SERO-PREVALENCE AND RISK FACTORS OF INFECTIOUS BOVINE RHINOTRACHEITIS IN THE SMALLHOLDER DAIRY FARMS OF NAARI SUB-LOCATION OF MERU COUNTY, KENYA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE AWARD OF THE MASTER OF VETERINARY MEDICINE (M. VET. MED), OF UNIVERSITY OF NAIROBI

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate my thesis work to my family and academic friends. A special gratitude to my loving father, Joseph Kipserem Seroney and mother, Susana Jeruto for their constant prayer for my wellbeing while undertaking my fieldwork and words of encouragement. I also dedicate my dissertation to Non-Governmental Organization, Farmers Helping Farmers, the University of Prince Edward Island and the University of Nairobi that has an existing developmental partnership with the Naari Dairy Cooperative Society, for providing me with a strong foundation for my study and also entry point to the community.

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LIST OF ABBREVIATIONS

IBR	Infectious Bovine Rhinotracheitis
BoHV	Bovine Herpesvirus Virus
BVDV	Bovine Viral Diarrhoea Virus
OIE	World Organization of Animal Health
SAS	Statistical Analysis Software
NDC	Naari Dairy Cooperative Society
gB	Glycoprotein B
ELISA	Enzyme linked immuno-Sorbent Assay
PCR	Polymerase Chain Reaction
VNT	Virus Neutralization Test
CpHV – 1	Caprine herpesvirus – 1
CvHV – 1	Cervine herpesvirus – 1
CvHV – 2	Cervine herpesvirus - 2
DNA	Deoxyribonucleic Acid
DIVA	Differentiation of Infected from Vaccinated Animals
OD	Optical Density
TMB	Tetramethylbenzidine substrate
LM	Light Microscope
IHC	Immunohistochemistry
IgM	M Immunoglobulin
IgG	Gamma Immunoglobulin
BPIV – 3	Bovine Para-Influenza Virus Type 3

ABSTRACT

The aim of the study was to analyze the sero-prevalence and risk factors of the Infectious Bovine Rhinotracheitis disease among organized small holder dairy farms in the Naari area of Meru County, Kenya.

A cross-sectional study was conducted in the Naari area of Meru County, Kenya between June-July 2018 and March-April 2018. The 149 farmers were randomly selected from members of the Naari Dairy Farmers Cooperative Society who were actively delivering milk to the society at the time of the study. Serum samples were obtained from 403 female dairy cattle. Farm level management and animal factors were collected through direct interviews with the owner or someone who was knowledgeable about the animals. All serum samples were processed with an indirect enzyme-linked immunosorbent assay (gB ELISA) to determine the presence of antibodies to BHV-1.

The results revealed that the sero-prevalence of IBR among the smallholder dairy cows' population in part of the large Meru County was 17.37% (95% CI: 13.80% to 21.43%). Among the categorical predictor, the sero-prevalence of the breeds of the dairy cows were Ayrshire (20.0%), Friesian (16.3%), Guernsey (17.9%) and Zebu (16.1%). The proportions positive for BoHV -1 among parity of the dairy cows were Heifer (parity 0) (15.0%), Primi-para (parity 1) (12.8%) and Multi-para (parity 2 – 8) (19.8%). The cow-heifer category was cow (18.7%) and heifer (12.2%). The feeding systems employed in the production of dairy cows were zero – grazing (17.5%), semi zero – grazing (18.9%) and grazing (12.1%). The study found out that the IBR infection was positively associated with the following factors; age of the dairy cattle (OR = 1.112, 95% CI = 1.012 – 1.222, P = 0.027), cows that were borrowed into farms (OR = 4.893, 95% CI, = 1.328 – 16.03,1 P = 0.017), rearing goats in the farms (OR, 1.438, 95% CI, 1.109 –

1.863, P = 0.006), cows that were given out of the farms (OR, = 2.486 95% CI, = 0.697 – 8.859 p = 0.014), Showed BVD signs OR = 1.243 95% CI, = 0.635 – 2.430, p = 0.526) and cows that had antibodies against Bovine Viral Diarrhea Virus (OR, = 2.262 95% CI, = 1.129 – 4.533, P = 0.021). The final multivariate analysis of individual level factors that were associated with those that tested positive to BoHV – 1 antibodies included; rearing goats on the farm (OR = 4.636, 95% CI = 2.053 – 10.467, P = 0.001), age of the dairy cattle, (OR = 1.113, 95%CI = 1.017 – 1.217, P = 0.020) and age of the female principal farmers (OR = 0.174, 95% CI = 0.082 – 0370, P = 0.001).

The study concluded that BoHV – 1 is naturally circulating among cattle population in Meru County, Kenya. There was a positive association between age of the dairy cattle, age of the principal female farmers and rearing of goats in the farm together with cattle, and BoHV – 1 sero-prevalence observed in the study. Thus, cattle population may be protected via punctual vaccination while considering the differentiation of infected from vaccinated cattle. Furthermore, there is need for a study to be carried out to identify long term effects of BoHV – 1 and access the potential ability of the viral cross infection with other four related Alpha-herpesviruses with BoHV – 1 among the cattle population.

Keywords: Infectious Bovine Rhinotracheitis (IBR), Bovine Herpesvirus type 1 (BoHV – 1) Cattle, Sero-Prevalence and risk factors.

CHAPTER ONE: INTRODUCTION

Infectious Bovine Rhinotracheitis disease is of significant economic importance worldwide caused by Bovine Herpes Virus – 1 (BoHV – 1) and it affects both domestic and wild ruminants (Bowland *et al.,* 2000; Muylkens *et al*., 2007). Bovine Herpes Virus – 1 is a virus of genus; *Varicellovirus*, subfamily; *Alphaherpesvirinae* and family; *Herpesviridae* is a highly contagious and infectious virus (King *et al.,* 2012; Biswas *et al.,* 2013; Newcomer and Givens, 2016). Various subtypes of the virus cause different syndromes in cattle. Infectious bovine rhinotracheitis in bovine is caused by BoHV – 1.1, the respiratory subtypes. Strain BoHV – 1.2a and BoHV – 1.2b are the genital subtype while BoHV 1.3 is the encephalitic subtype (Muylkens *et al.,* 2007).

Infectious bovine rhinotracheitis virus (IBRV) has potential for cross infection with other ruminant Alpha-herpes viruses; Cervine herpesvirus – 1, Cervine herpesvirus – 2, bovine herpesvirus – 1 and caprine herpesvirus – 1 (Yesilbag *et al.,* 2003). Bovine Herpes Virus – 1 is also closely related with elk herpesvirus and buffalo herpesvirus (Keuser *et al.,* 2004). The virus affects both domestic and wild ruminants; cattle, white-tailed deer, mule deer, water buffalo, African wildebeest, roe deer, red deer, woodland caribou and reindeer. Goats are naturally infected while pronghorn antelope and African buffalo are reservoirs of the disease. (Biswas *et al*., 2013).

Infected cattle are the source of infection to the susceptible herds. These cattle shed the virus through body secretions and excretions via nasal discharges or droplets, semen, genital secretions, fetal fluid and tissues (Takiuchi *et al.,* 2005; Constable *et al.,* 2017). The virus is transmitted through aerosol infection in the respiratory form and this is dependent on environmental factors such as humidity and temperature. Direct contact with contaminated

semen, mucosal discharges, fetal tissues and fluid and genital discharges can also lead to transmission (Mars *et al.,* 1999; Mars *et al.,* 2000; Kahrs, 2001).

Cattle of all breeds and ages are equally susceptible and the disease is common among cattle above 6 months of age due to increased chances of exposure to the BoHV – 1 (Majumder *et al*., 2015; Seyfi-Abad-Shapouri, *et al*., 2016; Constable *et al.,* 2017). The disease has no seasonal variability, though in temperate countries, during the months of fall and winter, the occurrence of the disease is high due to feedlot cattle assembling (Majumder *et al.,* 2015). Unvaccinated breeding cattle or feedlot cattle are susceptible to epidemics of abortion and respiratory diseases. The systemic type of the disease is common with newly born calves with inadequate colostrum antibodies or failure of passive immunity (Constable *et al.,* 2017). The managerial and environmental risk factors contributing to the spread of BoHV – 1 include; purchasing of infected cattle, participation in agricultural shows, increased herd size and production system. Uncontrolled movement of visitors and cattle within the farm, and unreliable records of vaccination dates also contributes to the spread of the disease (Boelaert *et al.,* 2005; González-Garcia *et al*., 2009).

1.1. General objective

The overall objective of this study was to investigate sero-prevalence and risk factors of Infectious Bovine Rhinotracheitis among smallholder dairy cattle in the Naari area of Meru County, Kenya

1.2. Specific objectives

This study was designed with the following specific objectives;

1. To determine the sero-prevalence of Infectious Bovine Rhinotracheitis virus in the Naari area of Meru County, Kenya.

2. To assess the risk factors associated with Infectious Bovine Rhinotracheitis virus infections in cattle in the Naari area of Meru County, Kenya.

1.3 Problem statement

Infectious Bovine Rhinotracheitis disease is of significant economic importance in the dairy industry causing losses due to respiratory diseases, calf mortalities up-to 100% and reproductive losses. Vaccination against the disease has probability to reduce the economic losses due to clinical disease and not the prevalence of BoHV – 1 infection. However, most infected cattle often show no clinical picture and it`s therefore difficult to correctly estimate the economic impact of the clinically sick dairy cattle. The spread of the disease may vary within the country, from one region or farm to farm due to stock densities, managerial differences, micro-climatic changes and other factors. The risk factors of BoHV – 1 infections include; herd size, introduction of new animals to the farm, season, production system, vaccination status and animal age. Thus, information on epidemiology of Infectious Bovine Rhinotracheitis is vital in development of prevention and control programmes. Although Infectious Bovine Rhinotracheitis disease has been reported all over the world, there is scarcity of information about the disease in Kenya and this was what this study was seeking to address.

1.4 Justification

Infectious Bovine Rhinotracheitis is a disease of economic importance in cattle. Cattle greatly contribute to the economy and welfare of most Kenyan rural populations. The economic losses are associated with treatment costs, inefficient feed conversion efficiency, reduced conception rates, loss of newborn calves, low milk yield, abortion and loss of body condition (Constable *et al.,* 2017). This study was prompted by scarcity of information about the disease in Kenya. The study aims at improving the knowledge on Infectious Bovine Rhinotracheitis in Eastern Africa

through estimation of sero-prevalence and the risk factors accompanying the disease. Some of the economic gains from this studies will include reduced clinical cases, increased reproductive and production turnover and forms part of epidemiological surveillance.

