ASSESSING PREVALENCE OF ENDOMETRITIS AND ASSOCIATED INFLUENCE ON PERFORMANCE OF SMALLHOLDER ZERO-GRAZED DAIRY COWS IN GASABO DISTRICT OF RWANDA

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

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DEDICATION

In Memoriam: my father, Mr. Bigaragara Joseph. God loved you more than us and took you very early. Know that I have accomplished what you started and I will honour you forever. RIP Daddy! I love you.

To my beloved sons (Manzi Nkusi King Paco Ivan and Manzi Nkusi Paco Brian), beloved wife (Noella), mother (Mrs. Mukankusi Laurence), brothers (Ildephonse, Jean Bosco and Eric), sisters (Epiphanie, Florence, Odette and Dative), grandmother (Mrs. Kamurehe Gricelia), uncle (Cyimana Gaspard), I love you all.

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ABSTRACT

Endometritis is a uterine disease that interrupts cow reproductive cycles as a clinical (CLE) or subclinical (SCLE) condition between 21st and 90th days postpartum (dpp), resulting in suboptimal fertility, production and profitability in a herd. This study tested the hypothesis that suboptimal fertility and production performance of zero-grazed dairy cows on smallholder farms result from prevalent CLE and SCLE cases and multiple risk factors (RFs) at the cow- and herd levels. The specific objectives were to determine: farmer perceived and observed endometritis prevalence; farmer perceived effective management interventions (MIs); risk factors; and endometritis influence on milk yield (MY) and reproductive performance. Sample farms (n=370) in Gasabo District of Rwanda were accessed through exponential non-discriminative snowball sampling in a cross-sectional survey. Zero-grazed dairy cows (n=466) in their 21 to 60 dpp were diagnosed for CLE and SCLE cases using Metricheck device and Cytotape, respectively, and prospectively observed for fertility performance up to 210 dpp, and MY for 30 days post-endometritis diagnosis. Data analysis used the Best-Worst Scaling choice method to determine effective MIs, path analysis model to determine RFs, and general linear model to determine influence of endometritis on MY and reproductive performance. Endometritis prevalence was 3.2% by farmer estimation, but 70.2% observed at the cow-level with 67.2% CLE and 31.8% SCLE while observed prevalence at herd-level was 71.1% with 68.1% CLE and 34.4% SCLE. Of the MIs (n=20) assessed, 60.0% were farmer perceived as the most effective prevention and control. The top four MIs were consulting animal health service (ANHS) providers, not sharing equipment between farms, keeping cows in a clean and dry shed, and selecting sires for calving ease. Some cow- and herd-levels RFs were specific for CLE or SCLE cases and some others were common for both CLE and SCLE cases in smallholder zero-grazed dairy cows. Compared to cows' negative for endometritis, the positive cows had longer days to first oestrus (median 85 vs 63 days), longer days-not pregnant (95.5 vs 63.0 days), lower pregnancy rate at first service (16.5% vs 32.7%), more services per pregnancy (1.3±0.1 vs. 1.1±0.0) and more anoestrus postpartum cows (48.4% vs. 11.7%). The milk loss during period of discarding was 7.3 ± 0.3 while decrease in MY resulting from endometritis was 1.4 ± 0.2 litres /cow/day. It is concluded that a combination of Metricheck device and Cytotape could optimise detection of endometritis, implementation of MIs reduces RFs and improves reproductive performance while treating endometritis positive cases using veterinary drugs having no residual effect in treated cows could be an alternative to minimise MY loss and associated economic loss.

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LIST OF ABBREVIATIONS AND ACRONYMS

AI	Artificial insemination
AMOS	Analysis of Moment Structures
ANHS	Animal Health Service
ANOVA	Analysis of Variance
ANPP	Anoestrous Postpartum
BCS	Body Condition Score
BWS	Best-Worst Scaling
CAHWs	Community-Based Animal Health Workers
CCS	Cow Cleanliness Score
CI	Confidence Interval
CLE	Clinical endometritis
CMT	California Mastitis Test
CP210	Cows Pregnant within 210 days postpartum
CRAS	Conception Rate to All Services
CRFMA	Conception rate at first natural mating or artificial insemination service
CYT	Cytotape
DFMA	Days to first natural mating or artificial insemination service
DFO	Days to first oestrus
DMY	Daily Milk Yield
DNP	Days-not pregnant
Dpp	Days postpartum
FAO	Food and Agriculture Organization of the United Nations
GDP	Gross Domestic Product
GIS	Geographic Information System
GLM	General Linear Model
GPS	Global Positioning System
HR	Hazard Ratio
IBM	International Business Machines
LTHs	Local Traditional Herbalists
MED	Metricheck Device
MINAGRI	Ministry of Agriculture and Animal Resources
MIs	Management Interventions
mm	Millimetre

MY	Milk yield
NISR	National Institute of Statistics of Rwanda
NPV	Negative Predictive Value
NSC	Number of Services (bull or artificial insemination) per Conception
OR	Odds Ratio
PMN	Polymorphonuclear inflammatory cells
PPV	Positive Predictive Value
QGIS	Quantum Geographic Information System
RFs	Risk factors
RF	Risk factor
SCLE	Subclinical endometritis
Se	Sensitivity
SEM	Standard error of the mean
Sp	Specificity
SPSS	Statistical Package for the Social Sciences
SSRP	Sani-Shield Rod Protector
US$	The United States dollar
VD	Veterinary drugs
VETs	Veterinarians
VMC	Vaginal Mucus

CHAPTER ONE
INTRODUCTION

1.1 Background information

Dairy production is a major component in the livestock sector in Rwanda. The dairy subsector is an essential source of livelihood to over 80.0% of households involved directly or indirectly throughout the agricultural value chain (IFAD, 2016). The dairy subsector contributes 28.0% to the agricultural Gross Domestic Product (GDP) and 4.0% to the national GDP (NISR, 2018). Rwanda has an estimated cattle population of 1,340,792, of which 45.0% are indigenous cattle, 33.0% are dairy crossbreds, and 22.0% are pure dairy breeds (IFAD, 2016). The dairy crossbreds and pure dairy breeds are of the Friesians, Jersey, and Fleckvieh breeds. Among the smallholder dairy farms, those practicing zero-grazing hold the majority (92.0%) of the cattle population and supply the bulk of the domestic milk market demand (IFAD, 2016). However, the supply has not satisfied the local demand. The average per capita milk consumption for both urban and rural areas estimates by the Rwanda Livestock Master Plan (RLMP, 2017) is 63.0 litres per person per annum. An increase of 3.5 fold would be necessary to achieve per capita consumption threshold of 220 litres recommended by the Food and Agriculture Organization of the United Nations (FAO, 2013).

The low per capita milk consumption is to a large extent due to low productivity of the national herd, and this is attributable to suboptimal performance of zero-grazed cows on smallholder dairy farms (Rukundo *et al*., 2018). The suboptimal fertility in smallholder farms is evidenced by long interval from calving to conception (298.7 ± 199.0 days), a high number of services per conception (NSC) (3.0 ± 1.3), long calving interval (18.3 ± 4.5 months), less conception rate at first natural mating or artificial insemination (AI) service (35.0%), and more occurrences of anoestrus postpartum (ANPP) (44.0%) (Manzi *et al*., 2019; Rukundo *et al*., 2018). The indigenous cattle, dairy crossbred and pure dairy breeds all exhibit suboptimal fertility in days to first oestrus, respectively, 8.8 ± 7.7; 8.9 ± 6.2 and 8.7 ± 7.8 months. The suboptimal reproductive performance can be associated with resulting low milk production estimated at 3.6 litres/cow/day for indigenous cattle, 5.5 litres/cow/day for dairy crossbreds, and 8.6 litres/cow/day for pure dairy breeds (Hirwa *et al*., 2017; Manzi *et al*., 2020; Rukundo *et al*., 2018). The suboptimal fertility and production performance observed in the Rwandan dairy herds has been explained as resulting from poor herd management practices but without identifying the underlying cause (s) involved (Nishimwe *et al*., 2015). One likely area of management failure could be in managing the uterine health of cows in the postpartum period for uninterrupted cow cyclic activities (Sharma *et al*., 2018a; Sheldon *et al*., 2020).

One uterine disease associated with suboptimal performance resulting from interrupted reproductive cycles, though often unnoticed, is endometritis (Hussein *et al*., 2017; Sharma *et al*., 2018a). It is, therefore, possible that smallholder zero-grazed dairy cows could be at risk of prevalent endometritis,a postpartum disease characterised by an inflammation of the uterine endometrium between 21st and 90th days postpartum (dpp) (Pothamann *et al*., 2015; Pascottini *et al*., 2017). The disease may manifest as clinical endometritis (CLE) and/or subclinical endometritis (SCLE). The CLE is characterised by presence of purulent or mucopurulent uterine discharge detectable in the vagina (Okawa *et al*., 2017; Tayebwa *et al*., 2015). In contrast, the SCLE is characterised by abnormal presence of the proportion of polymorphonuclear inflammatory cells (PMNs) in endometrial cytology samples (Melcher *et al*., 2014; Pascottini *et al*., 2017). Both CLE and SCLE are prevalent in dairy cows and disrupts reproductive cycles in positive cows resulting in reduced performance and profitability in the herd (Chan Lee *et al*., 2018; Mohammed *et al*., 2019).

The estimated prevalence rates of endometritis suggest large variability in commercial dairy herds, 6.7% to 89.0% for SCLE (Denis-Robichaud & Dubuc, 2015; Kelly *et al*., 2020) and 3.6% to 69.8% for CLE (McDougall *et al*., 2007; Tayebwa *et al*., 2015). For smallholder dairy herds, the prevalence varies from 38.0% to 86.7% for SCLE (Moges & Jebar, 2012; Moges, 2015), which suggests that there are opportunities to prevent and control endometritis with good understanding of the risk factors involved.

Several reports on the prevalence of CLE and SCLE are available for industrial dairy systems of America, Asia, and Europe, where dairy herds are managed in confinement in-group housing units (Chan Lee *et al*., 2018; Daros *et al*., 2017; Ryan *et al*., 2020) but few empirical literatures exist about endometritis in smallholder dairy farms, especially under zero-grazing conditions (Moges & Jebar, 2012; Moges, 2015). In Rwanda to date, where the smallholder dairy herds are managed under zero-grazing housing units, empirical evidence is yet to be presented to support the presence (or absence) of endometritis. In dairy herds, several studies (Juan Piñeiro, 2016; McDougall *et al*., 2011; Sharma *et al*., 2019) have associated the presence of reproductive disorders to significant losses in milk production. The milk losses arise from decrease in milk yield and milk discarded during the period of disease treatment (Ali, 2011; Angara & Elfadil, 2014; Cattaneo *et al*., 2015).

The risk factors associated with endometritis prevalence are those of cow- and herd-level sources. The main risks at the cow-level include cow parity, body condition score, twin births, dystocia, milk fever, ketosis, left displaced abomasum, age of cow, cow breed, calf sex, retained placenta, stillbirth, gestation length, season of calving, breeding services, dry period length, mastitis, and brucellosis (Adnane *et al*., 2017; Chan Lee *et al*., 2018; Kelly *et al*., 2020). In contrast, the potential risk factors at herd-level include bedding materials, cleanliness of the cowshed, housing of cows within the first 30 dpp, herd size, calving pen, and farm size (Cheong *et al*., 2011; Moges & Jebar, 2012; Tayebwa *et al*., 2015). These risks are relevant in the smallholder herds managed in zero-grazing units, and they could predispose dairy cows to endometritis. However, empirical evidence is lacking as to which ones of them pose high risks for CLE or SCLE. Such evidence would be valuable in informing management interventions to target high-risk factors for endometritis. Therefore, this study was designed to contribute to improved herd fertility and productivity through better-targeted management actions to prevent and control endometritis in smallholder zero-grazed dairy cows in Rwanda. Therefore, this study aimed to contribute to improved herd fertility and productivity through better-targeted management actions to prevent and control endometritis in smallholder zero-grazed dairy cows in Rwanda.

1.2 Statement of the problem

Suboptimal fertility of zero-grazed cows in smallholder dairy farms in Rwanda is indicative of interrupted reproductive cycles, of which CLE and SCLE prevalence are highly likely an underlying cause. The observed suboptimal fertility status is characterised by prevalent ANPP (44.0%), less conception rate at first natural mating or AI service (35.0%), many repeat services (3.0±1.3 services per conception), and long DNP (298.7±199.0). Endometritis prevalence could be contributing to the observed long calving interval (18.3±4.5 months) with low milk productivity (3.6 litres/day for indigenous cattle, 5.5 litres/cow/day for dairy crossbreds, and 8.6 litres/cow/day for pure dairy breeds against an average of 5, 14 and 21 litres/cow/day, respectively) (Hirwa *et al*., 2017; Manzi *et al*., 2020; Rukundo *et al*., 2018). This should attract targeted MIs for the prevention and control of endometritis.

Despite the high likelihood of CLE and SCLE prevalence, present herd fertility interventions ignore targeting endometritis because empirical evidence is lacking for presence, associated risk factors, and influence on the reproductive performance and milk yield. Prevalence rates of CLE and SCLE vary widely, from 3.6% to 89.0% in commercial and smallholder dairy herds. The large variability in prevalence can be associated with multiple risk factors at play, at the cow- and herd- levels, which could be as well present in zero-grazed cows on smallholder farms in Rwanda. However, there is a knowledge gap about existing prevalence, risk factors, associated influence on cow fertility performance, and milk yield that results from CLE and SCLE. The results would better inform targeted MIs for CLE and SCLE cases in smallholder zero-grazed cows on smallholder farms in Rwanda.

1.3 Objectives

1.3.1 General objective

This study was designed to contribute to improved herd fertility and productivity through better-targeted management actions to prevent and control endometritis in smallholder zero-grazed dairy cows in Rwanda.

1.3.2 Specific objectives

The specific objectives of this study were to:

(i) Estimate the perceived and observed prevalence of endometritis in smallholder zero-grazed dairy cows in Gasabo district.

(ii) Gather farmers' opinions on the effectiveness of management interventions for endometritis prevention and control on smallholder zero-grazed dairy farms in Gasabo district.

(iii) Determine the risk factors associated with the prevalence of CLE and SCLE in smallholder zero-grazed dairy cows in Gasabo district.

(iv) Determine the influence of endometritis on the number of services per conception, conception rate at first service, conception rate to all services, days- not pregnant, days to first oestrus, days to first natural mating or artificial insemination service, cows pregnant within 210 dpp, and anoestrus postpartum rate of smallholder zero-grazed dairy cows in Gasabo district.

(v) Determine the influence of endometritis on the volume of milk losses during the period of discarding milk and the decrease in milk yield of smallholder zero-grazed dairy cows in Gasabo district.

1.4 Research questions

(i) What is the perceived and observed prevalence of endometritis among zero-grazed cows in Gasabo district?

(ii) What are the management interventions that farmers consider most effective for endometritis prevention and control on smallholder zero-grazed dairy farms in Gasabo district?

(iii) What are the risk factors associated with the prevalence of CLE and SCLE in smallholder zero-grazed cows in Gasabo district?

(iv) Are the numbers of services per conception, conception rate at first service, conception rate to all services, days- not pregnant, days to first oestrus, days to first natural mating or artificial insemination service, cows pregnant within 210 dpp, and anoestrus postpartum rate significantly different between endometritis positive and negative cows in Gasabo district?

(v) What is the volume of milk losses during the period of discarding milk and the decrease in milk yield that results from endometritis infection in smallholder zero-grazed cows in Gasabo district?

1.5 Justification of the study

The dairy sub-sector is an integral part of the agricultural sector in Rwanda. The dairy subsector offers a pathway out of poverty for over 80.0% of households involved directly or indirectly in the dairy value chain. The dairy sub-sector contributes 28.0% to the agricultural GDP and 4.0% to the national GDP (NISR, 2018).In Rwanda, smallholder farms practicing zero-grazing hold the majority (92.0%) of the cattle population and supply the bulk of the milk for domestic market (IFAD, 2016). However, the supply has not satisfied the local demand. The average per capita milk consumption for both urban and rural areas is 63.0litres, which is below the 220 litres recommended by the Food and Agriculture Organization of the United Nations (FAO, 2013). To meet the demand, individual cow must produce milk more efficiently. Nevertheless, low fertility limits the potential of breeding services to improve breed quality in term of milk production. The suboptimal fertility and production performance of dairy cows results in low profitability that reduces food security, nutrition and income in smallholder farmers. The suboptimal performance could be due to the risk of endometritis, but there is a gap in confirming the presence (or absence) of endometritis. As a result, a large number of dairy cows remain non-pregnant having served several times and finally become a burden for the farmers (Nishimwe *et al*., 2015). Furthermore, in most cases, the suboptimal reproductive performance results in absence of heifer replacement which need to be introduced in order to maintain a stable herd size. Therefore, this study was designed to provide the empirical evidence of the prevalence of endometritis, its RF, and influence on fertility and production performance. This will inform MIs to improve herd fertility for increased productivity and profitability in smallholder zero-grazed cows in Rwanda.

This study will serve as a baseline for further study on endometritis in Rwanda. The methods developed and followed throughout this study will be adapted and adopted in other related studies aimed at finding sustainable management of endometritis on smallholder farms. Farmers and other actors of the dairy sub-sector, politicians and non-technical decision-makers, and researchers will benefit from the findings of this study to optimise fertility and productive efficiency of dairy cows towards the sustainability of the dairy herd. This will contribute to economic development, poverty reduction, food and nutritional security of smallholder dairy farmers. The findings of this thesis will also contribute to achieving seven sustainable development goals (SDG) which are now the currency of development across the world including Rwanda. Addressing the challenge posed by endometritis to dairy farmers would contribute to increase milk production, quality, and income of smallholders while contributing to health and well-being of the population. This is directly linked to SDG 1 (no poverty), SDG 2 (zero-hunger), SDG 3 (good health and well-being), SDG 8 (decent work and economic growth), SDG 12 (responsible consumption and production), SDG 13 (climate action), and SDG 15 (life on land).

1.6 Definition of terms

Abortion: Is defined as a loss of the foetus between the age of 42 days and approximately 260 days.

Clinical endometritis: is a postpartum uterine disease characterised by the presence of purulent or mucopurulent uterine discharge detectable in the vagina 21^{st} to 90^{th} dpp.

Crossbreed: This is a cross between local and exotic breeds.

Cut- and-carry feeding: is a method widely practiced by smallholder farmers in many countries where access to grazing land is in short supply. With this method, forage is harvested daily and fed to cows that are permanently housed indoors.

Cytotape: is a cytological sampling technique to diagnose subclinical endometritis.

Dystocia: Difficult calving or assistance at calving with calf puller, one or more person (s) pulling or veterinary assistance at calving either with manual traction, caesarean section or foetotomy.

Endometritis: is a postpartum uterine disease characterised by an inflammation of the uterine endometrium 21^{st} to 90^{th} dpp.

False-positive cases: Are the number of cows incorrectly diagnosed as having disease. Specifically, in this study, false-positive cases are the number of cows diagnosed as negative using Cytotape and positive using Metricheck device.

Fresh cows: are defined as the recently calved cows till 30 dpp.

Metricheck device: Is an intravaginal device of detecting clinical endometritis, which consists of 40 mm hemisphere of silicon attached to a 500 mm long stainless steel rod.

Milk offtake: is defined as the milk used for human consumption excluding that consumed by the calf.

Negative predictive value: Is the probability that an animal with a negative test result is a true negative.

Odds ratio: The ratio of two odds that measures the strength of the association between dependent and independent variables.

Odds: the ratio of the probability of an event occurring to that of it not occurring.

Polymorphonuclear inflammatory cells (PMNs): are the most important cells involved in the innate immune response of uterus of cows during the postpartum period and mostly found at sites of infection or inflammation.

Positive predictive value: Is the probability that an animal with a positive test is a true positive.

Post-partum period: Is defined as the period after calving.

Prevalence: Is the proportion of a particular population found to be affected by a disease or a risk factor) at a specific time (at any single point in time: point prevalence). It is derived by comparing the number of population found to have the condition with the total number of the population studied.

Repeat breeding: is defined as the failure to conceive from three or more successive breeding services.

Sensitivity of a diagnostic test: Is the percent of diseased individuals who have positive test results. It indicates the ability of the test to correctly identify disease cases.

Smallholder dairy farmer in the zero-grazing system: Is a farmer who own up to five cows.

Specificity of a diagnostic test: Is the percent of non-diseased individuals who have negative test results. It indicates the ability of the test to correctly identify disease-free cases.

Subclinical endometritis: is a postpartum uterus disease characterised by the abnormal presence of the PMNs cells in endometrial cytology samples collected between 21 and 90 dpp.

Transition period: Is defined as the period from three weeks before to three weeks after calving.

Twin births: Calving of two calves.

Withdrawal period for milk: Is the amount of time expressed in hours or days from the last administration of veterinary drugs to an animal you should wait to drink the milk from such animal to ensure that such milk does not contain drug residues in quantities in excess of the maximum residue limits laid down. This allows time for the dairy cows to eliminate the drug residues.

Zero-grazing: is a production system in which cattle are permanently housed indoors and fed on a cut-and-curry feeding system.

1.7 Organization of the study

This thesis is organized into eight chapters. Chapter one discusses the introduction comprising background information of the study, statement of the problem, objectives of the study, research questions, justification of the study, and definitions of terms. Chapter two presents a literature review of relevant studies related to this study and elaborates on the theoretical basis for this study. Chapter three presents the abstract, introduction, materials and methods, and discusses the findings related to perceived and estimated prevalence of endometritis in smallholder zero-grazed dairy cows. Abstract, introduction, methodology, and discussion of the findings related to effectiveness of management interventions for endometritis prevention and control in zero-grazed dairy cows on smallholder farms are presented in Chapter four. Chapter five presents the abstract, introduction, materials and methods, and discusses the findings related to cow- and herd-level risk factors associated with endometritis. Chapter six presents the abstract, introduction, materials and methods, and discusses the findings related to the influence of endometritis on reproductive performance of zero-grazed dairy cows on smallholder farms in Rwanda. The influence of endometritis on milk yield of zero-grazed dairy cows on smallholder farms in Rwanda is presented in Chapter seven. The last chapter presents a general discussion, conclusion, recommendations, and areas for further research.

CHAPTER TWO
LITERATURE REVIEW

This chapter presents a literature review of relevant studies related to this study and elaborates on the theoretical basis for this study. The literature dealing with the prevalence of endometritis and its estimation in dairy herds is covered. Further, it reviews studies on cow- and herd- levels risk factors for endometritis and their estimation, and the influence of endometritis on fertility performance and milk yield in dairy cows. Moreover, the literature on the empirical techniques used for the diagnosis of endometritis is highlighted. The knowledge gaps to be filled by this study are identified and pointed out in this chapter. The chapter ends with a conceptual framework showing the linkage between the objectives of this study.

2.1 Prevalence of endometritis in dairy herds

Endometritis is a prevalent disease in postpartum cows resulting in substantial economic losses due to decreases in both milk production and fertility performance (Chan Lee *et al*., 2018; Sharma *et al*., 2019). The contamination of the uterus by reproductive tract pathogens occurs at all stages of the reproduction cycle (Appiah *et al*., 2020; Karstrup *et al*., 2017), but the majority of cases is found mostly in the first two weeks of the postpartum period (Drillich & Wagener, 2018; Jeon *et al*., 2015). This contamination is attributed to the fluctuation and expansion of the microbial community diversity after calving (Pascottini *et al*., 2020; Wang *et al*., 2018; Williams *et al*., 2005). The reason for this is that the dilation of physical barriers such as vulvar sealing, vestibule-vaginal constriction, the cervix, cervicovaginal mucus secretion and the epithelial barriers allow the contamination and colonisation of the female reproductive tract with pathogenic micro-organisms from skin, faeces, bedding material heavily soiled, and vagina (Appiah *et al*., 2020; Dadarwal *et al*., 2017; Williams *et al*., 2005). Such microbial contamination of the uterus is common in 80.0% to100.0% of postpartum dairy cows (Bicalho *et al*., 2017; Ghanem *et al*., 2015; Plöntzke *et al*., 2011).

While a high proportion of cows clear naturally most of these pathogens from the uterine lumen, more than 40.0% of them develop uterine infection beyond three weeks postpartum (Cheong *et al*., 2011; Foley *et al*., 2015; Wagener *et al*., 2017). This is because of an unsuccessful immune response to resolve the inflammatory state postpartum. Therefore, maintaining the inflammatory response for a longer period occurs as a consequence (Foley *et al*., 2015; Sheldon *et al*., 2020). Persisting infections in the uterus lead to uterine diseases of which clinical endometritis (CLE) and subclinical endometritis (SCLE) are of importance.

These diseases impair subsequent fertility and production performance of dairy cows (Juan Piñeiro, 2016; Rinaudo *et al*., 2017).

In commercial dairy herds mostly with Holstein-Friesian cows, the cow-level prevalence of CLE ranges from 3.6% to 69.8% (McDougall *et al*., 2007; Tayebwa *et al*., 2015) and 6.7% to 89.0% for SCLE (Denis-Robichaud & Dubuc, 2015; Kelly *et al*., 2020) (Table 1). Although prevalence of endometritis is also reported for other dairy breeds, information is lacking for indigenous cattle breeds such as Ankole longhorn.

Table 1. Cow-level prevalence of clinical and subclinical endometritis in commercial dairy farms

Country	Breeds	Prevalence (%)	Dpp	Reference
Clinical endometritis				
Australia	HF	26.6	21 - 60	Okawa *et al*. (2017)
Egypt	HF	21.9	28	Hussein *et al*. (2017)
Estonia	HF	46.0	30 - 35	Jeremejeva *et al*. (2016)
Iran	HF	61.4	26 - 32	Ahmadi *et al*. (2016)
Ireland	HF	60.0	21	Ryan *et al*. (2020)
Italy	HF	7.0	28 - 35	Toni *et al*. (2015)
Japan	HF	67.8	29 - 60	Gautam *et al*. (2009)
Kenya	HF, J, AY	15.3	30 - 35	Gitonga (2010)
New Zealand	HF	69.8	33	McDougaall *et al*. (2007)
Uganda	Crossbred	3.6	60	Tayebwa *et al*. (2015)
Subclinical endometritis				
Argentina	HF	19.0	21 - 56	Rinaudo *et al*. (2017)
Australia	Crossbred	12.7	21	Pothamann *et al*. (2015)
Belgium	HF	27.8	90	Pascottini *et al*. (2017)
Canada	HF	89.0	28 - 42	Denis-Robichaud & Dubuc (2015)
Ecuador	Crossbred	50.0	35	Escandón *et al*. (2020)
Egypt	HF	29.7	28 - 42	Zyada *et al*. (2019)
Ethiopia	HF	80.0	30 - 60	Moges (2019)
Iran	HF	10.6	26 - 32	Ahmadi *et al*. (2016)
Ireland	HF	6.7	25 - 86	Kelly *et al*. (2020)
Japan	HF	60.0	27 - 40	Taniguchi *et al*. (2020)
Korea	HF	31.1	30	Chan Lee *et al*. (2018)
Turkey	Crossbred	31.3	30	Oruc *et al*. (2015)
Uganda	Crossbred	18.6	60	Tayebwa *et al*. (2015)

HF = Holstein-Friesian; J = Jersey, AY = Ayrshires, dpp = days postpartum at examination.

