | 93 | 50.3 | 230 | 59.9 |

**1 -** Statusduring the study visit

**12 -**Status for the 12 months prior to the visit (reported)

Table 4.4: Univariable mixed effects Poisson regression results of incidence risk ratios (IRR) for overall reported disease counts in cows in the last 12 months for farm-level binary predictor variables with a P < 0.1 for two visits among 384 cows on 292 smallholder dairy farms in Kenya

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Predictor Variable** | **Variable Yes (%) (#/#)** | **IRR** | **IRR 95% CI** | **P-Value** |
| Visit(Second visit=Y and First visit=N) 12 | 50.0 (292/584) | 0.78 | 0.686, 0.886 | <0.001 |
| Dairy cooperative (Buuri=Y/Naari=N) 12 | 47.6 (139/292) | 1.15 | 0.983, 1.346 | 0.080 |
| Dairy income dependency over 50% (Y/N) 12 | 56.5 (165/292) | 0.85 | 0.730, 0.998 | 0.047 |
| Total land three (3) acres or more(Y/N) 1 | 40.6 (119/292) | 0.79 | 0.672, 0.929 | 0.004 |
| More than two (2) calves sick12 | 14.7 (43/292) | 1.19 | 0.997, 1.425 | 0.054 |
| Artificial Insemination problems (Y/N) 12 | 56.9 (166/292) | 0.83 | 0.711, 0.974 | 0.022 |
| Acaricide within 2 weeks (Y/N) 12 | 51.4 (150/292) | 0.80 | 0.683, 0.931 | 0.004 |
| Post-calving disease condition(Y/N)12 | 46.9 (137/292) | 1.17 | 1.000, 1.369 | 0.049 |
| Attended seminars or training (Y/N) 12 | 51.9 (303/584) | 1.17 | 1.012, 1.360 | 0.034 |
| Wet season (Y/N) 12 | 34.6 (202/584) | 1.15 | 0.988, 1.330 | 0.071 |
| Fed maize silage (Y/N) 12 | 29.3 (171/584) | 1.14 | 0.976, 1.328 | 0.100 |
| Vary dairy meal by production (Y/N) 12 | 32.7 (191/584) | 0.83 | 0.707, 0.981 | 0.028 |
| Forage shortage (Y/N) 12 | 58.2 (340/584) | 1.12 | 0.980, 1.290 | 0.095 |
| Timed deworming (Y/N) 12 | 46.1 (269/584) | 1.25 | 1.095, 1.424 | 0.001 |
| Buy replacement heifers (Y/N)12 | 20.9 (122/584) | 0.78 | 0.653, 0.934 | 0.007 |
| Extra grain on calving (Y/N)12 | 24.8 (145/584) | 0.81 | 0.682, 0.975 | 0.025 |

Y/N - Binary variable code, Yes and No

IRR - Incidence risk ratio

**1 -** Statusduring the study visit

**12 -**Status for the 12 months prior to the visit

Table 4.5: Univariable mixed effects Poisson regression results of incidence risk ratios (IRR) for overall reported disease counts in cows in the last 12 months for animal-level binary predictor variables with a P < 0.1 for two visits among 384 cows on 292 smallholder dairy farms in Kenya

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Predictor Variable** | **Variable Yes (%) (#/#)** | **Mean count of reported disease when variable is Yes** | **Mean count of reported disease when variable is No** | **IRR** | **IRR 95% CI** | **P-Value** |
| 300 days in milk (Y/N) 1 | 50.3 (386/768) | 1.30 | 1.53 | 0.85 | 0.746, 0.963 | 0.011 |
| Bred previously (Y/N) 12 | 37.4 (287/768) | 1.29 | 1.49 | 0.86 | 0.746, 0.989 | 0.034 |
| Lactating(Y/N) 1 | 87.2 (670/768) | 1.46 | 1.08 | 1.36 | 0.059, 0.552 | 0.015 |
| Pregnant during visit (Y/N) 1 | 28.5 (219/768) | 1.00 | 1.56 | 0. 65 | 0.545, 0.767 | <0.001 |
| BVDV Vaccinated (Y/N) 12 | 48.2 (370/768) | 1.25 | 1.57 | 0.79 | 0.691, 0.913 | 0.001 |

IRR -Incidence risk ratio

**1 -** Statusduring the study visit

**12 -**Status for the 12 months prior to the visit

BVDV – Bovine Viral Diarrhoea Virus

Table 4.6: Univariable mixed effects Poisson regression results of incidence risk ratios (IRR) for overall reported disease counts in cows in the last 12 months for continuous predictor variables with a P < 0.1 for two visits among 384 cows on 292 smallholder dairy farms in Kenya

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Predictor variable** | **Number of observations** | **Mean** | **Range** | **IRR** | **IRR 95% CI** | **P-value** |
| Rented land proportion on fodder 1, | 292 | 0.231 | 0 - 1 | 1.280 | 1.033, 1.588 | 0.025 |
| Herd size1 | 584 | 5.770 | 1 - 34 | 0.980 | 0.958, 1.002 | 0.077 |
| Cow age in years (years) 1 | 714 | 6.800 | 1.5 - 16 | 1.026 | 0.996, 1.057 | 0.092 |
| Cow parity (number of calving)1 | 754 | 2.680 | 0 - 8 | 1.038 | 0.993, 1.085 | 0.099 |
| Cow number of times bred last1 | 737 | 1.537 | 0 - 7 | 1.107 | 1.031, 1.189 | 0.005 |
| Cow body condition score (BCS) 1 | 768 | 2.111 | 1.25 - 3.75 | 0.738 | 0.613, 0.888 | 0.001 |
| Cow shoulder height in centimeters1 | 768 | 121.0 | 97 - 145 | 1.010 | 1.003, 1.020 | 0.049 |

IRR -Incidence risk ratio

**1 -** Statusduring the study visit

Table 4.7: Univariable mixed effects Poisson regression results of incidence risk ratios (IRR) for overall reported disease counts in cows in the last 12 months for farm-level constant categorical predictor variables with a P < 0.1 for two visits among 384 cows on 292 smallholder dairy farm in Kenya

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Predictor variable** | **Category proportion**  **% (#/#)** | **Mean count of reported disease for cows** | **IRR** | **IRR 95% CI** | **P-Value** |
| **Udder cleaning towel use 12** | -- | -- | -- | -- | <0.001 gp |
| Single for all cows | 48.0 (140/292) | 1.52 | Baseline | Baseline | Baseline |
| Different towel per cow | 32.5 (95/292) | 1.63 | 1.137 | 0.950, 1.361 | 0.160 |
| Not using towel and had one cow | 19.7 (57/292) | 2.09 | 1.455 | 1.063, 1.760 | <0.001 |
| **Hand washing between milking 12** | -- | -- | -- | -- | <0.001 gp |
| Does not between cows | 71.6 (209/292) | 1.52 | Baseline | Baseline | Baseline |
| Wash between cows | 8.9 (26/292) | 1.33 | 0.809 | 0.580, 1.128 | 0.212 |
| Milking one cow | 19.5 (57/292) | 2.07 | 1.352 | 1.132, 1.615 | 0.001 |
| **Number of mastitis cases treated 12** | -- | -- | -- | -- | <0.005 gp |
| No case | 54.3 (157/292) | 1.41 | Baseline | Baseline | Baseline |
| One (1) case | 35.3 (103/292) | 1.77 | 1.290 | 1.091, 1.526 | 0.003 |
| More than one (1) case | 11.0 (32/292) | 1.79 | 1.304 | 1.038, 1.638 | 0.023 |

IRR-Incidence risk ratio

**12**Status for the 12 months prior to the study visit

gp Global P-Value for categorical variable

Table 4.8: Univariable mixed effects Poisson regression results of incidence risk ratios (IRR) for overall reported disease counts in cows in the last 12 months for farm-level varying categorical predictor variables with a P < 0.1 for two visits among 384 cows on 292 smallholder dairy farms in Kenya

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Predictor variable** | **Category proportion**  **% (#/#)** | **Mean count of reported disease for cows** | **IRR** | **IRR 95% CI** | **P-Value** |
| **Time mastitis cow milked 12** | -- | -- | -- | -- | 0.031gp |
| Not milked last | 11.8 (69/584) | 1.70 | Baseline | Baseline | Baseline |
| Milked last | 25.0 (146/584) | 1.60 | 0.972 | 0.772, 1.223 | 0.806 |
| No mastitis and had one cow | 63.2 (369/584) | 1.38 | 0.805 | 0.655, 0.989 | 0.039 |
| **Milk rejection and reasons 12** | -- | -- | -- | -- | 0.052 gp |
| Not had rejection | 74.5 (435/584) | 1.40 | Baseline | Baseline | Baseline |
| Due to mastitis | 16.6 (97/584) | 1.57 | 1.12 | 0.945, 1.338 | 0.187 |
| Other reasons | 8.9 (52/584) | 1.88 | 1.28 | 1.039, 1.570 | 0.020 |
| **Number of cows in the farm1** |  |  | -- | -- | <0.001 |
| With 1 and 2 cows (a) | 55.7 (325/584) | 1.66 | Baseline | Baseline | Baseline |
| With 3 and 4 cows (b) | 34.8 (203/584) | 1.23 | 0.747 | 0.637, 0.877 | <0.001 |
| With 5 or more (c) | 9.6 (56/584) | 1.25 | 0.762 | 0.582, 0.997 | 0.048 |

IRR-Incidence risk ratio

**12**- Status for the 12 months prior to the study visit

**1 -** Statusduring the study visit

gp Global P-Value for categorical variable

Table 4.9: Final multivariable Poisson regression model results showing associations with reported disease counts for the last 12 months among the 384 cows on 292 smallholder dairy farms in Kenya

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Predictor variable** | **Description** | **IRR** | **IRR 95% CI** | **P-value** |
| Model constant | -- | 1.75 | 1.13, 2.71 | 0.011 |
| Three acres of land | Y/N | 0.81 | 0.68, 0.93 | 0.004 |
| Buys in-calf heifers12 | Y/N | 0.80 | 0.68, 0.96 | 0.016 |
| Over two calf diseases12 | Y/N | 1.32 | 1.11, 1.55 | 0.001 |
| Bred 12 | Y/N | 1.43 | 1.21, 1.68 | <0.001 |
| Body Weight (kg) 1 | Range: 140 - 659 | 1.001 | 1.000, 1.002 | 0.008 |
| Mastitis cases treated **12,** | Categories | --- | --- | <0.001gp |
|  | *No case* | Baseline | Baseline | Baseline |
|  | *One case* | 1.27 | 1.10, 1.46 | 0.001 |
|  | *Two or more cases* | 1.50 | 1.19, 1.89 | 0.001 |
| Udder cleaning towel use12, | Categories | --- | --- | 0.006gp |
|  | *Single towel for cows* | Baseline | Baseline | Baseline |
|  | *Different towel per cow* | 1.00 | 0.86, 1.17 | 0.977 |
|  | *No use or only one cow* | 1.31 | 1.10, 1.54 | 0.002 |

Y/N **-** Binary variable code, Yes (1) and No (0)

IRR**-** Incidence risk ratio

gp Global P-Value for categorical variable

**12**Status for the 12 months prior to the study visit

**1** Statusduring the study visit

***i*** Main effects of interaction variable output - should not be interpreted without considering the interaction

Table 4.10: Final multivariable Poisson regression model results showing significant interactions for associations with reported disease counts for the last 12 months among the 384 cows on 292 smallholder dairy farms in Kenya

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Predictor variable** | **Description** | **IRR** | **IRR 95% CI** | **P-value** |
| Number of cows in the farm1 | Categories | --- | --- | 0.314gp |
|  | *With 1 and 2 cows (a)* | Baseline | Baseline | Baseline |
|  | *With 3 and 4 cows (b)* | 0.84 ***i*** | 0.67, 1.05 ***i*** | 0.126 ***i*** |
|  | *With 5 or more cows (c)* | 0.99 ***i*** | 0.69, 1.40 ***i*** | 0.946 ***i*** |
| 50% income reliance on dairy | Y/N | 0.92 ***i*** | 0.78, 1.08 ***i*** | 0.311 ***i*** |
| 50% income reliance on dairy and Number of cows | Interaction | --- | --- | <0.001gp |
| Pregnancy status 1 | Y/N | 0.49 ***i*** | 0.38, 0.63 ***i*** | <0.001 ***i*** |
| Fed Maize Silage 12 | Y/N | 1.11 ***i*** | 0.94, 1.30 ***i*** | 0.210 ***i*** |
| Fedmaize silage & Pregnant | Interaction | --- | --- | 0.006 |
| Extra grain 1.5 kg/d on calving 12 | Y/N | 1.93 ***i*** | 0.90, 4.14 ***i*** | 0.090 ***i*** |
| Body Condition Score (BCS) 1 | 1.25-3.75, | 0.74 ***i*** | 0.62, 0.89 ***i*** | 0.001 ***i*** |
| Extra grain on calving & BCS | Interaction | --- | --- | 0.034 |
| BVDV Vaccination | Vaccine/Placebo | 1.12***i*** | 0.96, 1.30***i*** | 0.145***i*** |
| Visit Number | 1st and 2nd visits | 1.23 ***i*** | 1.03, 1.47 ***i*** | 0.022 ***i*** |
| BVDV vaccination & Visit | Interaction | --- | --- | <0.001 |

Y/N **-** Binary variable code, Yes (1) and No (0)

IRR**-** Incidence risk ratio

gp Global P-Value for categorical variable

**12**Status for the 12 months prior to the study visit

**1** Statusduring the study visit

***i*** Main effects of interaction variable output - should not be interpreted without considering the interaction

**---**Interaction outputs - cannot be interpreted without considering the main effects

BVDV – Bovine Viral Diarrhoea Virus



Figure 4.3: Predicted incidence risk ratio for cow counts of reported disease in the last year for the multivariable model interaction between visit number (1st and 2nd) and intervention group (vaccination or placebo injection)

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Figure 4.4: Predicted incidence risk ratio for cow counts of reported disease in the last year for the multivariable model interaction between household income reliance on dairy farming and number of cows in the farm



Figure 4.5: Predicted incidence risk ratio for cow counts of reported disease in the last year for the multivariable model interaction between cow pregnancy status and farms feeding maize silage



Figure 4.6: Predicted incidence risk ratio for cow counts of reported disease in the last year for the multivariable model interaction between farms feeding extra grain post calving and cow body condition score during the visit

Table 4.11: Reported disease proportions in the last 12 months and physical exam findings, by vaccinated and placebo groups and overall, in 352 heifers on 260 smallholder dairy farms in Kenya

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Visit One** | | | | | | |  | **Visit Two** | | | | | |
| **Disease variable** | **Chi 95%** | **Placebo**  **(n=180)** | | **Vaccinates**  **(n=172)** | | **Overall (n=352)** | | **Chi 95%** | **Placebo**  **(n=199)** | | **Vaccinates**  **(n=185)** | | **Overall (n=352)** | |
| **P-value** | **#** | ***%*** | **#** | ***%*** | **#** | ***%*** | **P-value** | **#** | ***%*** | **#** | ***%*** | **#** | ***%*** |
| Pneumonia12 | 0.601 | 32 | 17.8 | 27 | 15.7 | 59 | 16.8 | **<0.001** | 44 | 24.4 | 10 | 5.8 | 54 | 15.3 |
| Diarrhoea12 | 0.629 | 21 | 11.7 | 23 | 13.4 | 44 | 12.5 | 0.176 | 12 | 6.7 | 6 | 3.5 | 18 | 5.1 |
| Other diseases12 | 0.198 | 39 | 21.7 | 28 | 16.3 | 67 | 19.0 | **0.039** | 55 | 30.6 | 36 | 20.9 | 91 | 25.9 |
| Physical exam1 | 0.714 | 6 | 3.3 | 7 | 4.1 | 13 | 3.7 | 0.544 | 18 | 10.0 | 14 | 8.1 | 34 | 4.7 |
| Overall Disease12 | 0.931 | 62 | 34.4 | 60 | 34.9 | 122 | 34.7 | **0.004** | 73 | 40.6 | 45 | 26.2 | 118 | 33.5 |

**1 -** Statusduring the study visit

**12 -**Status for the 12 months prior to the visit (reported)

Table 4.12: Univariable mixed effects logistic regression results of Odds Ratio (OR) for overall reported disease occurrence (Y/N) in the last 12 months for binary herd-level predictor variables with a P < 0.1 among the 352 heifers on 260 smallholder dairy farms in Kenya

Y/N **-** Binary variable, Yes and No

CI –Confidence interval

**1 -** Statusduring the study visit

**12 -**Status for the 12 months prior to the visit

AI – Artificial Insemination

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Predictor Variable** | **Proportion of the variable that is Yes**  **% (#/#)** | **Odds Ratio** | **Odds Ratio 95% CI** | **P-value** |
| Wet season (Y/N) 12 | 45.8 (238/520) | 1.79 | 1.13, 2.86 | 0.014 |
| Fed Napier grass (Y/N) 12 | 97.7 (508/520) | 0.20 | 0.06, 0.70 | 0.012 |
| Fed grass silage (Y/N) 12 | 3.7 (19/520) | 2.62 | 1.07, 6.44 | 0.036 |
| Fed high protein fodder (Y/N) 12 | 96.4 (501/520) | 1.66 | 0.46, 2.86 | 0.007 |
| Fed maize germ (Y/N) 12 | 17.1 (89/520) | 0.53 | 0.27, 1.05 | 0.068 |
| Fed mineral block (Y/N) 12 | 61.9 (322/520) | 0.65 | 0.40, 1.06 | 0.082 |
| Had enough feed (Y/N) 12 | 31.9 (166/520) | 0.60 | 0.38, 0.94 | 0.025 |
| Forage shortage (Y/N) 12 | 58.5 (304/520) | 1.54 | 0.99, 2.38 | 0.051 |
| Cows sick (Y/N)12 | 38.5 (100/260) | 1.93 | 1.14, 3.28 | 0.015 |
| Acaricide within 2 weeks (Y/N) 12 | 56.4 (147/260) | 1.92 | 1.13, 3.25 | 0.016 |
| Less AI problems (Y/N) 12 | 55.0 (143/260) | 0.60 | 0.35, 1.02 | 0.060 |
| 4 kg colostrum in 12 hours (Y/N) 12 | 22.7 (59/260) | 0.40 | 0.21, 0.74 | 0.004 |
| Buys replacement cattle (Y/N) 12 | 22.7 (59/260) | 1.95 | 1.08, 3.54 | 0.028 |
| Buys replacement heifers (Y/N) 12 | 33.5 (87/260) | 2.10 | 1.23, 3.61 | 0.007 |
| Grazing outside the farm (Y/N)12 | 41.9 (109/260) | 1.92 | 1.13, 3.27 | 0.016 |

Table 4.13: Univariable mixed effects logistic regression results of Odds Ratio (OR) for overall reported disease occurrence (Y/N) in the last 12 months for binary heifer-level predictor variables with a P < 0.1 among the 352 heifers on 260 smallholder dairy farms in Kenya

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Predictor Variable** | **Proportion of the variable that is Yes**  **% (#/#)** | **Reported disease in heifers when variable is Yes (%) (#/#) i** | **Reported disease in heifers when variable is No (%) (#/#) ii** | **Odds Ratio** | **Odds Ratio 95% CI** | **P-value** |
| Bred (Y/N) 12 | 19.6 (138/704) | 21.7 (30/138) | 37.1 (210/566) | 0.44 | 0.268, 0.727 | 0.001 |
| Pregnant during visit (Y/N) 1 | 16.7 (117/700) | 23.9 (28/117) | 36.2 (211/583) | 0.54 | 0.312, 0.941 | 0.029 |
| BVDV vaccination(Y/N)1 | 48.9 (172/352) | 26.2 (45/172) | 40.6 (73/180) | 0.60 | 0.406, 0.898 | 0.013 |
| Weight above 270 kg (Y/N) 1 | 23.7 (167/704) | 26.4 (44/167) | 36.5 (196/537) | 0.62 | 0.380, 0.999 | 0.050 |

Y/N **-** Binary variable, Yes and No

CI –Confidence interval

**1 -** Statusduring the study visit

**12 -**Status for the 12 months prior to the visit

i  The column denominator is the numerator for the the 1st column.

i  The column denominator is the denominator minus the numerator for the the 1st column.