CHAPTER TWO: LITERATURE REVIEW

2.1: Etiology of Infectious Bovine Rhinotracheitis

Bovine herpesvirus type 1 is an alpha-herpesvirus of family; *Herpesviridae*, subfamily; *Alpha-herpesvirinae* and genus *Varicellovirus.* Genetic analyses of clinical isolate found four distinct types; BoHV – 1.1, BoHV – 1.2a, BoHV – 1.2b and BoHV – 1.3. BoHV – 1.3 being neuropathic sero-type has about three genotypes; BoHV – 5a, BoHV – 5b and, BoHV – 5 non-a and non-b (Mahony, 2010). The observed antigenic differences among isolated viruses account for the diverse pathologic and epidemiologic pattern. However, vulvovaginitis/balanoposthitis or rhinotracheitis depend on the infection route rather than the subtype of the virus.

The four cud-chewing hoofed mammals alpha-herpesviruses related with BoHV – 1, may have potential for cross-infection with cattle include: bovine herpesvirus type 5 (BoHV – 5), cervine herpesvirus type 1 (CvHV – 1), caprine herpesvirus type 1 (CpHV – 1) and/or cervine herpesvirus -2 (CvHV – 2). Other alpha-herpesvirus which are closely related with BoHV – 1 include: elk herpesvirus and buffalo herpesvirus – 1. Bovine herpesvirus – 5 causes fatal type of meningoencephalitis among the calves while CpHV – 1 causes generalized infection among neonatal kids' and enteritis, Cervine herpesvirus – 1 may cause ocular diseases among red deer and is widespread in both free-living and farmed red deer and CvHV – 2 also has been isolated in Finland among the reindeer (Constable *et al.,* 2017). Bovine herpesvirus – 4 (BOHV – 1) has been isolated from case of bovine mastitis (Constable *et al.,* 2017.

2.2: Epidemiology of Infectious Bovine Rhinotracheitis

Infectious Bovine Rhinotracheitis disease has been recorded in various countries worldwide including; Germany, Sweden, Finland, Italy, Norway, United States of America, Switzerland Canada, Denmark and African countries (Muylkens *et al.,* 2007). Norway, Sweden, Switzerland,

Finland, parts of Italy and Germany have managed to eradicate the virus (Ackermann & Engels, 2006; Raaperi *et al.,* 2014). Denmark, Switzerland, Sweden, Finland and Austria are officially free of Infectious Bovine Rhinotracheitis (Ackermann and Engels, 2006). However, the sero-prevalence of Infectious Bovine Rhinotracheitis virus varies from continents, countries and regions and this is caused by differences in climates, stocking densities and management among other factors (Ackermann and Engels, 2006; Almeida *et al*., 2013). Sero-prevalence of BoHV – 1 varies from region to region with the rate of 64.4% being reported in North-Eastern Mexico (Segura-Correa *et al.,* 2016), 65.88% in Northern part of Tamil Nadu, India (Saravanajayam *et al.,* 2015), 36%–48% in South and Central America, 14%–86% in Africa (Straub, 1990; Ghirotti *et al.,* 1991; Mahmoud *et al.,* 2009), 43%, England (Woodbine *et al*., 2009) and 36%, China (Yan *et al.,* 2008).

Previous studies of the disease in Western part of Kenya revealed sero-prevalence rate of IBR was at 20.9% (Callaby *et al*., 2016). According to the study, they found out that, bovine para-influenza virus type 3, IBR and BVD have an association (Fulton *et al.,* 2000; Callaby *et al.,* 2016). Sero-prevalence of this disease among the small scale dairy cattle farms in coastal areas of Kenya was 28.6%, with significant increase in seroconversion rates with increasing age (Kenyanjui *et al.,* 2007).

The reproductive type of the disease due to BoHV – 1 was reported in Germany as infectious pustular balanoposthitis/vulvovaginitis (Segura-Correa *et al.*, 2006; Abu Elzein *et al.,* 2008; Graham, 2013). The virulent type of the disease is associated with BoHV – 1.1 which causes respiratory type of the disease. Morbidity and mortality of the disease is higher in feedlot cattle due to congregation of the susceptible population and introduction of new susceptible cattle from epizootic region (Graham, 2013). The case fatality and morbidity rates among the dairy cattle are

3% and 8% respectively. In feedlot cattle, morbidity rates of between 20 – 30% have been recorded (Majumder *et al*., 2015). A case fatality rate of less than 1% has been recorded in feedlot animals though it may reach 10% with secondary bacterial bronchopneumonia and tracheitis, (Graham, 2013; Constable *et al.*, 2017).

2.3: Immune Mechanism and Latency

Immune response to the virus consists of local and systemic cell-mediated and antibody immunity. The initial immune response to the virus in cattle exposed experimentally result in release of IgM or IgG antibodies. The second wave of immune response results from abortion following intra-amniotic inoculation of the virus leading to increase in IgM antibodies. Intranasal exposure of the virus does not produce IgM antibodies (Bahari *et al.,* 2013; Ghaemmaghami *et al.,* 2013; Haji-Hajikolaei and Seyfi-Abad-Shapouri, 2006). Once the cattle are infected naturally or vaccinated with modified live-viral vaccines, the humoral and cell-mediated immune systems are activated (Winkler *et al*., 1999; Jones, 2009).

Following intranasal infection or vaccination, antibodies and interferon production appears from the third day and persist up to ten days. Humoral immunity has been used to indicate previous bovine herpes virus – 1 infection (Constable *et al*., 2017). Cattle with low antibody may have immunity due to cell mediated immunity. Evaluation of the cell mediated immunity as per the previous studies may be done using delayed hyper-sensitivity test (Constable *et al.,* 2017). However, BoHV – 1 may become latent after primary infection or vaccinated with attenuated viral strains. The location of the viral latency in cattle body varies; localized on replication site, sacral or trigeminal ganglion (Seyfi-Abad-Shapouri, *et al*., 2016; Constable *et al.,* 2017). BoHV - 1 have been isolated in about 10% of clinically healthy cattle on the trigeminal ganglia at the

slaughter, 40% of the bovine had serum neutralizing antibodies to the BoHV – 1 (Winkler *et al*., 2000; Constable *et al.*, 2017).

Recrudescence, rise in neutralizing antibodies and viral shedding, occur following exposure of cattle to stress factors; high doses of corticosteroids, parturition, transportation and high ambient temperature (Radostits *et al*., 2000; OIE, 2008; Viu *et al.*, 2014). Detection of the latency form of the BoHV – 1 among cattle populations is vital in control and prevention practices, and international trading activities. Thus, tests to assay specific antibodies in the sera samples should have a higher sensitivity to detect low viral specific antibodies with emphasizes on international standardization of the tests (Moore *et al*., 2000; OIE, 2008). In endemic herds, transmission of the virus is non-continuous, however, its sufficient to obtain detectable quantities of antibodies. Latent infection result in negative serological test, since no animal reinfection to stimulate humoral immunity (Geraghty *et al.*, 2012).

2.4: Economic Importance

Infectious Bovine Rhinotracheitis causes significant economic impact in both beef and dairy cattle reproduction and production feedlots. The losses incurred as a result of infertilities are due to infectious pustular balanoposthitis in male cattle and infectious pustular vulvovaginitis in cows. Other losses may include; epidemic abortion, production loss, deaths due to respiratory disease among all ages of the cattle, deaths among newborn calves due to highly fatal form of systemic diseases and cost of disease management due to secondary bacterial infection of the respiratory system occurs (Bowland & Shewen, 2000; Saravanajayam *et al.*, 2015; Constable *et al.*, 2017).

2.5: Clinical Presentation of Infectious Bovine Rhinotracheitis

Clinically, IBR presents with fever ($39.9 – 42^0$C) and sudden drop in milk yield which eventually cease completely for 24 – 48 hours. Other signs include; hyper-salivation, inflamed mucous membranes with nasal discharge that is initially mucoid then later mucopurulent, short course of cough, apathy and anorexia (Mulyken *et al.,* 2007; Graham, 2013).

The respiratory disease affects oro-respiratory mucosa and the clinical picture includes; anorexia, fever, excessive salivation, coughing and nasal discharge, inflamed nares, conjunctivitis and lacrimal discharge. BoHV – 1.1 replicates on epithelial cells thus kills the respiratory mucosal cells thus, causing damage and necrosis of epithelial tissues (Jones, 2009).

The virus may also affect CD4+ T lymphocyte cells, affecting antigen production mechanism and CD8+ T lymphocyte cells recognition mechanism among the infected cells (Winkler *et al.,* 1999; Koppers-Lalic *et al.,* 2001). Furthermore, the dampening host-mounted an interferon response by employing the diverse modes of strategies (Henderson *et al.,* 2005; Saira *et al*., 2007; Saira *et al*., 2009; Da Silva *et al.,* 2011 and Da Silva *et al.,* 2012). The impaired non-specific immunity of the host provides opportunity where respiratory tract commensal bacteria of the family *Pasteurellaceae* may colonize the healthy lower respiratory tract system (Griffin *et al.,* 2010).

Abortion is a common sequela in reproductive disease. Abortion may occur several weeks after vaccination or clinical illness of unimmunized pregnant cow using modified live-virus vaccines of bovine tissue culture origin (Constable *et al.,* 2017). moreover, abortion may occur up-to 90 days of pregnancy especially when the virus goes latent within placenta thereafter infects foetus much later than usual. However, possibility of a vaccine to cause abortion even with safe vaccines may occur if the natural infection preceded vaccination, this commonly occur between

6 and 8 months of pregnant cow (Constable *et al.,* 2017). Retained after birth is often common and may be followed by residual infertility. Short estrus, endometritis and poor conception rates may occur if breed herd is inseminated with infected semen (Graham, 2013).

Infectious pustular vulvovaginitis manifests clinically with a mild vaginal discharge, elevated tail, and frequent urination. The vulva may present with small papules, swollen, mucosal ulceration and erosion on the surfaces. Ulcers on the mucosal surfaces may coalesce and slough off resulting to brown necrotizing tissue surfaces. Recovery may occur in about 10 – 14 days unless preceded by complications. On the other hand, balanoposthitis manifest clinically with small pustules, erosion and ulcers on glans penis and preputial mucosal (OIE, 2010; Bosco Cowley *et al.,* 2011; Gould *et al.,* 2013).