The mean within-herd prevalence of endometritis in commercial dairy farms varies widely, from 25.1% to 28.1% (Table 2). Herd prevalence ranged from 4.0% to 87.0% for CLE (Denis-Robichaud & Dubuc, 2015; Ryan *et al.*, 2020) and from 4.8% to 64.1% for SCLE (Chan Lee *et al.*, 2018; Cheong *et al.*, 2011). This wide range of prevalence suggests that herd-level RF exist that influence the prevalence of endometritis.

Table 2. Herd-level prevalence of clinical and subclinical endometritis in commercial dairy farms

Country	Herd prevalence (%)		Dpp	Reference
	Mean	Range		
Clinical endometritis				
New Zealand	25.1	5.0-65.0	41.0	McDougall *et al.* (2020)
Canada		4.0-29.0	35.0±7.0	Denis-Robichaud & Dubuc (2015)
Ireland		38.0-87.0	21	Ryan *et al.* (2020)
Subclinical endometritis				
New Zealand	27.1	5.0-63.6	41	McDougall *et al.* (2020)
Belgium	28.1	10.7-39.7	90	Pascottini *et al.* (2016)
Canada		13.0-64.0	35.0±7.0	Denis-Robichaud & Dubuc (2015)
New York	26.3	4.8-52.6	40-60	Cheong *et al.* (2011)
Iran		27.0-47.0	30.0±3.0	Dini *et al.* (2015)
Korea		15.7-64.1	28.3±0.1	Chan Lee *et al.* (2018)

Dpp = days postpartum at examination

In smallholder dairy herds, few studies on endometritis have been conducted, examples being in Ethiopia for SCLE (Moges & Jebar, 2012; Moges, 2015; Moges, 2019) and in Vietnam for CLE (Nguyen-Kien & Hanzen, 2017). The reported SCLE prevalence varies between 38.0% and 86.7%, whereas 19.2% was reported as CLE prevalence in smallholdings (Table 3).

Furthermore, several studies have shown that the estimated prevalence of different categories of endometritis varies widely among commercial dairy herds, from 7.2% to 35.7% for CLE only (Denis-Robichaud & Dubuc, 2015; Gobikrushanth *et al.*, 2016), 6.7% to 26.3% for SCLE only (Denis-Robichaud & Dubuc, 2015; Kelly *et al.*, 2020), and 2.6% to 23.8% for both CLE and SCLE (Gobikrushanth *et al.*, 2016; Kelly *et al.*, 2020).

Table 3. Prevalence of clinical and subclinical endometritis in smallholder dairy farms

Country	Breed	dpp	Prevalence (%)	Reference
Clinical endometritis				
Vietnam	Crossbred	21 -52	19.2	Nguyen-Kien & Hanzen (2017)
Subclinical endometritis				
Ethiopia	HF	40-60	38.0	Moges (2015)
Ethiopia	HF	28-56	46.3	Moges (2019)
Ethiopia	Crossbred	30-60	86.7	Moges & Jebar (2012)

dpp = days postpartum at examination, HF = Holstein-Friesian

These studies were mostly focused on commercial dairy herds mainly in Europe, Asia and America, and fewer in Africa. Therefore, it is more likely that smallholder zero-grazed dairy cows could be at high risk of endometritis, but empirical evidence is lacking in the literature search. The current study aimed to fill in this knowledge gap.

2.1.1 Diagnosis of endometritis

Two main approaches have been used for the diagnosis of endometritis: clinical or cytological (Kelly *et al*., 2020; Pascottini *et al*., 2017). Clinical diagnosis is based on the character of vagina discharge detectable between 21 and 90 dpp (Potter *et al*., 2010; Tayebwa *et al*., 2015). In their study, Williams *et al*. (2005) described a 4-point scoring method to classify vaginal mucus (0 = no mucus or clear mucus, 1 = clear or translucent mucus containing flecks of white pus, 2 = vaginal exudate containing white or off-white mucopurulent material, 3 = vaginal exudate containing purulent material, usually white or yellow, but occasionally sanguineous).

For cytological diagnosis, endometrial cytology is the most used technique in cattle in both field and research setups to diagnose SCLE, mainly for reasons of low cost and simplicity or easiest to achieve (Deguillaume *et al*., 2012; de Boer *et al*., 2014; Sofiane *et al*., 2020). This diagnosis is based on abnormal presence of the proportion of PMNs in endometrial cytological samples collected between 21 and 90 dpp (Melcher *et al*., 2014; Pascottini *et al*., 2017). This is because the PMN represents the first defence line and the primary white blood cell type for the elimination of infection in the uterus based on their ability for phagocytosis (Esposito *et al*., 2014; Jeon *et al*., 2015). In contrast, postpartum cows that fail to clear naturally uterine infection display continued PMNs infiltration into uterine lumen and endometrium (Chapwanya *et al*., 2012; Foley *et al*., 2015). This demonstrates that PMN is a good indicator of SCLE diagnosis criteria. Also, another advantage of PMN evaluation as an indicator for SCLE is because they are not significantly affected by the oestrous cycle stage or circulating progesterone concentration (Madoz *et al*., 2013).

The uterine body is mostly the location where the endometrial cytology sample is taken from the postpartum cow (Bicalho *et al*., 2016; de Boer *et al*., 2014; Hammson *et al*., 2006; Hartmann *et al*., 2015; Rana *et al*., 2020). According to the study of Pascottini (2016), the uterine body being the link between the uterine milieu and the external environment, enhances the sensitivity to detect endometrial inflammation since higher PMN counts were found in this region. Thus, to diagnose SCLE in dairy cows more accurately, endometrial cytology samples are recommended to be harvested from the uterine body (Pascottini, 2016; Zyda *et al*., 2019; Rana *et al*., 2020). Increased PMNs ratio in endometrial cytological samples is used as an indicator of uterine inflammation based on proportions of PMNs among the total number of endometrial epithelial cells and PMNs (Chan Lee *et al*., 2018; Zyada *et al*., 2019).

Several studies (Baranski *et al*., 2012; de Boer *et al*., 2014; McDougall *et al*., 2007; Pascottini *et al*., 2017; Pleticha *et al*., 2009) have been published on the use of different diagnostic techniques to determine cases of endometritis in dairy cows (Table 4). In their study, de Boer *et al*. (2014) and Pascottini *et al*. (2015) provided more in-depth insight into the diagnostic methods, diagnostic criteria and definitions, repeatability, and agreement among these methods for diagnosis of different categories of uterine diseases in dairy cows. The authors pointed out that the discrepancies among different diagnostic methods may indicate that those methods assess different aspects of reproductive health. This suggests that the combination of different methods would be more efficient in identifying cows with uterine diseases (Gobikrushanth *et al*., 2016; Kelly *et al*., 2020). Similarly, Šavc *et al*. (2016) found that a combination of both CLE and SCLE diagnostic techniques was better at predicting fertility outcomes than either method used in isolation or alone. Therefore, among endometritis diagnostic techniques (Table 4), Metricheck device (MED) and Cytotape (CYT) were the current practical, non-invasive, accurate, faster, easier to perform, and suitable tool to diagnose CLE and SCLE, respectively, in postpartum dairy cows (McDougall *et al*., 2007; de Boer *et al*., 2014; Pascottini *et al*., 2015; Rana *et al*., 2020).

In their study, Gobikrushanth *et al*. (2016) reported that cows that had combined category (CLE and SCLE) had an additive effect on subsequent reproductive performance compared to those that had either CLE only, SCLE only or cows without CLE and SCLE. Therefore, when CLE and SCLE are diagnosed concurrently, cows examined are classified into four categories based on their uterine health statuses: CLE only, SCLE only, both CLE and

SCLE, and cows not diagnosed to have CLE and SCLE (Denis-Robichaud & Dubuc, 2015; Gobikrushanth *et al*., 2016; Kelly *et al*., 2020). From the available studies, none of them used concurrently clinical and cytological criteria for endometritis diagnosis in zero-grazed dairy cows managed on smallholder farms. Therefore, this study will add to the empirical literature on diagnosing both CLE and SCLE simultaneously for a specific context such as Rwanda, where 92.0% of the national dairy herds are managed in smallholder zero-grazing feeding practice (Mupenzi *et al*., 2019).

Table 4. Endometritis diagnostic techniques

Endometritis	Diagnostic techniques	What is identified	Authors
Clinical endometritis	Metricheck device	Collect uterine discharge detectable in the vagina	McDougall *et al*., 2007; Pletichaet *al*., 2009; Tayebwa *et al*., 2015
	Manual vaginal examination/gloved-hand	Evaluate uterine discharge detectable in the vagina	Williams *et al*., 2005; Pleticha *et al*., 2009
	Vaginoscopy	Examination of the cervix/mucus (vaginal discharge)	Pleticha *et al*., 2009; Leutert *et al*., 2012
	Transrectal palpation of the uterus	Cervical diameter measurement, fluctuating content	Barlund *et al*., 2008; Hartmann *et al*., 2015
	Ultrasound examination	Uterine wall thickening, fluid volume, and appearance	Toni *et al*., 2015; Salah and Yimer, 2017
Subclinical endometritis	Cytotape	Collection of endometrial cytology samples for PMNs evaluation	Pascottini *et al*., 2015; Rana *et al*., 2020
	Low volume uterine lavage	Collection of uterine fluid for PMNs evaluation	Hammson *et al*., 2006; de Boeret *al*., 2014; Bicalho *et al*., 2016
	Cytobrush	Collection of uterine fluid for PMNs evaluation	Ledgard *et al*., 2015; Hartmann *et al*., 2015
	Endometrial biopsy	Endometrial tissue to build information on the tissue expression profiles	Chapwanya *et al*., 2010; Madoz *et al*., 2014
	Guarded cotton swab	Endometrial cytology samples for PMNs evaluation	Williams *et al*., 2005; Salah *et al*., 2017
	Uterine lavage sample optical density	Collection of uterine fluid for PMNs evaluation	Machado *et al*., 2012

PMNs = Polymorphonuclear inflammatory cells

2.1.2 Estimation of prevalence

Prevalence of endometritis is assessed during the postpartum period, between 21and 90 days postpartum. Clinical endometritis positive and negative cows are determined according to criteria proposed by Williams *et al*. (2005). Cows with vaginal mucus score ≥1 are recorded positive for CLE, otherwise are negative. The CLE prevalence is computed as the number of CLE positive cows divided by the total number of cows enrolled in the study times 100 (Okawa *et al*., 2017; Ryan *et al*., 2020; Williams *et al*., 2005). The estimated CLE prevalence suggests large variability in commercial and smallholder dairy herds, from 3.6% to 69.8% (McDougall *et al*., 2007; Nguyen-Kien & Hanzen, 2017; Tayebwa *et al*., 2015). The large variability in CLE prevalence can be associated with different postpartum periods at sampling and the magnitude of RF at cow- and herd- levels. However, these RF are relevant in the smallholder herds, and they could predispose dairy cows to CLE, but in the literature search, no attention was paid to the CLE in smallholder zero-grazed dairy herds. The current study was set to fill in this knowledge gap.

Diagnosis of SCLE relies on microscopic evaluation of the endometrial cytological samples by counting 300 cells (endometrial cells and PMNs) per slide and calculation of the proportion of PMNs (Dini *et al*., 2015; Melcher *et al*., 2014; Zyda *et al*., 2019). In general, a threshold of ≥5% PMNs was reported to be used for all cows between 21 and 62 dpp as an appropriate value for the diagnosis of SCLE when the applied method counts 300 cells per endometrial cytology slide (Chan Lee *et al*., 2018; Madoz *et al*., 2013; Melcher *et al*., 2014; Okawa *et al*., 2017). The SCLE prevalence is estimated as SCLE positive cows divided by the total number of cows enrolled in the study times 100 (Kelly *et al*., 2020; Pascottini *et al*., 2017). The prevalence of SCLE shows a wide variation (6.7% to 89.0%) in the reported cow-level prevalence of SCLE in commercial dairy herds (Denis-Robichaud & Dubuc, 2015; Kelly *et al*., 2020), and 38.0% to 86.7% in smallholder dairy herds (Moges, 2015; Moges, 2019). This disparity of SCLE prevalence among studies may be due to differences in the sampling time after calving, presence of RF, proportion PMNs above which an endometrial cytology sample was considered positive for SCLE, and the diagnostic technique used (Arias *et al*., 2018). However, to the best of our knowledge, no study exists about the prevalence of SCLE in zero-grazed dairy cows managed on smallholder farms. Thus, the present study aimed to fill in this knowledge gap.

2.2 Risk factors for endometritis

Endometritis is considered to be a multifactorial disease with many RF having a direct and/or indirect, determining, or predisposing influence (Potter *et al*., 2010). The disease disrupts fertility performance and reduces the productivity and profitability of the dairy herd (Chan Lee *et al*., 2018; Juan Piñeiro, 2016; Mohammed *et al*., 2019). Maintaining uterine health is essential for the creation of a uterine environment that is favourable for a successful pregnancy. Unfortunately, dairy cows are predisposed to endometritis during the transition period due to a wide variety of RF (Bohlen & Widener, 2019). Most studies (Adnane *et al*., 2017; Cheong *et al*., 2011; Giuliodori *et al*., 2017; Kelly *et al*., 2020; Potter *et al*., 2010) have identified some of the RF and found that they vary among different regions or countries because of the differences in general management, environmental and herd health control conditions. This implies a large variation among herds in the prevalence of endometritis (Moges, 2019; Ryan *et al*., 2020; Tayebwa *et al*., 2015).

The risk factors are classified at cow- and herd- levels, but all of them make endometritis more severe and frequent in postpartum cows (Adnane *et al*., 2017; Cheong *et al*., 2011). Most of these risks appear to be associated with the trauma of the female reproductive tract, the first exposure of the uterus to micro-organisms, human interventions, and disruption of the physical barriers of the vulva, vagina and cervix (Foley *et al*., 2015; Vieira-Neto *et al*., 2016). These conditions provide an opportunity for microorganisms to ascend female reproductive tract and increase the risk of endometritis occurrence (Giuliodori *et al*., 2017; Kelly *et al*., 2020). This is because the bacteria responsible for endometritis, such as *Escherichia coli*, *Trueperella pyogenes, Prevotella melaninogenicus, Dermacoccus spp, Enterococcus faecalis,* and *Fusobacterium necrophorum* are derived from the environment that cows are kept or from the cow's faeces, skin and vagina (Adnane *et al*., 2017; Appiah *et al*., 2020; Ricci *et al*., 2015; Sheldon *et al*., 2020). Other risk factors are associated with calving stress, mobilising tremendous energy reserves to support the change from a non-lactating state to peak milk production and inadequate feeding practices that gradually decrease the efficiency of the PMN function, including reduced killing capacity (Galvão *et al*., 2010). All these challenges after parturition make the cows more vulnerable to develop uterine diseases (Cunha *et al*., 2016; Esposito *et al*., 2014; Toni *et al*., 2015).

2.2.1 Cow-level risk factors

Cow-level risk factors are specific variable characteristics of the individual cow in the same herd (Adnane *et al*., 2017). These include cow parity, body condition score, twin births, dystocia, milk fever, ketosis, left displaced abomasum, age of cow, cow breed, calf sex, retained placenta, stillbirth, gestation length, calving season, breeding services, days dry, mastitis and brucellosis (Adnane *et al*., 2017; Cheong *et al*., 2011; Cunha *et al*., 2016; Kelly *et al*., 2020; Toni *et al*., 2015). These findings are meaningful to demonstrate that cow-level risk factors have been generally reported in commercial dairy farming systems. However, these risks are relevant in smallholder zero-grazing herds, and there could predispose dairy cows to endometritis, but the literature search did not yield similar studies. This demonstrates that smallholder dairy farmers are likely to incur considerable economic losses from endometritis because of lack of knowledge about cow-level RF involved in the occurrence of the disease for their prevention and control.

2.2.2 Herd-level risk factors

Herd-level RFs constitute all environmental conditions and management characteristics shared by cows in the same herd (Adnane *et al*., 2017). Because most previous studies (Adnane *et al*., 2017; Chan Lee *et al*., 2018; Kelly *et al*., 2020) have been focused on the cow-level RFs, the literature on herd-level RF associated with endometritis occurrence, however, is rather scarce. The examples are the study of Cheong *et al*. (2011) in Ithaca, United States of America, and Moges & Jebar (2012) in Ethiopia. The herd-level RFs reported by these authors include bedding materials, hygiene in the cowshed, housing of cows within the first 30 dpp, herd size, calving pen, and farm size. Furthermore, the limited information on herd-level RFs for endometritis was explained to be a consequence of the difficulty in obtaining data from enough herds to reach valid conclusions (Cheong *et al*., 2011). This suggests limited knowledge on the contribution of herd-level RFs to endometritis occurrence despite the economic losses that are substantial in herds with a high prevalence of endometritis (Galvão, 2012). From the literature search, there is no information available on the RF for endometritis in smallholder zero-grazed dairy cows in sub-Saharan African countries, specifically in Rwanda. Moreover, the implementation of MIs targeting cow-and herd-levels RF can result in better management of endometritis and thus increase fertility and production performance of dairy cows (Daros *et al*., 2017; Sheldon *et al*., 2020).

2.2.3 Estimation of risk factors

Different authors (Chan Lee *et al*., 2018; Giuliodori *et al*., 2017; Pascottini *et al*., 2017; Tayebwa *et al*., 2015) used a cross-sectional study to determine prevalence and RFs for endometritis with cows followed in a prospective observational study to determine reproductive outcomes. An observational study is used to identify RFs and to estimate the quantitative effects of various component causes that contribute to the occurrence of disease (Thrusfield & Christley, 2018). The investigations are typically based on analysis of disease occurrence in populations by comparing groups of individuals with respect to disease occurrence and exposure to hypothesised RFs. A cross-sectional study is a type of observational study, which classifies animals into those with and without the disease, and those exposed and unexposed to hypothesised risk factors, at the specified time of diagnosis. Therefore, they each generate a two by two contingency table for each factor/disease relationship, and prevalence is, therefore, calculated. Prospective observational is looking forward to the development of disease in the sample of animals exposed to the hypothesised RFs and quantify the outcome (Thrusfield & Christley, 2018).

For analysis of putative RFs, conventional logistic regression analysis is used to determine the significance and strength of association between the probability of disease and the presence (or absence) of one or more RFs (Dohoo *et al*., 2003; Thrusfield & Christley, 2018). Several limitations have been associated with this analysis method. First, it includes only the direct associations between the disease and individual risk factors without considering its relationship with other RFs. Second, it does not allow the model builder to make use of available a-priori information regarding known or plausible asymmetric relationships (Goldsmith, 1977). Therefore, a statistical analysis technique that overcomes these analysis deficiencies is path analysis model. Use of path analysis model has several advantages. It allows the model builder to specify hypothesised interrelationships among variables, including direct and indirect causal associations (Correa *et al*., 1993; Erb *et al*., 1985; Li *et al*., 2018; Rugoor *et al*., 1997). In path analysis models, indirect as well as direct associations can be observed. The path analysis model is often used to quantify the magnitudes of the cause and effect relationship between the variables as well as the mediated relationship of one or more variables for the other contributing variables by systematically inter-connected linear regressions (Dohoo *et al*., 2003; Neo *et al*., 2017; Rougoor *et al*., 2000). This has practical implications because information regarding the direct and indirect relationships among risk factors and between risk factors and the disease under study could be used to develop recommendations in herd health programs and the evaluation of disease control program (Correa *et al*., 1993).

The path analysis model approach has been used in a range of different disciplines and contexts to model the interrelationships of postpartum diseases within 30 dpp in livestock disease management (Correa *et al*., 1993); sexually transmitted diseases in animals (Lockhart *et al*., 1996); relationships between technical, economic and environmental effects on the level of individual dairy farms in dairy production management (Rugoor *et al*., 2000); effects of periparturient diseases on the reproductive performance of dairy cows (McDougall, 2001); pathological disease associated with fronto-striatal disconnection in human health (Cilia *et al*., 2011); and in an environmental context it has been used to quantify the contribution and interaction of different factors on the natural attenuation of sulfamethoxazole (Li *et al*., 2018). Despite these applications and advantages, the literature search revealed no application of the path analysis model to risk factors associated with endometritis. Therefore, the present study proposes to fill in this knowledge gap with an empirical study of the risk factors analysed using a path analysis model. This should inform management interventions to target high-risk factors for CLE and SCLE cases.

2.3 Influence of endometritis on reproductive and production performance

In their study, Gröhn *et al*. (2003) reported that diseases mainly affect dairy cow performance in three ways: (i) by reducing reproductive performance, (ii) by shortening the expected length of productive life through increased culling rate, and (iii) by lowering milk yield. Referring to this literature, several studies have shown that female genital tract diseases of which endometritis is of importance affect the reproductive performance significantly in general (Bromfield *et al*., 2015; Chan Lee *et al*., 2018; Ryan *et al*., 2020) and overall productivity of dairy cows by reducing milk yield (Juan Piñeiro, 2016; Sharama *et al*., 2019).

2.3.1 Influence of endometritis on reproductive performance

Optimal fertility performance in dairy cows is one key component to achieve a successful and economically efficient of a dairy farming system. This necessitates a resumption of ovarian cycles within 25 dpp and conception within 85 dpp to achieve a calving interval of 365 days (Noakes *et al*., 2001). Ensuring this optimal performance involves paying attention to many factors, among them uterine health in the postpartum period (Pascottini, 2016). It well known that uterine environment that is not healthy disrupts the uterine homeostasis (Bromfield *et al*., 2015) and significantly decreases the reproductive performance of the cow (Gilbert *et al*., 2005; Kasimanickam *et al*., 2004). Poor reproductive performance in dairy cows affects the genetic improvement and financial sustainability of the herd (Muller *et al*., 2018). It decreases the profit margin due to loss in milk yield, cost of replacing culled cows, increased veterinary costs, decreased calf sales per cow, and costs of heifer replacement in the herd (Ghavi Hossein-Zadeh, 2013; Inchaisri *et al*., 2010).

The mechanism by which endometritis adversely affects fertility performance in positive cows can take place in many ways: (i) through a delay in the resumption of ovarian cyclicity after parturition (Chaudhari *et al*., 2017; Salehi *et al*., 2016); (ii) through ovarian dysregulation resulting in reduced oocyte quality or in production of smaller corpora lutea with lower circulating concentrations of progesterone and this low progesterone prevents the uterine endometrium from properly preparing for a developing embryo, which will inhibit successful pregnancy even if fertilization occurs (Bohlen & Widener, 2019; Burke *et al*., 2010; Williams, 2013); (iii) through the disruption of the uterine environment to support embryonic development and implantation (Bromfield *et al*., 2015; Dickson *et al*., 2019; Drillich & Wagener, 2018); (iv) through disruption in the production of prostaglandin F2 alpha and prostaglandin E2 by endometrium by increasing the production of the latter relative to the former (Bidne *et al*., 2018; Bohlen & Widener, 2019); and (v) through an impairment of embryo development (Mokhtari *et al*., 2015; Sheldon & Owens, 2017).

Numerous indicators such as days to first oestrus (DFO), days to first natural mating or AI service (DFMA), days-not pregnant (DNP), anoestrus postpartum (ANPP), conception rate at first natural mating or AI service (CRFMA), conception rate to all services (CRAS), cows pregnant in a given period and number of services (bull or artificial insemination) per conception (NSC) are used to measure reproductive performance in dairy cows (Noakes *et al*., 2001). However, the results of previous studies indicate that these reproductive performance indicators are impaired in cows positive for endometritis when compared to negative ones (Table 5). The substantial impairment of reproductive performance of cows positive for endometritis is reflected in the extension of DFO and DNP (Hussein *et al*., 2017; Tayebwa *et al*., 2015; Wijayanto *et al*., 2019); in the reduction in the overall proportion of cows pregnant (Chan Lee *et al*., 2018), delayed DFMA (Sharma *et al*., 2018a), reduced CRFMA (Chaudhari *et al*., 2017; Pascottini *et al*., 2016) and CRAS (Marques *et al*., 2015; Pascottini *et al*., 2017), greater NSC (Hussein *et al*., 2017; Ryan *et al*., 2020), and a greater proportion of ANPP cows (Chan Lee *et al*., 2018; Tayebwa *et al*., 2015).

Table 5. Effects of endometritis on reproductive performance parameters

Measures of reproductive performance	Endometritis		Country	Reference
	Positive	**Negative**		
Days to first oestrus (days)	89.2±12.1	59.5±3.3	Egypt	Hussein *et al*. (2017)
	76.7±5.2	57.8±3.4	India	Chaudhari *et al*. (2017)
	51.3±8.9	49.8±6.6	Indonesia	Wijayanto *et al*. (2019)
	66.9±5.6	57.4±2.3	Iran	Ahmadi *et al*. (2016)
Days to first natural mating or AI service(days)	76.2±3.6	65.9±2.6	Egypt	Hussein *et al*. (2017)
	107.7±7.9	89.6±4.4	India	Sharma *et al*. (2018a)
	89.3±32.9	82.6±36.0	Japan	Okawa *et al*. (2017)
	221.8±91.1	133.3±82.5	Uganda	Tayebwa *et al*. (2015)
Days-not pregnant (Interval calving to conception) (days)	169.4±96.0	149.9±68.5	Brazil	Marques *et al*. (2015)
	210.9±21.8	109.4±12.6	Egypt	Hussein *et al*. (2017)
	121.7±10.9	101.3±5.9	India	Sharma *et al*. (2018a)
	184.8±34.6	163.3±24.3	Indonesia	Wijayanto *et al*. (2019)
	160.6±4.3	141.2±3.3	Korea	Chan Lee *et al*. (2018)
	109.0±9.8	100.2±10.2	Ireland	Ryan *et al*. (2020)
	155.3±61.3	129.5±76.7	Japan	Okawa *et al*. (2017)
	224.7±89.5	138.7±79.5	Uganda	Tayebwa *et al*. (2015)
	249.0±114.0	216.0±115.0	Vietnam	Nguyen-Kien *et al*., (2017)
Number of services per conception(number)	4.1±0.8	1.9±0.3	Egypt	Hussein *et al*. (2017)
	4.2±0.4	2.2±0.3	India	Chaudhari *et al*. (2017)
	3.8±0.9	3.6±0.8	Indonesia	Wijayanto *et al*. (2019)
	1.8±0.1	1.5±0.2	Ireland	Ryan *et al*. (2020)
	2.8±1.8	2.0±1.3	Japan	Okawa *et al*. (2017)
	4.7±2.7	3.9±2.5	Vietnam	Nguyen-Kien *et al*., (2017)
Anoestrous postpartum (%)	67.4	53.8	Korea	Chan Lee *et al*. (2018)
	22.8	12.8	Uganda	Tayebwa *et al*. (2015)
	32.7	47.0	Belgium	Pascottini *et al*. (2016)
	8.3	33.3	India	Chaudhari *et al*. (2017)

Conception rate at first natural mating or AI service(%)	25.0	41.1	Iran	Ahmadi *et al*. (2016)
	31.0	47.6	Japan	Okawa *et al*. (2017)
	26.3	35.5	Korea	Chan Lee *et al*. (2018)
	11.8	31.2	Uganda	Tayebwa *et al*. (2015)
	7.5	17.0	Vietnam	Nguyen-Kien *et al*. (2017)
Conception rate to all services (%)	38.5	62.8	Belgium	Pascottini *et al*. (2017)
	15.6	37.3	Brazil	Marques *et al*. (2015)
	33.3	45.1	Iran	Ahmadi *et al*. (2016)
	21.0	26.0	Vietnam	Nguyen-Kien *et al*. (2017)

Although many studies have reported different prevalence of endometritis categories when CLE and SCLE were diagnosed simultaneously, few of them have quantified the effect of those categories on subsequent reproductive performance in dairy cows (Denis-Robichaud & Dubuc, 2015; Gobikrushanth *et al*., 2016). For instance, Denis-Robichaud & Dubuc (2015) reported that the conception rate at first service was higher in healthy cows (35.0%) compared to cows that had SCLE only (16.3%) and CLE only (15.0%). Similarly, Gobikrushanth *et al*. (2016) reported that reproductive performance differs among categories of endometritis; cows that had both CLE and SCLE, and SCLE only were 4 and 3 times less likely to conceive to first service, respectively, compared to healthy cows. Cows that had CLE only (hazard ratio of 0.65), SCLE only (hazard ratio = 0.70) and both CLE and SCLE (hazard ratio = 0.48) had decreased likelihood of pregnancy at 250 dpp compared to healthy cows.