BVDV – Bovine Viral Diarrhoea Virus

Table 4.14: Univariable mixed effects logistic regression results of Odds Ratio (OR) for overall reported disease occurrence in the last 12 months for categorical predictor variables with a P < 0.1 among the 352 heifers on 260 smallholder dairy farms in Kenya

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Predictor variable** | **Category Proportion**  **% (#/#)** | **Reported disease in heifers per category (%) (#/#)** | **Odds Ratio (OR)** | **Odds Ratio 95% CI** | **P-Value** |
| **Colostrum feeding method12** | -- | -- | -- | -- | 0.020 gp |
| Suckling | 73.1 (190/260) | nr | Baseline | Baseline | Baseline |
| Nipple bottle | 8.1 (21/260) | nr | 0.250 | 0.094, 0.666 | 0.006 |
| Bucket feeding | 18.9 (49/260) | nr | 0.985 | 0.500, 1.941 | 0.966 |
| **Breed** | -- | -- | -- | -- | 0.013 gp |
| Holstein | 71.3 (251/352) | 33.9 (85/251) | Baseline | Baseline | Baseline |
| Ayrshire | 16.5 (58/352) | 46.6 (27/58) | 1.953 | 1.080, 3.534 | 0.027 |
| Others | 12.2 (43/352) | 23.3 (10/43) | 0.567 | 0.270, 1.190 | 0.134 |

CI –Confidence interval

***12***Status for the 12 months prior to the study visit

gp Global P-Value for categorical variable

**nr –** Not relevant (reporting animal-level disease proportion for farm-level categories)

Table 4. 15: Multivariable Logistic regression model results showing associations (P < 0.05) with reported disease occurrence in the last 12 months among the 352 heifers on 260 smallholder farms in Kenya

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Predictor variable** | **Description** | **Odds Ratio (OR)** | **Odds Ratio 95% CI** | **P-value** |
| Model constant | -- | 0.200 | 0.09, 0.43 | <0.001 |
| Fed grass silage 12 | Y/N | 3.27 | 1.50, 7.10 | 0.003 |
| Buys replacement heifers 12 | Y/N | 2.45 | 1.39, 3.32 | 0.002 |
| 4 kg colostrum in 12 hours 12 | Y/N | 0.30 | 0.15, 0.60 | 0.001 |
| Bred12 | Y/N | 0.31 | 0.16, 0.60 | <0.001 |
| Two years age | Y/N | 2.16 | 1.21. 3.83 | 0.009 |
| Breed\* | Categories | --- | --- | 0.012gp |
|  | Holstein | Baseline | Baseline | Baseline |
|  | Ayrshire | 1.88 | 1.01, 3.51 | 0.046 |
|  | Others | 0.45 | 0.20, 1.05 | 0.064 |
| Wet season 1 | Wet/Dry | 0.91***i*** | 0.46, 1.80***i*** | 0.787***i*** |
| Number of cows sick **12** | Count (0-10) | 1.10 ***i*** | 0.80, 1.51 ***i*** | 0.568 ***i*** |
| Wet season & Number of cows sick | Interaction | --- | --- | 0.008 |
| Vaccination (intervention) | Vaccine/Placebo | 0.95***i*** | 0.56, 1.63***i*** | 0.860***i*** |
| Visit Number | 1st and 2nd visits | 1.98 ***i*** | 1.01, 3.89 ***i*** | 0.046 ***i*** |
| Vaccination & Visit number | Interaction | --- | --- | <0.018 |

Y/N **-** Binary variable code, Yes (1) and No (0)

CI **–** Confidence interval

gp Global P-Value for categorical variable

**12**Status for the 12 months prior to the study visit

**1** Statusduring the study visit

***i*** Main effects of interaction variable output - should not be interpreted without considering the interaction

**---**Interaction outputs - cannot be interpreted without considering the main effects



Figure 4.7: Predicted disease occurrence of reported disease in the last year for heifers from the multivariable model interaction between visit number (1st and 2nd) and intervention (vaccination or placebo injection)



Figure 4.8:Predicted disease occurrence of reported disease in the last year for the heifers from the multivariable model interaction between number of sick cows on farm for the last 12 months and the season during the study visit

# Chapter 5: Bovine viral diarrhea virus antibody response to a single dose of modified live vaccine and associated factors in smallholder dairy cattle in Meru, Kenya

## 5.1 Abstract

**Background:** Good vaccination response is important in the prevention of bovine viral diarrhea virus (BVDV) where the infection is endemic. Bovine viral diarrhoea (BVD) is prevalent in Kenya but there is limited use of vaccination. Study objectives were to determine: 1) BVDV antibody response variability for BVDV vaccination in cows and heifers on smallholder dairy (SHD) farms in Kenya; and 2) factors associated with this vaccination variability in order to guide SHD farmers on strategic vaccination practices.

**Methods:** This study utilized a single modified live vaccine injection including BVDV in 128 cows and 109 heifers, all non-pregnant. The ELISA tests before and four weeks after vaccination determined pre- and post-vaccine sample-to-positive (S/P) ratios for BVDV antibody levels. Multivariable linear regression analysis modeling was used to determine factors associated with the change in antibody levels.

**Results:** Before vaccination, 40.6% and 7.3% of cows and heifers tested positive for BVDV antibodies (S/P ratio > 0.3), respectively. The mean increase in S/P ratio post-vaccination was 0.476 and 0.804 for cows and heifers, respectively. In the final cow model, the factors associated with the change in BVDV antibody S/P ratios included: cows’ body condition score (BCS) (Coefficient = 0.132), feeding cows protein supplements (Coefficient = -0.170), and post-partum disease in the farm (Coefficient = -0.137), along with abnormal physical exam findings (Coefficient = -0.160) and BVDV antibody test status at the time of vaccination (Coefficient = -0.507). There was also an interaction between: days in milk (DIM), age, and post-calving supplementation. For heifers, factors associated with the change in BVDV antibody S/P ratios included: heifer numbers (Coefficient = -0.059), regular acaricide application in the farm (Coefficient = 0.13), communal grazing, and BVDV antibody test is positive during vaccination (Coefficient = -0.574). There was an interaction between BCS and raising one’s own replacement heifers. Management type was also a significant categorical factor for both the heifers and cows. Zero-grazed cows had better antibody response compared to mixed management farms and communal grazing farms at (Coefficient = -0.137 and -0.124) respectively for the cows and (Coefficient = -0.33 and -0.34) respectively for heifers.

**Conclusions:** The results of this study support the recommendation of planning multivalent vaccination including BVDV component when cattle have a BCS of between 2 and 2.75, and a good health status, and when using more post-calving supplementation and ensuring regular ectoparasite control for maximum BVDV vaccine response on SHD farms.

**Key words: Bovine Viral Diarrhoea, vaccine, antibody response, smallholder dairy, Body Condition Score**

## 5.2 Introduction

Bovine viral diarrhea (BVD) is a very important viral disease of cattle with a global distribution and economic significance in the dairy industry (Pinior et al., 2017; Richter et al., 2017). Prevalence of BVD virus (BVDV) antibodies in herds globally is varied, ranging from very high (e.g. up to 53% in China and Botswana) to very low (e.g. less than 1% in Belgium and Germany) (Simon et al. 2017; Lysholm 2016; Ran et al. 2019; Scharnböck et al. 2018). Some European countries, such as Hungary, Spain, and Scandinavian states have been considered free of BVDV or having very low prevalence (Lindberg et al., 2006; Scharnböck et al., 2018; Simon et al., 2017). Economic losses to farmers are mainly direct through acute infection: pneumonia, diarrhea, reproductive disease, mortalities, and indirect losses through reduced milk production, poor growth, and poor reproductive performance (Scharnböck et al. 2018; Pinior et al. 2017; Richter et al. 2017). The main mode of transmission within and between farms is primarily through direct contact with super-shedding, persistently infected (PI) cattle or transiently infected (TI) cattle within farms or outside the herd if there is poor biosecurity. Other forms of transmission, such as contact with infected wildlife, natural breeding, or contact with aborted fetal materials, are considered of minor significance (Casaubon et al., 2012; Chamorro et al., 2011; Khodakaram-Tafti and Farjanikish, 2017; Richter et al., 2017; Tinsley et al., 2012).

Successful control of BVDV takes a three-pronged approach of biosecurity, testing for and identification of PIs, and vaccination (Walz et al., 2010). This success has been demonstrated particularly in countries, such as Ireland, that have had a sustainable control program in place and ensuring a systemic approach with all three prongs of vaccination, biosecurity, and PI identification (Graham et al. 2021; Moennig and Becher 2018; Thulke et al. 2018). Some countries, such as Austria and Switzerland, have successfully implemented their control programs without the use of vaccination as an option (Moennig & Becher, 2018), but this was made possible partly because there was limited contact of cattle with wild ruminants who can also be a reservoir for BVDV (Casaubon et al., 2012). In Kenya, there is weak biosecurity and contact between cattle and wild ruminants in many parts of the country. There is also very limited testing for PIs and no documented vaccination program by the public veterinary service for smallholder dairy farms. There is a possibility of BVDV vaccination in some large commercial farms in some parts of the country (Nthiwa et al., 2019).

Vaccination has been used with great success as a method of BVDV control in many countries (A. Lindberg et al., 2006). The practice has employed either modified live vaccines (MLV) or killed/inactivated vaccines (KV). The MLV vaccines elicit a stronger and broader protection than the inactivated vaccines (Kalaycioglu, 2007). Modified live vaccines also provide a better fetal protection through a stronger cellular and humoral responses. Many of the commercially available vaccines are multivalent with two genotypes, BVDV 1 and 2, and also in combination with other pathogens, especially those causing respiratory disease and abortion (Walz et al., 2020).

The advantage of MLV is the good cross-reactivity across the variants compared to KV, however the best vaccine antibody response and protection is against the homologous strain (Chowdhury et al., 2021; Xue et al., 2010). Both BVDV genotypes can have either of the two biotypes: cytopathic (CP) or non-cytopathic (NCP) (Bielefeldt-Ohmann, 1995). Non-cytopathic BVDV MLV should not be used for pregnant cattle due to the possibility for it to cross the placenta in pregnant animals and possibly affect the fetus or cause PI to the fetus unless the animal has received the MLV within the last 12 months (Brock et al., 2006; Walz et al., 2020)

Good antibody response to vaccines relies on the vaccinated animal being healthy and in a good plane of nutrition so that an appropriate immune response to the vaccine pathogen is achieved (Richeson et al., 2019). In Kenya, cattle are frequently underfed, especially during the dry season when quantity and quality of available forages are often reduced (Makau, 2019; Muraya, 2019). Vaccinating poorly fed cattle may lead to poor vaccine response and therefore poor protection against the pathogens in the vaccine. For some vaccines, such as foot-and-mouth disease vaccine, some animal and farm-level factors have been identified to be associated with vaccine response variability (Chowdhury et al., 2015; Gowane et al., 2013). However, there is no literature exploring the factors associated with BVDV vaccine response variability in the SDFs industry in eastern Africa. The varying response due to factors such as nutrition management and body condition score in the SHFs can be a cause of poor vaccine efficiency, thus the need to conduct this study.

Our research objectives were: 1) to determine antibody response variability from BVDV vaccination when given to cows and heifers on smallholder dairy farms in Kenya; 2) to determine factors associated with the BVDV antibody response variability; and 3) to determine criteria (e.g. BCS) for strategically utilizing BVDV vaccine effectively.

## 5.3 Methods

### 5.3.1 Study Area and Population

The study was done in Buuri sub-county of Meru County, Kenya. Meru County is located in central Kenya to the east of Mount Kenya and lies on the equator at between 0° 1′ South and 0° 6′ North and within longitudes 37° and 38° East. The county has an estimated population of 1.55 million people and is about 7,000 square kilometers, with animal and crop agriculture being the main economic activity.

Farm eligibility criteria included: 1) membership with the Buuri Dairy Farmers Cooperative Society; and 2) farms without swine or small ruminants currently or expected during the next month. The rationale for not recruiting farms with small ruminants and swine is that Border Disease Virus (BDV) or Classical Swine Fever Virus (CSFV) in these animals may confuse the interpretation of the titre results (Muasya et al., 2022). On eligible farms, eligible cattle included those: 1) with no previous BVDV vaccination; 2) above 6 months of age; and 3) not pregnant. These farms and cattle were a subset of the farms and cattle involved in the randomized controlled trial in chapter 4.

### 5.3.2 Study Design, and Laboratory Analyses

The included cattle were blood sampled via the coccygeal vein at the first farm visit (vaccination visit) and four weeks after vaccination. The blood samples were kept on ice until the end of the day, and then the serum was separated after being left for 12 hours at room temperature to allow clot formation. The serum samples were stored in serum storage vials and frozen at -20 degrees Celsius until all samples were collected. At the vaccination visit, the study animals were also injected with 2 ml of a commercial multivalent vaccine using the subcutaneous route in the neck. The vaccine used was a modified live vaccine (Pyramid® FP 5; Boehringer Ltd.) against four pathogens: BVDV, infectious bovine rhinotracheitis virus, bovine respiratory syncytial virus, and parainfluenza virus type 3. The BVDV components included BVDV type 1 (Singer 1a cytopathic) and BVDV type 2 (296 cytopathic). The vaccine was reconstituted and kept in a cooler, as recommended by the manufacturer, and used within 6 to 8 hours of reconstitution. Approximately 10 vaccine doses were used on a normal day and about 5 vaccine doses on a day with less farm visits depending on logistics and distance covered.

Also on the first farm visit, farm-level and animal-level information was collected using a questionnaire focusing on the general farm management, nutritional management, diseases, reproduction performance, and disease prevention practices for the last year (Table 5.2 and 5.3). Recruited animals were examined for various parameters including: weight (using a heart girth tape and reading the corresponding breed category), height (using a height meter), reproduction status (based on rectal palpation: stage of estrus for unbred animals; and stage of gestation for animals bred over two months ago), body condition score (BCS on a five-point scale and done by at least two people including the main researcher), California mastitis testing (CMT), and health status (based on a standard physical examination).

After all serum samples were collected, the frozen samples were thawed and tested for antibody to BVDV using the IDEXX Total Antibody ELISA (IDEXX Laboratories, Switzerland) and antigen BVDV ELISA (IDEXX Laboratories, Switzerland), at Clinical Studies Department, Immunology Laboratory at the University of Nairobi by a trained technician. All manufacturer protocols were followed during the ELISA testing. The BVDV antigen testing was conducted to detect animals persistently or transiently infected with BVDV at either visit.

### 5.3.3 Sample size calculation

The sample size for this study was determined at a minimum of 126 for both the cows and heifers, based on the calculation below. This calculated sample size was exceeded due to availability of kits and to take care of anticipated loss to follow-up during the second visit.

**n**=2{[(*Zα*+Z*β*)**/** (ES)] 2}= 2{31.4} = 63 (Charan & Biswas, 2013; Ledolter & Kardon, 2020)

**n**= Sample sizeforheifers or cow

**Zα** *=* 1.96 Z score for a type 1 error of 5%

**Zβ** *=* 0.842 Z score for power of 80%

**ES**= Effect size taken to be equivalent to 0.5 medium effect size (Cohen, 1988; Sawilowsky, 2009).

### 5.3.4 Statistical analysis

The ELISA results produced a sample-to-positive (S/P) ratio for each animal at each visit, indicating the reading that was adjusted for the positive control on the plate and ensuring the negative control was in the appropriate range. The outcome of interest in the study was the change in S/P ratio between samples collected at the first and second visit (sample 1 and 2). The normality of the outcome was determined using Shapiro–Wilk test. Descriptive statistical analyses determined the mean S/P ratio change for cows and heifers overall and for other variables of interest (e.g. BCS and nutritional management factors).

Analytical statistics started with univariable linear regression analysis to determine factors associated with the change in S/P ratio, and those associations with p-values < 0.2 were eligible for the multivariable modeling using Stata (Statacorp LCC, College Station, Texas, USA). Multivariable linear regression analysis modeling using the backward elimination method was used to determine the factors (e.g. nutritional management, BCS, milk production, diseases) associated with the change in S/P ratio. Confounding effects were assessed by checking regression coefficient changes greater than 20% with and without possible confounding variables but only where a confounder had a plausible relationship with both the outcome and the variable. Two-way and three-way interaction variables were explored among the significant variables in the final model. Standardized residuals were examined for the final model to determine the goodness of fit of the model.