The ocular form of the disease presents with reddened and edematous conjunctiva, profuse serous ocular discharge and diffuse edema. Calves of 6 months of age and below can develop encephalitis accompanied with incoordination, excitement, high mortality rates, hyper-salivation, bellowing, convulsion and blindness (Constable *et al*., 2017).

New born calves less than 10 days of age often come down with systemic type of the disease which is severe and invariably lethal. Clinical findings are varied and include; fever, hyper-salivation, sudden anorexia, rhinitis accompanied by either unilateral or bilateral conjunctivitis, hyperemia of buccal mucous membranes, erosion of the soft palate covered with tenacious mucus, acute pharyngitis covered with tenacious mucopurulent discharge, edematous larynx, bronchopneumonia, diarrhea and dehydration (Constable *et al.,* 2017).

2.6: Diagnosis of Infectious Bovine Rhinotracheitis

Experimental infection has revealed that the median period to shedding of the virus may be 2 days, median period towards peak viral shedding may be 4 days while the median period up to

shedding of the virus stops is 14 days (Grissett *et al.,* 2015). Viral isolation from the nasal swabs by employing tissue culture via combination of fourfold rise of antibodies either at acute or convalescent phase of the sera are the desirable state for the positive diagnosis. Cotton or polyester swabs samples are recommended for collecting nasal swabs compared to calcium alginate swab sample because the latter is virucidal within two hours (Constable *et al.,* 2017). Viral detection using nasal swabs may involve the use of direct and indirect immunofluorescence techniques, Enzyme Linked Immunosorbent Assay (ELISA), immune-peroxidase, or use of electronic microscopic examination which show the herpesvirus-like viral particles.

However, sensitivity of the direct immunofluorescence techniques therefore, is comparable to cell culture technique. In addition to, monoclonal antibodies detected by immunofluorescence assay technique may discriminate the 4 ruminant alpha-herpesvirus related to BoHV – 1. The ELISA technique used in detection of BoHV – 1 has a higher sensitivity compared to viral neutralization test (Saravanajayam *et al.,* 2015). Thus, combination of viral isolation and indirect immunofluorescence test from both nasal and ocular swab samples from several cattle samples will increase the chances of recovery rates (Roshtkhari *et al.,* 2012 and Constable *et al.,* 2017).

An alternative practical means of quick detection of the BoHV – 1 is the use of Polymerase Chain Reaction (PCR) assay since it is as sensitive as viral isolation (Mahajan *et al.,* 2013). PCR assay are considered equivalent to the standard dot blot hybridization and/or virus isolation thus, may be used also in viral detection in semen samples. The southern blot hybridization compared to PCR assay has high sensitivity and may detect virus in semen samples before the virus development of any detectable antibodies (Constable *et al.,* 2017). The PCR assay may be able to detect positive semen samples 5 times even in the virus isolated from egg yolk-extended semen.

Thus, PCR assay are considered the diagnostic test of choice especial in routine diagnosis of the BHV – 1 in aborted fetuses (Mahajan *et al*., 2013).

Employing the restricted endonuclease enzyme analysis of the viral DNA may be possible in differentiation of the field isolated virus from vaccine strains, thus, this is useful in investigation of vaccines-induced epidemics of the disease (Constable *et al*., 2017).

The bulk–tank milk testing for BoHV – 1 antibody is important in control program, eradication programs and monitoring programs since it provide a rapid inexpensive screening of the cattle. However, correlation between within herd prevalence and bulk milk testing seropositive of the cattle may be higher at about 0.86. When BoHV – 1 is detected in bulk milk test technique, thus there could be a probability that more than one cattle in a herd may be infected and the infections has spread (Constable *et al.,* 2017).

The following samples are collected for confirmatory diagnosis of BoHV – 1; histology samples include; formaldehyde fixed samples of neonate or abortion: liver, rumen, kidney, esophagus, trachea, lung, pharynx and adrenal glands, respiratory form: pharynx, trachea, nasal turbinate and lungs, encephalic form; half of the mid-sagittally sectioned brain for LM and IHC. Virology samples includes; neonate/abortion: kidney, rumen, lungs and liver, respiratory type; nasal swab, trachea and lung, encephalic type; half of the mid-sagittally brain section for fluorescent antibodies test, ISO and PCR (OIE, 2010; Constable *et al.*, 2017 and Barber *et al*., 2017).

2.7: Differential diagnosis

IBR is defined by anorexia, acute rhinotracheitis, excessive nasal discharges, coughing, bilateral conjunctivitis, nasal lesions, fever and gradual recovery within few days. However, secondary form of pneumonia and bacterial tracheitis may occur. Thus, IBR should be differentiated from bovine viral diarrhea, malignant head catarrh, pneumonic pasteurellosis, calf diphtheria, viral

pneumonia and allergic rhinitis (Griffin *et al.,* 2010 and Constable *et al*., 2017). It`s important to differentiate systemic disease of IBR in newborn calves among the following diseases; toxemias, septicemia and acute pneumonia (Constable *et al.,* 2017).

2.8: Treatment of Infectious Bovine Rhinotracheitis

Infected animals are isolated, identified and closely monitored especially for evidences of secondary pneumonia and bacterial disease which may be preceded by anorexia and toxemia. Treatment of tracheitis may be difficult, however broad – spectrum antibacterial are indicated for secondary pneumonia and bacterial tracheitis. The antibiotics should be administered daily and several days (Constable *et al.,* 2017).

2.9: Prevention and Control of Infectious Bovine Rhinotracheitis

IBR being viral disease, can set in unpredictably at any period, even in closed herds, then sudden outbreaks of the disease are experienced. The recent strategies of controlling IBR includes; biosecurity measures, natural exposure or vaccination may be effective in eradication of the virus in the herd of cattle population in a region or a country (Constable *et al.,* 2017).

2.9.1: Natural Exposure and Vaccination

The cattle which recovered from natural infection of BoHV – 1 are immune against further infections. However, naturally infected cattle are risky since all cattle population will not become infected and obtain immunity to further clinical disease (Constable *et al.,* 2017). Storm of abortion occur in unvaccinated herd, thus, vaccination is recommended in region with high prevalence and unfeasible eradication due to extensive nature of the cattle population and their movement from one point to another across various region (Graham, 2013). The vaccination rationale is normally determined by the following factors; the virus being ubiquitous and occurrence of the diseases is unpredictable, the economic impact due to respiratory disease,

neonatal disease and abortion may be higher, maternal antibodies in newborn calves start to wane at between 4 – 6 months of age and vaccination may prevent abortion and protect against respiratory form of the disease if given at least 10 days before an animal is infected with the virus naturally (Constable *et al.,* 2017). Thus, vaccination of cattle as a mode of control and eradication program as employed by the European countries was based on marker vaccine deleted in gE gene. The inactivated or live attenuated marker vaccine employed in serological diagnostic technique of detecting gE-specific antibodies, allowed discrimination of naturally infected animals from vaccinated cattle (Van Oirschot *et al*., 1997; Lehmann *et al*., 2002). This capacity employed in Differentiation of the Infected from Vaccinated Animals (DIVA) is key in world trade restriction (Mars *et al.,* 2001; Mulyken *et al*., 2007).

DIVA is demonstrated effectively with punctual vaccination of cattle at interval of six months' apart, however, this technique is associated with a few weaknesses. Thus, the sensitivity of the tool depends on capacity of the diagnostic test to detect the BoHV – 1 gE specific antibodies. However, the diagnostic test sensitivity is readily available at around 70% using gE specific ELISA technique (Perrin *et al*., 1996; Kramps *et al*., 2004). Another disadvantage is that the response of the immune level raised against BoHV – 1 gE antibodies is weak thus, the window period for the test ability to detect gE specific antibodies may be delayed up to 6 weeks (Beer *et al*., 2003; Mulyken *et al*., 2007).

2.9.2: Biosecurity

This is an important measure in a successful livestock production since it reduces risk and effects of introduction of infectious agents. The factors of biosecurity include; placement and management programs, immunization, decontamination, farm layout and pest control (Constable *et al*., 2017). Herpesviruses are sensitive to a number of disinfectants; 10% lugol`s iodine, 1%

phenolic derivatives and 1% quaternary ammonium bases (Constable et al., 2017). Introduction of new infectious agents into herd may be minimized or prevented by purchasing new cattle from farms known to be free of diseases in question. The adoption of this principles may require the awareness of any possibility of unknown infectious agents including testing the cattle for infectious agents before entry into the herd. It also involves quarantine of newly introduced cattle for several weeks from arrival time to avoid mixing with clean herd (Constable *et al.,* 2017).

Veterinarian play important role in development of specific disease control and biosecurity protocols as per the farm or regional requirements (Constable *et al*., 2017). They also facilitate development of methods of purchase of replacement stock and handling livestock through designing known protocols concentrating on specific and general aspects likes designing and putting up isolation rates (Constable *et al*., 2017).

2.9.3: Closed herd

Closed type of farming system facilitates prevention of emerging or re-emergence of infectious agents into dairy cattle farms. Closed dairy farming enterprise may minimize introduction of BoHV – 1, thus, this may form the baseline mechanism of eradication of infectious agents in dairy herd (Constable *et al*., 2017).

Movement of cattle from one point to another; cattle shows and sales, veterinary clinics, community grazing pasture, auction markets, club events, bull leasing and cattle commingling from adjacent herds, provide opportunities for transmission of important infectious agents (Constable *et al.,* 2017).

CHAPTER THREE: METHODS AND MATERIALS

3.1: Description of the study area

A cross-sectional study was conducted in 149 smallholder dairy farms, Naari Area, Meru County, Kenya (Figure 3.1). It lies at latitudes: 0°6'0" N and 37°34'60" E. Meru County is located in the former Eastern Province of Kenya, 37^{o} 18’37”to 37^{o} 28’33” E and 00^{o} 07’23” to 00^{o} 26’19” S, approximately 270 km North of Nairobi, the capital city of Kenya. Meru shares border with Isiolo County to the North, Laikipia County to the West, Tharaka Nithi and Nyeri to the South West. The climate in Meru is warm and temperate. The average annual temperature in Meru highlands ranges from14^{o}C to 17^{o}C in the highlands while in the lowlands it ranges from 22^{o}C to 27^{o}C. Precipitation in high altitude areas averages 2200 mm while low altitude areas averages 500 mm. The Naari sub-location is situated in highly agricultural potential region within an altitude of approximately 2000 m above the sea level. The main agricultural practice includes; lumbering, horticulture, crop production and dairy production (Makau *et al*., 2018).