Despite the advances in evaluating the effect of endometritis on subsequent fertility performance of dairy cows managed on commercial farms, there are knowledge gaps on influence of endometritis on the subsequent reproductive performance of zero-grazed cows on smallholder dairy farming to inform targeted management interventions. This study was set to fill in knowledge gaps.

2.3.2 Influence of endometritis on milk yield

In dairy herds, different studies (Bell & Roberts, 2007; Sharma *et al*., 2019; Sheldon *et al*., 2009) have reported that each case of postpartum uterine infection represents a significant milk production loss to the farmer. The potential production impact may result from a decrease in milk yield, milk loss due to treatment and reduced saleable milk due to withdrawal periods for milk (Burke *et al*., 2010; McDougall *et al*., 2011; Sharma *et al*., 2019). Several statistical approaches have been used to evaluate milk losses from diseases (Fourichon *et al*., 1999), but a basic method is to compare MY from cows with a specific disease with those without the disease (Angara & Elfadil, 2014; Can *et al*., 2016; Sharma *et al*., 2019).

Few studies on the effect of endometritis on MY in commercial dairy farms have been carried out. For instance, in India (Sharma *et al*., 2019), New Zealand (McDougall *et al*., 2011), and the United States of America (Juan Piñeiro, 2016). These studies estimated a decrease in milk yield between 0.6 and 2.4 litres/cow/day in cows positive for endometritis compared to cows negative for endometritis. However, in Rwanda and elsewhere in Sub-Saharan Africa, there have been no studies carried out in smallholder dairy herds to investigate the milk production loss that results from endometritis. Therefore, this study was set to fill in this knowledge gap.

2.4 Effect of endometritis on milk quality and safety

Endometritis is one of the most prevalent uterine diseases in the postpartum period and is associated with milk hygiene and quality among dairy cattle. Endometritis influences the total milk output, modifies milk composition and results in milk residues after treatment in positive cows (Burke *et al*., 2010; Yijun *et al*., 2006). In cows, the somatic cell count is an important component of milk in terms of quality and hygiene. Elevated milk somatic cell count is associated with altered protein quality, change in fatty acid composition, lactose, ion and mineral concentration, increased enzymatic activity, and a higher pH of raw milk (Ogola *et al*., 2007). In their study, Burke *et al*. (2010) reported that endometritis was associated with lower magnesium and protein. Magnesium was lower in endometritis positive cows (0.73±0.03 ml/L) than in negative ones (0.86±0.03 ml/L). Similarly, milk of endometritis negative cows had a higher protein percentage (4.3±0.1%) compared with endometritis positive cows (3.9±0.1%). Furthermore, Yijun *et al*. (2006) observed that milk that had drug's residues after treatment of endometritis using antibacterial therapy that includes oxytetracycline, chlortetracycline hydrochloride or gentamicin were not fermented normally. This implies that prudent conventional therapy use is of tremendous importance because each application of an antimicrobial drug implies a risk of contributing to the multiple resistances in pathogenic microbes and drug residues in milk and meat (Sharma *et al*., 2018b), which presents a challenge for public health (Oliver *et al*., 2005; Rana *et al*., 2019; Shaikh & Patil, 2020; Welch *et al*., 2007). Further studies on the effect of endometritis on milk quality and safety are required.

In summary, this study has made original contribution to the existing knowledge by (i) designing prospective observational study of clinical and subclinical endometritis under existing smallholder farms management conditions, otherwise studied under controlled experimental conditions, (ii) how to effectively diagnose clinical and subclinical endometritis using Metricheck device to detect presence and type of vaginal mucus and Cytotape to examine cows for subclinical endometritis on basis of polymorphonuclear inflammatory cells in the endometrial cytological samples collected between 21 and 60 days postpartum, (iii) application of Best-Worst Scaling choice method to identify management interventions that farmers consider effective for control and managing clinical and subclinical endometritis, (iv) application of path analysis model with logistic regression analysis to identify and quantify individual risk factors as being in direct and/or indirect association with clinical and subclinical endometritis cases, and (v) how to quantify milk loss and attribute the loss to decrease in milk yield and use of different conventional veterinary drugs that have withholding times for milk and long administration frequency.

2.5 Conceptual framework

Figure 1 illustrates the conceptual framework of the hypothesised relationships informing the objectives of this study. It was hypothesised that the cow- and herd-levels risk factors influence the occurrence of endometritis. In turn, the disease has some influence on milk yield and impairs subsequent fertility performance of dairy cows managed on smallholder zero-grazed farms.

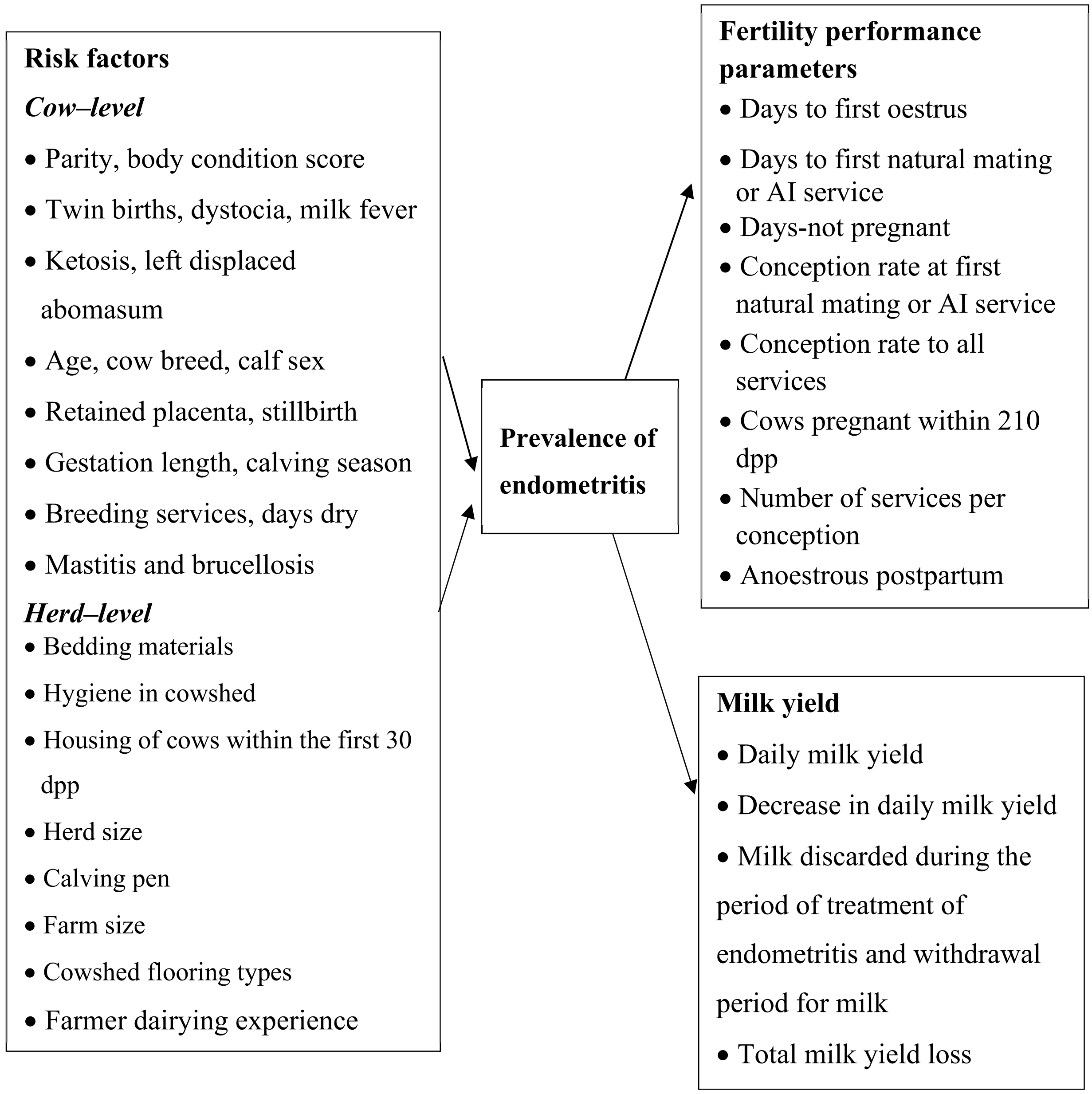


Figure 1. Conceptual framework of the hypothesised relationships in the study

CHAPTER THREE
ESTIMATING PREVALENCE OF ENDOMETRITIS IN SMALLHOLDER ZERO-GRAZED DAIRY COWS IN RWANDA

Abstract

Endometritis is a postpartum uterine disease of cows that interrupts reproductive cycles resulting in suboptimal fertility, reduced performance, and profitability of the dairy herd. The objective of the study was to estimate the perceived and observed prevalence of endometritis among zero-grazed dairy cows in smallholder farms in Rwanda. An exponential non-discriminative snowball sampling method was applied in a cross-sectional survey to obtain data from 370 farms on 466 cows within their 21 - 60 days postpartum (dpp). The survey, conducted from September 2018 to March 2019, concurrently examined cows using the Metricheck device (MED) to determine the presence and type of vaginal mucus (VMC) based on a score scale of 0 to 3. Cows scoring VMC≥1 were recorded clinical endometritis (CLE) positive. Cytotape (CYT) was used to examine cows for subclinical endometritis (SCLE). Cows with ≥5% polymorphonuclear inflammatory cells (PMN) in the endometrial cytological samples were recorded SCLE positive, whereas cows with VMC-0 and <5% PMNs were considered as not diagnosed to have CLE and SCLE. At cow-level, overall endometritis prevalence was 70.2% with 67.2% CLE and 31.8% SCLE while at the herd-level, overall prevalence was 71.1% with 68.1% CLE and 34.4% SCLE. The differences between the diagnostic performance of the MED and CYT were significant (p<0.001). Perceived prevalence by farmers was much lower (3.2%) and without agreement with the observed prevalence (Kappa = -0.02, p>0.05). The high-observed prevalence and farmer underestimation of endometritis prevalence indicate knowledge gaps about endometritis. The extension services, therefore, need to increase awareness and education among smallholder farmers about the detection and management of endometritis.

3.1 Introduction

Endometritis is a prevalent disease in postpartum cows that may occur in the form of CLE and/or SCLE. The disease can lead to substantial economic losses in a dairy herd (Dubuc *et al*., 2010). The CLE is characterised by the presence of mucopurulent or purulent discharge detectable in the vagina 21 - 90 dpp (Potter *et al*., 2010; Tayebwa *et al*., 2015). The CLE may be diagnosed using the MED (McDougall *et al*., 2007); vaginoscopy (Leutert *et al*., 2012), ultrasound (Toni *et al*., 2015), gloved-hand (Pleticha *et al*., 2009), or transrectal palpation (Hartmann *et al*., 2015). In contrast, the SCLE is characterised by the abnormal presence of the proportion of PMNs in endometrial cytology samples collected between 21st and 90th dpp (Kasimanickam *et al*., 2005; Pascottini *et al*., 2017). The diagnostic techniques of SCLE include CYT (Pascottini *et al*., 2015), cytobrush (Madoz *et al*., 2014), biopsy technique (Madoz *et al*., 2014), guarded cotton swab (Salah *et al*., 2017), or low volume uterine lavage (de Boer *et al*., 2014).

Both CLE and SCLE have been diagnosed separately between 21 - 90 dpp (Pascottini *et al*., 2017; Potter *et al*., 2010), and in most SCLE studies, CLE was used as an exclusion criterion for cows enrolled in the study (de Boer *et al*., 2014; Kasimanickam *et al*., 2005). Therefore, few studies have considered diagnosing both CLE and SCLE concurrently**,** yet this would provide better accuracy in identifying cows with endometrium inflammatory diseases (Denis-Robichaud & Dubuc, 2015; Kelly *et al*., 2020). This is because cows can be affected either by CLE only, SCLE only, or both CLE and SCLE (Dubuc *et al*., 2010; Gobikrushanth *et al*., 2016).

In developed countries, previous studies on endometritis were conducted in commercial farms where the Holstein-Friesian breed dominated. They report large variability in cow-level prevalence rates of endometritis, for instance, 6.7% to 89.0% for SCLE (Denis-Robichaud & Dubuc, 2015; Kelly *et al*., 2020) and 7.0% to 69.8% for CLE (McDougall *et al*., 2007; Toni *et al*., 2015). Within-herd level, prevalence ranges from 4.0% to 87.0% for CLE (Denis-Robichaud & Dubuc, 2015; Ryan *et al*., 2020), and from 4.8% to 64.1% for SCLE (Chan Lee *et al*., 2018; Cheong *et al*., 2011). Similarly, prevalence rates of different uterine disease statuses can vary widely, from 7.2% to 35.7% for CLE only (Denis-Robichaud & Dubuc, 2015; Gobikrushanth *et al*., 2016), from 6.7% to 26.3% for SCLE only (Denis-Robichaud & Dubuc, 2015; Kelly *et al*., 2020), and from 2.6% to23.8% for both CLE and SCLE (Gobikrushanth *et al*., 2016; Kelly *et al*., 2020).

Despite these several reports on the prevalence of CLE and SCLE for industrial dairy systems, few studies on CLE and SCLE in Sub-Saharan Africa have been conducted on commercial and smallholder dairy farms. Examples being Egypt (Hussein *et al*., 2017); Ethiopia **(**Jebar & Moges, 2012; Moges, 2019); Kenya (Gitonga, 2010); and Uganda (Tayebwa *et al*., 2015). In smallholder dairy herds, the reported SCLE prevalence rates range between 38.0% and 86.7% (Moges & Jebar, 2012; Moges, 2015), which suggest a high prevalence of SCLE in dairy cows. In contrast, the prevalence of CLE in the smallholder herds of Sub-Saharan Africa countries is lacking in the literature search.

In their study, Tayebwa *et al*. (2015) reported that farmers are likely to underestimate the disease in their dairy herds because they have limited knowledge and skills to diagnose the disease. This implies that farmers are unlikely to implement appropriate prevention measures and targeted treatment regime for the disease (Wolff *et al*., 2019). Despite this, farmer perception of endometritis cases in their herds has received little attention. Their perception indicates the level of awareness and interventions they would likely implement (John Christy & Kothandaraman, 2017; Sheldon *et al*., 2019). An exception is a study by Langford *et al*. (2009) who reported a perceived prevalence of 10.8% in non-organic farms and 6.1% in organic farms in the United Kingdom. This observation would suggest limited knowledge of endometritis among farmers, which implies attention to managing the disease is likely limited even in the industrial dairy herds (Paul *et al*., 2017; Subhash *et al*., 2012).

Rwanda is not any different regarding studies of endometritis in smallholder dairy herds. The country has about 92.0% of the smallholder dairy herds managed under zero-grazing housing units, and fertility performance is typically suboptimal (Bishop & Pfeiffer, 2008; Rukundo *et al*., 2018). However, the reported suboptimal fertility may be linked to endometritis, but empirical evidence is non-existent to inform targeted interventions. These zero-grazed dairy cows could be exposed to prevalent endometritis. This study is pioneering in Rwanda a simultaneous diagnosis study of CLE and SCLE, to fill knowledge gaps on perceived and observed prevalence of endometritis in smallholder zero-grazed dairy cows.

3.2 Materials and Methods

3.2.1 Data source

Data were collected from smallholder dairy farms with zero-grazed cows predominantly on cut- and-carry feeding systems with natural grasses, crop residues, banana (stems and leaves), and Napier grass (*Pennisetum purpureum*). All the farms were located in Gasabo district of Rwanda (1°52'S, 30°06'E) (Figure 2), at an altitude of 1800-meter above sea level, with an annual mean temperature of 22°C and a bimodal rainfall pattern that averages 1000 mm annually (RLMP, 2017). The total cattle population in the study area is about 12,414 comprising dairy crossbreds (52.0%), indigenous cattle breeds (29.0%), and pure dairy breeds (19.0%) (NISR, 2018).

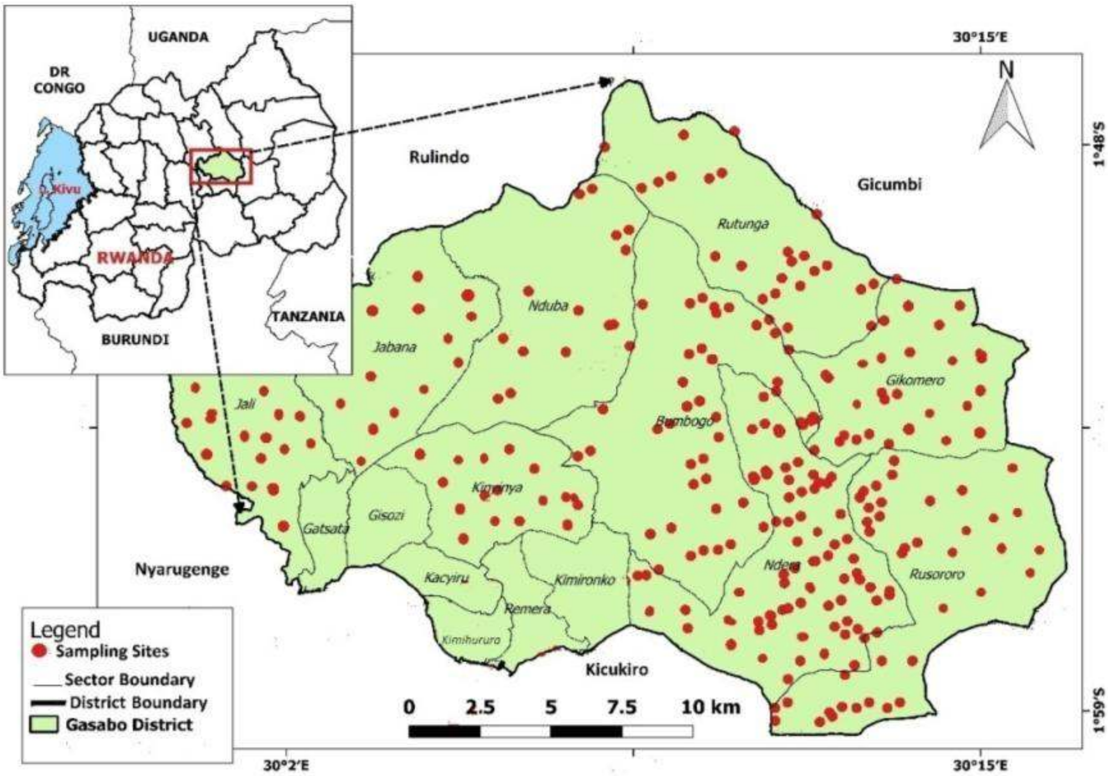


Figure 2. Map showing the study area and sampling sites. During data collection, Global Positioning System (GPS) data were collected on the location of each farmer's household using GPS eTrex 10 Garmin. Quantum GIS version 2.18.20-Las Palmas software was used to produce the map depicted in Figure 2 based on the GPS data.

The district was chosen because of more prevalent dairy zero-grazing farms than the other districts (MINAGRI, 2013). Under smallholder dairy zero-grazing conditions, muddy conditions are prevailing, hygienic standards are low, herd health management plan is absent (Ndahetuye *et al*., 2019), risk exposure to bacterial disease infections is high, and the likelihood of endometritis is high (Dutta *et al*., 2021; Sheldon & Owens, 2017).

The minimum sample size was determined according to Thrusfield (2007) as follows:

$$n = \frac{Z^2\,P\,(1-P)}{d^2}$$

Where$n$ is a sample size, Z is the value at 95% confidence level = 1.96, P is the expected prevalence of endometritis (47.9%) (Denis-Robichaud & Dubuc, 2015; Zobel, 2013). From the literature, the prevalence of CLE varies from 3.6 to 69.8%, while that of SCLE varies from 6.7 to 89.0%. Based on these prevalence estimates, the highest median value estimate of 47.9% was used to compute the needed sample size because the research interest was on both clinical and subclinical endometritis, d is precision level set at 0.05 for a 95% confidence interval. Thus, the minimum sample size was 384 dairy cows within their 21 - 60 dpp.

A cross-sectional study was conducted between September 2018 and March 2019 in 370 smallholder dairy farms recruited through exponential non-discriminative snowball sampling (i.e., sampling technique where existing study subjects recruit future subjects from among their acquaintances). This is because it was difficult to locate the smallholder zero-grazed dairy farms with the target criteria of the study and the choice of new farm depends on inclusion criteria defined in this study (Babbie, 2013). Identification and access to the initial farms were facilitated by officers of sector animal resources who provided at least two farms of which the researchers visited and asked for the next other farms in the neighbourhood. The two farms had to fit defined study criteria: (i) presence of at least one cow within 21 - 60dpp; (ii) the willingness of the farmer to participate in the study; and (iii) physical accessibility of the farm. For each recruited farmer, informed written consent was sought before engaging in the study activities. Four hundred sixty-six (466) dairy cows within 21 - 60 dpp at sampling were enrolled in the study. The breed distribution of the sample cows was 66.3% dairy crossbreds, 17.0% dairy pure breeds, and 16.7% indigenous cattle. The socio-demographic characteristics of each participating farm and farmer were recorded through a pre-tested structured questionnaire.

Before the examination of cows, an explanation of clinical signs of endometritis was presented to farmers and picture illustrations of endometritis signs (Figure 3) were used to complement, especially where farm records were not available and farmers' recall not possible. This was practiced to help farmers to estimate the number of cows positive for endometritis recognised in their herds in the past one year and on the day of the visit. Clinical signs of endometritis are the following: (i or C)white or whitish-yellow mucopurulent vaginal discharge comes out when the animal lies down, urinates, or defecates and visible externally on the tail, perineum, and vulva; (ii or A and B) mucopurulent or purulent discharge at the time of oestrus and visible externally on the tail, perineum and vulva, (iii) repeat breeding, and (iv) abortion (Abdullah *et al*., 2015; Kumar, 2009;). Therefore, using endometritis signs (i) and (ii), each farmer visited was asked how many endometritis positive cows were in her/his herd on the day of the visit, which informs about farmer perceived prevalence of endometritis. In contrast, using endometritis signs (i), (ii), (iii), and (iv), each farmer was asked the frequency of observation of each sign in cows within the herd for the past one year.

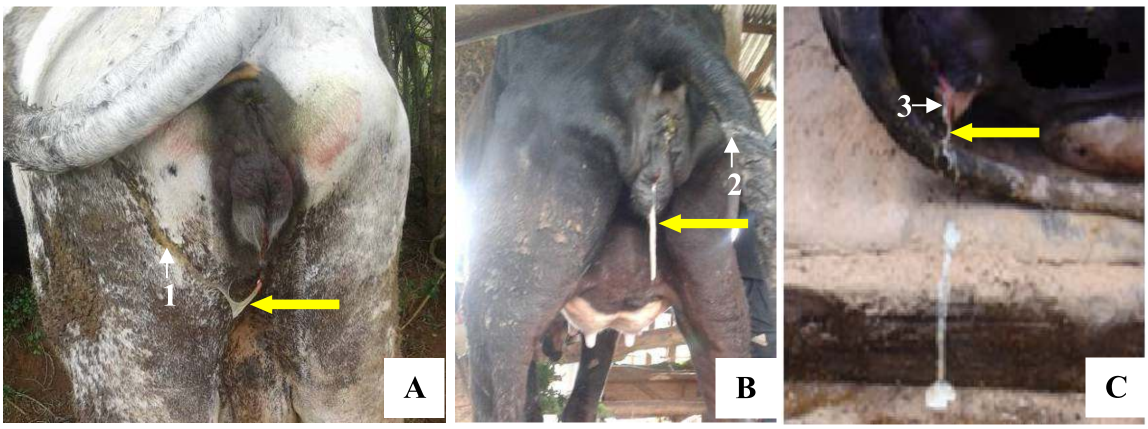


Figure 3. Some of the clinical signs of endometritis recognised by the farmers in cows in their herds. The yellow arrows indicate pus discharging from the vulva: Mucopurulent **(A)** or purulent discharge at the time of oestrus **(B)**, and white or whitish-yellow mucopurulent vaginal discharge comes out when the animal lies down**(C)**. These signs are visible externally on the perineum **(1)**, tail **(2)**, and vulva **(3)**.

3.2.2 Evaluation of vaginal mucus

All cows were assessed for the presence and type of VMC at 38.5±0.7 (median 35.0) dpp using MED (Metricheck, Simcro Limited, Hamilton, New Zealand) (McDougall *et al*., 2007). Briefly, after proper restraining the cow, the cow's vulva was washed with clean water, disinfected with 1.0% potassium permanganate solution and dried with a dry paper towel. Before inserting the MED into the vagina, the outside surface of the rubber examination cup was lubricated. The MED was carefully inserted into the vagina through the vulva and up to the luminal space around the cervix, after which the MED was slowly removed while raising the handle end slightly so the examination cup contacts the floor of the vagina and not to lose any VMC. The VMC was visualised within the examination cup's concave surface (Figure 4). Only one person gave the VMC score interpretation to reduce the error that may occur when many people were involved**.** The retrieved VMC were examined and scored on a 0 to 3 scale (Williams *et al*., 2005**)**: absence or clear mucus (VMC-0); clear mucus with flecks of pus (VMC-1); mucopurulent (VMC-2); or purulent white-coloured or sanguineous and fetid (VMC-3). Cows that had VMC score of ≥1 were recorded as CLE positive based on previously published work (Okawa *et al*., 2017; Ryan *et al*., 2020; Williams *et al*., 2005); otherwise recorded CLE negative, irrespective of the endometrial cytology result. A herd was considered positive for CLE if had at least one cow positive for CLE. The MED was cleaned and disinfected before testing each cow by rinsing it in a 1.0% potassium permanganate solution.