Separate analyses were conducted for the cows and heifers because heifers were managed in separate ways from the cows on most of the farms, and there were different variables available for analyses for the two strata (e.g. milk production, days in milk). A positive BVDV test on the first sample was also considered as an independent dichotomous variable to account for a substantial antibody titre at the beginning of the study.

## 5.4 Results

There were 134 cows and 114 heifers tested for BVDV antibodies at the first visit of the study, respectively, and of those animals, 128 cows and 109 heifers were again available for testing for BVDV antibodies at the second visit of the study, respectively. There were 6 cows and 5 heifers tested for BVDV on the first visit but not available on the second visit for the following reasons: death, sale, and relocation to a community pasture in the forest. There were 172 farms involved in the study, with 118 farms having cows and 96 farms having heifers.

The main breeds in the study appeared to be exotic (i.e. European origin dairy breeds), with Holstein-Friesian being the majority breed at 65% (155/237). The other breeds included: 43 Ayrshires, 29 primarily indigenous (East African Zebu and Boran), 6 Guernsey, 3 Jerseys, and 1 Brown Swiss. It was noted that records on breed were not kept and that most of the exotic breeds were crossbreeds. Only 4 of the cows were non-lactating and for the lactating cows, milk production ranged from 0.5 to 26 kg per day, with a mean production of 8.65 kg/d. California mastitis test (CMT) during the first visit examination revealed 37.9% of the lactating cows had subclinical mastitis in at least one quarter.

### 5.4.1 Bovine Viral Diarrhea Virus Test Results

There were 52 (40.6%) cows and 8 (7.3%) heifers that were positive for BVDV antibodies (above the 0.3 S/P ratio thresholds) on the 1st test before vaccination (25.3% overall). After vaccination, there were 15 (11.7%) cows and 7 (7.3%) heifers that still had S/P ratios that were below a positive case threshold of 0.3.

Table 5.1 shows descriptive statistics of the S/P ratios at visit 1 and visit 2 for cow and heifer strata, along with the S/P ratio changes for these groups when the S/P ratio for visit 1 is subtracted from the S/P ratio for visit 2. Given that a small number of animals (n=28: 25 cows and 3 heifers) had lower S/P ratios at visit 2 than visit 1, producing negative S/P changes (-0.597 for cows and -0.500 for heifers), a new variable was created, called S/P Difference that had no negative S/P ratio changes by adding 0.6 and 0.5 to the S/P ratios of the cows and heifers, respectively. This S/P Difference variable was utilized as the outcome variable in the subsequent regression analyses to ensure the outcome had no negative numbers.

The BVDV antigen tests identified seven cows being positive, four at the first sampling and three at the second sampling. One BVDV antigen-positive cow in the first sampling died suddenly before the second sampling, with reported signs of generalized weakness and a rough hair coat. It is possible that this cow was a PI, but without the second sample, we cannot confirm this PI status. Three of the seven positive cow samples had a suspect outcome upon testing and were reported as positive.

There were two heifers testing positive for BVDV antigens on the first sampling, with one heifer testing positive on her second sample, making it a likely PI and the other likely Transient Infected (TI). The heifer with two antigen positive tests was reported to have died two months later exhibiting general weakness, skin disease, and progressive diarrhoea. All nine antigen-positive animals came from nine different farms. All antigen-positive animals had an S/P Ratio greater than 0.3 on the second Ab test, indicating a positive antibody test, with three of these nine animals having an Ab-positive result on the first test.

### 5.4.2 Associations of S/P difference with animal and farm factors among cows

Tables 5.2 and 5.3 show the variables associated with S/P Difference considering a cut-off of P-value < 0.1. There were eight variables with a positive association (associated with an increased S/P Difference) and fifteen variables with a negative association. The final multivariable linear regression model (Table 5.4) had eight variables associated with S/P Difference (p≤ 0.05), with an adjusted R2 of 0.590. The model also includes a confounder (a three-category management system variable with over 20% confounding on the coefficient of total supplementation upon inclusion in the model), and an interaction between three variables: total supplementation, age in years, and days in milk (DIM), as described in Figure 5.1 and interpreted below.

Interpreting the model variables, cows that were BVDV Ab-positive on the first visit had a 0.51 lower S/P Difference than those that were negative. Cows with an abnormal finding during physical examination at the first visit had 0.16 lower S/P Difference than those without. Cows from farms that had recorded a post-calving disease condition in the last 12 months had lower S/P Difference of 0.13 versus cows on farms that did not record this problem. For every point increase in body condition score, there was an increase of 0.13 in S/P Difference (Figure 5.1). Cows on farms feeding high protein supplements had 0.17 lower S/P Difference than cows on farms not feeding these supplements. Looking at the management system variable, cows from farms that practiced zero-grazing had the highest antibody response. Cows from farms practicing both zero-grazing and communal grazing had a somewhat lower S/P Difference by 0.14, and those completely on communal grazing had a somewhat lower S/P Difference of 0.12, in reference to the zero-grazed cows. To assess the relationship between BCS and S/P Difference we compared the graphical trend of the response. The highest median was between BSC 2 and 2.75 in a box plot (Figure 5.2) concluding that the best response was above BCS of 2. At a higher BCS of 3 and more, there were however few outcomes as shown by fitted values in Figure 5.3

For the interaction variable, the coefficients of the three variables cannot be interpreted by themselves due to the effect modification by the other two variables in the interaction. In the young cows (Figure 5.1), the S/P Difference was higher for those fed a higher amount of supplements versus those fed a lower amount of supplements. With advanced age (over 8 years), the rate of reduction in S/P Difference by age was higher for the cows fed a higher amount of supplements. The S/P Differences for the cows that were below 30 DIM had a greater variability than those 30 DIM and above, with the variability diminishing towards the age of 7 and 10 years. Beyond 8 years of age, there were less than a quarter of the observations; therefore, there was more variability in those predictions and less confidence in those results, which appear to change direction, as shown in both graphs of Figure 5.1. Interpretation of the third variable in the interaction is through the differences in the two graphs in Figure 5.1. For the cows that were less than 30 days in milk (graph on the left), the variation in S/P Difference was greater than those that were 30 days in milk and beyond (graph on the right).

The standardized residuals were normally distributed when assessed graphically and with a Shapiro Wilk test p-value of 0.44. Assessment for independence of residuals shows homoscedastic data on both graphical presentation and Breusch Pagan/Cook–Weisberg test with a p-value of 0.8088. There were only 7 standardized residuals outside the -2 and 2 limits with none outside -3 and 3. The seven largest standardized residuals represented less than 5% of the 128 total observations; therefore, there was no concern for extreme observations.

### 5.4.3 Associations of S/P Difference with animal and farm factors among heifers

The heifers that were recruited to the study ranged in age from 6 months old to four and a half years old. The management of heifers in most of the farms, as observed and recorded in the questionnaire, was different with regards to housing and feeding; therefore, the heifer data were considered for separate analysis from the cow data. Tables 5.5 and 5.6 show the animal- and farm-level variables associated with S/P Difference considering a cut-off of P-value < 0.1. All fifteen variables had a negative association with S/P Difference at the designated cut-off.

The final multivariable linear regression model (Table 5.7) had seven variables associated with S/P Difference (p≤0.05) with an adjusted R2 of 0.419. The model includes an interaction between farms raising all replacements and body condition score as described in the Figure 5.4 and interpretation below. Heifers from farms with more people living on the farm had a lower S/P Difference than those on farms with fewer people. For every additional individual on the farm, the heifers had a 0.05 lower S/P Difference. Similarly, heifers from farms with many heifers had a lower S/P Difference than those with fewer heifers. With every additional heifer on a farm, there was a reduction of S/P Difference of 0.059. Heifers from farms practicing communal grazing partly or fully had lower S/P Differences by 0.33 and 0.34, respectively, compared to those from zero-grazed farms. Heifers that were BVDV antibody positive had a 0.57 lower S/P Difference compared to those that were negative. Farms that practiced regular acaricide use every 2 weeks had heifers with higher S/P Difference by 0.13 compared to those that did not use acaricides regularly.

For the interaction variable (Figure 5.4), at low body condition scores, there was no significant change in S/P Difference among heifers, regardless of whether farms raised all replacements or not. On farms not raising all their heifers, heifer S/P Difference increased as BCS increased, while this association was opposite on farms raising all their heifers. Conversely at high body condition scores for heifers (BCS 3 and 4), the S/P Difference was higher for the heifers on farms not raising all their replacements than those farms raising all heifers on the farm.

The standardized residuals were normally distributed when assessed graphically and on Shapiro Wilk test p= 0.39.) Assessment for independence of residuals shows homoscedastic data on both graphical presentation and Breusch Pagan/Cook–Weisberg test (p= 0.50.) There were only 5 standardized residuals outside the -2 and 2 limits with none outside -3 and 3. The seven largest standardized residuals represented less than 5% of the 109 total heifer observations; therefore, there was no concern for extreme observations.

## 5.5 **Discussion**

This study was an assessment of the antibody response to a single subcutaneous injection of MLV including BVDV in cows and heifers on SDFs in Meru, Kenya. Awareness and control programs for BVDV were virtually absent in this area of Kenya, despite the infection being present across Kenya (Callaby et al., 2016; Okumu et al., 2019; Olum et al., 2020; VanLeeuwen et al., 2021). Furthermore, there is little record of BVDV vaccine use in Kenya, and there are no studies on the benefits of vaccine use in the control of BVDV in Kenya. This study adds to the understanding of BVDV antibody response to vaccination in cows and heifers under varying management conditions of SDFs in Kenya.

This study recruited cattle from farms in the Buuri Dairy Cooperative Society where there has never been an history of BVDV vaccination in the past. Therefore, the BVDV antibody test results on the first visit (25% prevalence) likely represents the natural exposure to BVDV virus in the region, particularly with the farm exclusion factors of not having swine or small ruminants on the farm, which should have reduced the possibility for exposure to BDV or CSFV that have a potential diagnostic cross-reactivity (Muasya et al., 2022). This seroprevalence is not surprising considering the published results of BVDV exposure from other studies in Kenya ranged from 19% to 79% (Callaby et al., 2016; Okumu et al., 2019; Olum et al., 2020; VanLeeuwen et al., 2021). The antigen test results showed evidence of limited transient infections (TI) with BVDV and one confirmed PI in the sampled population. There have been many similar reports of low PI prevalence, with sporadic TI outbreaks (Kabir & Bishwabidyalay, 2017; Scharnböck et al., 2018).

### 5.5.1 Factors of BVDV Antibody Response Variability in Cows and Heifers

The vaccine response relationship with body condition score (BCS) was noted to have varying associations for both the heifers and cows with respect to the univariable and multivariable regression analysis. Body condition score has been shown to be closely associated with many management and animal factors, such as nutrition, feeding system, and cow weight (McCarthy et al., 2007). For the cows, there was a positive association in both BCS and the level of post-calving grain supplementation with the vaccine response, underlining the importance of vaccinating cows in higher BCS and higher levels of grain consumption. The importance of BCS with regards to immunity and cow transition health among other factors has been demonstrated in several studies.

A study in Ethiopia assessing the impact of drought on vaccination success found no significant advantage for vaccination during non-drought times; however, there were notable failures in the vaccination program’s execution (Catley et al., 2009; Roche et al., 2006, 2009). Data from a study assessing the effect of progesterone on cellular immunity in Holstein cows showed that there was a better immune response with good body condition score (Ohtsuka et al., 2008). In our study, the best vaccine antibody response was in cows between a score of 2 and 2.75, which may reflect the BCS perceived by farmers to be appropriate for a milking cow warranting grain supplementation. If a cow had a BCS of 3 or higher, farmers may have thought that they did not warrant higher grain supplementation, reducing the nutrient intake needed for a higher antibody response. We can draw similarity with a review study on BCS association with dairy cattle productivity and health that reported an optimum performance of dairy cows with a score of 3.0 to 3.25 (scale of 1-5) (Roche et al. 2009).

For heifers, there was a significant interaction for BCS with raising all replacements in the farm. The interaction showed that farms that raised all their replacements had higher S/P Difference at lower body condition score but lower S/P Difference at higher body condition score. Most of the farms with heifers did raise their replacements (94%). Therefore, these results might be a function of small numbers of heifers not raised on the farms who were responsible for this apparent relationship. This may also be attributed to feed shortage and herd size in the farms that raise all their replacement heifers. A review of health management of dairy replacements concluded that heifers are often overlooked because farmers do not have an immediate return on their efforts and prefer to focus on improving the management of their milking cows (Dutta, 2016). Further research is needed to confirm or clarify this possible interaction.

The type of management system was an important confounder for the total amount of grain supplementation post-calving in the final cow model. Zero-grazed cows had the highest S/P Difference, while the communally grazed and mixed system cows had somewhat lower S/P Differences. This finding can likely be attributed to the reduced stress of not needing to expend energy for grazing to eat, and the reduced exposure to infectious diseases, and their associated impacts on the immune system, with the zero-grazed animals (Carroll and Forsberg, 2007). Communally grazed animals often walk many kilometers per day for communal pastures, and encounter other cattle on the communal pastures, leading to potential transmission of infectious disease, along with the nutrient requirements and potential stress of long-distance locomotion (Arnott et al., 2017; Maurya et al., 2012). Farms with adequate forage supplies on-farm are less likely to need to send their cows onto communal pastures (FAO, 2012). A document titled “Feeding dairy cattle in East Africa” reports that there is not enough energy supply among communally grazed dairy cattle due to the communal pastures typically containing poor quality mature grasses and weeds (Gachuiri et al., 2012).

As with cows, the management system of keeping heifers had a similar significant association with the vaccine response, with farms that practiced grazing in communal grounds and farms doing a mixed form of nutrition management having lower S/P Difference compared to farms with purely zero-grazed heifers. In Estonia, the grazing management system was found to be associated with the health of dairy calves and cows, with grazing cattle suffering more mortalities than animals from farms that supplied all feed to their animals (Reimus et al., 2020). A similar observation was recorded in Ethiopia where response to tuberculin was higher in indoor confined cows than outdoor pasture-kept cows (Ameni et al., 2006).

The pre-vaccination antibody status for BVDV by an ELISA test had a substantial negative association with the S/P Difference for both the cows and heifers. It has been demonstrated that calves receiving colostrum rich in BVDV antibodies do not seroconvert well when a BVDV MLV vaccine is used (Endsley et al., 2003; Kirkpatrick et al., 2001). For BVDV, an age of 4 months for calves is recommended as a good time to start vaccination when the maternal antibodies wane (Chase et al., 2008). Similar to the acquired maternal antibodies, we can draw a comparison for cows and heifers with existing antibodies acquired from a previous natural exposure. It is unknown when these seropositive cows developed titers but if there were sufficient antibodies to lead to a positive test, there could be sufficient antibodies to inhibit an antibody response to the MLV vaccination.

A study assessing the effects of passive immunity in calves vaccinated with MLV BVDV vaccine showed that the vaccine seroconversion response was lower in calves remaining seropositive for a BVDV-1 strain than a BVDV-2 strain (Kirkpatrick et al., 2001). On the other hand, the use of killed BVDV vaccine on farms exposed to PI calves has been shown to produce higher mean titers (Houe et al., 1995). Infection with BVDV is known to cause immunosuppression in an acute, transient infection (Chase et al., 2004; Kelling, 2004; Walz et al., 2020). It has been demonstrated that adaptive immunity is impaired by compromised antigen-presenting cells after acute BVDV infections (Brodersen, 2014). The immunological response to MLV vaccine used in our study could have been affected by the presence of BVDV neutralizing antibodies and therefore the lower S/P Difference in our study (Lidia & Pedro, 2015).

### 5.5.2 Factors of BVDV Antibody Response Variability in Cows

The cow S/P Difference outcome was significantly associated with nine factors in the final model, with an interaction between three of the factors. Five of the factors were farm level while four were animal level, showing the critical role of management and environment on the health and immune system of cows.

Having a clinical condition detected during the first visit, such as swollen lymph nodes, fever, or skin disease, was associated with a lower S/P Difference than not having any condition. This cow-level finding can be attributed to a disease stress that could consequently lead to a weaker antibody response. It has been shown that stressors, such as diseases, can cause abnormal function of the innate immune system (Al-Kubati et al., 2021; Bronzo et al., 2020). It has also been demonstrated that when there is some level of system inflammation and chronic infection in cattle, that cellular and humoral immunity is impaired (Konnai et al., 2017). There is demonstrated evidence of immune exhaustion in anaplasmosis infection, impairing immune response in cattle (Okagawa et al., 2016). It has also been reported that MLV BVDV vaccine can cause immunosuppression, thereby potentiating other concurrent infections to flare up (Lidia & Pedro, 2015). Therefore, it is biologically plausible to find this lower antibody response in cows that had an abnormal finding during physical examination.

On farms with conditions affecting cows post-calving within the previous 12 months, such as retained afterbirth, metritis, acute mastitis, or udder edema, cows had a lower S/P Difference than those on farms that did not have such conditions. It has been shown that cows that suffer post-calving conditions were most likely overwhelmed by oxidative stress during the transition period (Sordillo, 2016). It has been reported that the innate immune system is affected by this stress, and that inflammation is impaired during the periparturient phase by the physiological changes (Mezzetti et al., 2020). Therefore, at the farm level, the lower S/P Differences for cows on farms that had incidences of post-calving conditions may be attributed to oxidative stress due to poor transition period management.

The interaction term in the cow model shows the dependency between age in years, DIM, and total amount of grain fed after calving and their association with vaccine response. In the young cows, the S/P Difference was higher for those fed a higher amount of supplements versus those fed a lower amount of supplements. However, this observation was more noticeable in cows with DIM below 30. These findings are consistent with other studies which show that immunity is negatively affected by poor body condition and poor supplementation during the periparturient period, along with the weaker immunity associated with stress of calving (Ingvartsen & Moyes, 2013) (Moyes et al., 2013). It was shown in a study in the USA that there was an interaction between days in milk and beta hydroxybutyrate (BHB) levels, with high post-partum BHB concentrations seen in cows with high dry period BCS (i.e. BCS>3.75), as well as a substantial drop in BCS (i.e. BCS drop > 0.5) post-partum (Rodriguez et al., 2021).