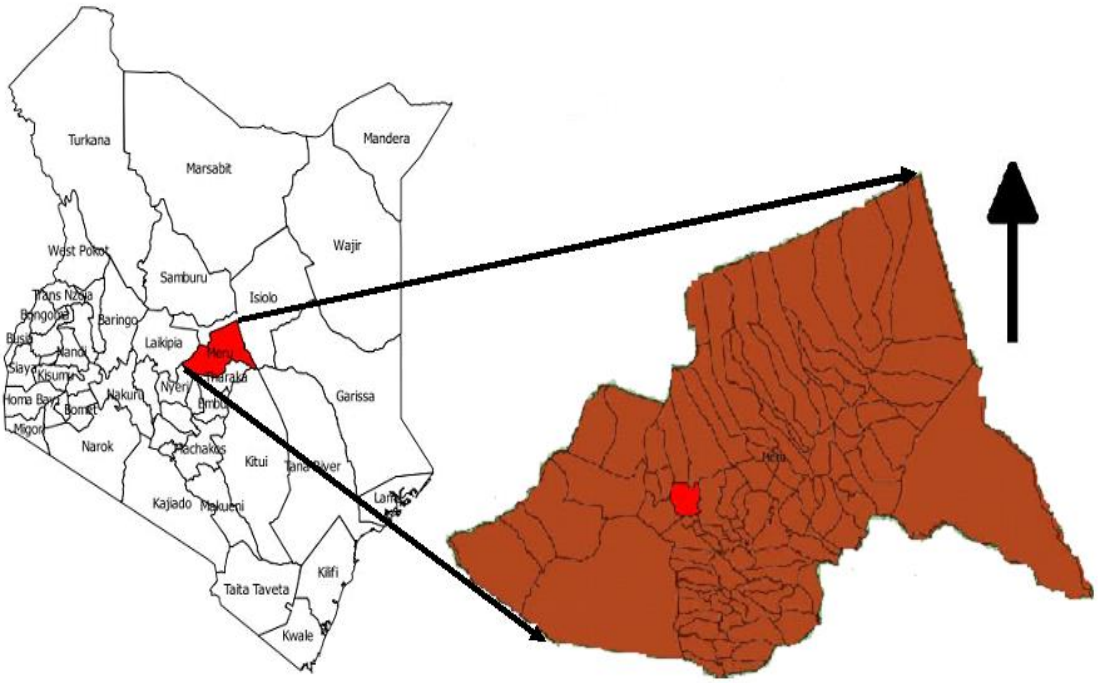

Figure 3-1: A map showing Naari Sub-location (middle of county) in Meru County of Kenya.

3.2: Selection of study area and farms

The study area was purposively selected since this research formed part of a larger study involving smallholder dairy farmers (Figure 3.1). A non-governmental organization, Farmers Helping Farmers, the University of Prince Edward Island and the University of Nairobi had an existing developmental partnership with the Naari Dairy Cooperative Society, which provided a strong foundation for this study and the entry point to the community.

The sampling frame for the study constituted of 568 farmers who were active members of the Naari Dairy Cooperative (NDC) and shipping milk to the cooperative. The dairy cattle sampled in the study was calculated based on an estimated prevalence of IBR of 50%, a precision of 5% and confidence level of 95%, giving a sample size of 385 (Dohoo *et al.*, 2009).

$$n = \frac{(1.96)^2 \times S^2}{L^2}$$

$$n = \frac{(1.96)^2 \times 0.5^2}{0.05^2}$$

$$n = 385$$

Since the average number of cattle per farm had been established as 2 to 3, then 149 farms were randomly selected from the 568 smallholder dairy farms from the registry of active members between January and June 2018 using software-based random number generation. The 149 farms randomly selected provided about 400 animals.

3.3: Data and sample collection

The selected farms were visited between March-April 2018 and June and July 2018, and a questionnaire was administered to capture farm and animal level factors (Appendix 1). The data

collected included collection of information about milking cows at the farm, systematic scrutiny of written records to obtain the age of the cattle, calving rates, history of respiratory and reproductive diseases, peri-parturient condition, and mastitis cases. Other information collected included; feed and mineral supplementation, vaccination status, cattle owner attendances to any dairy husbandry training, herd size, awareness and monitoring of heat signs, cow age and source of animals.

In addition, 5 ml of whole blood in plain tubes for sera preparation was collected via the tail vein of each dairy cattle, using 5 ml syringe and 21 gauge, 1.5mm needle. The blood tubes were placed under shade to allow clot separation and thereafter the serum was transferred to Eppendorf® vials which were labelled carefully, frozen and transported in ice to Heamatology and Biochemistry Laboratory, Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi and stored at -20^{0}C until testing.

3.4: Laboratory analysis of samples using ELISA kits

The frozen sera and ELISA kit from IDEXX Switzerland AG (Liebefeld-Bern, Switzerland) which was stored at 4^{0}C, BHV – 1 Antigen Coated Plate and all the reagents, were thawed carefully to room temperature (18 – 26^{0}C). The testing procedure was done following the protocol described by manufacturer. The IDEXX IBR gB X_3 was an indirect enzyme immunoassay which has been developed to detect presence of antibodies against IBR in individual bovine plasma, milk and serum samples. Antibody responses induced by vaccines which contains the glycoprotein B (gB) of BoHV - 1 are detected as well. A microtration format has been configured through immobilized IBR virus antigens on the plate. This kit is reported with a specificity of 95% and sensitivity of 100%, and is able to detect the majority of BoHV - 1 antibodies.

Bovine herpesvirus – 1 Antigen Coated Plates were obtained and sample position was recorded. Fifty microlitres of reconstituted wash solution was added to each well. Then 50 µl of Negative and Positive Control samples was dispensed into respective labelled duplicate wells, and 50 µl of each sample was dispensed into each respective sample well. The content of the micro-wells was mixed via gently tapping the plate. The wells were hermetically covered with microplate cover and incubated at 37^0C for 2 hours in a humid chamber. The solution was removed and each well was washed with approximately 300 µl of wash solution for 5 times. The plates were protected from drying between plate washings and prior to the addition of the next reagent. The final washing of the plates was tapped on the absorbent material to remove any residual wash fluid. Then gB specific monoclonal antibodies Horseradish Peroxidases conjugate was dispensed into each micro-well, and incubated at 18 – 26^0C for 1 hour. The plate was rinsed as described above and 100 µl of TMB Substrate N. 12 was dispensed into each micro-well.

The plate was then incubated at 18 – 26^0C for 10 minutes, and 100 µl of Stop Solution N. 3 was dispensed on each micro-well. The test samples on antigen-coated well were incubated, and antibodies specific to IBR virus formed complex with immobilized viral antigen. Unbound antigen materials in the well were washed away, a gB-specific monoclonal antibodies Horseradish Peroxidase conjugate was added. Thereafter, the unbound conjugate was washed away and a substrate solution was added. The enzyme acted on the substrate converting it into a product that reacted with chromogen to produce a blue color. The stop solution was thereafter added resulting to the formation of yellow color.

The results were read from microplate photometer, Mindray Microplate Reader (MR-96A), Shenzhen Mindray Bio-Medical Electronics Company Limited, where optical density (OD) was measured either at a single wavelength of 450 nm [A (450)] or dual wavelength of 450 nm and

650 nm [A (450/650)]. The blocking percentage was calculated by using the absorbance [A (450)] or [A (450/650)] obtained with the test sample and the negative control containing no specific antibodies.

$$\text{Blocking Percentage} = 100 \times \frac{\text{NCX A (450)} - \text{Sample A (450)}}{\text{NCX A (450)}}$$

Interpretation of the results was determined via sample blocking percentage in accordance's with manufacturers test instructions where; blocking % < 45 negatives, 45 ≤ blocking % < 55 suspect and blocking % ≥ 55 positives. The suspects were considered as positive in order to obtain a dichotomous outcome.

3.5: Data Entry and Statistical Analysis

Data collected through the questionnaires and the laboratory results were first entered into MS Excel (Microsoft Inc., Sacramento, California, USA) and then imported to Stata 15 (StataCorp LLC, College station, Texas, USA) for analyses. Initially, the data were checked for accuracy, coded and analyzed using descriptive statistics. Proportions were determined for categorical variables, breed, age category, parity category and history of abortion, and presented as a percentage of the overall number, along with a 95% confidence interval where applicable.

Mixed-effect logistic regression analysis was performed accounting for clustering of cows among herds, to determine associations between the categorical variables, breed, age category, parity category, history of an abortion, BVDV antibodies positive, showed BVDV signs, type of feeding system, rearing goats on the farm, rearing sheep on the farm, use of natural mating, fence-line contact with other cattle, grazing on community pasture, borrowed cows from other farms, lent cows to other farms, cattle bought into the farm, dichotomized age of the female principal farmers, dichotomized age of the male principal farmers, and continuous variables, age of the animal, dry cows, herd size and milking cows, and the dichotomized seropositivity

outcome (presence or absence of IBR antibody). In the first step, univariable multi-level mixed models for all the predictor variables were fitted into separate logistic regression models, employing the functional logit. In the second step, a multivariable mixed logistic regression analysis was fitted for all the univariable associations with $p \leq 0.30$ in the first step. Correlations between predictors variables were identified using paired-wise correlation, and where two or more variables were highly correlated (correlation coefficient >0.5), statistical significance and biological plausibility were used to identify which variable would be offered to the modeling process. The final models were built using backward stepwise elimination, leaving those variables which had a p-value ≤ 0.05.

CHAPTER FOUR: RESULTS

4.1: Farm and animal demographics

4.1.1: Farm Demographics

The farm demographics are summarized in Appendix 1. A total of 403 cattle and 149 farms were involved in the study. The principal farmer was mostly made of men (56.4%) while women were fewer (28.2%), and 15.4% of farms had male and female considered as jointly principal farmer (Figure 4-1). Most of the principal farmers were married (83.9%), but a few of them were young people who were single and establishing themselves as dairy farmers (5.3%). Among the principal farmers, a majority of the men were below 45 years (51.0%) while women also had the majority of them above the age of 45 years (50.3%) (Figure 4-2). The large majority of the female principal farmers had completed high school and tertiary school level of education (84.9%), while the proportion of male principal farmers having completed high school and tertiary school was slightly low at 79.8%.

The mean household size recorded in this study was 3.71 ± 1.54 with a minimum of 1 person and a maximum of 11. The mean total land holdings ownership among the respondents was 2.11 ± 2.04 acres. In addition, some of the farmers (0.51 ± 0.84 acres) also had an access to other pieces of land through leasing, borrowing and government owned lands lease to them.