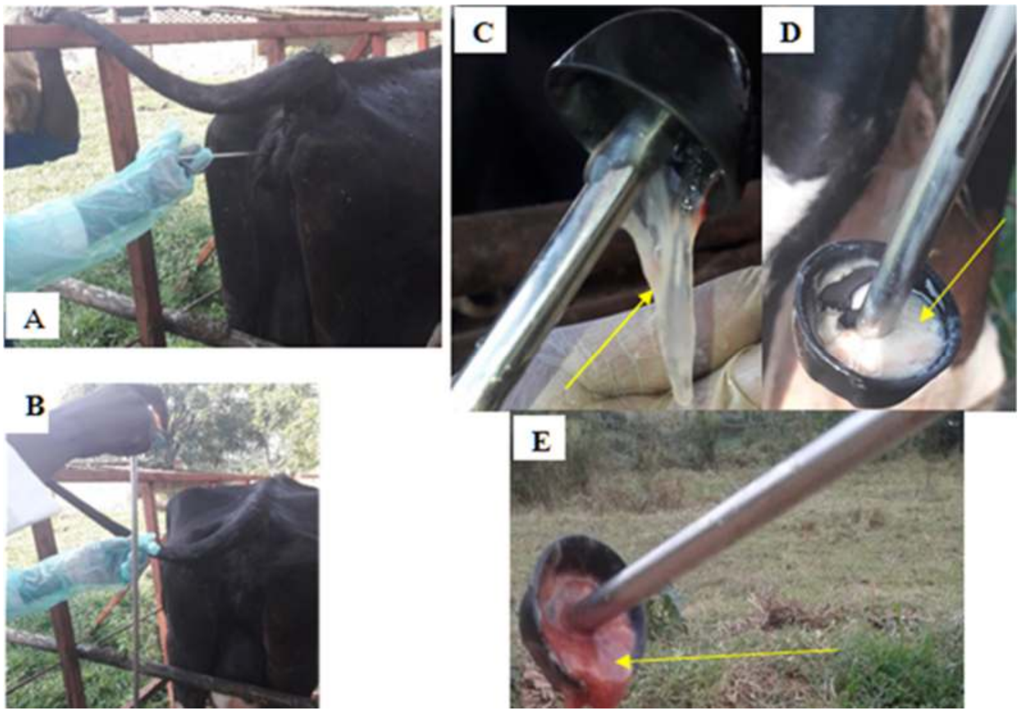


Figure 4. Evaluation of vaginal mucus. The VMC was retrieved with Metricheck device (**A** and **B**) and scored, VMC-2 (**C**) and VMC-3 (**D** and **E**).

3.2.3 Subclinical endometritis diagnosis

To avoid sampling of pregnant cows, the transrectal palpation method was used to diagnose those that are not pregnant based on diagnostic criteria previously described by Jaśkowski *et al*. (2018) so that they can be cytologically sampled. The diagnosis of SCLE consisted of two consecutive steps: (i) endometrial cytology sampling and preparation of slides at farm-level, and (ii) staining and evaluation of the endometrial cytology slides at laboratory-level.

(i) Endometrial cytology sampling and preparation of endometrial cytology slides

Immediately after VMC evaluation, samples of endometrial cytology were collected from the uterine body using CYT tool (Pascottini *et al*., 2015). In brief, the CYT was prepared by rolling a 1.5 cm piece of extremely stretchable paper masking tape (Tesa 4322, Klium, Ekkelgaarden, Hasselt, Belgium) on the top of a universal AI sheath (IMV technologies, l'Aigle, France). All universal AI sheath-Tesa 4322 were prepared in advance to be ready for use at the farm. At the farm level, the universal AI gun (CITO, Watertown, USA) was mounted with universal AI sheath-Tesa 4322 and covered with a 12- inch-long Sani-Shield Rod Protector (SSRP) (Agtech, Manhattan, USA). An illustration showing how the CYT was assembled and adapted to the universal AI gun is depicted in Figure 5.

Figure 5. Photographic illustrations showing step by step how the CYT was prepared. First, a 1.5 cm piece of Tesa 4322 (**1**) was rolled on the top of the universal AI sheath (**2** and **3**). Second, the universal AI sheath-Tesa 4322 (**3**) was adapted to the universal AI gun (**4** and **5**). Finally, the AI gun mounted with universal AI sheath-Tesa 4322 (**5**) was covered with a 12- inch-long Sani-Shield Rod Protector (**6** and **7**).

Before sampling, the perineal area was thoroughly disinfected with 1.0% potassium permanganate solution and dried with a dry paper towel. Next, the CYT was introduced into the uterine body following the procedures of AI technique in cattle (Dejarnette & Nebel, 2016). Once into the uterine body, the top of the CYT was released from the SSRP. Then, with some gentle pressure of the index finger through the rectum, the endometrial cytology sample was taken by rotating the top of the CYT in a clockwise direction on the wall of the uterine body. Once the top of the CYT was rotated, it was retracted into the SSRP to prevent contamination with cervical and vaginal cells (Figure 6). Finally, the device was gently removed from the cow's reproductive tract. Outside the reproductive tract, the top of CYT was released from the SSRP and gently rolled over a clean microscope slide (26 × 76 mm - 1.0/1.2 mm thick) (Nuova Aptaca Srl, Canelli, Italy). The smears were air-dried and kept in a slide-holder (Figure 6). Every two weeks, samples were brought to the Veterinary Laboratory of the School of Animal Sciences and Veterinary Medicine, Nyagatare Campus, University of Rwanda, for staining and evaluation. To prevent variability in sampling and interpretation, all the slides were collected by one person and were analysed by one laboratory technician trained in the endometrial cytological examination and had no information about the CLE results.

Figure 6. Photographic illustrations showing the process of endometrial cytology sampling. First, once the CYT reached into the uterine body **(1)**, its top was released from the SSRP **(2)**. Once the top of the CYT was rotated in a clockwise direction, it was retracted into the SSRP **(3)**, and the device was gently removed from the cow's genital tract **(4)**. The top of CYT was released from the SSRP and rolled over a clean microscopic slide **(5)**. Finally, the slide was air-dried and housed in a slide-holder **(6)**.

(i) Staining and evaluation of the endometrial cytological slides

At the laboratory facilities, all slides were stained with Differential-Quik stain kit (Modified Giemsa) (Polysciences Europe GmbH, Badener, Hirschberg, Germany), and once dried, were immediately mounted with Eukitt mounting medium (O. Kindler ORSAtec GmbH, Bobingen, Germany) according to the manufacturer's instructions. Eukitt mounting medium conserves smears unchanged for long periods. The slides were cover-slipped and evaluation was performed under a light microscope (Opta-Tech MB-5 series, Warszawa, Poland) mounted with a camera at 100x magnification to identify the areas of uniform cell distribution, and at 400x magnification for quantitative assessment of endometrial cells and PMN (Figure 7). For each slide, a total of 300 cells were counted by one observer (Melcher*et al*., 2014), and the proportion of PMNs was calculated based on (PMNs/PMNs plus endometrial cells) (Dini *et al*., 2015; Zyada *et al*., 2019). Based on previously published work (Chan Lee *et al*., 2018; Melcher *et al*., 2014; Rinaudo *et al*., 2017), cows that had ≥5% PMNs were considered SCLE positive, otherwise considered SCLE negative. A herd was considered positive for SCLE if had at least one cow positive for SCLE.

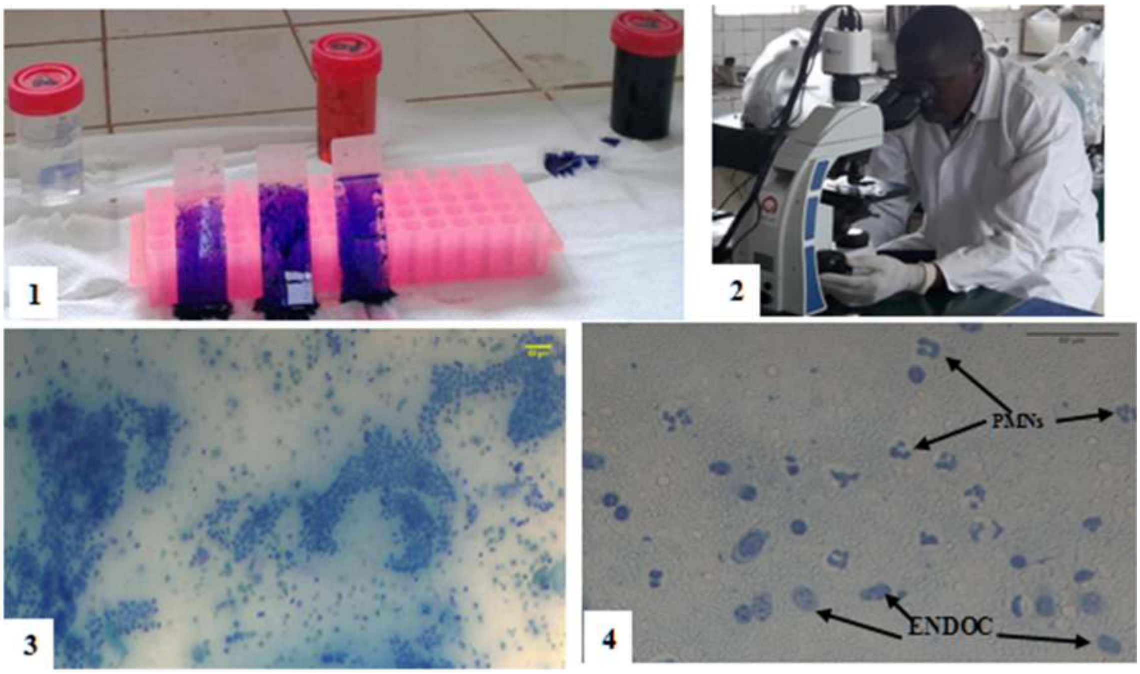


Figure 7. Staining and microscopic evaluation of the endometrial cytological slides: Endometrial cytology smears stained by Differential-Quik stains **(1)**, observed under light microscope **(2)** at x100 magnification to assess overall cellularity and to find areas within the smear that contain a monolayer of well preserved and adequately stained cells **(3)** and x400 magnifications to evaluate individual cells (polymorphonuclear inflammatory cells and endometrial cells)within the monolayer area **(4)**.*ENDOC = endometrial cells, *PMNs = polymorphonuclear inflammatory cells.

3.2.4 Statistical analysis

Of the 380 farms visited with 478 cows sampled, 12 cows from 10 farms could not be included in the analysis of CLE and SCLE prevalence due to poor quality of endometrial cytology slides. These were, therefore, not considered for subsequent statistical analysis. Thus, complete data for estimating the prevalence of endometritis was based on 466 cows from 370 farms.

The farmer perceived prevalence of endometritis was computed from the number of endometritis positive cows correctly estimated by farmers divided by the number of cows enrolled in the study (Leach *et al*., 2010). The data on 466 cows from 370 smallholder farms were cross-tabulated in Chi-square test statistic to estimate CLE and SCLE prevalence rates. The cow-level prevalence of CLE was calculated as the number of cows positive for CLE divided by the total number of cows enrolled in this study. In contrast, the cow-level prevalence of SCLE was calculated as the number of cows positive for SCLE divided by the total number of cows enrolled in the present study. Similarly, the herd prevalence of CLE was calculated as the number of herds positive for CLE divided by the total number of herds enrolled in this study. In contrast, the herd prevalence of SCLE was estimated as the number of herds positive for SCLE divided by the total number of herds enrolled. The reported sensitivity and specificity of the Rose Bengal Plate test is 81.2% and 86.3%, respectively (Gall & Nielsen, 2004).

Diagnostic performance tests setting reference test with MED because it has higher sensitivity (96.0%) and specificity (78.0%) (McDougall *et al*., 2007) in diagnosis endometritis and confirmatory test with Cytotape (Table 6) were used to compute sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) (Kumar *et al*., 2013; Pascottini, 2016).

Table 6. Possible results of diagnostic performance tests setting reference test with Metricheck device and confirmatory test with Cytotape

Diagnosis of endometritis		Metricheck Device	
		Positive	Negative
Cytotape	Positive	a	b
	Negative	c	d

$$Se = \frac{a}{a+c} \times 100 \quad Sp = \frac{d}{b+d} \times 100 \quad PPV = \frac{a}{a+b} \times 100 \quad NPV = \frac{d}{d+c} \times 100$$

The observed and perceived prevalence were compared using Wilcoxon signed ranks test and their agreement was tested using kappa statistic test. The agreement between VMC and %PMNs was also assessed using the kappa statistic test. The interpretation of kappa statistic (k) test was described as follows: k≤0.20 = poor agreement; 0.21 <k< 0.40 = fair agreement; 0.41 <k<0.60 = moderate agreement, 0.61<k< 0.80 = good agreement and k≥0.81= very good agreement (McHugh, 2012). The association between CLE and SCLE prevalence was tested using Pearson chi-square test. The correlation between VMC and %PMNs was tested using Spearman rank's correlation. The interpretation of Spearman rank's correlation (r) was described as follows: r ≤ 0.20 = low or no correlation; 0.21 < r < 0.5 = weak correlation; 0.51 <r< 0.8 = moderate correlation, r ≥ 0.81= strong correlation (Hartmann *et al*., 2015).

In this study, the sensitivity of Cytotape was defined as the likelihood of having ≥5% PMNs in CLE positive cows, whereas the specificity was the probability of founding <5% PMNs in CLE negative cows. The positive predictive value defines a sampled dairy cow's probability of having a positive result of SCLE once the results of CLE are positive. In contrast, the negative predictive value defines a sampled dairy cow's probability of having a negative result of SCLE once the results of CLE are negative (Thrusfield, 2007).

To investigate the point prevalence of endometritis according to the VMCs core and %PMN, data were analysed in four dpp groups: 21 to 30, 31 to 40, 41 to 50 and 51 to 60 days. In addition, vaginal mucus score and %PMN data were analysed for the overall dpp range (21 to 60 dpp). The prevalence of various degrees of endometritis, based on vaginal mucus score and % PMN, was expressed as a percentage of total cows examined during the same period. The data of %PMN was also categorised and analysed into four groups related to degree of endometrial inflammation based on the criteria previously described by Fuentes *et al*. (2017): (i) <5% PMN = no inflammatory infiltrate, (ii) 5% to 9% PMN = low endometrial inflammation, (iii) 10% to 14% PMN = moderate endometrial inflammation, (iv) >14% PMN= severe endometrial inflammation**.** Descriptive statistics were generated using frequency procedures and cross-tabulation. Data were analysed using Statistical Package for the Social Sciences (SPSS) software, version 22.0 for windows (SPSS, 2013). A p-value <0.05 was considered statistically significant.

3.3 Results

3.3.1 Farm characteristics

The sample farms had on average a herd size of 2.9±0.1 cows with a range of one to five cows and a median of two cows on a farm holding of 2.8±0.1 (median 2) acres with a range of 0.3 to 6.9 acres. Cows sampled on each farm ranged from one to three with a median of 1 and the average dpp (mean ± standard error) at sampling of 38.5±0.7 (median 35.0).

3.3.2 Knowledge of clinical signs of endometritis

Of the total 370 smallholder farmers, 41.6% (154/370, 95% confidence interval = 36.7 – 46.7) knew of some endometritis signs in cows in their herds for the past one year (Table 7). The most commonly recognised clinical sign of endometritis was repeat breeding (43.5%) followed by abortion (24.7%), mucopurulent or purulent discharge visible at the time of oestrus (18.8%), and white or whitish-yellow mucopurulent vaginal discharge (13.0%).

Table 7. Farmers' knowledge of clinical signs of endometritis in the study area

Knowledge of clinical signs of endometritis	
Clinical sign	**% (n)**
Repeat breeding	43.5 (67)
Abortion	24.7 (38)
Mucopurulent or purulent discharge at the time of oestrus and visible externally on the tail, perineum, and vulva	18.8 (29)
White or whitish-yellow mucopurulent vaginal discharge comes out when the animal lies down, urinates, or defecates and visible externally on the tail, perineum, and vulva	**2.0** (20)

3.3.3 Prevalence of endometritis

The overall point prevalence of endometritis at cow-level was 70.2% (95% CI (confidence interval) = 65.9 -74.2) with higher CLE (67.2%, 95% CI = 62.8 – 71.3) than SCLE (31.8%, 95% CI = 27.7 – 36.1). The corresponding herd-level prevalence was 71.1% (95% CI: 66.3 – 75.5), of which 68.1(95% CI = 63.2 – 72.7) and 34.9% (95% CI = 30.2 – 39.9) were CLE and SCLE cases, respectively. The prevalence was higher among dairy crossbred (60.7%) than among pure dairy breeds (20.1%) and indigenous cattle breeds (19.6%) (Table 8).

Table 8. Observed prevalence of endometritis in smallholder zero-grazed cows at 38.5±0.7 dpp

Prevalence of endometritis		Clinical % (n)	Subclinical % (n)	Overall % (n)	Chi-square test
Overall	Herd-level	68.1 (252)	34.9 (129)	71.1 (263)	***
	Cow-level	67.2 (313)	31.8 (148)	70.2 (327)	***
Cow breed	Indigenous	19.2 (60)	19.6 (29)	19.6 (64)	NS
	Crossbreds	60.7 (190)	58.1 (86)	60.9 (199)	***
	Pure	20.1 (63)	22.3 (33)	19.6 (64)	***

NS = not significant ($p > 0.05$); ***$p < 0.001$, *comparison was performed by row.

On the day of the herd visit, only 4.1% (95% CI = 2.5 – 6.6) of the sample farmers correctly estimated 15 cows positive for endometritis from 15 farms. Thus, at the herd-level, the farmer perceived prevalence of endometritis was 4.1% (95% CI = 2.5 – 6.6) compared with the observed prevalence of 68.1%. Their agreement was not statistically significant (k = 0.02, p>0.05) and Wilcoxon signed ranks test showed that they differed significantly (z = −15.3, p < 0.001)**.** Similarly, at the cow level**,** the farmer perceived prevalence of endometritis (3.2%) was much lower than the observed prevalence (67.2%). They had a poor agreement (k = 0.03, p<0.05) and Wilcoxon signed ranks test showed that they differed significantly (z = −17.3, p<0.001) (Table 9).

Table 9. Relationship between the prevalence of endometritis recorded by a researcher and the prevalence of endometritis reported by the farmer

Endometritis	**Cow-level, % (n)**	**Herd-level, % (n)**
Perceived prevalence by farmer	3.2 (15)	4.1 (15)
Observed prevalence by researcher	67.2 (313)	68.1 (252)
Kappa statistic	0.03*	0.02^{ns}
Wilcoxon signed-ran test	-17.5***	-15.3**

Table 10 summarises the results of MED and CYT. Of all cows positive for endometritis, 40.9% (134/327, 95% CI = 35.8 – 46.4) had both CLE and SCLE. Among cows that had SCLE, 90.5% (134/148, 95% CI = 84.8 – 94.3) had CLE at the same time, whereas among cows positive for CLE, only 42.8% (134/313, 95% CI = 37.5 – 48.4) had SCLE. Among cows negative for SCLE, 56.3% (179/318, 95% CI: 50.8 – 61.6) were positive for CLE. Table 10 also shows that based on the uterine health status of cows at 38.5 ± 0.7 dpp, the percentage of cows that had CLE only, SCLE only, and both CLE and SCLE were 38.4% (179/466, 95% CI = 34.1 - 42.9), 3.0% (14/466, 95% CI = 1.8 - 4.9), and 28.8% (134/466, 95% CI = 24.8 - 33.0), respectively, while 29.8% (139/466, 95% CI = 25.9 - 34.1) of sample cows were without CLE and SCLE (Table 10).

Table 10. Results of Metricheck device and Cytotape among the sample zero-grazed dairy cows at 38.5±0.7 dpp

Subclinical endometritis	**Clinical endometritis**	
	Positive, n	Negative, n
Positive	134	14
Negative	179	139

The level of agreement between diagnostic criteria for CLE (VMC score) and SCLE (%PMN) in 466 cows examined 38.5 ± 0.7 dpp was significantly poor (k= 0.10; 95% CI= -0.10 - 0.29; $p<0.001$). Also, the correlation between VMC and %PMN was positive and weak ($r = 0.47$; $p<0.001$) (Table 11).

Table 11. Number of cows within categories of vaginal mucus score (VMC; coded as VMC-0, 1, 2 and 3) and percentage of polymorphonuclear inflammatory cells (%PMN; coded as <5% and ≥5%)

%PMN	**VMC score**				**Total**
	0	**1**	**2**	**3**	
<5	139	114	28	37	318
≥5	14	49	25	60	148

Table 12 outlines the diagnostic performance of MED and CYT of diagnosis CLE and SCLE, respectively. The MED had higher sensitivity but low specificity of detecting CLE, whereas CYT had a lower sensitivity but higher specificity of detecting SCLE. The differences between the diagnostic performance of the MED and CYT were significant ($p<0.001$).

Table 12. Diagnostic performance of the Metricheck device and Cytotape

Diagnostic techniques	**Diagnostic performance criteria**			
	Sensitivity (%)	**Specificity (%)**	**Positive predictive value (%)**	**Negative predictive value (%)**
Cytotape	42.8	90.8	90.5	43.7
Metricheck Device	90.5	43.7	42.8	90.8
Chi-square test	***	***	***	***

***$p < 0.001$

Table 13 shows the prevalence of endometritis based on the VMC scores and %PMN. The average %PMN per slide was 4.6±0.4with a range between 0.0 and 66.7. The percent of cows with %PMN of 5 or more, 21 to 30, 31 to 40, 41 to 50, and 51 to 60 dpp, was 46.8%, 24.5%, 29.2%, and 17.4%, respectively. In contrast, the percent of cows with a VMC score of ≥ 1, 21 to 30, 31 to 40, 41 to 50 and 51 to 60 dpp, was 78.5%, 62.8%, 64.6% and 55.8%, respectively.

Table 13. Prevalence of endometritis at 21 to 60 dpp based on VMC score and %PMN

Criteria	**21-30 days (n = 186)**	**31-40 days (n = 94)**	**41-50 days (n = 48)**	**51-60 days (n = 138)**	**Overall (n = 466)**
Number of cows classified based on VMC score in each group at examination, %					
0	21.5	37.2	35.4	44.2	32.8
1	30.1	36.2	37.5	39.9	35.0
2	15.1	12.8	4.2	8.0	11.4
3	33.3	13.8	22.9	8.0	20.8
Number of cows classified based on %PMN in each group at examination, %					
< 5	53.2	75.5	70.8	82.6	68.2
5-9	21.5	13.8	20.8	12.3	17.2
10-14	8.1	5.3	4.2	2.2	5.4
> 14	17.2	5.3	4.2	2.9	9.2

VMC = Vaginal mucus; PMN = polymorphonuclear inflammatory cells

Table 14 shows the prevalence of endometritis according to farmers assisted calving with or without protective gloves, and per breeding services used. Of 299 total cases of dystocia observed in the present study, farmers assisted in 85.9% (257/299) cases in which 97.7% were with non-gloved hands. Cows with an assistance calving without gloves were associated with a high prevalence of CLE and SCLE (86.3% vs. 85.3%) compared to those where farmers wore protective gloves (13.7% vs. 14.7%). Moreover, bull serviced cows had a high prevalence of CLE and SCLE (56.9% vs. 55.4%) compared to artificial insemination served cows (43.1% vs.44.6%).

Table 14. Prevalence of endometritis associated with calving assistance and breeding services

Criteria	Prevalence of endometritis, % (n)	
	Clinical	Subclinical
Prevalence according to calving assistance using gloved or non-gloved hand		
Non-gloved hands	86.3 (176)	85.3 (93)
Gloved-hands	13.7 (28)	14.7 (16)
Prevalence according to the breeding services used		
Artificial insemination	43.1 (135)	44.6 (66)
Bull	56.9 (178)	**2.4 (82)**

3.4 Discussion

The farmer perceived prevalence being much lower than the prevalence observed by researchers at cow- and herd- levels, and with only a few farmers correctly estimating the number cows positive for endometritis demonstrate gross underestimation and low awareness of the disease by farmers. The problem is likely typical among farmers; corroborating observation is in the United Kingdom, where farmers were equally less knowledgeable about endometritis in organic farms (6.1%) and non-organic farms (10.8%) (Langford *et al*., 2009). Indeed, the current study revealed that less than half of farmers (41.6%) could recognize some of clinical signs of endometritis in cows in their herds for the past one year. Among them, only 13.0% could detect white or whitish-yellow mucopurulent vaginal discharge comes out when the animal lies down, urinates, or defecates, and 18.8% could detect mucopurulent or purulent discharge at the time of oestrus. In contrast, farmers demonstrated more awareness of repeat breeding (43.5%) and abortion (24.7%) though they could not solely attribute them to endometritis**.** This demonstrates knowledge gaps about endometritis. Other studies show more farmers awareness (19.0% to 47.0%) of the clinical signs of brucellosis in cattle; 47.0% in Kenya (Obonyo & Gufu, 2015), 30.0% in Ecuador (Ruano & Aguayo, 2017); 29.0% in Tanzania (Zhang *et al*., 2016), and 19.0% in Uganda (Kansiime *et al*., 2015).

The knowledge gaps found in this study has an implication on how farmers may manage endometritis. They are likely to engage in some high-risk practices such as non-use of gloves and providing unsanitary assistance during calving, and sharing or hiring breeding bulls from neighbouring farms (Ruano & Aguayo, 2017). Such practices increase the risk of exposure to the reproductive tract disease from microorganisms contamination as well as trauma to the vulvovaginal, urethra and/or cervix and endometritis may develop subsequently (de Boer *et al*., 2014; Hartmann *et al*., 2015; Vieiro-Neto *et al*., 2016). This can support the high disease prevalence observed in the sample dairy herds. Indeed, it was observed in this study among 257 calving assistance cases that 97.7% of the farmers were without gloves. These farmers had CLE and SCLE more prevalent (86.3% and 85.5%, respectively) than those who used gloves (13.7% and 14.7%, respectively). Therefore, to reduce physical trauma and bacterial contamination of the female reproductive tract and thus, decreasing CLE and SCLE positive cases, Rwandese farmers are advised to seek help from animal health service providers, use gloves and lubrication, clean the cow's vulva with antiseptic during calving assistance, and keep a cow in a clean cowshed in the transition period. Corroborating the current observation is the finding of Ruano & Aguayo (2017) in Ecuador for brucellosis that knowledge gaps explained 62.0% of unprotected parturition assistance and 71.0% of disposing of aborted foetuses without using protective gloves.