Farms practicing high levels of supplementation post-calving showed a better S/P Difference compared to non-supplemented cows. A study investigating the importance of physiological imbalances during the transition period showed that good nutrition post-calving prevents negative-energy balance and ensures immunocompetence (Ingvartsen & Moyes, 2013; Sordillo, 2016). It has been shown that when protein-rich supplements are fed to transition cows, there is better immune performance (Gandra et al., 2016). This finding means that cattle that are well supplemented after calving are also likely in a good energy status to mount a defense against health challenges (Sordillo, 2016). We also noted that in advanced ages in the study cows, the S/P Difference was lower than in younger cows, with non-supplementation being associated with similar S/P Differences across the ages. However, beyond eight years of age, there were limited numbers of observations to make a reliable interpretation and conclusion. A study assessing the association between ante-partum BCS, post-partum concentrates supplementation, and the hematological immune-cellular phenotypes concluded that there was no significant immune expression difference between adequate and high BCS (Bünemann et al., 2020).

Cows on farms feeding high protein supplements (e.g. cottonseed cake but not dairy meal) within the last 12 months showed a lower S/P Difference of 0.2 than cows on farms not feeding high protein supplements. This is opposite of what we would have been expected since high protein feeds should lead to better nutritional status and thus better immune response (Gandra et al., 2016; Sordillo, 2016). Speculatively, this may be related to the fact that farms feeding high protein supplements to cows may be having feed shortages in general. Most of the farms (31/40) feeding high protein supplements did not have enough feed supplies for the last one year. Another possible reason may be that farms supplementing high protein feeds were self-formulating dairy meal at home with no quality assessment. However, we did not collect data on home recipes for dairy meal. The report “Feeding dairy cattle in East Africa” documents the challenges in the quality of both the commercial supplements and homemade formulations (Gachuiri et al., 2012).

A study investigating feed management in SHFs of semi-arid eastern Kenya showed that supplementation was one of the feed shortages coping mechanisms. It also reported that most farms actually supplemented with very small amounts that would not make a large difference to production (Njarui et al., 2011). A study in the coastal region of Kenya demonstrated a similar situation where supplementation with concentrates was common but in small volumes, with feed shortages being also reported in most of the SDFs (Muraguri et al., 2004).

### 5.5.3 **Factors** of BVDV Antibody Response Variability in Heifers

The heifer S/P Difference outcome was significantly associated with seven factors in the final model, with an interaction between two of the factors. Five of the factors were farm level while two were animal level, again showing the critical role of management and environment to the health immunity of heifers. It has been shown that calf immunity and general performance is very much affected by environmental and management factors, and we can draw similar conclusions with heifers (Hulbert & Moisá, 2016). A study in Estonia showed that herd level factors were more associated with the health of dairy calves and cows with respect to mortalities (Reimus et al., 2020).

The number of persons living within the farm was associated with vaccine response. Every extra person in the farm was associated with a reduction of 0.05 S/P Difference in the heifers kept. It has been shown that there is a relationship between household size and the health of cattle, or livestock on smallholder farms (Thumbi et al., 2015). The competition for farm resources in big households and farms with more heifers could lead to poor animal health and subsequent poor vaccine response. A study in western Kenya showed a close relationship between livestock ownership and growth rate of children, with household member number and wealth score being significantly associated factors (Mosites et al., 2016). Similarly, farms with higher number of heifers had lower S/P Difference for their heifers, with every additional heifer having a 0.06 less S/P Difference for the heifers vaccinated. The farms with more heifers can be larger herds and this observation is similar to other studies. Farms with larger herd sizes are also shown to have a higher frequency of diseases and mortality (Reimus et al., 2020). Other studies have reported more diseases with an increase in herd size (Debebe and Haben, 2020; Ghebremariam et al., 2018).

The regular use of acaricides in the control of ecto-parasites was positively associated with S/P Difference. Heifers on farms that applied an acaricide at least every two weeks had a higher S/P Difference of 0.13 compared to less frequent application. Vector-borne disease prevention can minimize incidences of endemic diseases, such as anaplasmosis, babesiosis, and theileriosis, consequently leading to better health performance. Immune exhaustion in anaplasmosis infection leads to poor immune response in cattle (Okagawa et al., 2016). Ticks and other cattle ecto-parasites have been shown to express salivary proteins that cause immunosuppression and inflammatory cytokine down-regulation (Chmelař et al., 2017; Kotsyfakis et al., 2006; Sun et al., 2018).

### 5.5.4 Study limitations

This study was limited with respect to follow-up time, with only a single sampling post-vaccination. A longer follow-up sampling could provide more information about the maintenance of protection across the recommended time of 12 months. This study also had a single MLV injection only (as recommended by the manufacturer for adults) without a booster injection and therefore did not identify if the response may be better with a booster. Also, it should be remembered that vaccine-related seropositivity does not confirm protection, but that was not the objective of the study. Vaccine-related seropositivity is a good indicator of protection (Lidia and Pedro 2015; Xue et al. 2010).

This study did not explore the relationship between vaccination and certain important factors, such as season or different planes of nutrition, and did not have a control group. The trial was done during one season, and perhaps there are differences in vaccine response during different seasons, given the substantial differences in nutritional management on smallholder dairy farms in Kenya from rainy and dry seasons. Also, it may have been useful to have sentinel non-vaccinated animals tested on farms to determine the role of ongoing exposure to BVDV during the study. Ideally, for this purpose, you would match these sentinels on-farm. However, most farms only had one cow and/or one heifer, making this logistically difficult.

The final limitation is that the study drew most herd-level management information from the practices performed for the last 12 months. Therefore, this historical information was more prone to recall bias among the farmers. This bias was complicated by the challenges of poor record-keeping, especially on the age, parity, and reproduction data.

### 5.5.5 Future Research

There is a need to study further the relationship between the interplay between production stage in dairy cattle, age, and vaccine response to inform strategic time for vaccination. There is also need to investigate BVDV vaccine antibody titer trends for a longer period like 12 months in the smallholder dairy system in Kenya with keen interest on nutrition management and seasons. The circulating genetic variants in Kenya should also be investigated in the dairy cattle population as well as in pigs, sheep, and goats. The effectiveness and role of vaccine in protecting fetal infection should be investigated in the smallholder dairy system in combination with other methods of BVDV prevention.

## 5.6 Conclusion

This study identified the associations between farm-level and animal-level factors with antibody response to MLV vaccine including BVDV in the smallholder dairy system. The study established that better body condition score and more nutritional supplementation led to better vaccine response in cows. Active disease conditions, positive BVDV antibody test pre-vaccination, and history of post-calving disease conditions were associated with poor vaccine response. The study identified that there was an effect modification between days in milk, age, and daily supplementation post-calving in cows. There was also effect modification between body condition score and raising all replacement for heifers.

Regular ecto-parasite control was important for better BVDV vaccine response in heifers. Overall mean vaccine response in S/P Difference and the proportion of successful response above positive threshold was better for heifers than adult dairy cows. We can conclude that nutritional status, production stage, current disease status, and previous disease status are key determinants for BVDV vaccine response. We therefore recommend planning vaccination against BVDV to cows when having a good BCS of between 2 and 2.75, cows in farms that provide more supplementation post-calving and when cows do not have any disease condition for best vaccine benefits. For heifers, the study recommends priority to be given to early vaccination while young and that heifers be raised in farms where regular ecto-parasite control is practiced for maximum BVDV vaccine efficiency.

## 5.7 References

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Table 5.1: Descriptive statistics of bovine viral diarrhea virus antibody sample-to-positive (S/P) ratio results for 128 cows and 109 heifers on 172 smallholder dairy farms in Meru Kenya in 2020

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Outcome variable | Number | Mean | S. d. | Minimum | Maximum |
| S/P ratio 1c | 128 | 0.4641 | 0.6738 | -0.1166 | 2.1930 |
| S/P ratio 2c | 128 | 0.9402 | 0.5240 | -0.1304 | 2.4256 |
| S/P Change c | 128 | 0.4762 | 0.4747 | **-0.5965** | 1.6575 |
| S/P Difference c | 128 | 1.0761 | 0.4747 | 0.0035 | 2.2575 |
| S/P ratio 1h | 109 | 0.0848 | 0.2806 | -0.0750 | 2.2250 |
| S/P ratio 2h | 109 | 0.8888 | 0.3854 | -0.0179 | 1.7255 |
| S/P Change h | 109 | 0.8041 | 0.4109 | **-0.4995** | 1.6987 |
| S/P Difference h | 109 | 1.3041 | 0.4109 | 0.0005 | 2.1987 |

c: Cows

h: Heifers

1: First visit ELISA test

2: Second visit ELISA test

Table 5.2: Descriptive statistics for continuous predictor variables with a P-value less than 0.1 for associations with S/P Difference among the 128 cows on 118 smallholder dairy farms

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Predictor variable** | **Number** | **Mean** | **Range** | **Coefficient** | **P-value** |
| Age of male farmer (years) 1 | 110 | 51.35 | 32-86 | 0.008 | 0.048 |
| Land supporting livestock (acres)12 | 118 | 1.05 | 0-9 | 0.063 | 0.071 |
| Mineral lick amount (g)12Dry | 118 | 37.60 | 0-12 | 0.004 | 0.003 |
| Total daily supplement (kg)12Cal | 118 | 2.92 | 0-8 | 0.041 | 0.063 |
| Count of diseases in farm 12 | 118 | 2.40 | 0-7 | -0.049 | 0.049 |
| Age (years) 1 | 121 | 6.20 | 1.5-19 | -0.059 | <0.001 |
| Parity 1 | 128 | 2.563 | 0-11 | -0.080 | 0.001 |
| Diseases count for a cow 12 | 128 | 1.813 | 0-7 | -0.071 | 0.005 |
| Body condition score 1 | 128 | 2.271 | 1.5-3.5 | 0.294 | <0.001 |

**1**Statusduring the 1st visit (non-historical data)

**12**Status for a period of 12 months prior to the 1st visit

**12Cal**Post-calving practice for the 12 months prior to the 1st visit

**12Dry** Dry cow management practice for the 12 months prior to the 1st visit

Table 5.3: Descriptive statistics for categorical predictor variables with a P-value less than 0.1 for associations with S/P Difference among the 128 cows on 118 smallholder dairy farms of Meru Kenya

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Predictor variable** | **Categories** | **Number**  **Yes** | **Proportion**  **Yes** | **Coefficient** | **P-value** |
| Management system 12 | *Zero-graze* | 79 | 0.70 | Baseline | 0.006 **gp** |
|  | *Mixed* | 31 | 0.26 | -0.206 | 0.034 |
|  | *Communal* | 8 | 0.08 | -0.4156 | 0.005 |
| Fed Maize silage (Y/N)12 | Y/N | 38 | 0.03 | -0.4091 | 0.090 |
| Fed cows grass hay (Y/N)12 | Y/N | 91 | 0.77 | 0.2273 | 0.020 |
| Fed cows desmodium (Y/N)12 | Y/N | 22 | 0.19 | 0.2633 | 0.015 |
| Fed cows banana leaves (Y/N)12 | Y/N | 33 | 0.28 | -0.2038 | 0.030 |
| Stalls present for cows1 | Y/N | 97 | 0.76 | 0.1664 | 0.089 |
| If recently bred (Y/N)1 | Y/N | 18 | 0.14 | -0.2119 | 0.079 |
| History of mastitis (Y/N)12 | Y/N | 31 | 0.24 | -0.1864 | 0.057 |
| History of pneumonia (Y/N)12 | Y/N | 42 | 0.33 | -0.3017 | 0.001 |
| History of diarrhea (Y/N)12 | Y/N | 28 | 0.22 | -0.2765 | 0.006 |
| History of other disease (Y/N) 12 | Y/N | 12 | 0.09 | -0.2223 | 0.017 |
| Abnormal exam findings (Y/N)1 | Y/N | 54 | 0.42 | -0.3743 | <0.001 |
| BVDV ELISA test (Y/N) 1 | Y/N | 52 | 0.41 | -0.6280 | <0.001 |

**1**The statusduring the 1st visit for vaccination and taking the first serum sample (non-historical data)

**12**The status for a period of 12 months prior to the 1st visit

**gp** Global P-value for categorical variable association assessment

Table 5.4: Multivariable regression estimates for BVDV S/P Difference in 128 cows vaccinated against BVDV on 118 smallholder dairy farms of Meru, Kenya

|  |  |  |  |
| --- | --- | --- | --- |
| **Predictor variable** | **Coefficient** | **95% CI** | **P-value** |
| Model Constant | 0.415 | -0.26, 1.11 | 0.238 |
| BVDV ELISA test (Y/N)***1*** | -0.507 | -0.62, -0.35 | <0.001 |
| Abnormal findings (Y/N) ***1*** | -0.160 | -0.34. -0.07 | 0.015 |
| Post-calving problem (Y/N) ***12*** | -0.135 | -0.24, -0.01 | 0.022 |
| Body condition score***1*** | 0.132 | 0.03, 0.25 | 0.021 |
| Fed high protein supplements (Y/N) ***12*** | -0.170 | -0.32, -0.08 | 0.007 |
| Management system (i, ii and iii) 12 | -- | -- | 0.130 **gp** |
| *i. Zero-grazed* | Reference | Reference | Reference |
| *ii. Mixed practice* | -0.137 | -0.27, -0.005 | 0.051 |
| *iii. Communal Grazing* | -0.124 | -0.34, -0.09 | 0.258 |
| Total daily supplement (kg) ***12Cal*** | *0.280****i\*\**** | *0.11, 0.42****\*\**** | *0.001* |
| Age in years ***1*** | *0.120* ***i\*\**** | *0.03, 0.21****\*\**** | *0.011* |
| DIM <30 (Y/N) | *0.687* ***i\*\**** | *0.02, 1.34****\*\**** | *0.045* |
| Total daily supplement & Age in years *i2* | \*\* | \*\* | 0.008 |
| DIM >30 and Age in years *i2* | \*\* | \*\* | 0.017 |
| DIM >30 and & Total daily supplement *i2* | \*\* | \*\* | 0.035 |
| DIM >30, Age in years & Total daily supplement ***i3*** | \*\* | \*\* | 0.048 |

DIM - Days in Milk

***1***Statusduring the first visit for vaccination and taking the first serum sample (non-historical data)

***12***Status for a period of 12 months prior to the 1st visit

***gp*** Global P-value for categorical variable association assessment

***12Cal***Post-calving practice for the 12 months prior to the 1st visit

***\*\**** Coefficient main effects without considering the interaction – should not be interpreted without considering the coefficients of the other variables in the interaction

***i2***Results from the two-way interaction variables

***i3*** Results from the three-way interaction variable

BVDV – Bovine Viral Diarrhoea Virus

ELISA – Enzyme Linked Immunosorbent Assay

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Figure 5.1: Interaction between days in milk (DIM), age in years, and total grain supplement, from the multivariable regression model for S/P Difference in 128 cows vaccinated against BVDV on 118 smallholder dairy farms of Meru, Kenya

**Note: Red line shows most (85%) of the data points are on the left (less than 8 years cows)**



Body Condition Score (BCS)

Figure 5.2: The relationship between body condition score (BCS) and BVDV S/P Difference for cows

BVDV – Bovine Viral Diarrhoea Virus

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Figure 5.3 The relationship between body condition score (BCS) and BVDV S/P Difference fitted values for cows

BVDV – Bovine Viral Diarrhoea Virus

Table 5.2: Descriptive statistics for continuous predictor variables with a P-value less than 0.1 for associations with BVDV S/P Difference among the 109 heifers and 96 farms

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Predictor variable** | **Number** | **Mean** | **Range** | **Coefficient** | **P-value** |
| Number of people living on farm 12 | 96 | 3.65 | 1-8 | -0.062 | 0.028 |
| Total land size (acres) 12 | 96 | 0.73 | 0-6 | -0.072 | 0.098 |
| Rented land in fodder (acres) 12 | 96 | 0.22 | 0-1 | -0.192 | 0.095 |
| Total land in fodder (acres) 12 | 96 | 1.12 | 0-9 | -0.053 | 0.053 |
| Heifer number dead in herd12 | 96 | 1.12 | 0-2 | -0.257 | 0.067 |
| Total cattle in herd1 | 96 | 6.58 | 2-20 | -0.022 | 0.046 |
| Total heifers in the herd1 | 96 | 2.02 | 1-6 | -0.072 | 0.025 |
| Number of diseases per heifer12 | 96 | 0.51 | 0-3 | -0.086 | 0.079 |
| Body condition score 1 | 109 | 2.48 | 1.25-4 | -0.130 | 0.054 |

***1***Statusduring the 1st visit

***12***Status for the period of 12 months prior to the 1st visit

Table 5.3: Descriptive statistics for categorical predictor variables with a P-value less than 0.1 for associations with S/P Difference among the 109 heifers and 96 farms

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Predictor variable** | | **Category** | **Number**  **Yes** | **Proportion**  **Yes** | **Coefficient** | **P-value** |
| Farm location zone1 | |  |  | -- | -- | 0.095gp |
|  | *Zone 1* | | 36 | 0.39 | Baseline | 0.000 |
|  | *Zone 2* | | 25 | 0.27 | 0.090 | 0.370 |
|  | *Zone 3* | | 35 | 0.86 | -0.128 | 0.158 |
| Management System 12 | |  |  | -- | -- | 0.003***gp*** |
|  | *Zero-grazed* | | 59 | 0.63 | Baseline | <0.001 |
|  | *Mixed practice* | | 30 | 0.32 | -0.238 | 0.006 |
|  | *Common Grazing* | | 7 | 0.08 | -0.350 | 0.010 |
| Protein supplements 12 | | Y/N | 9 | 0.10 | -0.216 | 0.099 |
| Regularly deworming 12 | | Y/N | 19 | 0.21 | -0.216 | 0.024 |
| Raise all replacements ***co*** | | Y/N | 89 | 0.94 | -0.304 | 0.043 |
| BVDV ELISA test 1 | | Y/N | 109 | 0.07 | -0.5957 | <0.001 |

***1***The statusduring the 1st visit for vaccination and taking the first serum sample

***12***Status for the period of 12 months prior to the 1st visit

***co***Common practice for the period not limited to 12 months prior to the 1st visit

***gp***Global – Global P-value for categorical variable association assessment

BVDV – Bovine Viral Diarrhoea Virus

Table 5.4: Multivariable regression estimates for S/P Difference in 109 heifers vaccinated against BVDV on 96 smallholder dairy farms of Meru, Kenya

|  |  |  |  |
| --- | --- | --- | --- |
| **Predictor variable** | **Coefficient** | **95% CI** | **P-value** |
| Model Constant | 1.314 | 0.42, 2.21 | 0.004 |
| Number of people living on farm 12 | -0.053 | -0.1, -0.01 | 0.023 |
| Total heifers in the herd **1** | -0.059 | -0.11, -0.006 | -0.028 |
| Management system**1** | -- | -- | <0.001 **gp** |
| *i. Zero-grazed* | Reference | Reference | Reference |
| *ii. Mixed* | -0.33 | -0.47, -0.19 | <0.001 |
| *iii. Common Grazing* | -0.34 | -0.56, -0.12 | 0.003 |
| BVDV ELISA test**1** | -0.574 | -0.81, -0.34 | <0.001 |
| Acaricide use every 2 weeks**1** | 0.13 | 0.014, 0.26 | 0.029 |
| Raise all replacements ***co*** | 0.88 ***i\**** | -0.03, 1.8 | 0.059 ***i\**** |
| Body condition score**1** | 0. 25 ***i\**** | -.08, 0.58 | 0.14 ***i\**** |
| Raise all and BCS Interaction *i* | \*\* | \*\* | 0.026\*\* |

***1*** Statusduring the 1st visit

***12***Status for a period of 12 months prior to the 1st visit

***co*** Common practice not limited to the 12 months’ time prior to the 1st visit

***i*** Coefficient main effects without considering the interaction - should not be interpreted without considering the coefficients of the other variables in the interaction

**gp** Global – Global P-value for categorical variable association assessment

***12Cal***Post calving practice for the 12 months prior to the 1st visit

***\*\**** Interaction outputs

BVDV – Bovine Viral Diarrhoea Virus

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Figure 5.4: Interaction between body condition score (BCS) and raising all replacements on-farm (raisall), from the multivariable regression model for S/P Difference in 109 heifers vaccinated against BVDV on 96 smallholder dairy farms of Meru, Kenya

# Chapter 6: Chapter Summarizations, Conclusions, and Recommendations

This concluding chapter is for those who do not have time to read the four substantive chapters but want more information than the abstracts. This concluding chapter starts with a brief introduction and finishes with a section that interprets and integrates the substantive chapter results together, along with some overall recommendations from the thesis.