The distribution of the training on dairy production among the principal farmers included those with the dairy production training 81.2% (121/149) and those with no training on dairy production 18.8% (28/149) with the majority of the principal farmer having no training on dairy farming (Figure 4.3).

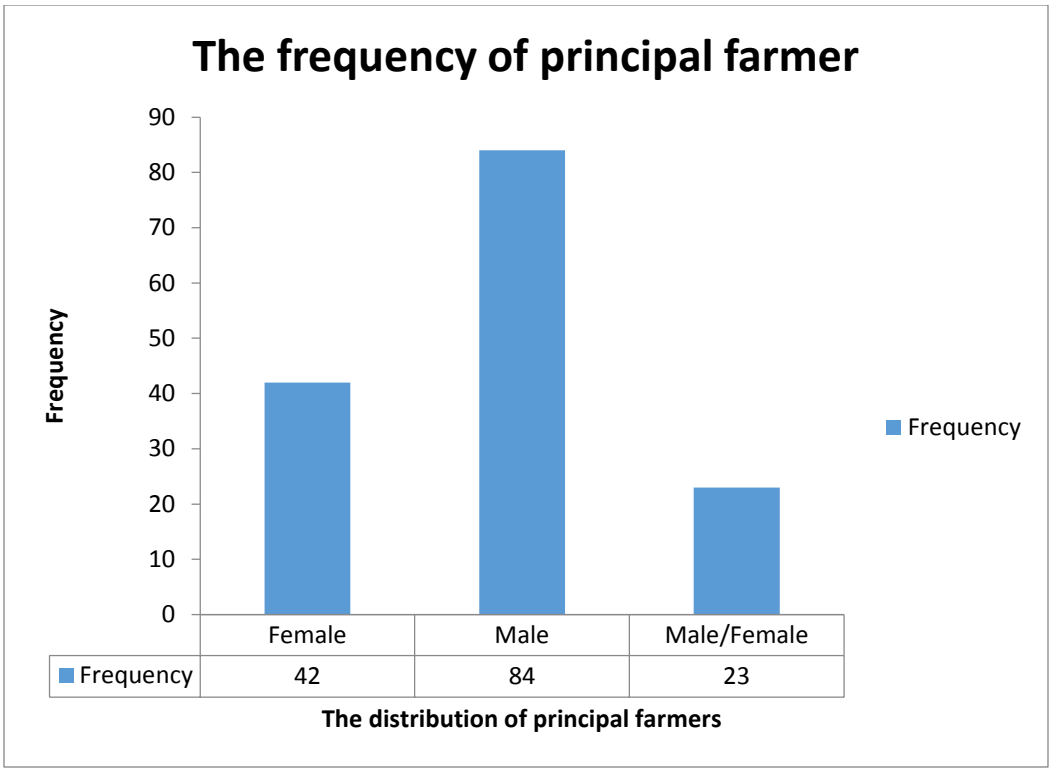

Figure 4-1 The distribution of the principal dairy farmers Naari sub-location, Meru County, Kenya in 2018

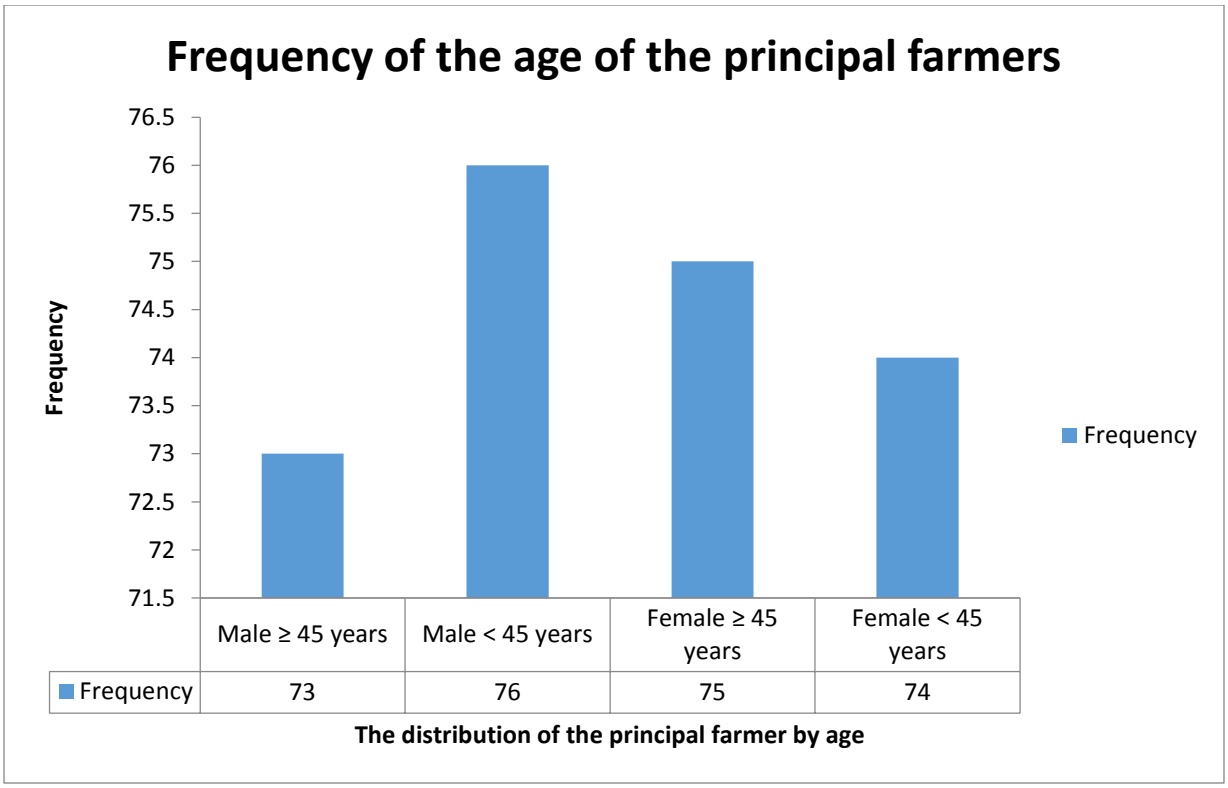

Figure 4-2 The distribution of principal dairy farmers by age, Naari area, Meru County, Kenya in 2018

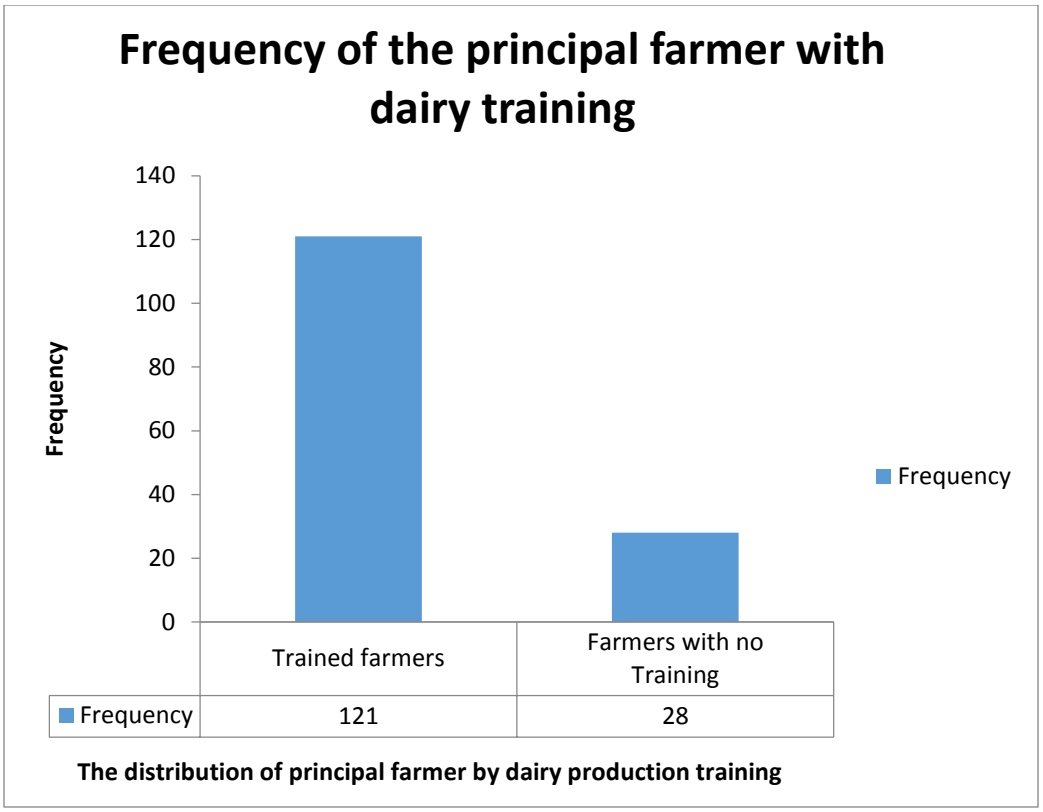

Figure 4-3 The distribution of the dairy farmers by training on dairy production in Naari area, Meru County, Kenya in 2018

4.1.2: Cow variables

Among the 149 smallholder dairy farms in the Naari Area, Meru County, 403 dairy cows were recruited for the study. The animal-level variables are summarized in Tables 4-1 and 4-2. The distribution of the study animals among the breeds kept in the region included; Friesian Holstein 47.2% (190/403), Guernsey 27.8% (112/403), Ayrshire 17.4% (70/403) and Zebu 7.7% (31/403). Friesian Holstein formed the majority of the dairy cattle reared in the region.

The mean herd size was 5 with a range of 1-16 animals. Majority of the dairy cows in the farms had a mean age of 5 years with a range of between 1 and 17 years. The average number of milking animals per herd was 2 with a range of between 0 and 7 cows. The lactating cows comprised the majority 79.7% (321/403) in the farms compared to replacement heifers/female calves 20.3% (82/403) (Table 4-2). The parity of the cow was classified between 0 to 8 and included, nulliparous heifer/female calves (parity 0) at 19.9% (80/403), primi-parous (parity 1) at 21.3% (86/403) and the multiparous (parity 2 and 8) at 58.8% (237/403). The farms had an average number of dry cows of 1 with a range of between 0-3 animals per herd. In addition, the farms had an average milking cows of 2 with a range of between 0-7. It was also reported that about 20.1% (81/403) of the animals were reported to have experienced an abortion.