Moreover, sharing or hiring bulls from neighbouring farms could attribute to the possibility of spreading reproductive diseases or pathogens transmission, because herd owners had no any control measures before using the bulls available locally (Duguma *et al*., 2012). This is supported by observations in this study where high disease prevalence was observed among bull serviced cows (CLE = 56.9%, SCLE = 55.4%) compared to artificial insemination (AI) serviced cows (CLE = 43.1%, SCLE = 44.6%). To manage this, farmers are advised to use artificial insemination as a breeding method or pre-screen cows and bulls for diseases before use. Corroborating this observation is the finding of Mushonga *et al*. (2017) in Rwanda and Shortall *et al*. (2017) in the United Kingdom. In contrast to this finding, a study in Ethiopia (Moges & Jebar, 2012) observed comparable SCLE prevalence among crossbreds dairy cows that were artificially inseminated (69.8%) and bull serviced (63.6%). This was attributed to exposure of cows to microorganisms contamination during AI (Moges & Jebar, 2012).

The reason for the knowledge gaps in the current study could be linked to non–existence of an extension programme and control strategy targeting endometritis in Rwandan dairy farming, which then limits farmer awareness about endometritis. This observation is in line with that reported by Tayebwa *et al*. (2015) in Uganda. They attributed the prevalence of SCLE (18.6%) in commercial dairy herds to limited knowledge of farmers and extension workers about the diagnosis of endometritis in their herds. In this study, because the prevalence is high and farmers are less aware of the disease, extension service needs to prioritise training to enhance knowledge about endometritis.

The results of the present study demonstrate that the diagnosis of CLE with the MED had a higher sensitivity (90.5%) but lower specificity (43.7%). This indicates that the MED identified more of the diseased cows among the study cows. The current finding is comparable with that observed in New Zealand (McDougall *et al*., 2007), where 43.0% of the cows were CLE positive by diagnosis with vaginoscopy, whereas the following diagnosis by MED recorded 60.0% of the cows with CLE with a sensitivity of 96.0% and specificity of 78.0%. Therefore, it concluded that by using MED, more examined cows could be diagnosed as affected with CLE because the VMC often accumulate in the fornix vagina are simply retrieved by MED compared to other CLE diagnostic techniques (Kumar *et al*., 2013; McDougall *et al*., 2007; Refaat *et al*., 2020; Pleticha *et al*., 2009). With a similar trend to the current study, Runciman *et al*. (2009) in Australia recorded the sensitivity and specificity of MED to be 64.5% and 60.4%, respectively. Also, Kumar *et al*. (2013) in India recorded sensitivity and specificity of 61.1% and 97.8%, respectively**.** Differences in the variation of MED performance may depend on farming systems, diagnostic criteria of CLE, and dpp at sampling. For instance, in the study of McDougall *et al*. (2007), VMC was scored on a 0 to 5 scale for cows at 33.0±16.0 dpp whereas Kumar *et al*. (2013) used a 0 to 3 scale to characterize VMC of cows within 25.0 to 40.0 dpp under commercial dairy farms.

The threshold value of ≥5%PMN indicating SCLE positive cow used in the current study was similar to some of the previously published work (Chan Lee *et al*., 2018; Melcher *et al*., 2014; Wagener *et al*., 2017). In the current study, the CYT had low sensitivity and negative predictive value, and high specificity and positive predictive value to diagnose SCLE. Similar results were reported by another author in Belgium (Pascottini, 2016). The positive predictive value (90.5%) found in this study, indicates that a cow that had a CYT positive result had 90.5% likelihood of having CLE, whereas the negative predictive value indicates that a cow negative for SCLE had a 43.7% probability of not having CLE. This indicates that CYT diagnosed significantly a larger proportion of cows that had SCLE in the study cows.

The level of agreement between %PMN and VMC score in the current study was significantly poor ($k = 0.10$, $p<0.001$). In other studies, the agreement has been reported to be between 0.01 and 0.23 (Dubuc *et al*., 2010; McDougall *et al*., 2020), which support the current findings. This indicates that %PMN and VMC score might reflect different conditions of the female genital reproductive tract (Dubuc *et al*., 2010; Kumar *et al*., 2013; Melcher *et al*., 2014; Pascottini, 2016). This is supported by the finding of this study that 56.3% of cows not diagnosed to have SCLE were positive for CLE. Despite the poor agreement between %PMN and VMC score, in their study, Runciman *et al*. (2009) observed that VMC score being quick, easy to undertake, and cheap to obtain can be the basis for further reproductive disorder test, and interventions that could improve the subsequent reproductive and productive performance of dairy cows.

In the present study, 56.3%cases not diagnosed positive with CYT were probably false-positive diagnoses commonly observed with CLE diagnosis techniques as previously reported by several authors (Leutert *et al*., 2012; McDougall *et al*., 2007; Westermann *et al*., 2010). This finding is an indication of poor correlation between %PMN and VMC score observed in this study, suggesting that the presence of mucopurulent or purulent discharge in the vagina may not be representative for uterine infections. Previous studies **(**Deguillaume *et al*., 2012; Hartmann *et al*., 2015; Vieira-Neto *et al*., 2016) have reported that cervicitis, vulvovaginitis, urethritis, CLE**,** or the combination of those conditions are all clinically expressed by a mucus detectable in the vagina. Therefore, the calving assistance without gloves observed in the current study could probably result in trauma and infection in vulvovaginal, cervix, uterus, and urethra, and contributed to those false-positive cases. Consequently, in accordance with the previously published data from Machado *et al*. (2012) and Westermann *et al*. (2010), MED did not allow differentiating the source and contribution of each condition to the retrieved VMC. Therefore, VMC of these cows (56.3%**)** could have originated from the vulvovaginal, urethra and/or cervical inflammation (s) instead of endometrium inflammation (Deguillaume *et al*., 2012; Hartmann *et al*., 2015; McDougall *et al*., 2011). This can explain the higher CLE prevalence (67.2%) and lower SCLE prevalence (31.8%) observed in the current study. These findings are in line to those observed in Australia (CLE = 27.3%; SCLE = 21.0%) (Prunner *et al*., 2014), Croatia (CLE = 15.3% ; SCLE = 7.8%) (Zobel, 2013) and in Canada (CLE = 15.0%; SCLE = 9.0%) (Denis-Robichaud & Dubuc, 2015). But findings contrast with those reported in India (CLE = 21.7%; SCLE = 29.4%) (Singh *et al*., 2016) and in Uganda (CLE = 3.6%; SCLE = 18.6%) (Tayebwa *et al*., 2015) probably due to dpp at examination, diagnostic methods used and dairying management practices.

In the present study, the prevalence of CLE and SCLE was higher in cows that were in their earlier dpp (21 to 30 days; 78.5% vs. 46.8%, respectively) than those were in later dpp (51 to 60 days; 55.8% vs. 17.4%, respectively) at the day of endometritis diagnosis. This indicates that uterine inflammation conditions are dynamic during the postpartum period, as has been previously reported (de Boer *et al*., 2014; Gilbert & Santos, 2015; Okawa *et al*., 2017). This also suggests that uterine infection persists longer in the postpartum period and affect subsequent fertility and productive performance (Gilbert &Santos, 2015).

The prevalence of CLE observed in this study (67.2%) was similar to that observed in commercial Holstein-Friesian cows in Japan (Gautam *et al*., 2009), but the prevalence was higher than some previously reported rate in Iran (61.4%) for cows between 26 and 32 dpp (Ahmadi *et al*., 2016); Belgium (27.0%) for cows between 31 and 37 dpp (Pascottini *et al*., 2015); Egypt (21.9%) for cows at 28 dpp (Hussein *et al*., 2017); Iran (60.0%) (Ryan *et al*., 2020), and in Uganda (3.6%) for cows at 60 dpp (Tayebwa *et al*., 2015). The estimated prevalence of CLE was lower than the prevalence rate (69.8%) reported in New Zealand for cows at 33 dpp (McDougall *et al*., 2007). Differences in variation in prevalence in these studies could be explained by the different dpp at sampling, diagnostic techniques, definition criteria of CLE positive cow, different characteristics of the cows, and varied environment and herd health management conditions (Kelly *et al*., 2020).

The prevalence of SCLE observed in this study (31.8%) is comparable with those observed in Turkey (31.3%) for cows at 30 dpp (Oruc *et al*., 2015) and in Korea (31.1%) for cows at 30 dpp (Chan Lee *et al*., 2018). Conversely, the prevalence of SCLE in the present study was higher than that reported in commercial dairy herds, for instance in Egypt (29.7%) for cows between 28 and 42 dpp (Zyada *et al*., 2019), Uganda (18.6%) for cows within 60 dpp (Tayebwa *et al*., 2015), Ethiopia (46.3%) for cows between 30 and 60 dpp (Moges, 2019), and Spain (14.9%) for cows between 30 and 45 dpp (Barrio *et al*., 2015). The prevalence of SCLE was lower than the rate of 89.0% for cows between 28 and 42 dpp reported by Denis-Robichaud & Dubuc (2015) in Canada and 80.0% for cows between 28 and 56 dpp in Ethiopia (Moges, 2019). The variation in the prevalence may be due to the differences in dpp at examination, different threshold values of %PMN that cytological sample should be considered positive, diagnostic techniques used, management practices, and hygienic conditions, which differ from dairying systems (Ahmadi *et al*., 2016; Madoz *et al*., 2014; Moges, 2019).

In agreement with some previous studies (Cheong *et al*., 2011; Denis-Robichaud & Dubuc, 2015; McDougall *et al*., 2020), a significant association between endometritis and herds was observed in this study. The present study revealed that 71.1% of the herds had at least a cow suffering from endometritis. The high prevalence of endometritis in the herds could be attributed to lack of implementation of endometritis prevention and control measures. The overall observed herd-prevalence in this study (71.1%) is within the range of herd-level endometritis prevalence (4.0% to 87.0%) recorded by some previous studies (Denis-Robichaud & Dubuc, 2015; Ryan *et al*., 2020). Herd prevalence of CLE in the current study was 68.1%. Such prevalence is higher than what has been reported in some previous studies (5.0% to 65.0%) (Denis-Robichaud & Dubuc, 2015; McDougall *et al*., 2020) and lower than the rate (87.0%) reported by Ryan *et al*. (2020). Similarly, the herd prevalence of SCLE in this study (34.9%) was higher than some previously reported rates (4.8% to 27.1%) (Cheong *et al*., 2011;

McDougall *et al*., 2020) and lower than the prevalence rates (39.7% to 64.1%) reported by Pascottini *et al*. (2016) in Belgium and Chan Lee *et al*. (2018) in Korea. These findings entail how serious endometritis is, in the dairy herd that warrant simple mentation of management practices for endometritis prevention and control during the transition period. The different herd prevalence of endometritis in these studies might be explained by the different diagnostic methods used, different characteristics of the cows, presence of risk factors for endometritis in these herds, and varied herd management conditions (Cheong *et al*., 2011; Denis-Robichaud & Dubuc, 2015; McDougall *et al*., 2020).

In the current study, the prevalence of CLE and SCLE were higher among dairy crossbreds compared with their indigenous cattle and dairy pure breeds' counterparts. In the context of endometritis, few studies in recent literature have considered cattle genotype diversity in studying its prevalence in cows, with the exception of a study involving both indigenous and pure dairy breeds in commercial dairy farms of Uganda (Tayebwa *et al*., 2015); and Holstein-Friesian and Brown Swiss cows in Ethiopia (Moges, 2019).Therefore, the current finding was higher than that recorded in indigenous cattle (CLE = 0.0%, SCLE = 3.8) and much lower than those recorded in dairy pure breeds (CLE = 80.0%, SCLE = 84.6%) (Tayebwa *et al*., 2015). The SCLE prevalence (58.1%) found in crossbred dairy cows in this study was higher than previously reported prevalence (26.0%) in Brazilian crossbred dairy cows (Carneiro *et al*., 2014), 29.7% in Ethiopia (Moges, 2019), and lower than the prevalence rate (68.3%) reported in Ethiopian crossbred dairy cows diagnosed between 30 to 60 dpp using low volume uterine lavage technique (Moges & Jebar, 2012). These findings could be associated with the lower degree of adaptation of improved breeds to tropical conditions of high temperature and humidity, inadequate feeding practices, and poor hygienic conditions than indigenous cattle (Mukasa-Mugerwa, 1989; Tekleye *et al*., 1991), making them more susceptible to disease than indigenous cattle (Tayebwa *et al*., 2015). Another reason may also be due to the fact that improved breeds require elaborated management practices, adequate feeding, and better health care to get better health status than indigenous cattle (Sarder *et al*., 2015). Those conditions are sometimes absent under smallholder dairy zero-grazing farming; for instance, inadequate feeding is partly influenced by their small size farm holding (2.8±0.1 acres) in which food crops and fodder compete for land location. The herd health management plan is also absent in smallholder farms (Ndahetuye *et al*., 2019). In their study, Sheldon *et al*. (2020) concluded that farmers could help prevent uterine disease by maintaining hygiene in the cows' environment and supplying adequate feeding during the transition period. Therefore, the farmers should be given attention to the appropriate feeding practices, hygiene in the cowshed, and training to reduce the prevalence of CLE and SCLE in their dairy herds.

In this study, the percentages of cows that had CLE only, SCLE only, and both CLE and SCLE were 38.4, 3.0, and 28.8%, respectively. Overall, only 29.8% of cows were healthy. The prevalence of CLE only was lower compared to 60.0% reported by Ryan *et al*. (2020) in Ireland. The greater prevalence reported in the study by Ryan *et al*. (2020) was probably because all cows included in their study were assessed for the presence of CLE at the early stage of the postpartum period (21 dpp). Conversely, the prevalence of CLE only reported in the current study was greater compared to some of the previous studies where the prevalence was reported to be 7.5 to 35.7% when cows were examined approximately between 24 and 86 dpp (Denis-Robichaud & Dubuc, 2015; Gobikrushanth *et al*., 2016; Kelly *et al*., 2020; Šavc *et al*., 2016). The higher prevalence of CLE only reported in this study was possibly due, firstly, to the inclusion of cows that had VMC≥1 as cases of CLE, whereas Šavc *et al*. (2016) and Kelly *et al*. (2020) in Ireland, and Denis-Robichaud & Dubuc (2015) in Canada only considered the cows with VMC≥2 as cases of CLE. Secondly, the higher prevalence of CLE only was probably due to the use of MED as a higher sensitive technique to diagnose CLE than vaginoscopy (McDougall *et al*., 2007). The vaginoscopy was used by Gobikrushanth *et al*. (2016) to diagnose CLE in cows within 25.0±1.0 dpp. The authors found a prevalence of CLE only of 35.7%, which is lower compared to the prevalence of CLE only observed in the present study. Thirdly, the high prevalence of CLE only could be associated with false-positive cases (56.3%).

The prevalence of SCLE only, in the present study, is comparatively lower than the 11.9% reported by Gobikrushanth *et al*. (2016), when a threshold of ≥8% PMN was used at 25.0±1.0 dpp. The prevalence of the combination of CLE and SCLE agreed to the 28.7% reported by Dubuc *et al*. (2010) in Canadian dairy cows; authors used a threshold of ≥6% PMN at 35.0±3.0 dpp. However, such prevalence is comparatively higher than the 23.8% reported by Gobikrushanth *et al*. (2016) in Canadian dairy cows. Overall, the variation in the prevalence of different categories of endometritis reported in those studies was probably influenced by the dpp at diagnosis; diagnostic techniques used, and varied categorization criteria of cows into different groups of endometritis based on their uterine health statuses.

The preferable diagnostic test for detecting endometritis in dairy cows are vaginoscopy, MED, gloved-hands, CYT, cytobrush, and low-volume uterine lavage (Barlund *et al*., 2008; de Boer *et al*., 2014; Pascottini, 2016). However, based on the findings of the current study, MED is a useful tool and cow-side diagnostic test to diagnose CLE with VMC ≥1 in cows within 21 and 60 dpp. In contrast, CYT is a good technique to diagnose SCLE with a threshold of ≥5% PMN in endometrial cytology sample of cows within 21 to 60 dpp, but it is not a cow-side diagnostic test. Thus, the result of MED and CYT should be combined to obtain an accurate diagnosis of endometritis status in cows within 21 to 60 dpp.

3.5 Conclusion

This study reveals that endometritis is highly prevalent in smallholder zero-grazed dairy herds, but farmers grossly underestimate the disease, reflecting they are much unaware and large knowledge gaps. The extension service, therefore, needs to increase awareness and education among smallholder farmers about endometritis, its prevention and control measures, and treatment regime. Further studies should determine the risk factors associated with the observed prevalence of endometritis, which would be valuable in informing management interventions to target the high-risk factors.

CHAPTER FOUR

PERCEPTION OF FARMERS ABOUT ENDOMETRITIS PREVENTION AND CONTROL MEASURES FOR ZERO-GRAZED DAIRY COWS ON SMALLHOLDER FARMS IN RWANDA

Abstract

Endometritis is a prevalent uterine disease in postpartum cows. The disease reduces fertility performance and MY, and subsequently, productivity and profitability of dairy farms. The reduction in performance is associated with considerable economic losses on dairy farms. Smallholder farmers are likely to incur considerable economic losses from the disease where they lack knowledge of effective prevention and control measures for the disease. However, empirical evidence is lacking for the effectiveness of management practices that are recommended on dairy farms. Therefore, the objective of this study was to gather farmer's perspectives on effectiveness of different management interventions (MIs) for endometritis prevention and control on smallholder farms in Rwanda practicing dairy zero-grazing. The best-worst scaling (BWS) choice method was applied to gather opinions from farmers that relied on past one-year recall data obtained from 154 farmers. These farmers were identified through exponential non-discriminative snowball sampling in a cross-sectional study. Of the 20 MIs evaluated, 12 scored highly for effectiveness. The top four most effective were: avoiding sharing equipment with neighbouring farms (45.5%), consulting animal health service provider about disease treatment (31.8%), keeping cows in a clean and dry shed (26.7%), and selecting sires based on calving ease (26.6%). The MIs considered least effective were: maintaining clean transition cow housing (35.1%), removal of foetal membrane immediately after passing (33.1%), disinfecting equipments of calving assistance before and after use (32.5%), and selecting sires with low percent stillbirths (29.2%). This study has demonstrated the application of BWS object case method in understanding the MIs that farmers consider are most effective in the prevention and control of endometritis in the dairy herds. The MIs are on-farm biosecurity and hygiene, seeking veterinary services for disease treatment and selecting sires for ease of calving. These MIs should be considered for prioritisation in extension services and research to continuously improve and enhance their practical application on smallholder dairy farms.

4.1 Introduction

Dairy production is a major component in the livestock sector in Rwanda. The dairy subsector is an essential source of livelihood to over 80.0% of households involved directly or indirectly throughout the agricultural value chain (IFAD, 2016). The dairy subsector contributes 28.0% to the agricultural Gross Domestic Product (GDP) and 4.0% to the national GDP (NISR, 2018). Rwanda has an estimated cattle population of 1,340,792, of which 45.0% are indigenous cattle, 33.0% are dairy crossbreds, and 22.0% are pure dairy breeds (IFAD, 2016). The dairy crossbreds and pure dairy breeds are of the Friesians, Jersey, and Fleckvieh breeds. Among the smallholder dairy farms, those practicing zero-grazing hold the majority (92.0%) of the cattle population and supply the bulk of the domestic milk market demand (IFAD, 2016). However, the supply has not satisfied the local demand. The average per capita milk consumption for both urban and rural areas estimates by the Rwanda Livestock Master Plan (RLMP, 2017) is 63.0 litres per person per annum. An increase of 3.5 fold would be necessary to achieve per capita consumption threshold of 220 litres recommended by the Food and Agriculture Organization of the United Nations (FAO, 2013).

One disease associated with suboptimal fertility, though often unnoticed, is endometritis disease (Hussein *et al*., 2017; Sharma *et al*., 2019). Endometritis is a prevalent uterine disease in postpartum cows that results in considerable economic losses through the reduction in production and fertility performance, culling of cows, and veterinary costs (Hussein *et al*., 2017; Sharma *et al*., 2019). The contamination of uterus by endometrial microbiota occurs at all stages of the reproduction cycle (Karstrup *et al*., 2017), but the majority of cases are found mostly during the first two weeks of postpartum (Drillich & Wagener, 2018). This contamination is attributed to the fluctuation and expansion of the microbial community diversity after calving. The reason for this is because of the dilation of physical barriers such as vulvar sealing, vestibule-vaginal constriction, the cervix, cervicovaginal mucus secretion, and the epithelial barrier (Sheldon *et al*., 2020). These allow contamination and colonisation of the female genital tract with pathogenic bacteria from bedding materials, skin, faeces, and vagina (Appiah *et al*., 2020; Sheldon *et al*., 2020). These pathogenic bacteria such as *Escherichia coli, Trueperella pyogenes, Prevotella melaninogenicus, Dermacoccus spp, Enterococcus faecalis,* and *Fusobacterium necrophorum* are the common causes of endometritis in dairy cattle (Appiah *et al*., 2020; Wang *et al*., 2018).

Good management practices in the pre-and postpartum periods can minimize or even avoid cow uterine infections and prevent the prevalence of endometritis (Ganaie *et al*., 2018). In contrast, suboptimal management of transition cows exposes them to postpartum uterine diseases in which endometritis is of importance (Karstrup *et al*., 2017; Sheldon *et al*., 2020). Sadly, effective treatment options for endometritis remain limite, yet the disease can persist even after treatment and recovery (Jeremejeva *et al*., 2016; Makki *et al*., 2017). This means that treating the condition is not a sustainable solution; it is necessary to implement effective prevention and control measures (Ganaie *et al*., 2018; Sheldon *et al*., 2020). Management interventions (MIs) that prevent the introduction and reduce the spread of disease-causing agents into and off the herd are critical components of the herd health program (Crowe *et al*., 2018; Renault *et al*., 2017; Wolff *et al*., 2019).

The implementation of MIs could significantly minimise endometritis prevalence and consequently improve animal welfare and increase productivity and profitability of dairy herds (Renault *et al*., 2017). This is supported by observations that improved extension service and advisory support in the pre- during and post-partum periods improve the prevention and control of endometritis in the dairy herds (Tayebwa *et al*., 2015). In extension service delivery, farmers are essential in implementing MIs and evaluating the effectiveness of the different MIs for disease prevention and control (Renault *et al*., 2017; Shortall *et al*., 2017).

The best-worst scaling (BWS) choice is a preferred technique to gather opinions from different experts on the effectiveness of varying biosecurity measures on dairy farms (Hansson & Lagerkvist, 2016; Shortall *et al*., 2017). The BWS has been used in market research (Nunes *et al*., 2016); human health (Cheung *et al*., 2016); agriculture (Shittu & Kehinde, 2018), and livestock management science (Guinat *et al*., 2017; Jones *et al*., 2013). The literature search revealed no application of BWS choice to endometritis management studies. Empirical evidence on how dairy farmers perceive the effectiveness of MIs for endometritis prevention and control is yet to be documented. However, BWS holds great potential in determining effective MIs from farmers' perspectives; they are the first implementers in dairy value chain. Advances in this knowledge gap would be informative and educative to actors in the dairy sector towards reducing the prevalence rate of endometritis in the dairy herds. In particular, extension service and farmers stand to benefit from the immediate application of effective MIs. In Rwanda, smallholder dairy zero-grazing is a priority development intervention towards hunger eradication and attaining food and nutrition security (RLMP, 2017). High prevalence of endometritis in the herd could, however, impede the achievement of these development goals due to the economic loss associated with the disease. Unfortunately, in literature search, several studies have focused on effectiveness of management interventions for stopping the spread of diseases (Guinat *et al*., 2017; Shortall *et al*., 2017) unlike endometritis, the disease associated with considerable economic losses on dairy farms. This indicates that empirical evidence is lacking for the effectiveness of MIs that are recommended to manage endometritis on dairy farms. For this reason, this study evaluated the opinion of farmers about the effectiveness of different MIs for endometritis prevention and control under field conditions in Rwanda. The research will inform prioritisation of MIs in extension service and for on-farm implementation.

2.2 Methodology

4.2.1 Study area

Data was collected from smallholder dairy farms with zero-grazed cows in Gasabo district of Rwanda (Figure 8). The full detailed description of the study area is described in Section 3.2.1.

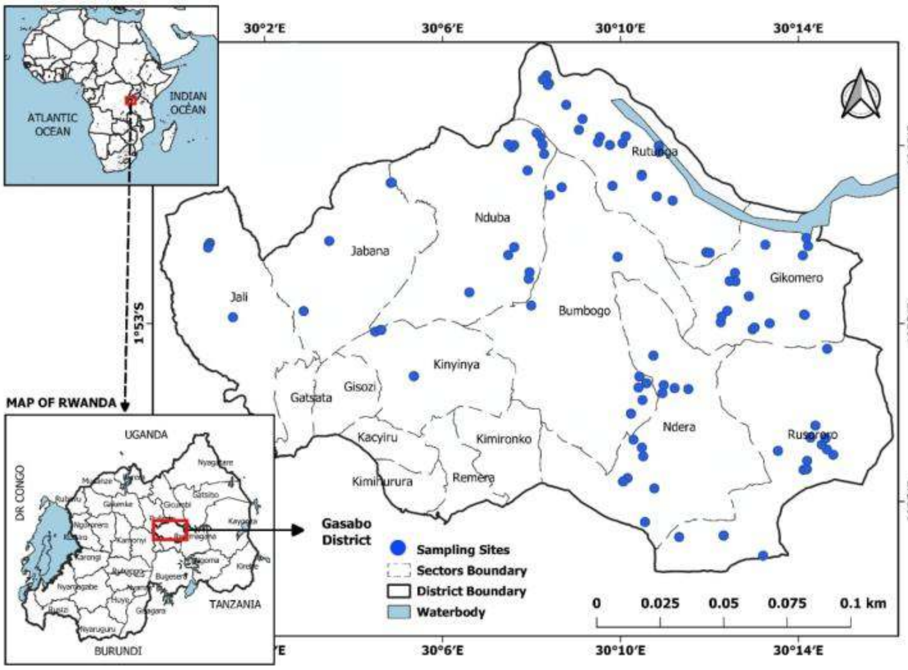


Figure 8. Map of Gasabo district showing the sampling sites. During this study, GPS data were collected on the location of each farmer's household using GPS eTrex 10 Garmin. QGIS version 2.18.20-Las Palmas software was used to produce the map depicted in Figure 8 based on the GPS data.

4.2.2 Survey design

This cross-sectional study recruited 154 farmers through an exponential non-discriminative snowball sampling procedure. These farmers were a subset of the sample farmers with knowledge of clinical signs of endometritis as described in section 3.3. Briefly, officers of sector animal resources aided identification and access to the initial farmers in the sampling process. The researchers visited these farmers and, through exponential non-discriminative snowball sampling, identified other farmers with the help of initial farmers. The initial farmers had to meet defined criteria. One, being aware of at least one clinical symptom of endometritis in cows (repeat breeding, abortion, or purulent or mucopurulent vaginal discharge) observed in the herd within the past one year. Two, granting informed written consent to participate in the study. Three, ease of accessing the farm. All the recruited farmers were visited from September 2018 to March 2019.