## 6.1 Introduction

Bovine viral diarrhoea virus (BVDV) vaccination as a key inexpensive method of BVDV prevention where there are substantial exposure risks, and the vaccine can mitigate the losses from BVDV infection (e.g. infertility, pneumonia, diarrhoea, immunosuppression) for better productivity (Walz et al. 2020; Richter et al. 2017). A previous study in Meru, Kenya, reported a high prevalence of BVDV and reproductive challenges among the smallholder dairy (SHD) farmers, making a study on BVDV vaccination very relevant to SHD farmers, as well as researchers and livestock sector policymakers (VanLeeuwen et al. 2021). The SHD farms are a very important part of the dairy industry in Kenya, supporting many livelihoods (Waitituh 2017; FAO 2011). There is little information on the benefit of BVDV prevention by vaccination, BVDV diagnostic challenges, and factors that can affect successful vaccination on SHD farms in Kenya.

In this thesis, a cross-sectional study was conducted to evaluate the possibility of classical swine fever virus (CSFV) cross-reactivity with BVDV test results when using both antigen and antibody ELISA tests (Chapter 2). To determine the benefits of vaccination among herd-mates in SHD farmers using a multivalent modified live viral (MLV) vaccine including BVDV, a retrospective cohort study (Chapter 3) and randomized controlled trial (Chapter 4) were conducted. Finally, a cross-sectional study to evaluate BVDV antibody response variability and its associated factors in SHD farms was conducted (Chapter 5). This concluding chapter summarizes these four research chapters’ methods, results, and conclusions, and outlines linkages between the findings. In addition, we make recommendations and identify future research areas to improve awareness and understanding of BVDV vaccination and diagnosis.

The four studies utilized the following four hypotheses: 1) the diagnosis of BVDV using both antibody and antigen ELISA in cattle is not affected or complicated by potential cross-reactivity with CSFV among cattle on SDH farms in Kenya; 2) there are health benefits and lower disease occurrence in the vaccinated cohort compared to the non-vaccinated cohort, with good management practices complementing the benefits of vaccination among cattle on SDH farms in Kenya; 3) there are health benefits and lower disease occurrence in the vaccinated group compared to the placebo group after a one-year follow-up, with other preventive management practices complementing the benefits of vaccination among cattle on SDH farms in Kenya; 4) the BVDV antibody response to BVDV vaccination varies under different planes of nutrition, management, and Body Condition Score (BCS) of cattle on smallholder dairy farms (SDFs) in Kenya.

The research was undertaken in collaboration with Buuri and Naari Dairy Farmer's Cooperative Societies, the Farmers Helping farmers program in Meru, Atlantic Veterinary College at the University of Prince Edward, and the University of Nairobi. The funding for the research was made possible by the Rideau Hall Foundation, Community Foundations of Canada, and other donors to the Queen Elizabeth II Diamond Jubilee Scholarships, administered by Universities Canada.

To ensure that the results of the studies reflect real-world circumstances, a commercially available vaccine was used (Pyramid® donated by Boehringer Animal Health Inc.), containing two strains of BVDV. This multivalent vaccine for cattle contains a number of common pathogens for cattle, as do most of its competitors for this niche of the marketplace. The vaccine includes two strains of BVDV, plus bovine herpesvirus type 1 virus, bovine respiratory syncytial virus, and parainfluenza virus type 3. Therefore, the reader should be aware that the benefits of the vaccine cannot necessarily be attributed to BVDV prevention only.

## 6.2 Evaluation of antibody and antigen cross-reaction in Kenyan dairy cattle naturally infected with two Pestivirus: Bovine Viral Diarrhea virus and classical swine fever virus

The correct diagnosis of BVDV, especially persistently infected (PI) cattle, is critical for a successful control program in SDFs. Knowing the challenge posed by related Pestiviruses, such as CSFV, is also important, especially where mixed livestock keeping is common, which is often the case on SDFs in Kenya.

In this study, the investigation was based on a previous study of a single visit to a random sample of SHD farms in the Naari Dairy Farmer’s Cooperative Society to collect serum samples and other descriptive farm-level and animal-level information (VanLeeuwen et al. 2021). Testing for BVDV antigens (Ag) and antibodies (Ab) was conducted on serum samples from 320 dairy cows and heifers. Classical Swine Fever Virus (CSFV) Ag and Ab testing were conducted on a subset of 133 and 74 serum samples, respectively. The CSFV testing was on a reduced number of samples based on BVDV test results, sample volume availability, and some farms keeping pigs. The Ag and Ab tests utilized IDEXX ELISA for both BVDV and CSFV, with the results being presented as positive, negatives, or suspects for the BVDV antibody test. The testing was carried out as recommended by the manufacturer and samples handled as guided by the laboratory Standard Operating Procedures and research approval.

There were 74 samples tested for antibodies against both viruses with 40 (54.0%) being BVDV Ab positive, while 63 (85.1%) were CSFV Ab positive. Among the 40 BVDV Ab positive samples, 36 cattle (90.0%) tested positive for CSFV Ab. However, among the 34 BVDV Ab negative samples, 27 (79.4%) were also CSFV Ab test positive. For antigens, 133 samples were tested for both viruses, with 125 (94.0%) being BVDV Ag positive, while 2 (1.5%) samples were CSFV Ag positive. None of the eight BVDV Ag negative samples was positive for CSFV Ag and only two (1.6%) of the 125 BVDV Ag positive samples were positive for CSFV Ag.

The use of antibody testing to diagnose BVDV in the SDFs where cattle comingle with pigs is not able to determine a reliable disease burden measure. There is the possibility of combined infections with the two viruses, or reactivity with some other Pestiviruses in the samples, such as CSFV or Border Disease Virus (BDV) (Kaiser et al. 2017; Burton 2016). This finding is very important for screening diagnosis as well as monitoring vaccine responses, which would mostly rely on antibody testing. More research is needed to confirm and determine the exact proportion of cross-reactivity between BVDV and CSFV using other tests, such as a specific antibody neutralization test or a novel specific antibody ELISA (Ji et al. 2018).

There was only limited evidence for cross-reactivity of the two Ag ELISA tests. We, therefore, recommend testing for both BVDV Ag and Ab when sampling cattle for BVDV, and using other methods, such as virus neutralization, PCR, sequencing or a combination of methods on Ag-positive samples, to clarify study results where multiple species are housed in proximity. The antigen ELISA tests not being cross-reactive should, however, be confirmed by the testing for more than the two Pestiviruses to ascertain this finding. The antigen capture ELISA using ear notch samples or serum has been shown to have a 100% agreement with a PCR test; therefore, it is a superior test to identify PIs and confirm active cases or outbreaks (Lanyon et al. 2014). The findings of this research in SDFs are in agreement with the available information on the reliability of antigen ELISA testing in other production systems (Yitagesu et al. 2021; Khodakaram and Farjanikish 2017). If confirmed that BVDV antigen ELISA testing is not cross-reactive to other Pestiviruses, such as CSFV, then this becomes a very key monitoring tool that is not only easy to perform for most laboratories but also cheap.

This study concludes that the use of antibody ELISA for diagnosis of BVDV in cattle where pigs are also located on the farm should be used with caution, because there is possible cross-reactivity with CSFV. The use of antigen ELISA, especially for the identification of BVDV PIs, can be utilized in the SDFs in Kenya with little doubt of cross-reactivity with CSFV.

## 6.3 A retrospective cohort study of disease among vaccinates and non-vaccinates after a single injection of multivalent vaccine including modified live viral Bovine viral diarrhoea virus on smallholder dairy farms in Kenya

Knowing the benefits related to reduced disease associated with vaccination with a multivalent vaccine including BVDV on SDFs in Kenya would be helpful to provide evidence specific to SDFs for convincing farmers on the importance of utilizing this vaccine. This study compared 226 cows and 85 heifers given a single multivalent modified live vaccine including BVDV against 215 cows and 60 heifers in non-vaccinate cohorts. One year after vaccination, a follow-up visit recorded reported disease outcomes, and farm and animal factors. The farm factors included: feeding and nutrition practices, disease prevention practices, farm structures and comfort status. The animal factors included: reproduction performance, production performance, current health status, and other animal biometrics. For the practices and reported animal diseases, the period of interest was one year plus or minus 2 months between vaccination and our study visit. Mixed multivariable logistic and Poisson regression modeling was used for the heifers and cows, respectively, to determine factors associated with the disease outcomes.

The study established that vaccination was significantly associated with a reduction of overall disease, pneumonia, and diarrhea in both the cows and heifers reported in the last year. In the heifers, there was a significant association with other reported diseases, which included any other conditions such as tick-borne diseases and skin diseases reported for the last year. Additionally, vaccination in cows was significantly associated with reduced poor appetite, tick-borne disease, and uterine disease reported for the past year. These results are similar to other studies that have shown health benefits and consequent production and economic benefits (Grooms 2004; Pinior et al. 2017).

The cow model included three positively associated variables and six protective factors. Increased reported disease counts in cows for the last year were associated with: feeding grass weeds with an incidence risk ratio (IRR) of 1.29 (i.e. 29% higher rate of reported disease when feeding grass weeds compared to not feeding grass weeds); farms that had more than two reported diseases at the farm level within the last year, with an IRR of 1.41, compared to 2 or fewer reported diseases; and cows that had over three calvings, at an IRR of 1.34, compared to cows with three or fewer calvings. Cows were associated with reduced reported diseases counts during the last year for the following factors: farms that practiced milking of mastitis cows last at an IRR of 0.67, farms with more than six cows at an IRR of 0.80; cows being only bred once in the last year at an IRR of 0.78, compared to cows requiring multiple breedings to get pregnant; and cows with a BCS above 2.25 with an IRR of 0.81.

Pregnancy checking status as a cow-level factor and BVDV vaccination status had a significant interaction association with reported disease count for the last one year. Both interacting factors had a protective effect at the univariable association. The reported disease counts for the last year for vaccinated cows that were pregnancy checked and those not pregnancy checked were not different, while the non-vaccinated cows that were not pregnancy checked had higher reported disease counts during the last year than vaccinated cows that were pregnancy checked.

The heifer model included four protective factors and five positively associated variables. Vaccinated heifers were 0.11 times as likely to have a reported disease within the last 12 months compared to the non-vaccinate heifers. Reduced reported disease likelihood in heifers was also associated with: farms managed by a woman older than 55 years of age, at an Odds Ratio (OR) of 0.23; farms with over 2 acres of land, at an OR of 0.13; farms feeding Napier grass that was less than 1 meter in height during the dry season, at an OR of 6.5; and farms whose dairy cattle stalls had concrete floors, at an OR of 0.02. Increased reported disease likelihood in heifers was associated with: farms that had lost (death) a cow vaccinated against BVDV within the last 12 months, at an OR of 8.9; farms that bought replacement heifers, at an OR of 3.3; and heifers above 30 months of age, at an OR of 6.1.

Body condition score (BCS) was categorized into 3 categories based on the linear trend function and showed an increase in the likelihood of reported disease with higher BCS. Compared to the baseline BCS of 2.25 and below, heifers with a moderate BCS of 2.25 to 2.75 were 3.9 times more likely to have a reported disease in the last 12 months, while heifers with a BCS of 3 and above were 10.7 times more likely to have had a reported disease within the last 12 months.

The farm management factors associated with lower reported disease in the cows and heifers were good milking hygiene practices and having a concrete floor in the cattle stalls, respectively. This underlines the critical complementary feature of biosecurity to disease prevention in SHD farms.

Our findings identified other farm characteristics that were associated with lower reported disease, including larger herds and larger land size, good reproductive performance, and older female farmers. These findings could be taken to mean that farms with higher fodder production resources of land allowed for more cows to be kept, while maintaining better health and productivity performance. We can also conclude that the experience that usually comes with age is a key indicator of overall farm performance that can complement vaccination in reducing the occurrence of diseases. Management features are shown to be very critical confounders to disease prevention in other studies (Newcomer and Givens 2016; Grooms 2004).

At the cow level, good BCS was associated with lower reported disease counts, while in the heifers, it was related to a higher likelihood of reported disease. This contradicting result shows that body condition score is an important confounder of vaccination in disease prevention. For the cows, we conclude that a higher body condition score is correlated with good health performance and therefore complementary to vaccination. For the heifers, the unexpected finding of higher categories of BCS being associated with higher odds of reported disease could be associated with other BCS factors, such as: age, seasonal feed availability, and prioritization of available high-quality feeds to cows. Further research is needed to clarify this finding.

The study identified several farm-level factors that were associated with higher reported diseases in cows and heifers. Farms reporting more disease conditions also had more disease counts for their cows. Furthermore, heifers on farms that had lost (death) vaccinated cattle through death had a higher likelihood of disease for the last year. We conclude that the farm-level health status is important for successful disease prevention by vaccination.

Feed supply and scarcity are very important to the SDFs and can affect the health performance in cattle. Cows that were on farms feeding grass weeds had more reported diseases as did heifers on the farms using shorter Napier grass in the dry season. Both the feeding of short Napier grass in the dry season and utilizing of weeds as fodder indicate feed supply challenges (Balehegn et al. 2022; Maleko et al. 2018; Ntakyo et al. 2020). Related to these factors, the introduction of new animals to the farm is an important factor associated with more disease likelihood. The relationship between biosecurity and nutrition of dairy farms has been shown by other studies to be very important for health performance (Singh et al. 2020; Brennan and Christley 2013).

At the farm level, older heifers were more likely to have reported disease occurrence, and the cows that were above third parity reported more disease counts for the last year despite vaccination. Older heifers and cows have a longer time to be exposed to infectious diseases than younger animals. Longer exposure time to disease conditions is a common factor seen in other studies (Abera et al., 2019; Lasser et al. 2021).

The practice of pregnancy checking had a significant effect modification of vaccination status for the cows, was associated with reduced disease counts for the cows. It is unlikely that the practice of pregnancy checking is leading to reduced disease. The study speculates that farms practicing pregnancy checking could also undertake better management in other ways that do lead to reduced disease, and thus better health performance.

In summary, there was an evident benefit of vaccination using a multivalent vaccine including BVDV on SHD cattle in Kenya in the reduction of overall disease outcomes in both the cows and heifers, while controlling for other important farm- and animal-level variables.

## 6.4 A randomized controlled trial of a multivalent vaccine including modified live viral Bovine viral diarrhoea virus on smallholder dairy farms in Kenya

Cohort study results of the benefits related to reduced disease associated with vaccination with a multivalent modified live viral (MLV) vaccine including BVDV on SDFs in Kenya would be helpful to provide evidence specific to SDFs for convincing farmers on the importance of utilizing this vaccine. However, randomized controlled trial (RCT) results of the vaccine would be even more helpful because an RCT would likely have less selection bias, information bias, and confounding bias (Dohoo et al., 2009)

This study was done over a period of one year with two visits twelve months apart to compare vaccinated group and a placebo group at each time point, and to compare results over time within each group.The study recruited a total of 384 and 352 randomly selectedcows andheifers on 292 and 260 SDFs, respectively. During the first farm visit, we recorded the baseline level of diseases reported in the last 12 months, along with other cow- and farm-level data through a questionnaire and animal examinations. With random block allocation, we vaccinated 185 cows and 172 heifers using a single injection dose of a multivalent MLV vaccine including BVDV, while 199 cows and 180 heifers were given a placebo injection. After one year, we returned to the farms to record the same data, for time-varying variables. Lost to follow-up were 132 heifers (75 vaccinated and 57 placebo), 171 cows (89 vaccinated and 82 placebo) from a total of 95 farms. Among the reasons for the losses were: sale, death, and farms lending out animals for various reasons. Mixed multivariable logistic and Poisson regression modeling were used for the heifers and cows, respectively, to determine factors associated with the reported disease in the last 12 months. The proportionate change in reported diseases outcome between the first and second visit was checked and tested for significance.

For both the cows and heifers, there was a significant interaction variable between visit number and intervention group, confirming fewer diseasesreported in the last 12 monthsin the vaccinated group versus the placebo group on the second visit, adjusting for the reported disease counts on the first visit. Other studies have shown similar benefits of multivalent vaccination containing the BVDV component (Richter et al. 2017; Dubovi et al. 2000; Walz et al. 2017). The study concludes that the intervention with multivalent MLV BVDV vaccination is beneficial in reported disease prevention in both cows and heifers over 6 months of age, and therefore it is recommended in these age groups on smallholder dairy farms like those in Kenya.