Table 4-1: Description of categorical variables for animal level factors for 403 dairy cattle on 149 smallholder dairy farms Naari area, Meru County, Kenya in 2018

Variable	Category	Category Frequency	Percent by Category
Breed	Ayrshire	70	17.4
	Friesian	190	47.2
	Guernsey	112	27.8
	Zebu	31	07.7
Age category	Cow	321	79.7
	Heifer	82	20.3
Parity category	Parity 0	80	19.9
	Parity1	86	21.3
	Parity >1	237	58.8
History of an abortion	No history of abortion	322	79.9
	History of abortion	81	20.1

Table 4-2: Description of continuous variables for animal and farm level factors (n = 403) on 149 smallholder dairy farms in Naari area, Meru County, Kenya in 2018

Variable	Range	Mean	S d	Variance	OR	95% CI_{OR}
Age of the animal	5 - 17	5.521	3.240	10.496	1.112	1.012 – 1.222
Dry cows	0 - 3	0.149	0.516	0.266	1.446	0.685 – 3.049
Herd size	1 - 16	5.754	2.989	8.937	1.446	0.830 – 1.463
Milking cows	0 - 7	1.531	1.493	2.230	1.072	0.931 – 1.235

S – Standard deviation, IBR – Infectious Bovine Rhinotracheitis, OR – Odd Ratio & 95% CI_{OR} – 95% Confidents Intervals of OR

4.2: Management practices at the farms

A summary of managerial practices found among the 149 smallholder dairy farms recruited in the study are summarized in Table 4.3. The distribution of various types of feeding systems among the smallholder dairy farms included; zero grazing system 42.2% (63/149), semi-zero grazing system 35.7% (53/149) and grazing 22.2% (33/149). The zero-grazing and semi-zero grazing systems formed the majority of smallholder dairy farms. Majority of the farmers interviewed practiced zero-grazing with a few of the farmers grazing their cows in community pastures 22.2% (33/149). Movement of the animals across the region were captured as follows: cows that were borrowed into the farm 6.7% (10/149), cows that were lend out of the farm 8.1% (12/149) and cows that were introduced into the farm 8.7% (13/149). Artificial insemination was readily available in the region at 57.7% (86/149) and was offered by government veterinary officers, veterinary technicians and private practitioners. Majority of the smallholder dairy farms however used artificial insemination at 57.7% (86/149) while a few employed the natural mating method at 42.3% (63/149). This may be associated with the high prices of semen, perceived low conception rates and repeat breeding service. Among the farmers interviewed, other than dairy production, they also practiced other production systems which included sheep 38.3% (57/149) and goat 15.4% (23/149).

Table 4-3: Description of categorical variables for farm level factors, 403 dairy cattle in 149 smallholder dairy farms in Naari area, Meru County, Kenya in 2018

Variable	Category	Category Frequency	Percent by Category
Type of feeding system	Zero– grazing	63	42.2
	Semi-zero-grazing	53	35.6
	Grazing	33	22.2
Rearing goats on the farm	Yes	23	15.4
	No	126	84.6
Rearing sheep on the farm	Yes	57	38.3
	No	92	61.7
Use of natural mating	Yes	63	42.3
	No	86	57.7
Fence-line contact with other cattle	Yes	120	80.5
	No	29	19.5
Grazing on community pasture	Yes	98	65.8
	No	51	34.2
Borrowed from other farms	Yes	10	06.7
	No	139	93.3
Lent to other farms	Yes	12	08.1
	No	137	91.9
Cows bought into the farm	Yes	13	08.7
	No	136	91.3
BVDV ab +ve[a]	Positive	157	53.4
	Negative	137	46.6
Showed BVD signs[b]	Positive	254	63.2
	Negative	148	36.8

a Bovine Viral Diarrhea Disease Virus Antibodies positive cows

b Bovine Viral Diarrhea Disease

4.3: Sero-prevalence of Infectious Bovine Rhinotracheitis

The overall sero-prevalence of BoHV – 1 was 17.37% (70/403; 95% CI: 13.80% to 21.43%). The sero-prevalence of the antibodies to the IBR virus in association to cow variables are summarized in Table 4-4. For the categorical variables, the sero-prevalence for BHV-1 infection inbreeds of the dairy cows were; Ayrshire 20.0% (14/70), Guernsey 17.9% (20/112), Friesian 16.3% (31/190) and Zebu 16.1% (5/31).

The sero-prevalence for BoHV-1 by parity of the dairy cows were primi-paraous 19.8% (47/237), heifer 15.0% (12/80) and multi-parous 12.8% (11/86). Majority of categories detected with IBR were cows 18.7% (60/321) while a few were heifers 12.2% (10/82). Majority of the sero-positive animals were mainly reared under the zero-grazing and semi zero-grazing type of production systems at 18.1% (21/116) and the sero-prevalence for open grazing was 12.1% (4/33). The summary of the sero-prevalence to IBR to the other categorical variables are shown in Table 4.4. The highest recorded sero-prevalence to IBR antibodies were observed in the following variables: farms rearing goats 39.1% (9/23), farms rearing sheep 19.3% (11/57), cattle grazing on the community pastures 19.4% (19/98), new animals introduced in the farms 23.1% (3/13) and cows with negative antibodies against Bovine Viral Diarrhea Virus 23.4% (32/137).

Table 4-4: Description of categorical variables for animal and farm level factors for 403 dairy cattle on 149 smallholder dairy farms, Naari area, Meru County, Kenya in 2018

Variable	Category	Category Frequency (Percent)	IBR Percent + by Category
Animal level factors			
Breed	Ayrshire	70	14(20.0)
	Friesian	190	31(16.3)
	Guernsey	112	20(17.9)
	Zebu	31	05(16.1)
Age category	Cow	321	60(18.7)
	Heifer	82	10(12.2)
Parity category	Parity 0	80	12(15.0)
	Parity1	86	11(12.8)
	Parity >1	237	47(19.8)
History of an abortion	No history of abortion	322	55(17.1)
	History of abortion	81	15(18.5)
Farm level factors			
Type of feeding system	Zero– grazing	63	11(17.5)
	Semi-zero-grazing	53	10(18.9)
	Grazing	33	4(12.1)
Rearing goats on the farm	Yes	23	09(39.1)
	No	126	17(13.5)
Rearing sheep on the farm	Yes	57	11(19.3)
	No	92	15(16.3)
Use of natural mating	Yes	63	12(19.0)
	No	86	14(16.3)
Fence-line contact with other cattle	Yes	120	23(19.2)
	No	29	03(10.3)
Grazing on community pasture	Yes	98	19(19.4)
	No	51	07(13.7)
Borrowed from other farms	Yes	10	05(50.0)
	No	139	21(15.1)
Lent to other farms	Yes	12	04(33.3)
	No	137	22(16.1)
Cows bought into the farm	Yes	13	03(23.1)
	No	136	23(16.9)
BVDV ab +ve[a]	Positive	157	20(12.7)
	Negative	137	32(23.4)
Showed BVD signs[b]	Positive	254	41(16.1)
	Negative	148	29(19.6)

4.4: Factors associated with Infectious Bovine Rhinotracheitis sero-prevalence in univariable analysis

The study found out that the IBR sero-prevalence was positively associated with the following factors; age of the dairy cattle (OR = 1.112, 95% CI = 1.012 – 1.222, P = 0.027), cows that were borrowed into farms (OR = 4.893, 95% CI, = 1.328 – 16.03,1 P = 0.017), age of the female principal farmers (OR = 0.230, 95% CI = 0.108 – 0.498, P = 0.000), age of the male principal farmers (OR = 0.394 95% CI = 0.181 – 0.898, P = 0.019), rearing goats in the farms (OR, 1.438, 95% CI, 1.109 – 1.863, P = 0.006), cows that were given out of the farms (OR, = 2.486 95% CI, = 0.697 – 8.859 p = 0.014) Showed BVD signs OR = 1.243 95% CI, = 0.635 – 2.430, p = 0.526) and cows that had antibodies against Bovine Viral Diarrhea Virus (OR, = 2.262 95% CI, = 1.129 – 4.533, P = 0.021) (Table 4–5).

Table 4-5: Univariable logistic mixed models of the outcome variable IBR type 1 antibody seropositivity, while accounting for clustering of 403 dairy cattle among 149 smallholder dairy farms, Naari area, Meru County, Kenya in 2018, for variables of interest with P – Value ≤ 0.3

Variable and category	Categories	OR	95% CI_{OR}	P–Value
Parity category				0.206!
	Parity 0			
	Parity 1	1.059	0.361 – 3.103	
	Parity 2-8	1.414	0.803 – 4.428	
Age category	heifer			
	Cows	2.181	0.913 – 5.211	0.079
Fence-line contact with other cattle	No			
	Yes	2.850	0.924 – 8.790	0.068
Grazing on community pasture	No			
	Yes	2.150	0.918 – 5.033	0.078
Borrowed from the farms	No			
	Yes	4.893	1.328–16.031	0.017
Lent to other farms	No			
	Yes	2.486	0.697 – 8.859	0.014
Rearing Goats on the farm	No			
	Yes	1.438	1.109 – 1.863	0.006
Rearing sheep on the farm	No			
	Yes	1.076	0.969 – 1.192	0.173
Use of natural mating	No			
	Yes	1.706	0.769 – 3.786	0.150
BVDV ab +ve[a]	No			
	Yes	2.262	1.129 – 4.533	0.021
Showed BVD signs[b]	No			
	Yes	1.243	0.635 – 2.430	0.526
Age of the female principal farmers		0.230	0.108 – 0.498	0.001
Age of the male principal farmers		0.394	0.181 – 0.898	0.019
Age of the dairy cattle		1.112	1.012 – 1.222	0.027
Dry cows		1.446	0.685 – 3.049	0.249

! Overall P-values for variables with >2 categories, [a] Bovine Viral Diarrhea Disease Virus antibody positive cows, [b] Bovine Viral Diarrhea Disease, 95% CI_{OR}: 95% Confidence Interval of OR, OR: Odds Ratio

4.5: Factors associated with Infectious Bovine Rhinotracheitis sero-prevalence in multivariable analysis

The final multivariate analysis of individual level factors that were associated with sero-positivity to BoHV – 1 antibodies included; rearing goats on the farm (OR = 4.636, 95% CI = 2.053 – 10.467, P = 0.001), age of the dairy cattle, (OR = 1.113, 95%CI = 1.017 – 1.217, P = 0.020) and age of the female principal farmers (OR = 0.174, 95% CI = 0.082 – 0370, P = 0.001). The likelihood of a older dairy cattle to have BoHV – 1 antibody is 1.113 times compared to young dairy cattle. Farms that are rearing goats are 4.636 times more likely to have BoHV – 1 antibodies compared to farms not rearing goats. In addition, as the age of the women principal farmer's increases, the likelihood of a cow to have BoHV – 1 antibody were lower by 0.174 times compared with the younger women principal farmers (Table 4-6).