The study used the best-worst scaling (BWS) object case method to gather the perspectives of the farm owner about the effectiveness of MIs for endometritis prevention and control (Shortall *et al.*, 2017; Wittenberg *et al.*, 2016). BWS is a choice method about how respondents make the best and worst choices from choice sets containing three or more items and is considered better than rating and ranking scales because it provides discriminating results and sufficient information to calculate the choices scores of each item being evaluated (Kiritchenko & Mohammad, 2017; Parvin *et al.*, 2016). Moreover, BWS holds advantages of handling a long list of items, generating accurate preferences of all items, provides information on the magnitude of the importance of each of the items, BWS questionnaires are easy for respondents to understand, requiring a shorter time to answer BWS questions and avoiding scale (Guinat *et al.*, 2017; Marley & Flynn, 2015; Orme, 2018). This approach has been used for livestock disease management in many countries. In the United States of America on dairy farm management priorities and implications (Li *et al.*, 2018), in Colorado on consumer priorities for corporate social responsibility in milk production (Costanigro *et al.*, 2016), in Sweden on dairy farmer's use and non-use of values in animal welfare (Hansson & Lagerkvist, 2016). Other applications were in Western European regions on effectiveness and control strategies for African swine fever (Guinat *et al.*, 2017) and in the United Kingdom on effectiveness of biosecurity measures of common animal disease on dairy herds (Shortall *et al.*, 2017). In this study, the BWS object case was applied to 20 MIs considered important in the prevention and control of endometritis (Table 15), based on the literature review (Crowe *et al.*, 2018; El-Khadrawy *et al.*, 2015; Ganaie *et al.*, 2018; Gantner *et al.*, 2016; Purohit *et al.*, 2015; Rossi *et al.*, 2017; Sheldon *et al.*, 2020; Wittenberg *et al.*, 2016).

Table 15. Management interventions examined in the study

MIs codes	Management interventions (MIs)
1	Avoid equipment (water tubs, feeders, animal health equipment, milking machine, calving equipment)-sharing between cows within the farm
2	Avoid equipment (tractor, fodder chopper machine, water tubs, feeders, animal health equipment, milking machine, calving equipment)-sharing with neighbouring farms
3	Avoid housing fresh cows with diseased cows or those with chronic illnesses such as mastitis
4	Avoid off-farm bedding materials and maintain adequate bedding materials per cow
5	Avoid sharing or hiring a breeding bull
6	Consult ANHS provider about the treatment and prevention of diseases such as mastitis and metabolic diseases
7	Consult ANHS providers about the treatment of endometritis positive cases
8	Cull of persistently endometritis positive cows
9	Disinfect equipments of calving assistance before and after use
10	Keep the cows in a clean and dry shed
11	Maintain adequate feeding per cow
12	Maintain a clean transition cow housing
13	Maintain regular contact with ANHS providers for advisory support on endometritis prevention in dairy farm
14	Select sires based on calving ease
15	Select sires based on low percent stillbirths
16	Remove foetal membranes immediately after passing
17	Use gloves during calving assistance
18	Use artificial insemination as a breeding method
19	Use sexed semen during artificial insemination service
20	Wash the hands and udder before each milking

MIs = Management interventions, ANHS = Animal Health Service

In this study, MI was defined as an action that reduces or targets risk factors for endometritis in the dairy herds. The effectiveness of MI was the extent to which the MI prevents or controls endometritis-causing agents on-farm. The check-list for best-worst scaling choice was designed in Sawtooth Software, Version 8 (Sawtooth Software, 2013) based on a balanced incomplete block design for 20 management interventions generating four replicates (versions), each containing four-choice cards of size five (e.g. five management interventions per choice card). In this balanced incomplete block design, each MI appeared in an equal number of times in a different choice card; each MI paired with any other MI an equal number of times; each MI appeared four times in total across all choice cards; every pair of MIs appears once and the order of MIs within each choice card was randomly assigned (Orme, 2018; Sawtooth Software, 2013). The questionnaire was designed in such a way that each dairy farmer had to respond to a total of 16 choice cards of five MIs each. The questionnaire was developed in English, and the researchers conducted the interviews in the local language (Kinyarwanda). The questionnaire was pre-tested on 30 farmers to ensure the objective of the study is clear, to determine the time needed to complete the survey per farmer and the obstacle that could arise, and to improve the clarity of the questions to respondents (Perneger *et al*., 2015). The 30 farmers used in the pre-testing survey were not part of the farmers recruited for the study being reported in this Chapter.

For each recruited farmer, an explanation of each MI was presented and subsequently, for each choice card, the farmer was asked to choose first the most effective and then the least effective MI for endometritis prevention and control. To increase variation and combination of MIs across dairy farmers, the order of 16 choice cards was randomly assigned for each dairy farmer using Microsoft Office Excel (version 2016) (Wittenberg *et al*., 2016). The socio-demographic characteristics of each participating farm and farmer were recorded.

4.2.3 Statistical analysis

Data analysis of the best-worst choices were performed using two approaches: (i) best-worst percentages for the number of times each MI was selected as most effective, least effective or not chosen across a sequence of 16 choice cards divided by the availability of each MI; and (ii) best–worst score as the standardised score for each MI (UTS: CenSoc, 2018; Wittenberg *et al*., 2016). The effectiveness score was computed as the difference between the number of times chosen as the most and least effective divided by the availability of each MI (Costanigro *et al*., 2015; Wittenberg *et al*., 2016). The availability of each MI was computed as the number of times it has appeared in total across the 16 choice cards multiplied by the sample size (Marley & Flynn, 2015; Wittenberg *et al*., 2016).

The computed standardised score indicates the effectiveness of MI on a scale from −1.0 to +1.0. Scores toward +1.0 indicate that the MIs were chosen as most effective more often than as least effective. In contrast, scores toward −1.0 suggest that the MI was chosen as least effective more often than as most effective (Jones *et al*., 2013; Wittenberg *et al*., 2016). Thus, the higher the score, the more the MI is effective. In this study, different MIs were categorised into four-group endometritis prevention and control plan relating to (i) equipment-sharing between cows within a farm and/or with neighbouring farms and hygiene in a cowshed, (ii) control of breeding services, (iii) animal health interventions, and (iv) reduce the risks of contamination within and between farms.

Frequency distribution describing farmer characteristics was generated from cross-tabulation and frequency tested with Chi-square test statistics. Hypothesis testing was with the Non-parametric Kruskal-Wallis H test for whether the sources of ANHS providers, education level, or poverty level differ by farmer's choices. A Binomial test was used to analyse the significance of the differences in cowshed flooring (earthen or concrete), cowshed types (covered with or without a roof), breeding services (artificial insemination or bull), and herds recording (not practiced or incomplete). All statistical analyses were performed in IBM SPSS Statistics 22.0 for Windows (SPSS, 2013), and the statistical significance level was set at alpha <0.05.

4.3 Results

4.3.1 Socio-economic characteristics of the dairy farmers

The socio-economic characteristics of the sample farmers in the study area are presented in Table 16**.** The sampled farmers were between 25 and 85 years old and on average, were of middle-aged (41.5±1.1 years) with an average of 9.6±0.5 (median 10.0) years of dairy farming experience. The majority were males (71.4%). About half of the farmers had attained primary level education (48.7%) while a few had attained secondary (14.9%) or university (2.6%) level education. However, over a third of the sample farmers had not acquired any formal education (33.8%). The family size was, on average, 5.0±0.1 members in a household. By poverty classification of the Government of Rwanda, farmers in the category of poor (63.0%) dominated over those in the category of wealthy farmers (28.6%). On average, a household kept a herd of less than three cattle on a farm of less than four acres.

Table 16. Socio-economic characteristic of the sample smallholder dairy farmers (n=154)

Variables	Frequency (%)	Mean±SEM	Median	Minimum	Maximum
Gender					
Male (%)	71.4				
Female (%)	28.6				
Educational level					
No schooling (%)	33.8				
Primary (%)	48.7				
Secondary (%)	14.9				
University (%)	2.6				
Poverty level					
Very poor (%)	8.4				
Poor (%)	63.0				
Rich (%)	28.6				
Age (years)		41.5±1.1	40.0	25.0	85.0
Dairying experience (years)		9.6±0.5	10.0	1.0	25.0
Household size (number)		5.0±0.1	5.0	1.0	9.0
Herd size (number)		2.9±0.8	3.0	1.0	4.0
Farm size (acres)		3.8±0.1	3.7	0.6	6.9

The herds were predominantly crossbreeds (63.6%) with some pure breeds (29.0%), and indigenous cattle breeds (7.4%) kept to provide milk for domestic consumption, sale, and to provide manure for fertilizing the farms. The cows were kept in zero-grazing housing units, on the cut-and-carry feeding system, with over half servicing with bulls (53.9%). At the time of this study, the breeding services were at a cost: US$5.6 per service when using artificial insemination service and US$ 3.3 per service when using bull service. Dairy farmers sourced animal health services (ANHS) from veterinarians (VETs) (39.6%), community-based animal health workers (CAHWs) (37.7%), and local traditional herbalists (LTHs) (22.7%). Bedding materials were natural green grasses (85.5%), and the leftovers or waste feeds from feeding troughs (14.5%). The frequency of removal of bedding materials was, on average, twice a week. The flooring of cowshed was typically earthen, and only a few had concrete (Table 17). The majority of farmers had a cowshed with a roof, and few had a cowshed without a roof. The herd recording was not practiced (78.6%) or incomplete (21.4%).

Table 17. Herd characteristics in the study area (n=154)

Characteristics	Level	Frequency	Percentage (%)	Chi-square test
Cowshed flooring	Earthen	139	90.3	***
	Concrete	15	9.7	
Types of cowshed	With a roof	101	65.6	***
	Without a roof	53	34.4	
Breeding services	Artificial insemination service	71	46.1	NS
	Bull service	83	53.9	
Herd records keeping	Incomplete	33	21.4	***
	Not practised	121	78.6	
Calving pen	Presence	11	7.1	***
	Absence	143	92.9	

^{NS} not significant (p>0.05), ***p<0.001

Figure 9 depicts cows kept in different types of cowshed and flooring types in smallholder zero-grazed dairy farms. The majority of cows were kept in cowsheds covered with a roof and a few cows were kept in cowsheds without a roof.

Figure 9. Types of cowshed and their flooring in smallholder zero-grazed dairy farms. **(A)** cows in the cowshed with a roof, **(B)** cows kept in cowshed without a roof, **(C)**cow kept in cowshed with concrete floor, and cows kept in cowshed with earthen floor **(D)**.

4.3.2 Effectiveness of the management interventions

Figure 10 shows the percentage of MIs that farmers considered the most effective, least effective, and not considered the most or least effective. In decreasing order of probability of being considered most effective, the top four MIs were: avoiding equipment-sharing with neighbouring farms (45.5%), consulting ANHS provider about the treatment of positive endometritis case (31.8%), keeping cows in a clean and dry shed (26.7%), and selecting sires based on calving ease (26.6%). The MIs considered least effective were: maintaining clean transition cow housing (35.1%), removal of foetal membrane immediately after passing (33.1%), disinfecting calving assistance's equipment before and after use (32.5%), and selecting sires with a lower percentage of stillbirths (29.2%). The MIs not considered to be effective were: using gloved-hands during calving assistance (76.6%), washing the hands and udder before each milking (70.1%), consulting ANHS providers about dairy cattle diseases prevention (68.8%), and culling persistently endometritis positive cows (67.5%). The study revealed that neither dairying experience, poverty level, education level, nor the source of ANHS providers influenced ($p>0.05$) the considerations by farmers about the MIs.

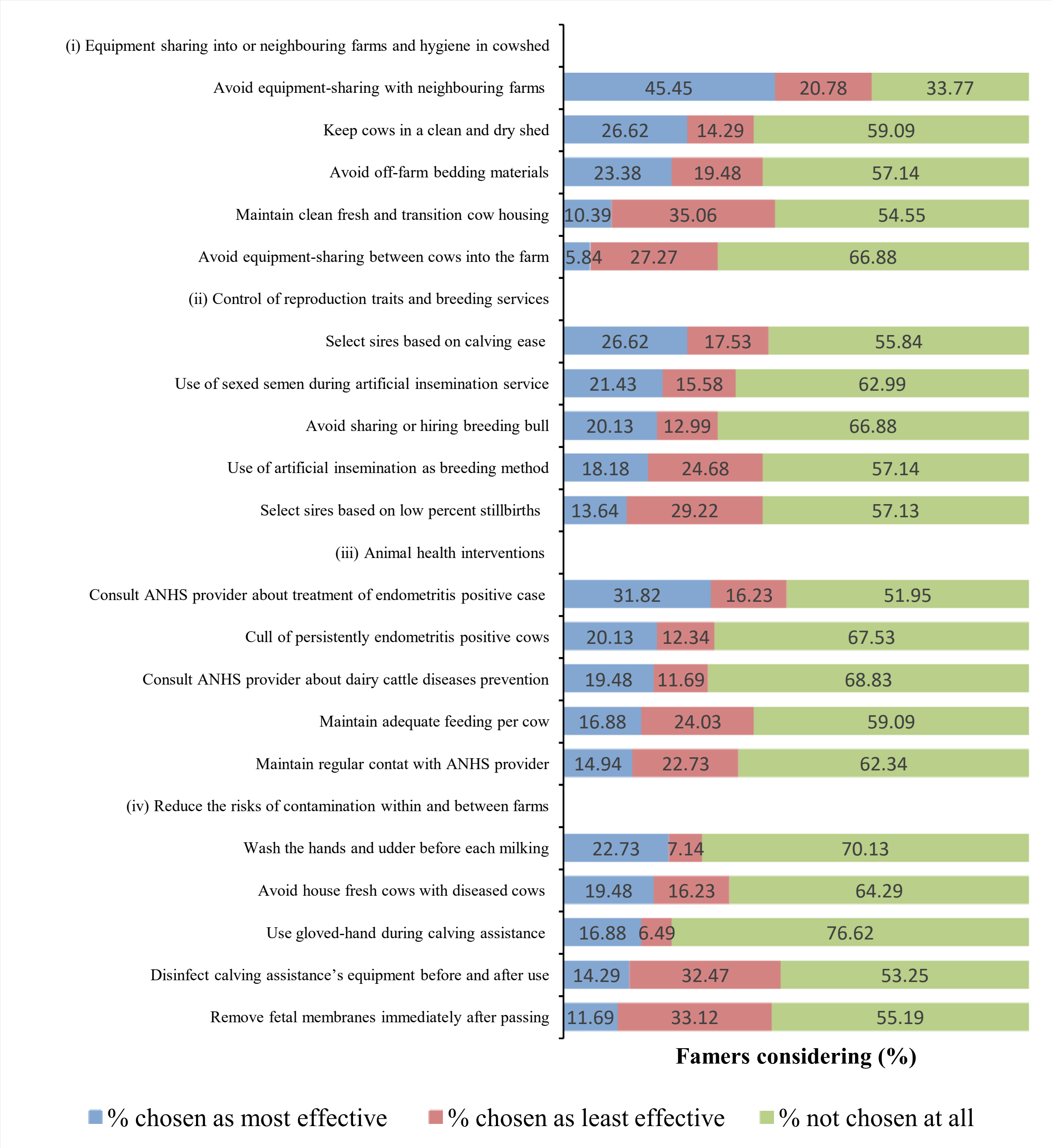
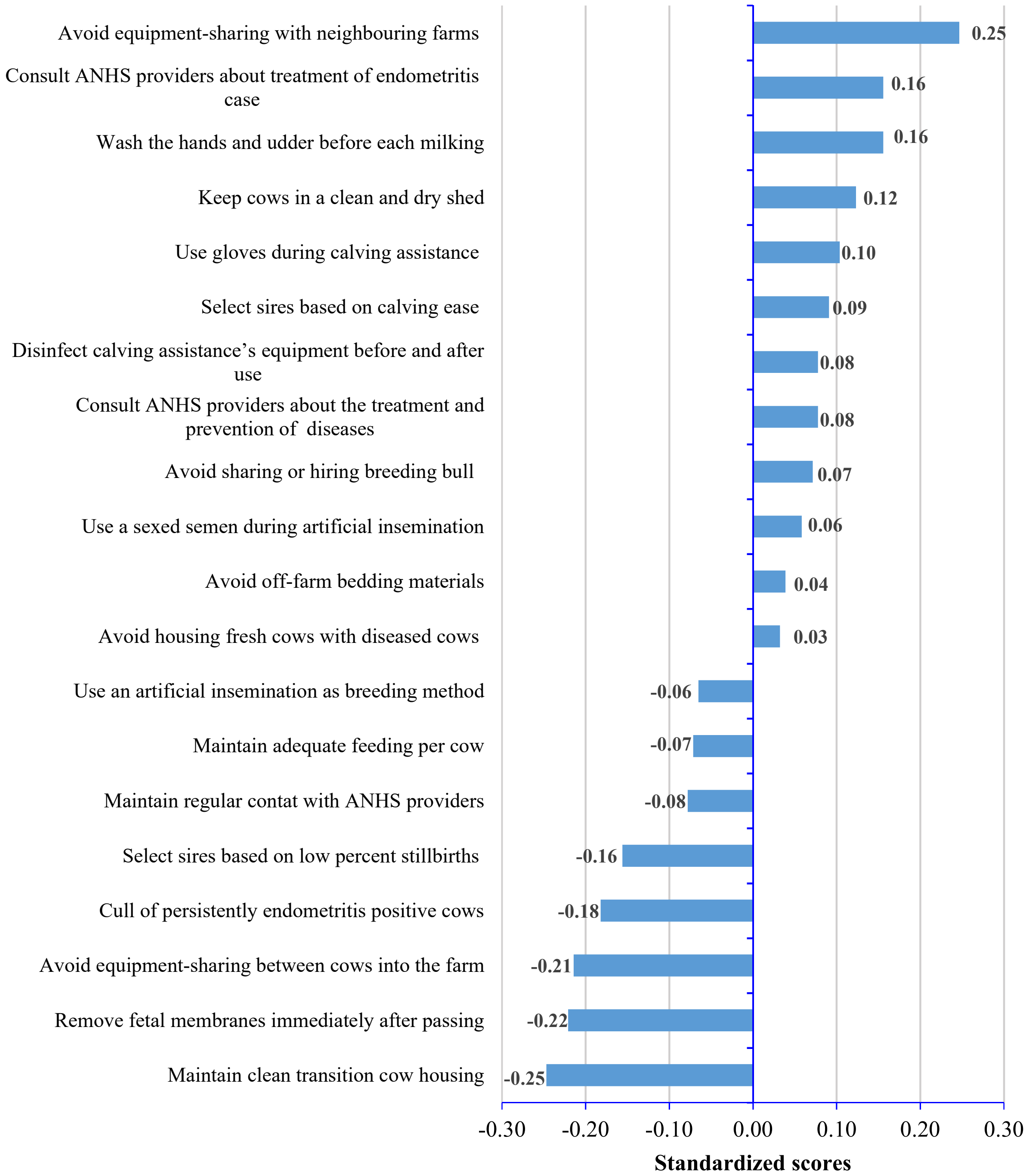


Figure 10. Best-worst percentages of farmers' opinions on the effectiveness of 20 management interventions towards endometritis prevention and control on dairy farms (n = 154).

The standardised scores illustrated in Figure 11 represent the computed effectiveness scores assigned to each MI. The y-axis represents the effectiveness scores of all 20 MIs that were examined. The right of the x-axis shows the MIs that were scored highly for effectiveness, whereas the left of the x-axis represents the MIs that were scored low for effectiveness. Of the 20 MIs, 60.0% (n=12) were scored highly for effectiveness, and these are located in the upper right-hand quadrant.

These MIs belong to equipment-sharing between cows within farms and/or with neighbouring farms and hygiene in a cowshed (group 1 of MIs: 02, 04, and 10), control of breeding services (group 2 of MIs: 05, 14 and 19), animal health interventions (group 3 of MIs: 06 and 07), and reduce the risks of contamination within and between farms (group 4 of MIs: 03, 09, 17 and 20). Based on standardised scores, 20.0%, 15.0%, 15.0%, and 10.0% of MIs were considered as the most effective MIs in group 4, 1, 2, and 3, respectively. Avoiding equipment sharing with neighbouring farms (MI 02), consulting ANHS provider about the treatment of positive endometritis case (MI 07), and washing the hands and udder before each milking (MI 20) were the perceived most effective MIs. However, there were no significant differences (Chi-square = 1.583, $p = 0.663$) found among the four groups of MIs. The MIs scored low for effectiveness were: maintaining clean transition cow housing (MI 12), removing foetal membranes immediately after passing (MI 17), and avoiding equipment sharing between cows within the farm (MI 01).



*ANHS = Animal Health Service

Figure 11. Effectiveness scores for the 20 management interventions (MIs).

4.4 Discussion

This study is a pioneer application of best-worst scaling (BWS) choice method in analysing farmers' perspectives on the effectiveness of different MIs in the prevention and control of endometritis among zero-grazed dairy cows on smallholder farms. The approach has not been used previously in evaluating how dairy farmers perceive the effectiveness of different

MIs for endometritis in dairy farms. The application of BWS method enabled the identification of 12 (60.0%) most effective MIs and 8 (40.0%) least effective MIs on smallholder farms practicing cut-and-carry feeding system in Rwanda.

As in the present study, the application of BWS with experts on opinion about the effectiveness of biosecurity measures on dairy farms informed livestock management practices in the United Kingdom (Shortall *et al*., 2017). In that study, preventing contact with neighbouring animals, and implementing rapid culling of persistently infected animals were rated the most effective biosecurity measures. In contrast, minimising the number of visitors entering the farms, and avoiding equipment-sharing between farms were rated the least effective. Similarly, BWS was applied (Guinat *et al*., 2017) to identify the effectiveness of intervention strategies for African swine fever in Western Europe. Findings suggest that the culling of all infected pigs and restricting movement for neighbouring farms were the most effective interventions to control the disease. These findings reflect farmer experiences with MIs that effectively work for them despite their scientifically proven. Therefore, farmer's experiences with MIs indicate that farmers require a basket of choices of management practices from which to choose what suits their farming circumstances. Their practical skills can be integrated into extension strategies and veterinary service delivery to farmers and research attention to enhance herd health management (Hansson & Lagerkvist, 2016).

In the current study, MIs (02, 04, and 10) related to equipment-sharing within the farm or with neighbouring farms and hygiene in cowshed were scored the most effective for preventing and controlling endometritis in dairy farms. The practice of sharing or borrowing farm equipment can be a media of disease and/or pathogens transmission (Rossi *et al*., 2017). This finding corroborates with the observations (Wapenaar *et al*., 2017) in the United Kingdom that sharing equipment and materials between farms without appropriate biosecurity measures increases the risk of microorganisms contamination or disease transmission.

There are implications on hygiene practices for farmer consideration that clean and dry cowshed (MI 10) and avoiding off-farm bedding materials (MI 04) are effective for prevention and control of endometritis. It is an indication that it is crucial to practice frequent removal of any soiled or damp bedding before adding fresh bedding materials. On the sampled farms, this was practiced on average twice a week. Unhygienic bedding materials and heavily soiled cowsheds are potential risk factors for transmission of causal microorganisms for disease in postpartum cows, of which endometritis is a prevalent disease (Adnane *et al*., 2017; Dutta *et al*., 2021). Similarly, high mastitis prevalence (76.2%) has been attributed to inadequate biosecurity measures on zero-grazing dairy farms in Rwanda (Ndahetuye *et al*., 2019).

Therefore, the implementation of biosecurity measures is essential to improving cow welfare as well as their production and fertility performance. This observation concurs with observations made in Ethiopia (Getie *et al*., 2015), in India (Dutta *et al*., 2021), and in California, United States of America (Espadamala *et al*., 2016).

The MIs (05, 14, and 19) related to the control of breeding services scored highly for effectiveness. In the sampled farms, over half were using shared breeding bulls for service without precautions for potential risks of disease transmission. Farmers used bull service because breeding bulls were readily available and affordable within the communities and about twice cheaper compared to artificial insemination (AI) (US$3.3 vs. US$5.6 per service). In this practice, the breeding bulls or the cows on heat are moved from one place to another for mating. The bulls and cows are not pre-screened for diseases before use. This way, the bull mating practice presents a risk of spreading the reproductive diseases or pathogens transmission, as corroborated by finding (Mushonga *et al*., 2017) in Nyagatare district, Rwanda, and Shortall *et al*. (2017) in the United Kingdom.

Farmers using AI services can be advised by their AI technicians about the advantages of selecting sires with easy - calving and can be assisted in choosing AI semen for their cows. The birth of a male calf may increase the risk of dystocia cases (Yehualaw *et al*., 2017). In such cases, giving calving assistance may also increase the likelihood of trauma and contamination of the reproductive tract and increase the risk of endometritis infections. Farmers can reduce the risk of dystocia by using sexed semen from sires with calving ease and low percent stillbirths to produce female calves (Diers *et al*., 2020).

The MIs (06 and 07) related to animal health intervention scored highly for effectiveness, implying that proper veterinary service delivery is essential for farmers in the prevention and control of endometritis (Sumner *et al*., 2018). In their study, Daros *et al*. (2017) in Brazil, reported that metabolic disorders are important in the transition period because they predispose cows to reproductive disorders. Mastitis disease is also a significant risk factor for endometritis (Bacha & Regassa, 2010). Proper veterinary service delivery facilitates prompt veterinary intervention for these risk factors.

In the present study, hardly half of the farmers accessed ANHS from VETs, CAHWs or LTHs. Basically, ANHS providers visit dairy herds at farmer's request mostly when a health problem is noticed. In that case, the services offered target the diseases with commonly noticeable symptoms in dairy cows. This supports the earlier findings that endometritis received less attention because the sampled farmers could not attribute the symptoms they observed to endometritis (Chapter three). This calls for capacity building program on endometritis diagnosis, extension advisory, and management on dairy farms to apply effective MIs for prevention and control of endometritis. This recommendation aligns with the observation (Tayebwa *et al*., 2015) in Uganda that improved extension service and advisory support in pre, during and postpartum periods are effective ways to manage endometritis in the dairy herds.

Other MIs (03, 09, 17, and 20) that farmers considered highly effective for the prevention and control of endometritis related to reducing risks of contamination within and between farms. At high risk of contamination are fresh cows and cows with trauma in the reproductive tract because fresh cows are immunosuppressed, and housing them with mastitic cows exposes them to disease-causing pathogens (Bradtmueller &Amaral-phillips, 2019). Therefore, on-farm biosecurity measures are important to minimise disease transmission in the dairy herds. Knowledge gaps might lead to widespread high-risk practices for both animals and humans (Ruano & Aguayo, 2017). From the literature (Bradtmueller & Amaral-phillips, 2019; Ruano & Aguayo, 2017), it is advisable to assist calving only when needed and always using gloved hands, disinfecting calving equipments, and keeping cows in a clean cowshed and calving area to reduce trauma and contamination of female genital tract. Further, studies in France (Duval *et al*., 2016) and the United States of America (Havlin *et al*., 2017) demonstrate the importance of good housing conditions, disinfection, and disease prevention in minimising disease entry and spread within a dairy herd.