Regarding specific diseases, in both the heifers and cows, there was a significant reduction in the occurrence of reported pneumonia and diarrhea for the follow-up period compared to the 12 months before the study. Both diseases are common conditions caused directly by BVDV and the other components of the multivalent vaccine, or secondarily by other pathogens that could lead to clinical infections in cattle with immunosuppression from BVDV. This study, therefore, concludes that the use of the multivalent vaccine is beneficial in reducing the negative effects of the two common diseases.

In the final heifer model, there was a significant association between vaccination group and other reported diseases, including tick-borne diseases and skin diseases, reported for the last year compared to the period before the study. Additionally, vaccination in cows was significantly associated with reduced poor appetite, tick-borne disease, and other diseases reported in the follow-up period compared to the 12 months before the trial. Since these similar trends in reported disease outcomes were seen in both cows and heifers, there is evidence to conclude that the use of the multivalent vaccine may also be beneficial in reducing the negative effects of these diseases.

The final cow model had five binary variables, two categorical variables, and eight variables involved in four interactions. Cow associations with lower reported disease counts for the last year included: farms having more than three acres of land, at an Incidence Risk Ratio (IRR) of 0.82; and farms that bought in-calf replacement heifers, at an IRR of 0.80. Cows were associated with higher reported disease in the last year for the following factors: farms that reported calves having more than two diseases, at an IRR of 1.32; cows that had been bred within the last 12 months, at an IRR of 1.43; and heavier cows, at an of IRR=1.01. The cows on farms that had not reported any case of mastitis during the 12 months before the visit had lower reported disease counts than the cows on farms that had reported one or more mastitis cases, with the IRR increasing numerically with more mastitis cases. The cows on farms that had only one cow or reported using no towel when cleaning the udder before milking had higher reported disease counts than cows on farms using the same towel when cleaning the udder for multiple cows. There was no difference between using a single towel or different towels between cows; however, most farmers with multiple cows did not use different towels for udder cleaning.

In the interaction between herd size and household reliance on dairy enterprise, herd size was not associated with reported disease count when the household relied on dairy farming for less than 50% of the household income, but when the household relied on dairy farming for more than 50% of the income, there were lower reported disease counts for cows on farms with three or more cows compared to the smaller herd size farms. For the interaction between feeding maize silage and pregnancy status, feeding maize silage was not associated with reported disease count in the last 12 months when cows were not pregnant on the day of the farm visit, but when cows were pregnant on the day of the visit, the reported count of disease was lower in the last 12 months, but more so on the farms not feeding maize silage. The last interaction was between extra grain supplementation post-calving and BCS. Feeding extra grain post-calving was not associated with reported disease count in the last 12 months when cows had low BCS, but when cows had higher BCS (2.25 or higher), they had lower disease counts, especially on farms feeding extra grain post-calving.

The final heifer multivariable logistic model had five binary variable factors, one categorical variable, and four variables involved in two interactions. There was higher reported disease likelihood for the last year in heifers associated with the following variables: farms that were feeding heifers grass silage with Odds Ratio (OR) of 3.3, and farms that were buying replacement heifers at 2.5 times the odds. Factors associated with lower reported disease likelihood for the last year were: farms that administered at least four kg of colostrum within the first 12 hours of a calf’s life, with an OR of 0.3; heifers that had been bred within the last 12 months, with an OR of 0.3; and heifers that were more than two years old, at an OR of 2.2. For the categorical breed variable, Holstein-Friesians, other breed heifers (Jerseys and Zebu etc.) had marginally (p=0.064) lower odds of reported disease in the last year (OR=0.45), while Ayrshires had higher odds of reported disease in the last year, at OR of 1.9.

The study found that some farm characteristics and management factors are important to consider for the success of a disease prevention program that might contain the studied vaccine. Farms that have more land resources reported lower reported disease counts. More land availability for the farm means that there will be a better fodder supply guarantee for the animals. Farms that purchased in-calf heifers had fewer reported counts of disease for their cows, while the farms that bought non-pregnant heifers had more disease likelihoods in their heifers. The study can conclude that there is an element of the quality or status of replacement heifers brought to the farms that is related to the health performance of both cows and heifers.

The study found that the farms that did not have a case of mastitis 12 months before the first visit reported fewer disease counts for their cows during the follow-up period. We can conclude that mastitis is a key indicator of health performance for farms which can be a result of good management. Ensuring at least 4 kilograms of colostrum for the first 12 hours of the calf’s life was associated with good health performance of heifers later in life. At the animal level, the heifers that had been bred showed a lower likelihood of reported disease within the follow-up period. There are many other studies that have reported similar relationship between management and disease occurrence in different management systems (Debebe and Haben 2020; Chepkwony et al. 2021; Gizaw et al. 2020).

The effect of breeding status on reported disease was confounded by the age of heifers, with the likelihood of reported disease occurrence increasing with their age. On the other hand, the breeding status at the time of the visit for the cows was associated with more reported disease counts. The study concludes that the heifers that were bred were likely to be in good health performance, which could be a result of good management and nutrition.

The farms that had reported more than one sick calf had more reported disease counts in their cows during the follow-up period. Likewise, in the heifers, there was more disease likelihood on farms that had reported more sick cows within the last year. These findings underline the importance of whole-farm consideration when planning vaccination programs in the smallholder system.

Other management factors that affected reported disease outcomes were the use of udder cleaning towels in the milking cows and feeding of maize silage to the cows. There was higher disease likelihood in heifers that were in farms feeding grass silage and there were higher reported disease counts in cows within farms not using a towel to clean the udder before milking. There was no difference between using a single towel or different towels between cows, with most farmers with multiple cows not using different towels. The study concludes that hygiene, as part of biosecurity, is very important and an indicator of cow health performance. However, the association with the feeding of grass silage is against expectations; we recommend further evaluation of the role of nutrition and feeding in vaccination, with consideration of the seasonal variations and coping strategies.

Animal-level factors that were associated with the reported disease outcome in the follow-up period were the weight of cows and the breed of heifers. Heavier cows are more likely to report more diseases while Holstein and Ayrshire heifers had a higher likelihood of reporting disease. The study concludes that it is important to pay attention to some animal-level factors confounders when putting in place a vaccination program in the SHD farms. The interplay between animal and farm level factor association with health outcome has been reported by other studies (Alvåsen et al. 2018; Reimus et al. 2020).

In this study, there were several significant effect modification interactions between factors associated with reported disease outcomes within the follow-up period. There was a higher likelihood of reported disease in heifers associated with a visit during wet months and a higher number of reported diseases in cows. Cows that were fed extra grain post-calving and had a better body condition score during the visit had lower reported disease counts. There were lower reported disease counts for cows that were pregnant during the visit and within farms that fed maize silage. Farms that had more cows and relied more on the dairy enterprise income had lower reported disease counts. These interactions should be considered carefully so the results are not over-interpreted.

The farm-level management factors associated with reported disease occurrence underline the importance of other complementary and supplementary components of consideration for a successful disease prevention effort in the SHD farms. Animal-level factors associated with reported disease occurrence show the importance of variation of specific stages of production, age, and breed in the success of disease prevention by vaccination. Similar to the reported disease occurrence association with various preventive factors, we can draw similar or stronger association for BVDV vaccination in the SHD farms. We recommend further studies to specifically evaluate the benefits and associated factors to the prevention of BVDV incidences within and between farms in the SHD sector.

## 6.5 Bovine viral diarrhea virus antibody response to a single dose of modified live viral vaccine and associated factors in smallholder dairy cattle in Meru, Kenya

This study evaluated the associations between farm-level and animal-level factors and BVDV antibody response to BVDV vaccine in heifers and cows on SDFs. The study evaluated variability in antibody response in 128 cows and 109 heifers, all non-pregnant, after a single multivalent modified live vaccine injection including BVDV. The antibody response was determined by testing cattle before and four weeks after vaccination, with the difference being used as the outcome measure. Antibody ELISA testing was used to compute the pre- and post-vaccine sample-to-positive (S/P) ratios for BVDV antibody levels. Antigen ELISA testing was also used on the blood samples from both visits. A questionnaire and animal examination were used to collect information on animal health status, management practices, and reported disease outcomes for the last 12 months. Multivariable linear regression analysis modeling was used to determine factors associated with the change in antibody levels.

Before vaccination, 40.6% and 7.3% of cows and heifers tested positive for BVDV antibodies (S/P ratio > 0.3), respectively. The mean increase in S/P ratio post-vaccination was 0.476 and 0.804 for cows and heifers, respectively. Therefore, the overall mean vaccine response in S/P Difference and the proportion of successful response above a positive threshold was better for heifers than that of adult dairy cows. After vaccination, there were 15 (11.7%) cows and 7 (7.3%) heifers that still had antibody S/P ratios that were below a positive case threshold of 0.3.

The BVDV antigen tests identified seven cows being positive, four at the pre-vaccination sampling and three at the post-vaccination sampling. One BVDV antigen-positive cow in the first sampling died suddenly before the second sampling, with reported signs of generalized weakness and a rough hair coat. It is possible that this cow was a PI, but without the second sample, we cannot confirm this PI status. Three of the seven positive cow samples had a suspect outcome upon testing and were reported as positive.

The final heifer multivariable linear regression model had seven variable factors associated with S/P Difference (p≤0.05) and an interaction between farms raising all replacements and body condition score. For every additional individual on the farm, the heifers had a 0.05 lower S/P Difference. Similarly, heifers from farms with many heifers had a lower S/P Difference than those with fewer heifers. With every additional heifer on a farm, there was a reduction of S/P Difference of 0.059. Heifers from farms practicing communal grazing partly or fully had lower S/P Differences by 0.33 and 0.34, respectively, compared to those from zero-grazed farms. Heifers that were BVDV antibody positive had a 0.57 lower S/P Difference compared to those that were negative. Farms that practiced regular acaricide use every 2 weeks had heifers with higher S/P Difference by 0.13 compared to those that did not use acaricides regularly. For the interaction, at low body condition scores, there was no significant difference in S/P Difference among heifers, regardless of whether farms raised all replacements or not. At high body condition scores for heifers (BCS 3 and 4), the S/P Difference was higher for the heifers on farms not raising all their replacements than those farms raising all heifers on the farm.

The final multivariable linear regression model for cows had eight variables associated with S/P Difference (p≤ 0.05). The model also included a confounder (a three-category management system variable with over 20% confounding on the total supplementation coefficient upon inclusion in the model), and an interaction between three variables: total supplementation, age in years, and days in milk (DIM). The following were the specific variables associated with S/P difference for the cows. Cows that were BVDV Ab-positive on the first visit had a 0.51 lower S/P Difference than those that were negative. Cows with an abnormal finding during physical examination at the first visit had 0.16 lower S/P Difference than those without. Cows from farms that had recorded a post-calving disease condition in the last 12 months had lower S/P Difference of 0.13 versus cows on farms that did not record this problem.

For every point increase in body condition score, there was an increase of 0.13 in S/P Difference. For the cows, optimum response was between a BCS of 2 and 2.75 with the low and high BCS showing lower S/P difference. Cows on farms feeding high protein supplements had 0.17 lower S/P Difference than cows on farms not feeding these supplements. Looking at the management system variable, cows from farms that practiced zero-grazing had the highest antibody response. Cows from farms practicing both zero-grazing and communal grazing had a somewhat lower S/P Difference by 0.14, and those completely on communal grazing had a somewhat lower S/P Difference of 0.12, in reference to the zero-grazed cows. For the interaction, in the younger cows, the S/P Difference was higher for those fed a higher amount of supplements versus those fed a lower amount of supplements.

With advanced age (over 8 years), the rate of reduction in S/P Difference by age was higher for the cows fed a higher amount of supplements. The S/P Differences for the cows that were below 30 DIM had a greater variability than those 30 DIM and above, with the variability diminishing towards the age of 7 and 10 years. Beyond 8 years of age, there were less than a quarter of the observations; therefore, there was more variability in those predictions and less confidence in the results.

The study established that better BCS, more post-calving grain supplementation, and being on zero grazing managed farms led to better vaccine response in cows. Active disease conditions, positive BVDV antibody test pre-vaccination, history of post-calving disease conditions, and being on farms practicing communal or semi-zero grazing management were associated with poor vaccine response for the cows. The study identified that there was an effect modification between days in milk, age, and daily supplementation post-calving in cows. For the heifers, good BVDV antibody response was associated with being on a zero-grazed farm, being on farms that practice acaricide control at least every 2 weeks, and being on a farm that raised all the replacements. Poor BVDV antibody response was associated with heifers in farms with more household members, farms with more heifers, being on farms practicing communal or semi-zero-grazing management, and BVDV-positive heifers. There was an effect modification between BCS and raising all replacements for heifers’ BVDV antibody response.

This study found that management type was a very important factor associated with the response of BVDV antibodies. Zero grazing of both heifers and cows, where animals are provided with feed, water, and shelter without having to walk or mingle with other animals is recommended for good antibody response to BVD vaccination in the SHD farms. Biosecurity-conscious management factors, such as raising all the replacement within the farm and practicing ectoparasite control, are key components of a successful vaccination response. At the animal level, it is evident that vaccination of cattle when having good BCS is beneficial and associated with a better response than poor BCS. The closely associated practice of grain supplementation of cows post-calving was found to be beneficial for a good antibody response.

On the other hand, a poor BVDV antibody response was significantly related to disease factors. The cows and heifers that tested positive for BVDV antibodies had a poorer antibody response than those who were not. Cows that had signs of disease during vaccination and cows on farms that had reported post-calving diseases within the last 12 months had poorer BVDV antibody response.

The study concludes that health status is very important at the time of BVDV vaccination and considerations should be made to target times of good health performance periods. The study also identified that DIM and transition period nutritional status are important considerations for vaccination. We recommend avoiding the first 30 days post-calving and ensuring the proper amount of supplementation of cows after calving for better vaccine response in the SHD farms. Further investigation is needed in the future to determine and measure the specific role of larger farms and household size on the immune response to vaccination. We can conclude that nutritional status, production stage, current disease status, and previous disease status are key determinants for BVDV vaccine response.

## 6.5 Linked conclusions

Similar to the previous study that preceded this research and that provided the samples for the antibody and antigen cross-reactivity study (VanLeeuwen et al. 2021), the BVDV antibody response study confirmed the presence of BVDV antibodies in the herds. There were however very low BVDV antigen-positive samples in the chapter 5 study of this research compared to the previous study and the chapter two study. The differences could be due to the varying degrees of disease transmission at different times, and also the possible awareness created by the previous research, leading to reduced transmission.

Vaccination with a multivalent vaccine that includes a BVDV component proved to be antibody responsive in both the heifers and cows with variations across multiple associated factors. Using the vaccine as an intervention method for disease prevention in the SHD farms in both the retrospective cohort and RCT studies was beneficial in reducing reported disease occurrence when compared with unvaccinated groups.

A good body condition score was significantly associated with good BVDV antibody response in the cows in the response variability study (chapter 5) and associated with lower disease counts for both the cohort study (chapter 3) and RCT study (chapter 4). The three studies indicate the importance of BCS in the realization of vaccination benefits on reported disease and BVDV antibody response. For the heifers, the BCS association was not consistent in all three research studies. Body condition score was not significantly associated with disease outcome for the RCT, while in the cohort study the lower BCS was associated with lower disease likelihood. Heifers on SHD farms are often given lower quality feed than the milking cows because they are not giving milk, leading to possible differences in protein and immunity levels. In the BVDV antibody response study, BCS interacted with the status of farms raising replacement. It however should be noted that there can be variation of BCS across different stages of production, health status, and seasonality with feed availability changes. The role of BCS in vaccine benefits and antibody response should be studied further in the SHD farming sector with a keen focus on other potential effect modifiers and seasonal variation trends.

The age of both cows and heifers demonstrated its importance in both the benefits of vaccination and vaccine response. For the vaccine response, the heifers had a better response than the cows whose response also reduced with age. In both the retrospective cohort and randomized controlled trial, the older heifers had a higher likelihood of reported disease outcomes. In the retrospective cohort study, higher parity cows had more reported disease counts compared to younger cows. These findings lead to the conclusion that with increased age the cattle are more likely to be exposed to health challenges than when they are younger. This means farmers in the SHD farming system should target to start vaccinations and implementation of preventive measures from an early age to realize maximum benefits. Other animal-level factors, such as weight, height, breed, and production stages, were not linked to vaccine antibody response or disease outcomes in the three studies.

Regarding the reproductive stage and performance, there was a similarity in the outcome. In the randomized controlled trial, the cows that had been bred within the follow-up period had more disease counts while the ones confirmed pregnant had lower disease counts. In the retrospective cohort study, the cows that had been bred only once had lower disease counts for the last 12 months compared to those who were bred multiple times. It is important to note that we recruited non-pregnant cattle only for the BVDV antibody response study and the RCT study; therefore, the follow-up pregnancy status was only relevant for these two studies, whereas the non-vaccinates in the cohort study could be pregnant or not pregnant. Pregnancy is expected to cause lower immunity to cattle due to the progesterone effect (Ingvartsen and Moyes 2013). However, pregnancy could not be investigated directly in our research due to having given the vaccine to only non-pregnant cattle in the three vaccine studies. Interestingly, pregnancy checking practice was associated with fewer disease counts and had a significant interaction with vaccination status in the retrospective cohort study. Also, for the BVDV antibody response study, cows in early days in milk showed greater variability than later days in milk over 30 days, likely due to the large stress of calving on some cows (Sordillo 2016; Gowane et al. 2013). The reproductive performance and stage of lactation are thus critical factors of consideration in the realization of the benefits of vaccination against BVDV and the other antigen components in the multivalent vaccine.

Feed and nutrition for heifers and cows had several associated factors in all three vaccine studies. Different feed materials available and used in the farms were associated with both the BVDV response and reported diseases outcome in both directions. In the BVDV antibody response study, there was a better response when cows were fed more grain supplementation post-calving and there was a poor response for cows on farms feeding high protein supplements. For the retrospective cohort study, there were more disease counts for cows on farms feeding grass weeds and there was more likelihood of disease for heifers on farms using short Napier grass fodder in the dry season. In the RCT study, extra grain supplementation at the farm level was associated with lower reported disease counts, with a cow on farms feeding maize silage having more reported disease counts, while the heifers on farms feeding grass silage had higher disease likelihood. These studies lead us to conclude that the post-calving supplementation of cows is generally a complementary practice for better benefits of vaccination. We further note the inconclusive finding of defining the negative association between silage use and disease outcome. Further research will be needed to evaluate the specific relationship between various feed materials’ impact on the health outcome and vaccine benefit realization of cattle in the smallholder dairy system.