Table 4-6: Final multivariable logistic mixed model for variables associated with IBR antibody seropositivity for 403 dairy cattle on 149 smallholder dairy farms, Naari area, Meru County, Kenya in 2018

Variable	OR	95% CI_{OR}	P – Value
Rearing goats on the farm	4.636	2.053 – 10.467	0.001
Age of the dairy cattle	1.113	1.017 – 1.217	0.020
Age of the female principal farmers	0.174	0.082 - 0370	0.001

OR: Odd Ratio, 95% CI_{OR}: 95% Confidence Interval of OR

CHAPTER FIVE: DISCUSSION

The findings of this study confirmed the existences of IBR infection through sero-prevalence of BoHV-1 antibodies among the smallholder dairy cattle reared in Meru County. Past studies in Kenya have reported varying sero-prevalence's to IBR for example a 20.9% in the western part of Kenya and 28.0% in the former Malindi District of the Coastal Region were recorded (Kenyanjui *et al.*, 2007; Callaby *et al*., 2016). However, the sero-prevalence in this study was lower than those reported and this could have been due to the different livestock production systems studied and the way the studies were designed.

The observed sero-prevalence to Infectious Bovine Rhinotracheitis Disease of 17.4% was within the range of 16% - 54% that was observed by McDermott *et al*. (1997) estimated in former Districts in 1991 – 1992 in Kenya. However, the sero-prevalence observed in parts of Meru County was lower than that which was observed in traditionally managed herds in Zambia, which ranged from 42% -76% (Ghirotti *et al.*, 1991) and in Egypt ranged from 63%–86% (Mahmoud *et al.*, 2009).

It has been observed that higher prevalence's recorded in larger herd sizes and intensively farmed cattle could be associated with a high level of contact between individual animals within a herd (Snowder *et al*., 2006). For extensively managed farms with median herd size of 5 animals, the risk of contact between a susceptible individual with infected or persistently infected animal is lower (Callaby *et al.*, 2016). The studies reported above involved animals of various ages for example 51 weeks old (Callaby *et al.*, 2016), age 3 months to adults (Ghirotti *et al.*, 1991), zebu adults (Kenyanjui *et al*., 2007), and this study involved adult dairy cows and heifers. The age differences among the animals studied may have explained some of the variations in the sero-prevalence. In addition, Ghirotti *et al*. (1991) and Kenyanjui *et al.* (2007) employed virus

neutralization tests (VNT) compared to the ELISA tests used in this study and the differences in test specificity and sensitivity may also have contributed to observed differences in prevalence (Graham *et al*., 1998). In the studies done by Saravanajayam *et al.* (2015) found out that ELISA has a higher specificity and sensitivity compared to VNT, thus ELISA is the rapid, reliable and technically superior test for detection of BoHV – 1 antibody. Callaby *et al*. (2016) and IDEAL study employed indirect ELISA test thus may have contributed to observe close proximity prevalence's. This observed close proximity prevalence's may be attributed by use of ELISA test, therefore similar in test sensitivity and specificity.

In other studies, in Africa, the IBR estimated prevalence among the cattle population ranged from 14% to 86%, (Straub, 1990; Ghirotti *et al.,* 1991; Mahmoud *et al.,* 2009), whereas this study revealed prevalence of 17.37% which is within the range. The observed differences in antibodies prevalence in different regions, countries and counties could be explained by factors likes production systems, differences in herd sizes; small, median or large herds, type of breeding methods, differences in disease-control measures and age of the cattle, these are important since they indicate diseases permanence in an environment (Orjuela *et al.,* 1991; Mainar-Jaime *et al.*, 2001). Moreover, the observed differences in the IDEAL study and across the world could be attributed by the breeding managements and geographical positions of the considered in various regions and countries (Ackermann and Engels, 2006).

Cross-reaction may be observed between virus and its related viruses, for instances, BVDV has potential for cross-reaction with pestiviruses like Classical Swine Fever and Border Diseases Virus of sheep. Infectious Bovine Rhinotracheitis has the potential for cross-reaction with four herpesviruses from other animals including goats and buffalo while the Para-Influenza Virus type 3 (PIV3) can cross react with human strains of virus (Handel *et al.,* 2011; Callaby *et al.,*

2016). Thus, since most of such viruses have not been exhaustively tested in cattle in Meru County, the majority of sero-positive cases were likely to be due to exposure to IBR virus.

The fact that mixed rearing of sheep, goats and cattle is a common livestock production practice in the region, there could be possibility of cross-reaction therefore the need for a study to be carried out in the region in order to obtain more information on viral cross-infection. The present study revealed that rearing of goats in the farm was highly associated with BoHV-1 sero-prevalence. This may be attributed by the potential ability of the viral cross-infection with related Alpha-herpesvirus (Biswas *et al.*, 2013). In previous studies done by Whetstone and Everman (1988), it was demonstrated that BoHV-1 has potential to infect and cause disease in goats and sheep.

Based on past epidemiological studies, this study showed that IBR and BVD are closely associated since one disease predispose to the other disease (Callaby *et al.,* 2016). This finding agrees with those reported earlier (Martin and Bohac, 1986; Durham and Hassard, 1990; Fulton *et al*., 2000; Callaby *et al*., 2016). However, BoHV-1 infection is determined by stressor factors such as use of steroids, transportation, parturition and high ambient temperature and these may induce carrier animals to start shedding the virus (Six *et al.,* 2001). Thus the clinical manifestation of the disease depends on primary factors that lower the immunity system of the animals (Seyfi Abad Shapouri, *et al.,* 2016). This is because of the latency nature of the BoHV – 1 after primary infection or exposure to the virus or vaccination with attenuated viral strains (Radostits *et al*., 2000; Constable *et al.,* 2017).

The study also showed that BVDV sero-prevalence was significantly associated with BoHV-1. Other studies found out positive correlation between IBRV, BPIV3 and BVDV (Callaby *et al.,* 2016). Martin and Bohac, (1986); Durham and Hassard, (1990) and Fulton *et al*., (2000), found

out that there was an association between the three viruses; BoHV-1, BVDV and PIV3. However, Callaby *et al*. (2016) further noted that by including the environmental confounders, the model quantified relationship between sero-positivity of IBRV, BPIV3 and BVDV but had little effects on the association observed among the three viruses.

The findings of this study revealed that a number of risk factors such as age of the cattle, cattle that were borrowed into farms, rearing goats in the farms, cows that were given out of the farms and cows that had antibodies against Bovine Viral Diarrhea were associated with sero-prevalence of BoHV – 1 antibody. The latter agree with several serological studies carried out worldwide to identify risk factors associated with BoHV – 1 sero-positivity (Mulyken *et al.,* 2007). The current study also agrees with other studies which reported a number of risk factors in their studies such as large herd size, housing, intensive production systems and managerial practices like cattle movement and hygiene, age, sex with male frequently positive than females, direct cattle contact especially participation in animal shows, purchasing of cattle and large herd sizes (Van Schaik, 2001; Van Schaik *et al*., 2002; Solis-Calderon *et al.*, 2003; Vonk Noordegraaf *et al*., 2004; Boelaert *et al.*, 2005).

Age of the cattle is a frequent risk factor reported in IBRV seropositive cattle, Solis-Calderon *et al.* (2003), Cabonero *et al.* (2011), Romero-Salas *et al.* (2013), Saravanajayam *et al.* (2015) and Segura-Correa *et al.* (2016) findings showed that older animals had a higher prevalence compared to young animals while Saravanajayam *et al*. (2015) further reported that cattle over 3 years of age may have a higher sero-positivity than those of less than 3 years of age. The findings of this study revealed that age of the cow is a significant risk factor to sero-prevalence of BoHV – 1 antibody. This similarity could be attributed by the physiology of the cattle since the ability of aging animal's immunity system to defend against infection wears off compared to

young animals. Callaby *et al.* (2016) reported sero-positivity of 20.9% among the East African Shorthorn Zebu calves, thus maternal antibodies against IBR are transferred to calves which provides initial protection before development of the innate immunity. These observed differences may be associated to animal inclusion criteria in the studies, for instance Callaby *et al.* (2016) in his studies selection was based on calves age between 3 to 7 days old, the dam must have been in the farm for not less than one year, and calf was due to natural mating.

The present study showed that introduction of new animals into the herd was significantly associated sero-positivity of BoHV-1 hence this was an important risk factor. This difference may be explained by differences in geographical location, disease-control programs employed and herd sizes. Other past studies by Solis-Calderon *et al*., (2003) and Segura-Correa *et al.,* (2016) reported that introduction or non-introduction of animal into the herd was not significantly associated with sero-prevalence of BoHV-1. Furthermore, when the age of the female principal farmer was over 45 years, the odds of cattle on the farm to have BHV-1 antibodies were lower by 0.174 times compared with cattle reared by younger women principal farmers under or equal to 45 years (Table 3). This could be associated to majority of the female principal farmers attend dairy production training sessions on husbandry, production, reproduction and disease control training, thus, they obtain an understand on general disease management in herds.

This study was carried out in smallholder farms with approximate herd size of 3 animals, as a result, the study did not had enough power to identify herd size as risk factor for seropositivity. Other studies suggested that at herd level, herd size is the main important risk factor for BHV-1 infection (Snowder *et al*., 2006; Segura-Correa *et al.*, 2016). Our herd sizes were small, making it less likely for our herds to be aggregates of animals from other farms.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

1. In Meru County, BoHV-1 is naturally circulating among the cattle population.
2. There was a positive association between IBR and age of the dairy cattle and rearing of goats in the farm together with cattle.
3. Older women principal farmers had good experience with disease control methods, thus the risk of diseases spread is reduced.

6.2: Recommendations

1. A study should be carried out to establish if the clinical IBR occurs in Naari sub location in order to formulate prevention and control measures such as vaccination.
2. A study is also needed to be carried out to determine if their long-term effects of BoHV-1 sero-prevalence in cattle production.
3. Further study is required to access the potential ability of viral cross-infection with other four related Alpha- herpesviruses with BoHV -1 which include; Cervine herpesvirus – 1, Cervine herpesvirus – 2, bovine herpesvirus – 1 and caprine herpesvirus – 1.