The adoption and implementation of some MIs that farmers perceived are most effective for the prevention and control of endometritis remain limited on the smallholder zero-grazed dairy farms in Rwanda. This situation applies as well to the United Kingdom (Shortall *et al*., 2017) and Switzerland (Kuster*et al*., 2015). The reasons are likely related to some MIs being impractical to implement. This has to be addressed in in-depth research to enhance the practical application of MIs for the prevention and control of endometritis.

For the MIs that farmers considered the least effective for the prevention and control of endometritis, local contextual issues are likely to be at play. The sampled farmers had a low level of information about endometritis diagnosis and management. Recent studies conducted in Switzerland (Kuster *et al*., 2015) and Canada (Denis-Robichaud *et al*., 2019) showed that farmers' awareness of the disease and a better understanding of the transmission of disease influence their perceptions on the effectiveness of biosecurity measures. Another study (Channappagouda *et al*., 2016) with Indian farmers indicated that knowledge gaps about cattle diseases and how to prevent them limited the adoption of animal genetic improvement and health care practices.

In the study area, the VETs cover a wide area in the mountainous region with many farmers to attend to, which hinder prompt access in remote areas. CAHWs and LTHs could serve remotely accessible areas for better and timely service delivery to farmers. In Indonesia (Lestari *et al.*, 2018) and Ghana (Adams & Kwasi, 2015), studies concluded that insufficient VETs and lack of capital hindered farmers from accessing prompt veterinary services and adopting and implementing biosecurity measures. In Canada, a study indicated that farmers who discussed biosecurity measures with a VET were more likely to perceive biosecurity measures as more effective than farmers who did not (Denis-Robichaud *et al.*, 2019). This is an indication that it is the best practice that farmers regularly consult with the ANHS providers on a plan of dairy herd health management.

Of the sampled farmers, about a third had a cowshed without a roof, and therefore, cows are not protected from environmental stresses such as the muddy floor in the zero-grazing unit. This condition favours the proliferation and transmission of disease-causing organisms, where disease management practices are not implemented. It is because of the association between the lack of implementation of disease management practices and the high prevalence of diseases such as uterine infections (Dutta *et al.*, 2021) and mastitis in the dairy herds (Amer *et al.*, 2018; Ndahetuye *et al.*, 2019; Suleiman *et al.*, 2017).

In Rwandan comprehensive wealth-ranking system criteria (Cho & Kim, 2017), the sample farmers were in the group classified as poor to very poor. Because they are the most vulnerable, resource-poor farmers, they have low capacity to implement the MIs (Wolff *et al.*, 2019). This is corroborated (Ritter *et al.*, 2017) in Canadian dairy farms where a significant barrier to implementing prevention strategies for Johne's disease was the cost to build facilities, hire more labour, and purchase the recommended equipments. In the present study farms, the land size owned was a resource constraint to farmers, necessitating practicing cut-and-carry feeding system in crowded cowshed units, with poor standards of hygiene. This condition exposes cows to bacterial contamination (Mpatswenumugabo *et al.*, 2017).

4.5 Conclusion

This study has demonstrated the application of the BWS object case method in understanding the MIs that farmers consider are most effective in the prevention and control of endometritis in the dairy herds. The identified most effective MIs can be prioritised for extension dissemination to farmers for effective prevention and control of endometritis. The top four are: avoiding sharing equipment with neighbouring farms, consulting ANHS providers about the treatment of endometritis positive cases, keeping cows in a clean and dry shed, and selecting sires based on calving ease. Furthermore, the MIs have to be applied in combination: no one of the MIs would be singly effective as there are multiple risk factors. In-depth research on these MIs is, however, necessary to enhance their practical application on smallholder dairy farms.

CHAPTER FIVE

RISK FACTORS ASSOCIATED WITH ENDOMETRITIS IN ZERO-GRAZED DAIRY COWS ON SMALLHOLDER FARMS IN RWANDA

Abstract

Clinical endometritis (CLE) and subclinical endometritis (SCLE) manifesting at the cow- and herd-levels have been associated with multiple risk factors (RFs), but hardly are RFs with direct influences separated from those with mediated indirect influences. This study identified and quantified the direct and indirect associations of cow- and herd-levels RFs with CLE and SCLE cases observed among 466 zero-grazed dairy cows that were in their 21 to 60 days postpartum (dpp). The cases were observed in a cross-sectional survey of smallholder farms (n = 370) in Rwanda. The direct and indirect associations were constructed with odds ratio (OR) derived from multiple logistic regression modelling. The cow-level RFs that had direct positive association with CLE and SCLE were the season of calving (OR: 5.0, 2.1), dystocia (OR: 1.9, 2.2), poor body condition score (OR: 4.1, 2.2), stillbirth (OR: 3.5, 3.3), and retained placenta (OR: 1.4, 1.8) while mastitis (OR: 2.5) and parity (OR: 1.5) had a direct positive association with SCLE. Breed and parity of cow, sex of calf, and twin births had indirect positive association with both CLE and SCLE cases. At the herd level, unhygienic cowshed (OR: 25.1, 8.9) had direct positive association with both CLE and SCLE cases. In contrast, earthen floor cowshed (OR: 6.6) and large herd size (OR: 3.1) had direct positive association with CLE and not using bedding materials (OR: 1.5) had direct positive association with SCLE. Herd-level RFs that showed indirect positive association with both CLE and SCLE cases were farm size (OR: 2.9) and farmer's experience in dairying (OR: 1.7) while housing cows within the first 30 dpp (OR: 0.1) showed indirect negative association. These results show which RFs have strong direct and indirect influences on CLE and SCLE cases at the cow- and herd- levels. Effective management of those RFs should be a priority in extension education and services to enable smallholder farmers effectively manage them to prevent and control endometritis among their zero-grazed dairy cows.

5.1 Introduction

Endometritis is an important postpartum uterine disease of economic importance in dairy cows. The disease disrupts cows' fertility performance and reduces dairy herd productivity and profitability (Chaudhari *et al*., 2017; Sharma *et al*., 2019). The disease may manifest as clinical endometritis (CLE) and/or subclinical endometritis (SCLE) between 21st and 90th days postpartum (dpp) period (Kelly *et al*., 2020; Okawa *et al*., 2017). The CLE is characterized by presence of mucopurulent or purulent uterine discharge detectable in the vagina between 21 and 90 dpp (Potter *et al*., 2010; Tayebwa *et al*., 2015). In contrast, the SCLE is characterized by abnormal presence of the proportion of polymorphonuclear inflammatory cells (≥5%) in endometrial cytology samples collected in the period 21 to 90 dpp (Kasimanickam *et al*., 2005; Pascottini *et al*., 2017). In diagnosis of the endometritis disease, Okawa *et al*. (2017) was successful with diagnosis of the disease between 21 to 60 dpp, whereas Kelly *et al*. (2020) did diagnose the disease between 25 and 86 dpp. These are evidences that CLE and SCLE cases can be effectively diagnosed between 21 and 90 dpp period.

The prevalence of CLE and SCLE shows large variation at the cow- and herd- levels as well as in smallholder and large dairy herds. At the cow- level, the SCLE prevalence has varied from 6.7 to 89.0% for cows examined between 25 and 86 dpp (Denis-Robichaud & Dubuc, 2015; Kelly *et al*., 2020), and CLE prevalence has ranged from 3.6 to 69.8% for cows examined between 33 and 60 dpp (McDougall *et al*., 2007; Tayebwa *et al*., 2015). At the herd- level, the SCLE prevalence has varied from 4.8 to 64.1% (Cheong *et al*., 2011; Lee *et al*., 2018) while CLE prevalence has ranged from 4.0 to 87.0% (Denis-Robichaud & Dubuc, 2015; Ryan *et al*., 2020).

The prevalence of CLE and SCLE seems higher in large dairy herds than in smallholder dairy herds (Chan Lee *et al*., 2018; Moges, 2015; Ryan *et al*., 2020). The large variation in CLE and SCLE prevalence at the cow- and herd- levels and in large and smallholder dairy herds could be a reflection of multiple RFs, some with direct influences and some mediated indirect influences. This could be resulting from the large heterogeneity of management practices in the dairy herds (Adnane *et al*., 2017; Chan Lee *et al*., 2018).

Most studies of RFs for endometritis are available for commercial dairy systems, and they show differences between countries, reflecting differences in herd health management practices and environmental conditions (Adnane *et al*., 2017; Chan Lee *et al*., 2018; Kelly *et al*., 2020). The cow-level RFs studied and reported include cow parity, body condition score, cow breed, twins, breeding services, retained placenta, dystocia, gestational length, days dry, left displaced abomasum, offspring sex, calving season, stillbirth, brucellosis, mastitis; milk fever, and ketosis (Adnane *et al*., 2017; Chan Lee *et al*., 2018; Kelly *et al*., 2020). For the herd-level RFs, those reported include hygiene conditions (cleanliness of cowshed, housing cows within the first 30 dpp, bedding materials, calving pen), herd size, and farm size (Cheong *et al*., 2011; Moges & Jebar, 2012).

These RFs could be relevance in the smallholder herds for the observed high prevalent endometritis cases. A recent study of endometritis with cows in their 21 to 60 dpp managed on smallholder dairy herds in Rwanda (Chapter three) showed that the prevalence of endometritis is as high as 71.1% at herd-level and 70.2% at cow-level, with CLE reaching 68.1% at herd-level and 67.2% at cow-level. The SCLE prevalence is 34.4% at herd-level and 31.8% at cow-level. Despite this high prevalence, literature search suggests that CLE and SCLE have received little research attention in smallholder herds. An exception is the study of Nguyen-Kien & Hanzen (2017) in Vietnam, which reported significant contribution of the season of calving, dystocia, and retained placenta to the occurrence of CLE in smallholder herds. This study revealed the presence of multiple RFs for endometritis in smallholder dairy farms, but empirical evidence is lacking in sub-Saharan African countries, specifically in Rwanda, where smallholder zero-grazed farming is among the high prioritised livelihood strategies.

In Rwanda, smallholder dairy farming accounts for 92.0% of the national dairy herd, predominantly managed under zero-grazing systems and characterized by suboptimal fertility performance and milk production (Manzi *et al*., 2019; Rukundo *et al*., 2018). Because this suboptimal performance has been associated with CLE and SCLE cases in dairy cows (Chapter six and seven), a good understanding of the RFs involved would inform management of the disease on the farms. Despite the high prevalence of endometritis, present herd fertility interventions ignore targeting CLE and SCLE because empirical evidence is lacking for the presence of RFs involved and as to which ones of them pose high risks for CLE and/or SCLE. Though CLE and SCLE manifesting at the cow- and herd-levels have been associated with multiple risk factors (RFs), hardly are RFs with direct influences separated from those with mediated indirect influences. Such evidence can inform management practices targeted to RFs at the cow- and herd-levels and high-risk cows in the transition period for early intervention against the occurrence of CLE and SCLE cases.

A path analysis model is relevant for studying RFs with possible direct and indirect influences on a disease occurrence. The model comprises multiple regression techniques that allow modelling of the dynamic process and interactions between a dependent variable and two or more independent variables (Curtis *et al*., 1985). The path analysis model has several advantages. It allows making use of available a priori information regarding known or plausible hypothesized interrelationships among variables, including direct and indirect causal associations. This is an important advantage over conventional regression analysis, which only allows quantification of conditional direct relationships (Neo *et al*., 2017; Rougoor *et al*., 1997). The path analysis model has been used to study livestock disease management (Correa *et al*., 1993; McDougall, 2001; Oltenacu *et al*., 1990), production in dairy farms (Rougoor *et al*., 1997); human health (Cilia *et al*., 2011), and environmental management (Li *et al*., 2018).

This study applied a path analysis model with multiple logistic regression in estimating the OR to identify and quantify cow- and herd-levels RFs associated with CLE and SCLE cases in smallholder zero-grazed dairy cows in Rwanda.

5.2 Materials and methods

5.2.1 Study population and farm

This study was conducted from September 2018 to March 2019 in smallholder zero-grazed dairy farms of Gasabo district, Rwanda. A detailed description of the study area has been presented in Section 3.2. The study applied a cross-sectional design involving a total of 370 dairy farmers selected through exponential non-discriminative snowball sampling. This was initiated with a list of two to three farmers provided to the research team by officers of sector animal resources. These initial farmers had to fit defined study criteria: (i) having at least one cow within 21 – 60 dpp, (ii) willingness of the farmer to participate, and (iii) physical accessibility of the farm.

For each recruited farmer, an explanation of the objective of the study was presented, and written informed consent was sought before starting data collection. The sample farms granted access to enrolment of four hundred sixty-six (466) cows within their 21 - 60 dpp at sampling. Their breed distribution was 66.3% dairy crossbreds, 17.0% dairy pure breeds, and 16.7% indigenous cattle. These cows were kept in zero-grazing housing units, and the dominant feeding practice was the cut-and-carry system. Their diet consisted of fodder (*Pennisetum purpureum*, banana fodder, and natural grass) and sometimes supplemented with a commercial dairy meal and mineral licks during milking.

5.2.2 Data collection

A pre-tested structured questionnaire developed in English was administered by trained enumerators able to conduct the interviews in the local language (Kinyarwanda). The questionnaire pre-testing was with 30 farmers that were not part of the farmers recruited for the study. On each farm, all sampled cows were examined for individual cow-level RF. In contrast, the farm was examined for the status of herd-level RF hypothesised to influence the CLE and SCLE cases (Table 18). A definition in this study is that cow-level RF are specific variable characteristics of the individual cow in the same herd while herd-level RF are environmental conditions and management characteristics shared by cows within the same herd (Adnane *et al*., 2017; Chan Lee *et al*., 2018; Kelly *et al*., 2020).

Table 18. Cow- and herd-levels risk factors evaluated in the study

Risk factors	Measures
Cow-level	
Cow breed	Indigenous cattle, dairy crossbred or pure dairy breed
Breeding services	Artificial insemination or bull
Stillbirth	Occurrence or non-occurrence
Dystocia	Occurrence or non-occurrence
Retained placenta	Occurrence or non-occurrence
Milk fever	Occurrence or non-occurrence
Ketosis	Occurrence or non-occurrence
Left displaced abomasum	Occurrence or non-occurrence
Cow parity	Primiparous or multiparous
Mastitis	Positive or negative
Brucellosis	Positive or negative
Dry period length	Days (≥90 or <90)
Gestation length	days (≥283 or <283)
Cow age	Years (≥5 or <5)
Body condition score at sampling	Score (≥3 or <3)
Calf sex	Female or male
Calving season	Rainy or dry
Twin births	Yes or non
Herd-level	
Housing cows within the first 30 dpp	Yes or no
Calving pen	Yes or no
Cowshed flooring	Concrete or earthen
Bedding materials	Using or not using
Cleanliness of the cowshed	Clean or dirty/unhygienic
Herd size	Numbers (≥3 or <3)
Farmer dairying experience	Years (≥8 or <8)
Farm size	Acres (≥3 or <3)

dpp = days postpartum

Some cow-level RF data such as days dry, gestation length, cow parity, sex of the calf, season of calving, breeding services, dystocia, retained placenta, twin births, milk fever, ketosis, left displaced abomasums, and status of a calf at birth were collected retrospectively from the animal health service (ANHS) providers' records, farm records or farmer recall through an interview, and direct observations. In this study, cases of twins in which one was male, and the other was female, or one was alive, and the other dead were excluded from the analysis because the effect of stillbirth or sex of the calf could not be assigned uniquely to only one member of the twin pair (Correa *et al*., 1993).

The researchers explained to the farmers and ANHS providers the definitions of RFs that are not obvious in an attempt to standardise the diagnostic process. Dystocia was defined as assisted calving either by the farmers who normally pull the foetus or by the ANHS providers who may pull the foetus or apply caesarean section or foetotomy (Funnell & Hilton, 2016). Therefore, diagnoses of dystocia were made by asking farmers whether anyone had assisted the cow during calving. Left displaced abomasum was defined as the presence of a combination of the following clinical signs: reduction in the intensity of rumen movements and a fluid-gas interface when struck sharply with a finger on the left side of the abdominal cavity (Toni *et al*., 2015). The retained placenta was defined as the failure to expel foetal membranes for more than 12 h after calving (Patel & Parmar, 2016). Ketosis was a case related to observing clinical signs that include: abnormal licking, excess salivation, nervousness, odor of acetone on the breath, and sternal recumbency (Biswal *et al*., 2016). Milk fever was a case related to observing clinical signs that include: weakness, nervousness, cold skin, off-feed behaviour, cows becomes too weak to stand and eventually becomes comatose over a matter of time, and favourable response to calcium therapy (Fikadu *et al*., 2016). The stillbirth included either delivery of a dead single calf, both dead twin calves between 260 days and full-term (283 days), or death in the first 12 h after calving (Mahnani *et al*., 2017). The breeding service was either artificial insemination (AI) or bull. Only one breeding service (AI or bull) was used to serve the cow in a given farm. A twin birth was defined as the calving of two calves. The female calf was defined as either single or both female twins, while the male calf was either singleton or both male twins (Correa *et al*., 1993). In this study, cases of twins in which one was male, and the other was female, or one was alive and the other dead were excluded from the analysis because the effect of stillbirth or sex of the calf could not be assigned uniquely to only one member of the twin pair (Correa *et al*., 1993). The breed of the cows was identified based on phenotype (Hirwa *et al*., 2017), history from farmers and the available records. Briefly, the local breed was Ankole longhorn, and crossbreds were Ankole longhorn crossed with Jersey, Friesian, Sahiwal, and Brown Swiss. The pure dairy breed was Holstein Friesian with black and white or brown and white colour, short haired coat, and short horns; and Jersey characterized by light brown in colour or grey to dull black with black nose bordered by an almost white muzzle, and they have protruding eyes. In the sample farms, flooring refers to the lower enclosing surface of spaces within buildings where the cow is lying. Herd size includes cows and youngstock as well.

The cow age was estimated using dentition (Parish & Karisch, 2013), as depicted in Figure 12. Briefly, cows with fully developed corners and the second intermediate incisors were considered to have <5 years old, whereas cows that had the permanent pinchers or central pair of incisors that become leveled were regarded as having ≥5 years' old (Parish & Karisch, 2013).

Figure 12. Trained enumerator performing the estimation of cow age using dentition

In this study, cows enrolled were tested for endometritis, mastitis and brucellosis. Thereafter, cows were body condition, and cleanliness scored. The data on herd-level RF were obtained from visual observation and by face-to-face interviews with farmers.

5.2.3 Endometritis diagnosis

This was as given in Chapter three. In brief, a cow that had VMC≥1 were recorded as CLE positive otherwise was CLE negative (Williams *et al*., 2005). Correspondingly, a cow that had endometrial cytology sample with ≥5% PMNs was recorded as SCLE positive otherwise was SCLE negative (Melcher *et al*., 2014). A herd was considered as positive for CLE if had at least one cow tested positive for CLE; whereas a herd was recorded as positive for SCLE if had at least one cow tested positive for SCLE.

5.2.4 Determination of mastitis

Mastitis was tested using California Mastitis Test as described by Leach *et al*. (2008). In brief, two millilitres of milk from each quarter of the udder were collected in each of the four shallow cups of the California Mastitis Test paddle after foremilk is removed. An equal amount of California Mastitis Test reagent (BOVIVET CMT Liquid, Denmark) was added to each cup in the paddle, and the results were observed and recorded by a single trained observer on a 5-point ordered categorical scale, ranging from 0 = Mixture remains unchanged to 4 = almost-solid gel forms (using 1-point increment). The California Mastitis Test was repeated twice for each sample to have the accuracy of the test results. Cows that had one or more quarter (s) with a reaction of ≥ 1 then were recorded mastitis positive (Figure 13).

Figure 13. California Mastitis test results

5.2.5 Brucellosis screening

Cows were blood sampled by puncture of the middle coccygeal vein using a sterile needle (Vacutest, Kima Srl, Italy) and a vacutainer blood collection tube (PharmaLab Ltd, Kigali, Rwanda). Therefore, each sample was labelled using codes describing the sampling date, cow's identification (eartag or name), sector and herd, and kept in insulated cool box. The following day, blood samples were transported to the National Veterinary Laboratory of Rwanda Agriculture and Animal Resources Development Board for sera preparation and brucellosis analysis. Serum samples were separated by centrifugation at 3,000 revolutions per minute (rpm) for 15 minutes and stored in 2ml cryovials at −20°C until analysis. Harvested sera were tested for the presence of brucellosis antibodies using Rose Bengal test (Idvet, rue Louis Pasteur-Grabels, France) according to the World Organisation for Animal Health procedures (OIE, 2018). The Rose Bengal Plate test was selected among other tests because it is the most widely used screening test for brucellosis in animals (OIE, 2018). The reported sensitivity and specificity of the Rose Bengal Plate test is 81.2% and 86.3%, respectively (Gall & Nielsen, 2004). Equal volumes (30µL or 0.03 ml) of Rose Bengal antigen and test serum were thoroughly mixed on a slide, and the reaction was observed after 4 minutes and recorded. The presence of agglutination was recorded as a positive case, whereas the absence of agglutination was considered as a negative case (Figure 14).

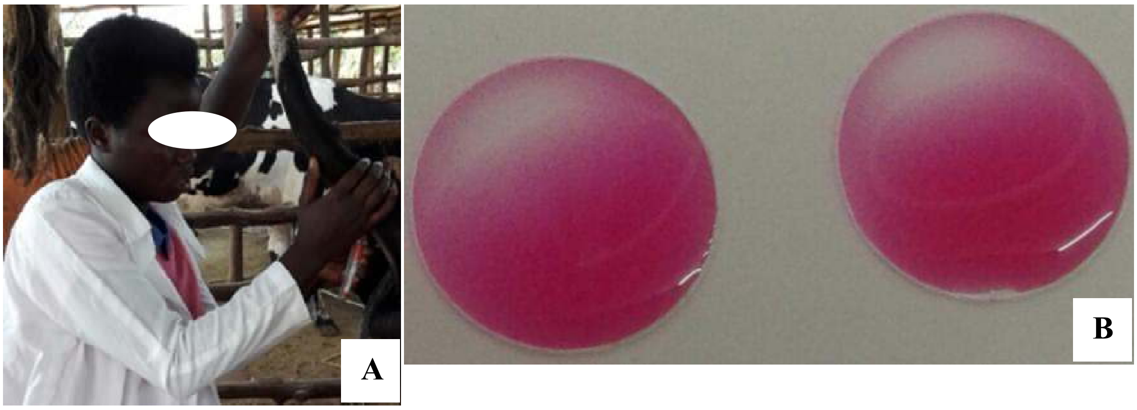


Figure 14. Trained enumerator performing blood sampling on middle coccygeal vein (**A**), and appearance of mixture Rose Bengal antigen and test serum result (negative) (**B**).

5.2.6 Body condition scoring

In this study, three trained enumerators using the visual technique guided by a short description of the anatomical areas (vertebrae at the middle of the back and rear view of the hook bones) to be scored (Klopčič *et al.*, 2011) assessed BCS following the procedure described previously by Edmonson *et al.* (1989). Therefore, cows were body condition scored in a 1 to 5 scale with 1-unit increment (1-point score being "very thin cow" and 5 being "fat cow"). All scores from the three assessors were averaged to find a single BCS for each sample cow. The BCS was dichotomised (BCS≥3 indicated cow in good body condition, BCS<3 meant cow in poor body condition) (Figure 15).

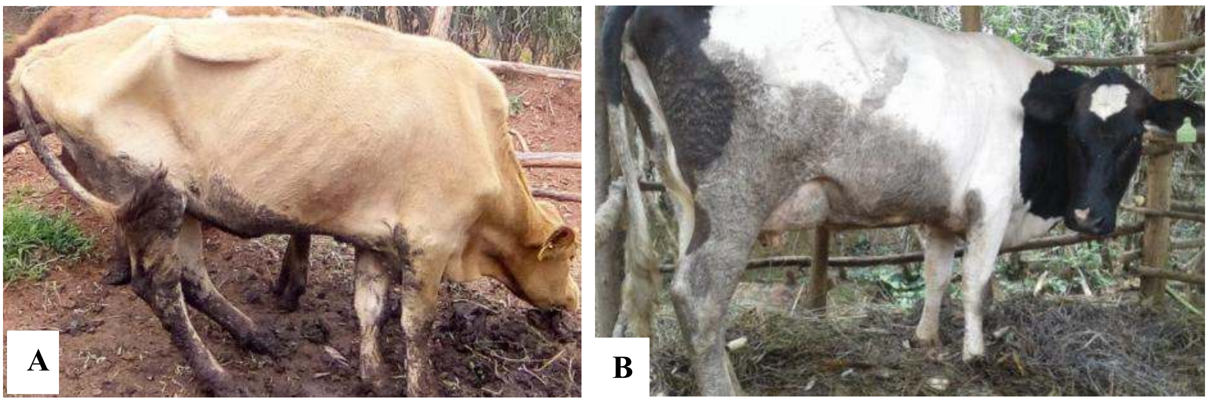


Figure 15. Cow in poor body condition (**A**) and cow in good body condition (**B**)

5.2.7 Evaluation of hygiene in the cowshed

To determine the level of hygiene in the cowshed, each sampled cow was assessed for cleanliness. During an assessment, a modified cow cleanliness scoring method based on previous studies (Ellis *et al*., 2006; Hughes, 2001) was used in this study. Briefly, five anatomical sites (Figure 16) which considered to indicate cleanliness were observed on each sampled dairy cow (Hulsen, 2016; Reneau *et al*., 2005): (i) the lower hind legs, (ii) the udder, (iii) the pelvis including the upper part of the tail, (iv) the flanks including the lower part of the tail, and (v) the ventral aspect of the abdomen including the knee.

To facilitate the assessment exercise, a cow cleanliness scorecard was provided to convey the degree of cleanliness associated with scores (Figure 16). The cow cleanliness score was recorded on a 3-point scale (Hulsen, 2016) ranging from 1 = the area was very clean (no dirt, bedding, or faeces) to 3 = the area was heavily soiled with dirt, bedding or faeces (using 1-point increments). Both sides of the cow were assessed by three observers and all scores were averaged to find a single score for each side of the anatomical area scored. Therefore, if scores of both sides of anatomical area were different, the higher score was recorded. Next, cow cleanliness score of all anatomical areas was summated to find a single score for each sampled cow, thus giving the whole-cow cleanliness score from 5 to 15. In the case of 2 or more cows sampled on a farm, a cow with a higher score was considered and represented the farm. Finally, the cow cleanliness score was dichotomised: cow cleanliness score = 5 indicated that the cow was kept in a clean cowshed, whereas cow cleanliness score>5 meant that the cow was kept in the dirty cowshed.

Figure 16. Cow cleanliness scorecard describing the scores (1 = no dirt, bedding, or faeces, 2 = moderate soiled with dirt, bedding or faeces, 3 = heavily soiled with dirt, bedding and/or faeces**)** per each anatomical area [Green (A): the pelvis including the upper part of the tail, Orange (B): the flanks including the lower part of the tail, Dark red (C): Udder, Blue (D): the lower hind legs, and Pink (E): the ventral aspect of the abdomen including the knee]
Source: Adapted from Hulsen (2016) and Ruud *et al*. (2010).