Herd size and herd structure factors were associated with the disease outcome and BVDV antibody response, making cattle numbers a key component to consider when planning disease prevention in the SDFs. In the BVDV antibody response study, a higher number of heifers on a farm was associated with poor antibody response. For the RCT and retrospective cohort studies, more cows in a herd were associated with lower reported disease counts. The conflicting findings concerning the direction of association between herd size and the outcomes can likely be attributed to both the experience of farmers and the competition for feed resources, which differ among the SHD farms. Since heifers are not giving milk, they are often fed lower quality feed than the milking cows on SHD farms with limited resources, and lower protein intake can lead to reduced immunity and vaccine antibody response, which could be exacerbated with larger numbers of heifers. The type of management for the BVDV antibody response study had a similar outcome for both the heifers and the cows, with a good response for zero-grazed cattle than in the other forms of management. Farms that had more land had lower reported disease counts for their cows in the RCT study and likewise lower reported disease likelihood for the retrospective cohort study. The study concludes that there is a potentially strong interrelationship between SHD farms' herd sizes and land resource availability and the management system.

The studies identified several biosecurity practices that were associated with disease prevention benefits, which could potentially be complementary to vaccination intervention. Mastitis prevention and good hygiene practices in cows were associated with disease outcomes in the RCT and retrospective cohort studies. Farms that milked the mastitis cow last and the farms that were using a towel to clean the udder before milking had lower reported disease counts for their cows. However, we noted that there was no difference between the use of a single towel or different towels per cow. This could be due to the use of soap and detergents preventing the spread of pathogens between cows. The regular use of acaricides every 2 weeks and the raising of replacements within the farm were associated with lower disease likelihood for the heifers in the retrospective cohort study. In the retrospective cohort study, heifers in farms with cattle structures with concrete floors were associated with lower reported disease likelihoods. In the RCT study, there was a higher disease likelihood for heifers in farms that bought replacement heifers; however, for the farms that bought in-calf heifers, the cows were associated with lower reported disease counts. This difference can lead to a conclusion that in-calf heifers are better quality replacements and possibly beneficial to the health performance of farms. These biosecurity practices can be described as potential complementary disease prevention measures to vaccination; however, their consistency on the SHD farms should be further assessed.

Disease burden and occurrence in different groups of the herd structure were noted to be important and associated with the health outcome of other herd groups on the farm. In the RCT, the occurrence of reported disease in the calves was associated with more disease counts in the cows during the follow-up period. Similar higher reported disease likelihood in the heifers was associated with higher reported diseases in the cows within the farms. The retrospective cohort study showed that there was a higher reported disease count for the cows on farms that had more disease conditions within the farm level. In the antibody response study, there was a reduced response when the vaccinated cows had any symptoms of disease, such as swollen lymph nodes or harsh lung sounds during the time of vaccination. The impacts of exposure to diseases on farms, and within the vaccinated animals specifically, are likely to modify the effects of the multivalent vaccine.

## 6.6 Overall recommendations

Considering the findings, research analysis, and overall conclusions, this research leads us to make the following recommendations to SHD farms in Kenya and elsewhere where SHD farms have similar conditions:

1. Upon this research we highly recommend vaccination with a multivalent vaccine that includes a BVDV component on SHD farms in Kenya. The vaccine proved responsive and beneficial in both the heifers and cows. Using the vaccine as an intervention method for disease prevention in SHD farms can lead to reduced disease, which should improve efficiency of production and better income to support dairy farming livelihoods.
2. The research identified that good health and good nutrition especially after calving to be associated with good BVDV vaccination response. We, therefore, recommend that farmers in the SHD farms plan and ensure good nutrition, especially in the cows’ transition period and ensure they vaccinate cattle when in good health. The cows on farms that gave more supplementation after calving had better antibody response and the cows on farms that provided extra grain supplementation had lower disease counts. The awareness of this fact can be created by veterinarians while doing the vaccinations and possibly delay the vaccination of sick cows.
3. In this research, it was evident that better BCS for cows and younger age were associated with good vaccine response and lower disease occurrence. We recommend planning multivalent vaccination including BVDV for cows that have a good BCS on SHD farms of between 2 and 2.75 for the cows. However, there was a different outcome concerning BCS for heifers; there is a need for more research to understand and evaluate the relationship between BCS and vaccine benefits in heifers while controlling for the potential variation with seasons and nutrition. Based on this research recommendation can be made that vaccination should be started early in the life of cattle to realize more benefits and good responses. This will ensure a cumulative effect in preventing the spread and maintenance of BVDV as well as realizing the other benefits of preventing other diseases in the multivalent vaccine.
4. We recommend the consideration of regular herd-level screening diagnosis to identify potential PI animals and prevent the possibility of continuous transmission or maintenance of BVDV. However, this practice would be challenging for smallholder dairy farmers who cannot afford regular screening for BVDV. Through the dairy cooperatives and county veterinary services, dairy farmers can benefit from the awareness of our research findings and should have access to antigen ELISA testing at the Central Veterinary Laboratories (CVL), particularly for working up cases of abortion, but also potentially for cases of pneumonia and diarrhea. However, our study concludes that there is potential cross-reactivity between CSFV and BVDV antibody testing, while there could be little or no cross-reactivity when using antigen ELISA. With regards to the circulating variants and the possibility of the other related Pestiviruses causing diagnostic challenges, it can be recommended that genetic identification of Pestiviruses variants affecting cattle in the SHD herds should be researched in Kenya. The genetic variant identification will also benefit vaccination strains choice to ensure specific and efficient antibody response.
5. The vital place of other complementary practices and measures such as biosecurity in the prevention of BVDV and other diseases has been highlighted in this research. The SHD farmers need to be taught the importance of biosecurity practices, in addition to the adoption of the MLV vaccine including BVDV. A number of specific preventive practices were identified in our research, such as good milking hygiene, regular tick control, and the proper handling of new introductions or heifer replacements. The control of movement and preventing the comingling of cattle with other herds in communal grazing grounds should also be promoted by practicing zero-grazing. Proper stall structures that are comfortable and easy to clean, such as having well-drained concrete floors, were shown to be associated with lower disease likelihood too.

Together, these recommendations should help SHD farms to reduce disease and enhance productivity, with vaccination against BVDV and the other components of the multivalent vaccine being an important part of infectious disease prevention.

### 6.6.1 Future research suggestions

Upon conducting this study, analyzing the finding for the four substantive chapters, and making recommendations, we also provide the following suggestions for possible future research.

1. The identification of circulating genotypes and variants by doing a study that includes molecular investigation on antigen-positive cases. An investigation drawing upon positive samples from different parts across Kenya and in different production/management systems would be informative. This investigation can involve testing of stored wildlife samples for BVDV and other Pestiviruses.

2. Investigating the role of other animal species in carrying BVDV infections in Kenya and understanding the possibility of diagnostic cross reactivity with different Pestiviruses. Due to the close resemblance in antigenic proteins of Pestiviruses, cross reactivity in Pestivirus diagnosis could be assessed by experimental infections and confirmations in multiple species, using a superior test, such as viral neutralization or PCR test, instead of ELISA. The research could also involve testing of wildlife in areas where livestock interact with game species that are likely to harbor Pestiviruses.

3. Investigating the efficiency and efficacy of vaccines within the SHD farms by vaccination and BVDV challenge experimentation. The uniqueness and abundance of SHD farming calls for specific research that shows benefits of vaccination in that sector in the attempt to improve productivity and reduce the losses due to BVDV disease.

4. Determining the benefits of all the MLV vaccine components on the SHD farms and comparing the results with possible antibody response variability. This recommendation could include investigation on the role of natural infection effects on the use of MLV for all the pathogens components.

5. Economic benefits of using an MLV vaccine including BVDV in the SHD farming system. This recommendation could be conducted together with a benefits or antibody response research study and in a collaborative investigation with livestock economics experts to compute an estimate of loss due to BVDV in SDFs. This investigation can be used to provide awareness to the farmers on the cost benefit of BVDV vaccination.

6. Investigating BVDV burden in Kenya for continuous surveillance. Surveys to determine prevalence of persistently infected cattle across the SHD farms in Kenya as well as in commercial farms would clarify the extent of the problem. Surveys on farm-level prevalence or cooperative-level prevalence through antigen screening of pooled milk samples at collection points could be done through the dairy cooperative societies. This will provide the government and other policy makers, information for control-planning.

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# Chapter 7: Appendices

## 7.1 Questionnaire visit one (1)

*Naari/Buuri*: Farmer #: Phone: *Survey Visit Date*: .

***Cohort Questionnaire for Management and Disease on Vaccinated Naari Smallholder Dairy Farms***

***ASK QUESTIONS AS OPEN-ENDED (NOT GIVING ANSWERS); GIVE OPTIONS IF NEEDED***

**A. Farm overview:**

1. How many people live in this household for more than 5 days per week? \_\_\_\_\_

2. Gender of **principal farmer (person who manages/takes care of the cows)**: male/female/both

3. Woman’s education completed: \_\_\_\_ primary \_\_\_ secondary \_\_\_\_ college/university \_\_\_ n/a

4. Man’s education completed: \_\_\_\_ primary \_\_\_ secondary \_\_\_\_ college/university \_\_\_ n/a

5. Woman’s age: \_\_\_\_\_ years \_\_\_ n/a

6. Man’s age: \_\_\_\_\_ years \_\_\_ n/a

7. a) Percent of total income coming from dairy production: \_\_\_< 50% \_\_\_50-75% \_\_\_> 75%

8a. Area of land owned: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ acres / hectares (circle units)

8b. Percent of land used for crop and fodder production for cattle?

8c. Area of land rented/used (unpaid): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ acres / hectares (circle units)

8d. Percent of land rented/used for crop and fodder production for cattle?

9a. Have you attended any training on milk production in the last year ? Y/N

9b. If yes, what was this training about ? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**B. Feeding - Normal feeding**: Some feeds are only given seasonally. **Over the last year**, which of the following did you feed to your cattle (***amounts not needed***).

|  |  |  |
| --- | --- | --- |
| **Feed name** | **Heifers over 6 mo (10)** | **Cows (11)** |
| a. Napier grass |  |  |
| b. Grass silage |  |  |
| c. Maize silage |  |  |
| d. Grass hay |  |  |
| e. Desmodium |  |  |
| f. Sweet potato vines |  |  |
| g. Tree fodders –specify \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  |  |
| h. Other high protein forages – lucerne, leucana, –  identify which one(s) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  |  |
| i. Maize stover |  |  |
| j. Banana leaves |  |  |
| k. Other fodder – specify (eg. weeds) \_\_\_\_\_\_\_\_\_ |  |  |
| l. Dairy meal |  |  |
| m. Wheat bran |  |  |
| n. Maize Germ |  |  |
| o. Vitamin/mineral powder |  |  |
| p. Vitamin/mineral block |  |  |
| q. Calf meal/pellets/calf pencils. If yes, until what age? |  |  |
| r. Other feeds –specify (eg. meal or cake) \_\_\_\_\_\_\_\_\_\_\_\_ |  |  |
| s. Water available (always/sometimes ) | A/S | A/S |

12a. Do you usually feed **dairy meal or grain** to cows for the **month before calving**? \_\_YES \_\_NO

12b. If yes, do you increase the amount of dairy meal or grain during this month? \_\_YES \_\_NO

13a. Do you feed **minerals** to cows during the **month before calving**? \_\_\_YES \_\_\_NO

13b and c. If yes, what brand?

*Brand: \_\_\_\_\_\_\_\_\_\_\_\_\_\_ (from bag: Ca:P ratio(b): \_\_\_\_ Selenium amount & unit (c): \_\_\_\_\_)*

13d. If yes, how much is given to the cow? *Amount (in spoons or grams per day): \_\_\_\_\_\_\_\_\_\_*

*(figure out if flat or heaped spoon, and size of spoon or container; and then measuring with measuring cup or true level TBSP=15g)*

14. How much **dairy meal and/or grain** (eg. maize “jam”) do you give cows on the **day they calve**?

a) dairy meal \_\_\_\_\_\_ kg in morning \_\_\_\_\_\_ kg in evening (*enter total\_\_\_\_\_)*

b) other grain \_\_\_\_\_\_ kg in morning \_\_\_\_\_\_ kg in evening *(enter total\_\_\_\_\_)*

*(note size of container; 1 kg or 2 kg; and then know conversion: 2 kg kasuku is 1.3kg dairy meal)*

15a. In general, during the first **5 months after calving**, do you normally feed the same amount of **dairy**

**meal or grain** per day to your cows? \_\_\_\_\_YES \_\_\_\_\_NO

b. If no, 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 1.0 - 2.0 meters \_\_\_\_\_\_\_ 2. mostly 1.0 - 2.0 meters \_\_\_\_\_\_\_

3. mostly > 2.0 meters \_\_\_\_\_\_\_ 3. mostly > 2.0 meters \_\_\_\_\_\_\_

c. Are these the same heights for Napier grass fed to heifers > 6 mo too? Yes\_\_\_ No\_\_\_

If no, what is different? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

17a. For your **cows,** did you always have enough feeds over the last year? Yes\_\_\_\_\_ No\_\_\_\_\_

17b. If no, which feeds were inadequate *(check all that apply)?*

\_\_\_Forages1 \_\_\_Grain or meals2 \_\_\_Vitamin-minerals3 \_\_\_Water4 \_\_\_Other(specify)5\_\_\_\_\_\_

18a. How frequently do you normally deworm your cows in a year?

Every \_\_\_ months \_\_when suspect a problem (-1) \_\_\_when not pregnant (-2) \_\_ Other (-3)?

18b. How frequently do you normally deworm your heifers > 6 mo in a year?

Every \_\_\_ months \_\_when suspect a problem (-1) \_\_ Other (-2)?

18c. Who normally deworms your cattle? Self (1):\_\_ Vet service provider (2): \_\_\_

18d. What do you usually use to deworm your animals? \_\_\_\_\_\_\_\_\_\_\_\_ (get container if not know)

19. Deaths 19a. How many **calves** died in the last year? \_\_\_ 19b.Out of how many calves last year? \_\_\_\_

19c. If some, from what causes\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

19d. How many **heifers > 6 mo** died in last year? \_\_\_ 19

e. Out of how many heifers last year? \_\_\_\_

19f. If some, from what causes \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

19g. How many **cows** died in the last year? \_\_\_\_\_ 19h.Out of how many cows last year? \_\_\_\_

19i If some, from what causes \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

19j Were any of the deaths in the last year in animals you purchased? Yes \_\_ No \_\_

19k Were any of the deaths in the last year in animals vaccinated for BVD? Yes \_\_ No \_\_

20a. In the past year, did a vet service provider visit your farm for a sick animal? Yes \_\_ No \_\_

20b. If yes, for what reason(s)? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

21a. When you have a cow or heifer in heat, do you always use an AI service? Y\_\_N\_\_

21.b How often do you spray for ticks? Every \_\_\_\_\_\_\_\_\_ weeks

**C. Mastitis Prevention Management**

22. a) If more than 1 milking cow, is a different wash cloth used for each milking cow? Yes \_\_No \_\_

b) If more than 1 milking cow, do you wash your hands between milking cows? Yes \_\_No \_\_

c) Do you use a teat dip post milking? Yes \_\_No \_\_

d) Do you give fresh feed after milking? Yes \_\_No \_\_

e) Do you use dry cow treatment when drying cows off prior to calving? Yes \_\_No \_\_

f) How many cows leaked milk in the last year? \_\_\_\_\_

23. a) How many cases of mastitis did you have in the last year? \_\_\_\_\_ *(if zero skip to 24)*

b) If you had mastitis, how many cases of mastitis did you treatin the last year? \_\_\_\_\_

c) If you treated for mastitis, how many cases of mastitis did not resolve after 1 treatment? \_\_

d) If >1 cow, did you milk the mastitic cow after other cows? Yes \_\_ No \_\_ n/a\_\_\_ *(only 1 cow)*

24. a) How many times did you have milk rejected in the last year? \_\_\_\_\_

b) If you had rejected milk, what were the reasons for rejection? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**D. Cow Stall Design and Management**

25a. Do your milking cows: 1) full-time zero-graze; 2) part-time zero-graze; 3) no zero-graze? \_\_\_\_\_\_\_

25b. If 3) go to question 28

25b. If 1) or 2), how often do you **remove manure** from where the **milking cows** lie down?

1) at least once a day

2) a few times per week

3) once a week or less

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26. How often do you **add new bedding** to where **milking** cows lie down?