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APPENDICES

Appendix 1: Descriptive statistics of continuous variables and proportion among the principal farmers in 149 smallholder dairy farms, Naari area, Meru County, Kenya in 2018

Variable	**Category**	**Frequency**	**Proportion**
Principal farmer	Female	42	28.2
	Male	84	56.4
	Male and Female	23	15.4
Age of Males in the farm	≥ 45 years	73	49.0
	< 45 years	76	51.0
Age of Females in the farm	≥ 45 years	75	50.3
	< 45 years	74	49.7
Marital status	Married	125	83.9
	single/Separated/Divorced/Widowed	24	16.1
Highest level of education male in the farm	N/a/Primary school	30	20.2
	High school	61	40.9
	Tertiary school	58	38.9
Highest level of education female in the farm	N/a/Primary school	24	16.1
	High school	71	47.7
	Tertiary school	54	36.2
Principal farmer with Dairy training	No training	28	18.8
	Trained on dairy	121	81.2

Appendix 2: Questionnaire for Management and Feeding Practices on Naari Smallholder Dairy Farms

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

Farmer Name: _____

Farm Number: _____

GPS lat: _____

GPS long: _____

Phone #: _____

Survey Visit Date: _____

Interviewer Initials: _____

1. **Level of Education:**
    a) Primary [ ]
    b) Secondary [ ]
    c) College [ ]
    d) University [ ]
    e) N/A [ ]
2. **Occupation:**
    a) Government employee
    b) Self – employed
    c)
3. Area of land owned: _____ acres / hectares (circle units)
    a) Percent of land used for crop and fodder production for cattle? [ ] acres/ hectares

b) Area of land rented/used (unpaid): _____ acres / hectares (circle units)

c) Percent of land used for crop and fodder production for cattle?

d) Do you practice: Stall feeding _____ or Semi – stall feed _____?

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

   If yes, what was this training about? _____

5. What are the sources of information about dairy production in your farm?

   a) Farmers training session [ ]

   b) Internet [ ]

   c) Other(specify)_____

II. Feeding – Part A – Normal feeding:

6. Some feeds are only given seasonally. **Over the last year**, please check which of the following you fed to your cattle (**amounts not needed**) and indicate the source (purchased, shamba, neighbor, river, etc.).

Feed name	Calves/Heifers	Cows	Source
a. Napier grass			
b. Grass silage			
c. Whole plant maize silage			
c. Grass hay			
d. Desmodium			
e. Sweet potato vines			
f. Other high protein forages – Lucerne, leucana, –			
g. Tree fodders – specify			
h. Maize stover			
i. Banana leaves			
j. Other fodder – specify (eg. Weeds) _____			
k. Dairy meal			
l. Wheat bran			
m. Maize "Jam"			

n. Vitamin/mineral powder			
o. Vitamin/mineral block			
p. Calf pellets/calf pencils. If yes, until what age?			
q. Other feeds –specify (eg. Meal or cake)			
r. Water available (always/sometimes)	A/S	A/S	

7. Do you usually feed **dairy meal or grain** to cows for the **month before calving**?

__YES __NO

If yes, do you increase the amount of dairy meal or grain during this month?

__YES __NO

8. Do you feed **vitamins/minerals** to cows during the **month before calving**?

___YES ___NO

If yes, what brand?

Brand: _____ *(from bag: Ca:P ratio: ___ Selenium amount & unit: _____)*

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

---

9. For your **cows,** did you always have enough feeds over the last year? Yes_____ No_____

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

Forages ___Grain or meals __Vitamin-minerals ___Water__ Other (specify)

10. <u>Cattle deworming regime</u>

a) How frequently do you normally deworm your cows?

Every ___ month's ____when suspect it is a problem ___when not pregnant __ other?

b) How frequently do you normally deworm your calves/heifers?

Every ___ months ___ when suspect it is a problem ___ other (specify: ____)

c) Who normally deworms your cattle? Self: _ Vet service provider: ___ other: ___ who? __

d) What do you usually use to deworm your animals? _____

11. <u>Farm anima Deaths</u>

a) How many **calves** died in the last year? _____

If some, from what causes _____

b) How many **heifers** died in the last year? _____

If some, from what causes _____

c) How many **cows** died in the last year? _____

If some, from what causes _____

12. <u>Veterinary Services</u>

a) How far is the nearest vet service provider? _____km

b) In the past year did a vet service provider visit your farm? Yes __ No __

If yes, for what reason? _____

c) Do you use an AI service? Y__N__

If yes, who provides your AI service? ___ Vet __Vet tech ___ AI tech ___ other_____

d) Do they come on time when you call them? Y [ ] N [ ]

e) Do they have the bulls you want to use? Y [ ] N [ ]

f) How do you decide what bull to use?

_____

13. **Cow Stall Design and Management**

a) How often do you **remove manure** from where the **milking cows** lie down?

a) Two times a day

b) Every day

c) Every other day

d) two times a week

e) Every week

f) Less than one time a week

b) How often do you **add new bedding** to where **milking** cows lie down?

a) Every other day

b) Every day

c) Two times a week

d) One time a week

e) Less than one time a week

c) How often do you add new bedding to where dry cows lie down?

a) Every other day

b) Every day

c) Two times a week

d) Once a week

e) Less than one time a week

d) How often do you trim your cow's feet?

a) Every 4 months

b) Every 8 months

c) Every 12 months

d) Less often or never

14. For observation:

a) What kind of bedding is used where the milking cows lie down?

a) grass/hay b) straw c) sawdust d) crop waste e) soil f) none g) other (specify _____)

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

c) What is the type of the floor where the milking cows lie down?

1) Concrete 2) dirt 3) other (please specify: _____)

d) Is the floor (observe – check all that apply):

1) Lumpy (have to lie on sticks, rocks, dirt chunks, etc.) Yes __ No __

2) Hard (fails Knee impact test) Yes __ No __

3) Wet in the udder area (fails the Knee wetness test) Yes __ No __

e) Are there any sharp objects in the cow shed that may risk injuring the cattle (eg nails)?

Yes __ No ___

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

Yes ___No ____

15. **<u>Young-stock health and productivity checks</u>** (calves and heifers that have never

calved yet):

a) How do your calves usually receive their **first colostrum**? _____

b) Choose only **ONE** of the options that is MOST commonly used

Free choice suckle [ ]

Assisted suckle [ ]

Nursing bottle [ ]

Bucket [ ]

Other -specify:_____

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

< 6 hours ___6 - 12 hour's ___ 12 - 24 hours ____ > 24 hours ____ unknown ____

d) What do you usually do if a calf is weak and unable to drink colostrum during the first day of life? Bottle feed ___Tube feed ____ Call Vet ____Other (specify) _____

e) Do you have a pen for pre-weaned calves? Y_____ N_____

If yes, type of flooring? _____ 23c. Adequate roof? Y____ N____

	Calf/Heifer #1 ID_____	Calf/Heifer#2 ID_____	Calf/Heifer#3 ID_____	Calf/Heifer#4 ID_____
a. "Birthdate or Age (months)"				
b. Sex				
c. Breed				
d. "First breeding date" (date or n/a)				
d. "Latest breeding date" (date or n/a)				
e." Number of breeding's to date" (# or n/a)				
f. "Had diarrhea"	Y/N	Y/N	Y/N	Y/N
g. "Had pneumonia"	Y/N	Y/N	Y/N	Y/N
h. "Had navel-ill"	Y/N	Y/N	Y/N	Y/N

i. Weight				
j. Height				
k. Body condition score				
l. TPR/physical exam Normal / Abnormal? (manure, feet, skin, lymph nodes, eyes, rumen)	N / A	N / A	N / A	N / A
m. Reproductive status (preg days confirmed?)	Preg: Y/N	Preg: Y/N	Preg: Y/N	Preg: Y/N
n. Month of last deworming				
o. When last sprayed/dipped for ticks?				

16. Health and Productivity of Cows

Examination of Cows:	Cow1 (Q45) ID_____	Cow2 (Q46) ID_____	Cow3 (Q47) ID_____	Cow4 (Q48) ID_____
a. "Approximate age (years)"				
b. Breed				
c. "Number of calvings"				
d. "Last calving date"				
e. "First breeding date after last calving"				
f. "Latest breeding date after last calving"				
g. "Latest observed heat seen"				
h. "Number of breedings for last pregnancy"				
i. "# of times used hormones for breeding"				
j." Current daily milk yield (kg/day)"				
k. "Is this what she produced last week?"	Y/N	Y/N	Y/N	Y/N
m. "Abortion/stillbirths in last 12 months"	Y/N	Y/N	Y/N	Y/N
n. "Other disease (RP) in last 12 months"____	Y/N	Y/N	Y/N	Y/N
o. Weight				
p. Height				
q. Body condition score				
r. TPR/physical exam Normal / Abnormal? (manure, feet, skin, lymph nodes, eyes, rumen)	N / A	N / A	N / A	N / A
s. Reproductive status (preg days confirmed?)	Preg: Y/N	Preg: Y/N	Preg: Y/N	Preg: Y/N
t. Normally get pregnancy confirmation?	Y/N	Y/N	Y/N	Y/N
u. Reproductive disease reported				
v. Month of last deworming				
w. When last sprayed/dipped for ticks?				
x. Diseases vaccinated against (frequency)				

a. In the last year, how frequently did your **cows** have an abrupt feed change? (For example, you completely run out of one type of feed one day, such as Napier grass, so you switched to a different type of feed the next day, such as weeds, banana leaves or maize stover) Choose ONE

Never ____Occasionally in the dry season ____ 1 time/month _____more than 1 time/month_____

b. In the last year, how frequently did your **calves** have an abrupt feed change? Choose ONE

Never ____Occasionally in the dry season ____ 1 time/month _____more than 1 time/month_____

c. What is the source of your cattle?

Buy as in-calf heifers [ ]

Buy as adult cows [ ]

Raise from young ones on my farm [ ]

d. Have you had a cow with difficult calving requiring assistance in the past? Yes No

If yes, who came to help? _____

e. Have you had a cow that could not stand up before or after calving? Yes No

If yes, who came to help? _____

f. Have you had a cow that had any other problem after calving? Yes No

If yes, what was it? _____

If yes, who came to help? _____

g. Any other problems on the farm? Yes No

If yes, what was it? _____

If yes, did anyone come to help? _____

If yes, still a problem? _____

17. Digital photo file range: _____ to _____