1) at least once a day

2) a few times per week

3) once a week or less

27. Do your cows do any of the following behaviours (circle all that apply – observe to confirm)?

a) lying somewhere other than in the stall Yes \_\_No \_\_

b) other unusual behaviours such as standing partly in the stall or standing backwards in the stall

Yes \_\_No \_\_ (if yes please specify \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_)

28. How do your calves usually receive their **first colostrum**?

*Choose the* ***ONE*** *option that is MOST commonly used*

Suckle on cow (1) nipple bottle (2) bucket (3)

29. **How soon** **would most of your calves** receive **4L** of colostrum? Choose **ONE** answer only

< 6 hours1 6 - 12 hours2 12 - 24 hours3 > 24 hours4 unknown5

30. What is the source of your cattle (*circle all that apply – each is yes/no question for coding*)?

a. Buy heifer b. Buy as in-calf heifers? c. Buy as adult cows d. Raise from young ones on my farm

31a. Have you had a cow with difficult calving requiring assistance in the last year? Yes \_\_ No \_\_

31b. If yes, who came to help? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

32a. Have you had a cow that could not stand up during the days just before or after calving in the last year? Yes \_\_ No \_\_

32b. If yes, who came to help? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

33a. Have you had a cow that had any other problem after calving in the last year? Yes \_\_ No \_\_

33b If yes, what was it? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

34a. Any other health problems in cattle on the farm? Yes \_\_ No \_\_

34b If yes, what was it? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**For observation:**

35. What kind of bedding is used where the milking cows lie down?

1) grass/hay 2) straw 3) sawdust 4) crop waste 5) soil 6) none 7) other (specify \_\_\_\_\_\_\_\_\_)

36. What is the type of the floor where the milking cows lie down?

1) concrete 2) dirt 3) other (please specify: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_)

37. Is the floor (observe - check all that apply):

a) lumpy (have to lie on sticks, rocks, dirt chunks , etc.) Yes \_\_ No \_\_

b) hard (fails Knee impact test) Yes \_\_ No \_\_

c) wet in the udder area (fails the Knee wetness test) Yes \_\_ No \_\_

38. Is water/urine/feces able to flow (by gravity) under udder where milking cows lie down? Y \_N \_

39. Digital photo file range: \_\_\_\_\_\_\_\_\_\_\_\_\_\_ to \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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Farm Number \_\_\_\_\_\_\_\_ Naari/Buuri: \_\_\_\_\_\_\_\_

**E. Youngstock Health and Productivity Checks** (calves and heifers that have never calved yet):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Calf/Heifer #1 (Q40)  ID\_\_\_\_\_\_\_ | Calf/Heifer #2 (Q41)  ID\_\_\_\_\_\_\_\_ | Calf/Heifer #3 (Q42)  ID\_\_\_\_\_\_\_\_\_ | Calf/Heifer #4 (Q43)  ID\_\_\_\_\_\_\_\_\_ |
| *a. “Birthdate or approximate age (months)”* |  |  |  |  |
| b. Sex |  |  |  |  |
| c1. Breed |  |  |  |  |
| *c2. Is this animal bred? If no, go to g.* |  |  |  |  |
| *d. “First breeding date” (date or n/a if go to g)* |  |  |  |  |
| *e. “Latest breeding date” (date or n/a if go to g)* |  |  |  |  |
| *f.” Number of breedings to date” (# or n/a “)* |  |  |  |  |
| *g. “Had diarrhea in last year”* | Y/N | Y/N | Y/N | Y/N |
| *h. “Had pneumonia in last year”* | Y/N | Y/N | Y/N | Y/N |
| *i. “Had navel-ill* *in last year”* | Y/N | Y/N | Y/N | Y/N |
| j. “*Had any other disease in last year*” specify | Y/N | Y/N | Y/N | Y/N |
| k. Weight (kg) |  |  |  |  |
| l. Height (cm or inches) |  |  |  |  |
| m. Body condition score (1-5) |  |  |  |  |
| n. TPR/physical exam Normal / Abnormal?  (manure, feet, skin, lymph nodes, eyes, rumen) | N / A | N / A | N / A | N / A |
| o. Disease #1 found on physical exam |  |  |  |  |
| p. Disease #2 found on physical exam |  |  |  |  |
| r. Reproductive status (*preg days confirmed?)* | Preg: y/n/m | Preg: y/n/m | Preg: y/n/m | Preg: y/n/m |
| *t. Vaccinated for BVDV in 2018.* | Y/N | Y/N | Y/N | Y/N |
| *u. Vaccinated for BVDV in 2019* | Y/N | Y/N | Y/N | Y/N |
| v. Vacc or Plabebo (A or B or n/a) |  |  |  |  |
|  |  |  |  |  |

**44. Blood taken?** Y/N Y/N Y/N Y/N

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Farm Number \_\_\_\_\_\_\_\_ Naari/Buuri: \_\_\_\_\_\_\_\_

**F. Cow Health and Productivity Checks**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Examination of Cows: | Cow1 (Q46) ID\_\_\_\_\_\_\_\_\_ | Cow2 (Q47)  ID\_\_\_\_\_\_\_\_ | Cow3 (Q48)  ID\_\_\_\_\_\_\_\_\_ | Cow4 (Q49)  ID\_\_\_\_\_\_\_\_\_ |
| *a. “Approximate age (years)”* |  |  |  |  |
| b. Breed |  |  |  |  |
| *c. “Approximate number of calvings”* |  |  |  |  |
| *d. “Approximate last calving date”* |  |  |  |  |
| *e. Bred since last calving? If no, go to h.* | Y/N | Y/N | Y/N | Y/N |
| *f. “First breeding date after last calving” (mo)* |  |  |  |  |
| *g.“Latest breeding after last calving” (date/mo)* |  |  |  |  |
| *h. “Number of breedings for last pregnancy”* |  |  |  |  |
| *i.” Current daily milk yield (kg/day)”* |  |  |  |  |
| *j. “Is this what she produced last week?”* | Y/N | Y/N | Y/N | Y/N |
| *k. “Mastitis in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *l. “Abortion/stillbirths in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *m. “Pneumonia in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *n. “Diarrhea in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *o. “Poor appetite in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *p. “Tick-borne disease in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *q. “Skin disease in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *r. “Uterus infection (RP) in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *s. “Other disease in last 12 months”\_\_\_\_\_\_\_\_* | Y/N | Y/N | Y/N | Y/N |
| t. Weight (kg) |  |  |  |  |
| u. Height (cm of inches) |  |  |  |  |
| v. Body condition score (1-5) |  |  |  |  |
| w. TPR/physical exam Normal / Abnormal?  (manure, feet, skin, lymph nodes, eyes, rumen) | N / A | N / A | N / A | N / A |
| x. Disease #1 found on physical exam |  |  |  |  |
| y. Disease #2 found on physical exam |  |  |  |  |
| z. CMT (circle CMT result if milk looks abnormal as well) (0,T,1,2,3) | LF LH RF RH  \_\_ \_\_ \_\_ \_\_ | LF LH RF RH  \_\_ \_\_ \_\_ \_\_ | LF LH RF RH  \_\_ \_\_ \_\_ \_\_ | LF LH RF RH  \_\_ \_\_ \_\_ \_\_ |
| aa. Reproductive status (*preg days confirmed?)* | Preg: Y/N | Preg: Y/N | Preg: Y/N | Preg: Y/N |
| ab. Normally get pregnancy confirmation? | Y/N | Y/N | Y/N | Y/N |
| ac. Vacc or Plabebo (A or B or n/a) |  |  |  |  |
| *ad. Vaccinated for BVDV in 2018?* | Y/N | Y/N | Y/N | Y/N |
| *ae. Vaccinated for BVDV in 2019?* | Y/N | Y/N | Y/N | Y/N |

**50. Blood taken?** Y/N Y/N Y/N Y/N

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Farm Number \_\_\_\_\_\_\_\_ Naari/Buuri: \_\_\_\_\_\_\_\_

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## 7.2 Questionnaire visit two (2)

Farmer Number: Survey Visit Date: Interviewer Initials: .

Area Naari/Buuri: .

**TRIAL Questionnaire for Management and Disease on Vaccinated Naari Smallholder Dairy Farms**

**ASK QUESTIONS AS OPEN-ENDED (NOT GIVING ANSWERS); GIVE OPTIONS IF NEEDED**

**A. Farmer information**

1a. Have you attended any training on milk production in the last year ? Y/N

1b. If yes, what was this training about? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**B. Feeding - Normal feeding**:. 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** | **Heifers over 6 mo (2)**  **If heifer(s) in RCT** | **Cows (3)**  **If cow(s) in RCT** |
| a. Napier grass |  |  |
| b. Grass silage |  |  |
| c. Maize silage |  |  |
| d. Grass hay |  |  |
| e. Desmodium |  |  |
| f. Sweet potato vines |  |  |
| g. Tree fodders –specify \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  |  |
| h. Other high protein forages – lucerne, leucana, –  identify which one(s) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  |  |
| i. Maize stover |  |  |
| j. Banana leaves |  |  |
| k. Other fodder – specify (eg. weeds) \_\_\_\_\_\_\_\_\_ |  |  |
| l. Dairy meal |  |  |
| m. Wheat bran |  |  |
| n. Maize “Germ” |  |  |
| o. Vitamin/mineral powder |  |  |
| p. Vitamin/mineral block |  |  |
| q. Calf pellets/calf pencils. If yes, until what age? |  |  |
| r. Other feeds –specify (eg. meal or cake) \_\_\_\_\_\_\_\_\_\_\_\_ |  |  |
| s. Water available (always/sometimes ) | A/S | A/S |

ONLY DO QUESTIONS 4-10 IF FARMER HAS COWS IN RCT

4a. Do you usually feed **dairy meal or grain** to cows for the **month before calving**? \_\_YES \_\_NO

4b. If yes, do you increase the amount of dairy meal or grain during this month? \_\_YES \_\_NO

5a. Do you feed **vitamins/minerals** to cows during the **month before calving**? \_\_\_YES \_\_\_NO

5b. If yes, what brand?

Brand: \_\_\_\_\_\_\_\_\_\_\_\_\_\_ *(from bag: Ca:P ratio: \_\_\_\_ Selenium amount & unit: \_\_\_\_\_\_)*

5c. If yes, how much is given to the cow? Amount (in spoons or grams per day): \_\_\_\_\_\_\_\_\_\_

6. How much **dairy meal and/or grain** (eg. maize “Germ”) do you give cows on the **day they calve**?

a) dairy meal \_\_\_\_\_\_ kg in morning \_\_\_\_\_\_ kg in evening (enter total)

b) other grain \_\_\_\_\_\_ kg in morning \_\_\_\_\_\_ kg in evening (enter total)

7a. In general, during the first **5 months after calving**, do you normally feed the same amount of **dairy**

**meal or grain** per day to your cows? \_\_\_\_\_YES \_\_\_\_\_NO

b. If no, what factors affect how much dairy meal or grain you feed per day?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

8. 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 heifers > 6 mo too? Yes\_\_\_ No\_\_\_

If no, what is different? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

9a. For your **cows,** did you always have enough feeds over the last year? Yes\_\_\_\_\_ No\_\_\_\_\_

9b. If no, which feeds were inadequate (check all that apply)?

\_\_\_Forages \_\_\_Grain or meals \_\_\_Vitamin-minerals \_\_\_Water \_\_\_Other(specify)\_\_\_\_\_\_\_

10a. How frequently do you normally deworm your cows?

Every \_\_\_ months \_\_\_\_when suspect it is a problem \_\_\_when not pregnant \_\_ Other?

10b. How frequently do you normally deworm your heifers > 6 mo?

Every \_\_\_ months \_\_\_\_when suspect it is a problem \_\_\_other (specify:\_\_\_\_\_\_\_\_\_\_\_\_)

10c. Who normally deworms your cattle? Self :\_\_ Vet service provider: \_\_\_ Other:\_\_\_ Who? \_\_

10d. What do you usually use to deworm your cattle? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**C. Population and Sickness**

11a. How many **calves** **< 6 mo** did you have in the last year? \_\_\_\_

11b. How many of these calves were sick in the last year? \_\_\_\_

11c. If some, from what sickness\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

11d. How many **heifers > 6 mo** did you have in the last year? \_\_\_\_

11e. How many of these heifers were sick in the last year? \_\_\_\_

11f. If some, from what sickness \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

11g. How many **cows** did you have in the last year? \_\_\_\_

11h. How many of these cows were sick in the last year? \_\_\_\_

11i If some, from what sickness \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

11j Were any of the sicknesses in the last year in cattle you purchased? Yes \_\_ No \_\_

12a. In the past year, did a vet service provider visit your farm for sick cattle? Yes \_\_ No \_\_

12b. If yes, for what reason(s)? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

13. Deaths 13a. How many **calves** **< 6 mo** died in the last year? \_\_\_\_

13b. If some, give identities \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

13c. If some, from what causes\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

13d. How many **heifers > 6 mo** died in the last year? \_\_\_\_\_

13e. If some, give identities \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

13f. If some, from what causes \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

13g. How many **cows** died in the last year? \_\_\_\_\_

13h. If some, give identities \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

13i If some, from what causes \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

13j Were any of the deaths in the last year in cattle you purchased? Yes \_\_ No \_\_

14a. In the past year, did a vet service provider visit your farm for dead cattle? Yes \_\_ No \_\_

14b. If yes, for what reason(s)? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

15. In the past year, did you use an AI service? Y\_\_N\_\_

15b. Did you have less trouble getting cows pregnant in the last year than other years? Y\_\_N\_\_

**Some questions on specific diseases:**

16. In the past year, did you have any **abortions?** Y\_\_N\_\_

16b. If yes, did you have **more** trouble with abortion in the last year than other years? Y\_\_N\_\_

16c. If no, did you have **less** trouble with abortion in the last year than other years? Y\_\_N\_\_

17. In the past year, did you have any cattle **with diarrhea**? Y\_\_N\_\_

17b. If yes, did you have **more** trouble with diarrhea in the last year than other years? Y\_\_N\_\_

17c. If no, did you have **less** trouble with diarrhea in the last year than other years? Y\_\_N\_\_

18. In the past year, did you have any cattle with **pneumonia**? Y\_\_N\_\_

18b. If yes, did you have **more** trouble with pneumonia in the last year than other years? Y\_\_N\_\_

18c. If no, did you have **less** trouble with pneumonia in the last year than other years? Y\_\_N\_\_

19. In the past year, did you have any calves born with **birth defects**? Y\_\_N\_\_

19b. If yes, did you have **more** trouble with birth defects in the last year than other years? Y\_\_N\_\_

19c. If no, did you have **less** trouble with birth defects in the last year than other years? Y\_\_N\_\_

20. a) How many cases of **mastitis** did you have in the last year? \_\_\_\_\_

20b. If some, did you have **more** trouble with mastitis in the last year than other years? Y\_\_N\_\_

20c. If none, did you have **less** trouble with mastitis in the last year than other years? Y\_\_N\_\_

20d. If you treated for mastitis, how many cases **did not resolve after 1 treatment**? \_\_

20e. If more than 1 cow, is the mastitic cow milked before or after other cows? Before \_\_ After\_\_\_

20f. Do you use teatdip after milking your cows? Y\_\_N\_\_

21. a) How many times did you have **milk rejected** in the last year? \_\_\_\_\_

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b) If you had rejected milk, what were the **reasons** for rejection? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

22a. Any **other health problems** on the farm in the last year? Yes\_\_ No\_\_

22b If yes, what was it? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

22b. If yes, did you have **more** of this problem in the last year than other years? Y\_\_N\_\_

**D. Other Management**

23. **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

24. Did you purchase any cattle in the last year? Yes \_\_ No\_\_

25. Did you borrow/lend any cattle in the last year? Yes \_\_ No\_\_

26. Did any of your cattle graze outside the farm in the last year? Yes \_\_ No\_\_

27. What is the source of your cattle?

a. Buy as in-calf heifers? \_\_ b. Buy as adult cows \_\_ c. Raise from young ones on my farm, \_\_

**E. Youngstock Health and Productivity** (calves and heifers that have never calved yet – only if in RCT ):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Calf/Heifer #1 (Q28)  ID\_\_\_\_\_\_\_ | Calf/Heifer #2 (Q29)  ID\_\_\_\_\_\_\_\_ | Calf/Heifer #3 (Q30)  ID\_\_\_\_\_\_\_\_\_ | Calf/Heifer #4 (Q31)  ID\_\_\_\_\_\_\_\_\_ |
| *a. “Birthdate or Age (months)”* |  |  |  |  |
| b. Sex |  |  |  |  |
| c. Breed |  |  |  |  |
| *d. “First breeding date” (date or n/a)* |  |  |  |  |
| *e. “Latest breeding date” (date or n/a)* |  |  |  |  |
| *f.” Number of breedings to date” (# or n/a)* |  |  |  |  |
| *g. “Had diarrhea in last year”* | Y/N | Y/N | Y/N | Y/N |
| *h. “Had pneumonia in last year”* | Y/N | Y/N | Y/N | Y/N |
| *i. “Had navel-ill* *in last year”* | Y/N | Y/N | Y/N | Y/N |
| j. “*Had any other disease in last year*” specify | Y/N | Y/N | Y/N | Y/N |
| k. Weight |  |  |  |  |
| l. Height |  |  |  |  |
| m. Body condition score |  |  |  |  |
| n. TPR/physical exam Normal / Abnormal?  (manure, feet, skin, lymph nodes, eyes, rumen) | N / A | N / A | N / A | N / A |
| o. Udder hygiene score (1-5) |  |  |  |  |
| p. Reproductive status (*preg days confirmed?)* | Preg: Y / N | Preg: Y / N | Preg: Y / N | Preg: Y / N |
| q. Ovary status (CL, follicle, in heat, anestrus): | L R | L R | L R | L R |
| *r. Month of last deworming* |  |  |  |  |

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**F. Cow Health and Productivity Checks (only for cows that are in the RCT)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Examination of Cows: | Cow1 (Q32) ID\_\_\_\_\_\_\_\_\_ | Cow2 (Q33)  ID\_\_\_\_\_\_\_\_ | Cow3 (Q34)  ID\_\_\_\_\_\_\_\_\_ | Cow4 (Q35)  ID\_\_\_\_\_\_\_\_\_ |
| *a. “Approximate age (years)”* |  |  |  |  |
| b. Breed |  |  |  |  |
| *c. “Number of calvings”* |  |  |  |  |
| *d. “Last calving date” (or month if unsure)* |  |  |  |  |
| *e. “First breeding date after last calving”* |  |  |  |  |
| *f. “Latest breeding date after last calving”* |  |  |  |  |
| *g. “Number of breedings for last pregnancy”* |  |  |  |  |
| *h.” Current daily milk yield (kg/day)”* |  |  |  |  |
| *i. “Is this what she produced last week?”* | Y/N | Y/N | Y/N | Y/N |
| *j. “Mastitis in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *k. “Abortion/stillbirths in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *l. “Pneumonia in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *m. “Diarrhea in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *n. “Poor appetite in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *o. “Tick-borne disease in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *p. “Skin disease in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *q. “Uterus infection (RP) in last 12 months”* | Y/N | Y/N | Y/N | Y/N |
| *r. “Other disease in last 12 months”\_\_\_\_* | Y/N | Y/N | Y/N | Y/N |
| s. Weight |  |  |  |  |
| t. Height |  |  |  |  |
| u. Body condition score |  |  |  |  |
| v. TPR/physical exam Normal / Abnormal?  (manure, feet, skin, lymph nodes, eyes, rumen) | N / A | N / A | N / A | N / A |
| w. 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  \_\_ \_\_ \_\_ \_\_ |
| x. Reproductive status (*preg days confirmed?)* | Preg: Y / N | Preg: Y / N | Preg: Y / N | Preg: Y / N |
| y. Disease #1 found on physical exam |  |  |  |  |
| z. Disease #2 found on physical exam |  |  |  |  |
| *aa.* Udder score |  |  |  |  |
| *ab.* ***Month of last deworming*** |  |  |  |  |

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