5.2.8 Data analysis

The cow- and herd-levels risk factors for CLE and SCLE cases were determined using the path analysis model, fitting multiple regression models for a dependent variable and two or more independent variables (Curtis *et al*., 1985) hypothesised in the null path model (Figure 17 and Figure 18).

Null path analysis model for cow-level risk factors

The hypothesised direct and indirect causal pathways were constructed with unidirectional arrows, producing the null hypothesis path model (Figure 17). The arrows indicate the hypothesised relationships among variables and are read from left to right. Association only flows along the unidirectional arrows extending from presumed RF to the possible effects. The arrows that go from one hypothesised risk factors to the outcome through one or more intervening risk factor (s) denote the indirect path. For instance, gestational length was expected to influence indirectly (mediated through stillbirth, dystocia, retained placenta, and left displaced abomasums) the risk of CLE or SCLE. In contrast, for a direct causal path, there must be no intervening variable between the RF and the outcome (Dohoo *et al*., 2003). Example being retained placenta was expected to influence the risk of CLE or SCLE directly. Feedback loops were not possible because RF can occur only once per postpartum period, and also paths only could go from left to right (Correa *et al*., 1993). Only cow-level RF with biological justification or previously documented (Adnane *et al*., 2017; Chan Lee *et al*., 2018; Kelly *et al*., 2020) were included in the null path analysis model. Cow-level RF considered are presented in Table 18.

Statistical testing of the null hypothesis path model was performed using multiple logistic regression techniques. All variables in the path model at the end of an arrow were dependent variables regressed on all the preceding variables along the direct paths (arrows) (Correa *et al*., 1993; Rougoor *et al*., 1997). For instance, body condition score was regressed on season of calving, cow breed, cow parity, and days dry but not on breeding services. In this path analysis model, a variable can act as the dependent variable in one relationship, while it concomitantly acts as the independent variable in another relationship. For example, calf sex acts as a dependent variable in relationship with twin births and breeding services. In turn, it acts as an independent variable in relationship with milk fever, ketosis, stillbirth, mastitis, retained placenta, left displaced abomasums, and endometritis (CLE or SCLE).

The possible paths between milk fever, ketosis, stillbirth, twin births, and gestational length, age of cows, endometritis, and left displaced abomasums were not analysed because there were no cases of the left displaced abomasums. Similarly, the paths between breeding services, milk fever, dystocia, endometritis, retained placenta, and brucellosis were noted analysed because there were no cases of brucellosis. Therefore, a total of twelve multivariable models were analysed for each form of endometritis (CLE or SCLE) as part of the null hypothesis path model (Figure 17).

The model building involved two steps: In the first step, univariable logistic regression analysis was performed to explore the relationship between the individual independent variable and the dependent variable. Statistical significance in this step was assessed at $p<0.25$ by Wald's test (Abebe *et al*., 2016) to account for potential confounders affecting both the predictors and the response variable. The RFs that were significant in the first step were checked for collinearity using Spearman's rank correlation. When two variables were highly correlated ($r\geq0.70$), the one with the lowest P-value was brought forward for multivariable analysis. In the second step, initially, the multivariable mixed-effect logistic regression model, with herd included as a random variable, was used. However, herd as a random variable effect was not significant (p>0.05); thus, the ordinary logistic regression model was used. The final multivariable logistic regression model was built using the backward stepwise variable selection method, and variables with a significant probability (p<0.05) were retained in the model. In parallel, confounding was assessed if removal of the variable in the final model substantially (>25%) changed the regression path coefficients of the remaining variables. The model fit was assessed basing on Hosmer-Lemeshow goodness-of-fit test (Hosmer *et al.*, 2013). The general equation of the logistic regression model was defined as follows:

$$log\left(\frac{\pi}{1-\pi}\right) = \beta_0+\beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \cdots + \beta_n X_n + \varepsilon$$

where π indicates the probability of an event (dependent variable); $\frac{\pi}{1-\pi}$ is the odds ratio, β_0 is the common intercept parameter, β_1 to β_n are the logistic regression path coefficients for X_1 to X_n (independent variables) included in the model, n is the number of independent variables included in the statistical model, and ε is the random error term.

The odds ratio (OR) in the final multivariable logistic regression models indicated the strength and significance of the association between the potential predictor and outcome (Dohoo *et al.*, 2003). An OR>1 implies a positive association, OR<1 means a negative association, and OR = 1 indicates no association. The final path analysis model for CLE and SCLE was constructed using the results from the final multivariable logistic regression analysis. While constructing, only cow-level RF directly or indirectly significantly associated with CLE or SCLE were considered.

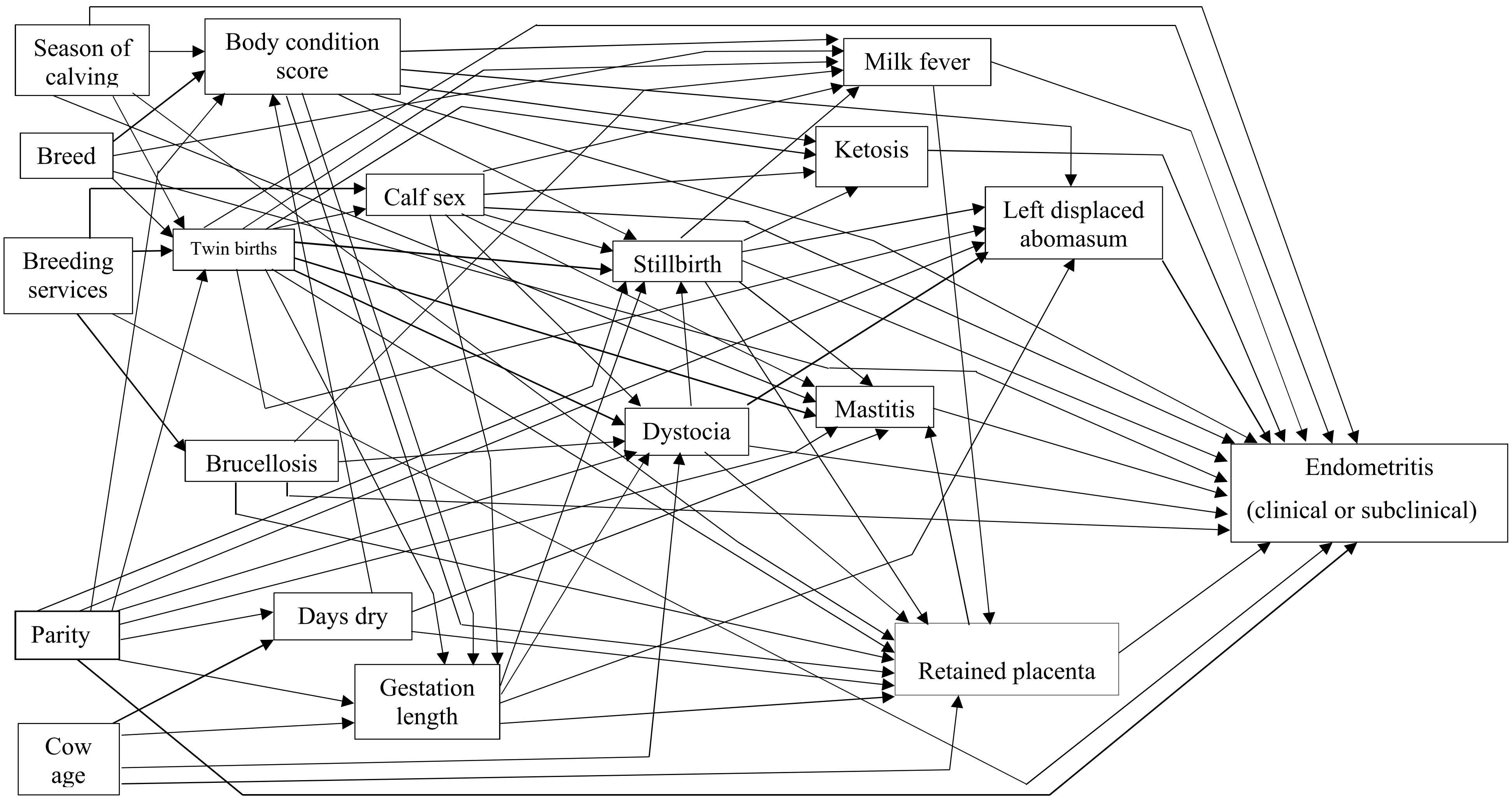


Figure 17. Null hypothesis path analysis model for cow-level RF associated with endometritis (clinical or subclinical) in dairy cows

Source: Adapted from Correa *et al*. (1993) and Rougoor *et al*. (1997).

Null path analysis model for herd-level risk factors

Construction of the null hypothesis path model (Figure 18) for the herd-level RF considered documented interrelationships (Bacha & Regassa, 2010; Cheong *et al.*, 2011; Dutta *et al.*, 2021; Moges & Jebar, 2012). The herd-level RF considered are presented in Table 18. The null path analysis model was performed in a similar manner, as was with cow-level risk factors. In total, seven multivariable models were analysed for each form of endometritis (CLE or SCLE) to test the null hypothesis path model (Figure 18).

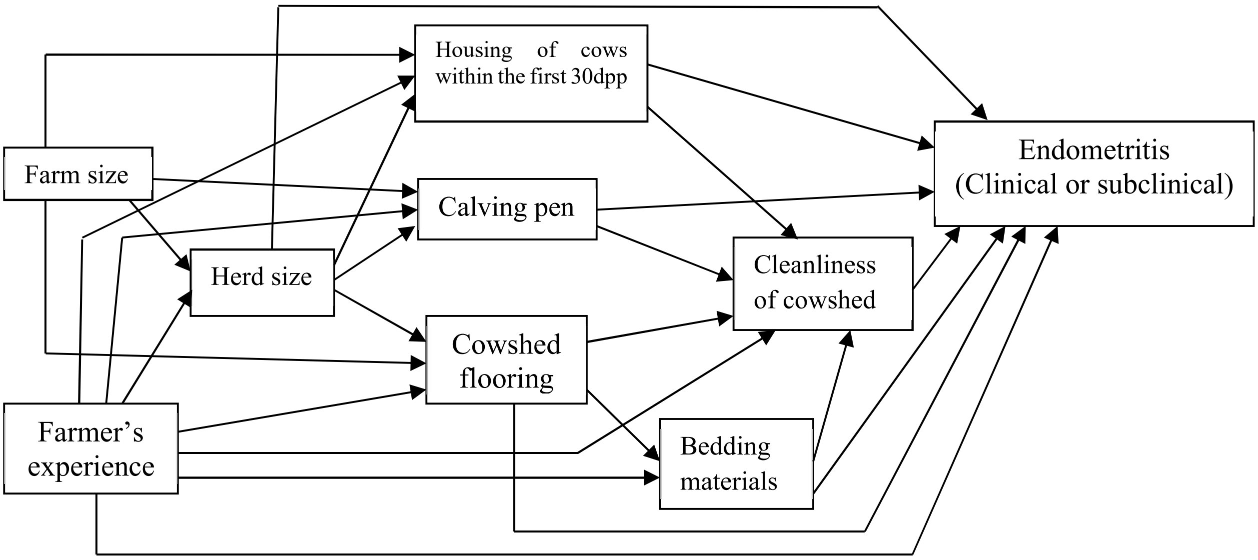


Figure 18. Null hypothesis path analysis model for herd-level RF associated with clinical and subclinical endometritis in dairy cows.

The final path analysis model for herd CLE and SCLE was constructed using only the directly or indirectly significant RF. Descriptive statistics were generated using frequency procedures and cross-tabulation. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows package software (SPSS, 2013). The graphical output was performed in IBM*SPSS*AMOS version 26.0 (Arbuckle, 2014).

5.3 Results

5.3.1 Socio-economic characteristics of the farmer and farm management

Table 19 presents the socio-economic characteristics of the sample farmers and herd management. In poverty classification of the Government of Rwanda (Cho & Kim, 2017), more than half (62.4%) of the farmers were in the poor category, with less than half (42.2%) without formal education, and males were dominating (61.1%) dairy farming. Farmers kept cows in zero-grazing housing units, mostly earthen floor (79.7%) without calving pen (95.9%), though with a roof (54.3%) but in a dirty condition (78.4%) despite the supply of bedding materials (63.5%). In the sample farms, majority of the cows (98.1%) were not housed within the first 30 dpp.

Table 19. Socio-economic characteristics of the sample farmers and herd management

Characteristics	**Level**	**Frequency**	**Percentage (%)**
Gender	Male	226	61.1
	Female	144	38.9
Educational level	No schooling	156	42.2
	Primary	148	40.0
	Secondary	58	15.7
	University	8	2.2
Poverty level	Very poor	46	12.4
	Poor	231	62.4
	Rich	93	25.1
Cowshed flooring	Concrete	75	20.3
	Earthen	295	79.7
Housing of cows within the first	Yes	7	1.9
30 dpp	No	363	98.1
Calving pen	Yes	15	4.1
	No	355	95.9
Cleanliness of the cowshed	Clean cowshed	80	21.6
	Dirty cowshed	290	78.4
Bedding materials	Using	235	63.5
	Not using	135	36.5
Cowshed roofing	With a roof	201	54.3
	Without a roof	169	45.7

dpp = days postpartum.

The dominant feeding practice was a cut- and -carry utilising Napier grass (*Pennisetum purpureum*) fodder with limited supplemental maize forage, mineral licks, and dairy meal (21.6%), more for pure dairy breeds (14.1%) than for dairy crossbreds (6.8%) or indigenous cattle breeds (0.8%). Cows had limited access to water, offered twice a day (noon and evening).

The mean estimates for farmer and herd characteristics presented in Table 20 reflect that the family size was on average 5.1±0.1 members per household, and the mean age was 41.6±0.7 years (median 40.0) with 8.8±0.3 (median 8.0) years of experience in dairy farming. The average herd size was small, 2.9±0.1 (median 2.0) cattle on small farm holding of 2.8±0.1 (median 2.0) acres in which food crops and fodder competed for land use.

Table 20. Mean estimates for farmer and herd characteristics in the study area (n=370)

Variables	Mean±SEM	Median	Minimum	Maximum
Age (years)	41.6±0.7	40.0	24.0	85.0
Experience in dairy farming (years)	8.8±0.3	8.0	1.0	25.0
Household size (number)	5.1±0.1	5.0	1.0	8.0
Herd size (number)	2.9±0.1	2.0	1.0	5.0
Farm size (acres)	2.8±0.1	2.0	0.3	6.9

*SEM = standard error of the mean

5.3.2 Cow-level risk factors associated with clinical and subclinical endometritis

The univariable logistic regression analysis (Table 21) found poor body condition (BCS<3), season of calving, stillbirth, mastitis, dystocia, retained placenta, and cow breed directly and significantly ($p<0.25$) associated with CLE cases. All these RF, in addition to cow parity, were also found to be directly and significantly ($p<0.25$) associated with SCLE cases. On the other hand, milk fever, ketosis, breeding services, twin birth, and sex of the calf were not directly and significantly associated with CLE or SCLE ($p\geq0.25$) in the univariable models and therefore were not eligible for multivariable logistic regression. There was no evidence of any high correlation among the tested RF.

Cases of the left displaced abomasums and brucellosis were not observed. Among 299 dystocia cases, 11.0% (33/299) were assisted with gloves and 89.0% (266/299) without gloves. The results revealed that not wearing gloves during calving assistance was significantly associated with CLE and SCLE cases. Compared to dystocias assisted with gloves, those without gloves were associated with a high prevalence of CLE (58.9% vs. 9.4%) and SCLE (31.1% vs. 5.4%). Furthermore, cow with dystocia assisted with gloves was less likely to have CLE (OR = 0.3, CI = 0.1 - 0.9, $p = 0.036$) and SCLE (OR = 0.6, CI = 0.3-1.2, $p = 0.041$) compared to cow that was assisted without gloves.

Table 21. Descriptive statistics and results from univariable logistic regression model of the association between cow-level RF and clinical or subclinical endometritis cases in smallholder zero-grazed dairy cows in Gasabo district, Rwanda

Risk factors	Level	Clinical endometritis				Subclinical endometritis			
		Cases, % (n)	OR	95%CI	P-value	Cases, % (n)	OR	95%CI	P-value
Milk fever	Occurrence	66.7 (6)	1.0	0.2-5.4	0.979	50.0 (6)	2.2	0.4-10.9	0.334
	Non-occurrence	67.2 (460)	Reference			31.5 (460)	Reference		
Ketosis	Occurrence	50.0 (2)	0.5	0.0-7.8	0.604	0.0 (2)	1.5	1.4-1.6	0.334
	Non-occurrence	67.2 (464)	Reference			31.9 (464)	Reference		
Body condition score[1]	<3	71.0 (404)	3.4	2.0-5.9	0.001	33.9 (404)	2.4	1.2-4.7	0.011
	≥3	41.9 (62)	Reference			17.7 (62)	Reference		
Breeding services	AI	66.5 (203)	0.9	0.6-1.0	0.788	32.5 (203)	1.1	0.7-1.6	0.759
	Bull	67.7 (263)	Reference			31.2 (263)	Reference		
Season of calving[1]	Rainy season	79.3 (237)	3.2	2.1-4.8	0.001	36.7 (237)	1.6	1.1 -2.4	0.020
	Dry season	54.6 (229)	Reference			26.6 (229)	Reference		
Stillbirth[1]	Occurrence	84.8 (33)	2.9	1.1-7.7	0.025	57.6 (33)	3.2	1.6-6.6	0.001
	Non-occurrence	65.8 (433)	Reference			29.8 (433)	Reference		
Mastitis[1]	Positive	72.2 (259)	1.7	1.1-2.5	0.010	40.2 (259)	2.5	1.6-3.8	0.001
	Negative	60.9 (207)	Reference			21.3 (207)	Reference		
Twin births	No	67.1 (462)	0.7	0.1-6.6	0.738	31.8 (462)	1.4	0.1-13.6	0.771

	Yes	75.0 (4)	Reference			25.0 (4)	Reference		
Dystocia[1]	Occurrence	72.9 (299)	2.0	1.4-3.0	0.001	37.8 (299)	2.3	1.5-3.6	0.001
	Non-occurrence	56.9 (167)	Reference			21.0 (167)	Reference		
Cow parity[2]	Primiparous	69.6 (161)	1.2	0.8-1.8	0.423	37.3 (161)	1.5	1.0-2.2	0.064
	Multiparous	65.9 (305)	Reference			28.9 (305)	Reference		
Retained	Occurrence	78.4 (88)	1.9	1.2-3.5	0.013	43.2 (88)	1.9	1.2-2.9	0.011
placenta[1]	Non-occurrence	64.6 (438)	Reference			29.1 (378)	Reference		
Cow breed[1]	Pure breeds	79.7 (79)	2.5	1.4-4.5	0.003	41.8 (79)	1.5	0.9-2.6	0.108
	Indigenous	76.9 (78)	1.2	0.6-2.5	0.668	37.2 (78)	0.8	0.4-1.6	0.556
	Crossbreds	61.5 (309)	Reference			27.8 (309)	Reference		
Sex of the calf	Male	67.1 (213)	1.0	0.7-1.5	0.989	33.8 (213)	1.2	0.8-1.8	0.385
	Female	67.2 (253)	Reference			30.0 (253)	Reference		

[1] RF associated ($p<0.25$) with clinical and subclinical endometritis, [2]RF only associated ($p<0.25$) with subclinical endometritis, were offered to build the final model, OR = odds ratio, 95% CI = 95% confidence interval of OR.

The final path analysis model from multivariable logistic regression analysis for CLE (Table 22) had a good model fit of the data: Hosmer-Lemeshow goodness-of-fit test ($p = 0.977$). Only cow breed, sex of the calf, twin births, and cow parity had multiple indirect associations. Sex of the calf was associated with stillbirth with male calves more likely to be stillborn (OR = 2.7) than female calves. Cow parity was an RF for stillbirths, with calf from a primiparous cow more likely (OR=1.9) a stillbirth than calf from a multiparous cow. The risk of retained placenta was higher in cows with twins (OR = 14.4) and stillbirth (OR = 6.3) than cows without these conditions. The odds of CLE were 3.2 times higher in cows that had poor BCS (BCS<3) than in those that had good BCS (BCS≥3) ($p<0.05$). Cow-level prevalence of CLE was significantly higher (OR=5.0) in cows that calved in the rainy season than in those that calved in the dry season. Postpartum records indicated that cows that had retained placenta had a higher risk of CLE (OR=1.4) than cows without a retained placenta. Dystocia and stillbirths were also RF ($p<0.05$) for CLE cases.

Table 22. Results from the final multivariable logistic regression model used to identify cow-level risk factors associated with clinical endometritis.

Model Dependent	Model Independent	Cases, % (n)	Odds ratio	95% CI	P-value
BCS	Cow breed				
	Dairy crossbreds	86.4 (309)	5.9	1.4 - 25.3	0.015
	Dairy pure breeds	77.2 (79)	11.2	2.5 - 50.2	0.002
	Indigenous cattle	97.4 (78)	Reference		
Gestation length	Twin births				
	Yes	75.0 (4)	0.1	0.0 - 0.9	0.046
	Non	97.0 (462)	Reference		
Milk fever	Stillbirth				
	Occurrence	9.1 (33)	14.3	2.8 - 74.1	0.001
	Non-occurrence	0.7 (433)	Reference		
Stillbirth	Calf sex				
	Male	10.3 (213)	2.7	1.3 - 5.6	0.011
	Female	4.3 (253)	Reference		
	Cow parity				
	Primiparous	9.9 (161)	1.9	0.9 - 4.1	0.061
	Multiparous	5.6 (305)	Reference		
Mastitis	Cow parity				
	Multiparous	58.7 (305)	1.4	0.4 - 1.9	0.037
	Primiparous	49.7 (161)	Reference		
	Cow breed				
	Dairy crossbreds	51.5 (309)	0.5	0.3 - 0.9	0.017
	Indigenous cattle	61.5 (78)	0.6	0.4 - 1.1	0.082
	Dairy pure breeds	65.8 (79)	Reference		
Retained placenta	Stillbirth				
	Occurrence	24.2 (33)	6.3	2.5 - 16.2	0.001
	Non-occurrence	4.6 (433)	Reference		
	Twin births				
	Yes	50.0 (4)	14.4	1.7 - 22.1	0.014
	Non	5.6 (462)	Reference		
Clinical endometritis	Body condition score				
	<3	74.0 (404)	4.1	2.2 - 7.8	0.001
	≥3	45.2 (62)	Reference		
	Season of calving				
	Rainy	79.3 (237)	5.0	3.1 - 8.0	0.001
	Dry	54.6 (229)	Reference		
	Dystocia				
	Occurrence	72.9 (299)	1.9	1.2 - 3.0	0.004
	Non-occurrence	56.9 (167)	Reference		
	Retained placenta				
	Occurrence	78.4 (88)	1.4	1.0 - 3.5	0.040
	Non-occurrence	64.6 (438)	Reference		
	Stillbirth				
	Yes	84.8 (33)	3.5	1.2 - 10.1	0.019
	No	65.8 (433)	Reference		

*95% CI= 95% confidence interval of odds ratio

The cow-level risk factors directly and/or indirectly associated (p<0.05) with CLE were then used to construct the path model presented in Figure 19. In a decreasing magnitude, the RF that directly associated with increased (p<0.05) CLE cases were rainy-season of calving (OR = 5.0 times), poor body condition (BCS<3) (OR = 4.1 times), stillbirth (OR = 3.5 times), dystocia (OR = 1.9 times) and retained placenta (OR = 1.4 times). Some RF indirectly associated with increased CLE cases. The breed of a cow was associated with CLE cases through the BCS. The sex of the calf and cow parity had an association with CLE cases through stillbirths. Also, stillbirth had an indirect association with CLE through a retained placenta. Twin birth indirectly associated with CLE cases through a retained placenta as well.

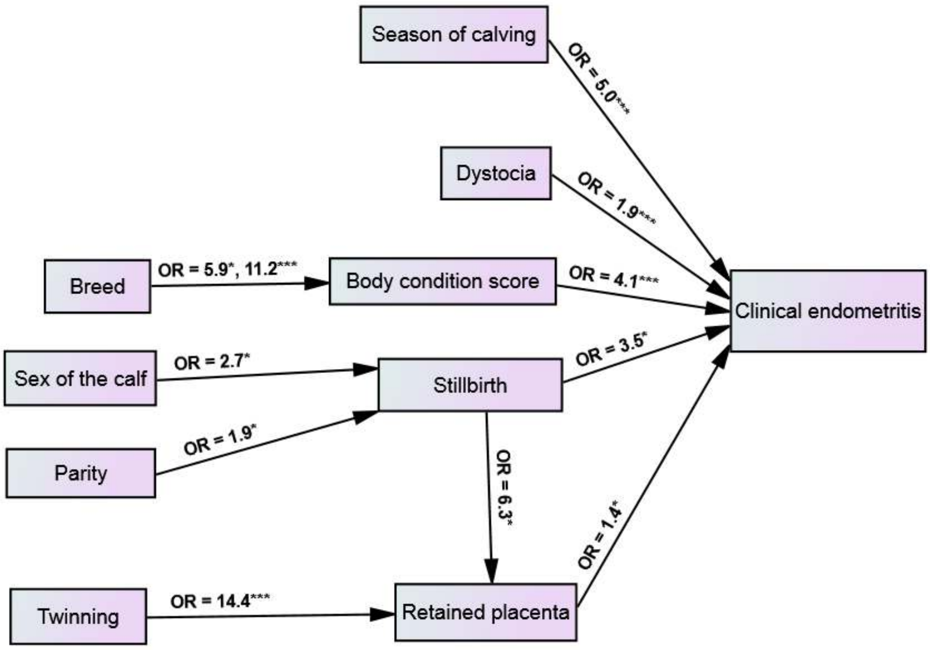


Figure 19. Final path analysis model for cow-level RF associated with clinical endometritis in smallholder zero-grazed dairy cows, *p<0.05; ***p<0.001. Arrow weight = OR = Odds ratio. An OR>1 indicates a positive association.

The final path analysis model from multivariable logistic regression analysis for cow-level RF associated with SCLE (Table 23) had adequate goodness of fit test (p = 0.595). Several RF had direct (p<0.05) associations with increased risk of SCLE. These were a cow in poor body condition (BCS<3), rainy season, mastitis, dystocia, retained placenta, stillbirth, and multiparous cows. The risk of SCLE increased (p<0.05) with BCS<3 compared to BCS≥3 (OR=2.1), calving in the rainy season than in the dry season (OR = 2.1), when a cow had retained placenta (OR = 1.8) and dystocia (OR=2.2) and after stillbirth (OR = 3.3) and for primiparous cows (OR = 1.5). Finally, the presence of SCLE at 38.5±0.7 dpp was directly (p<0.05) associated with mastitis (OR = 2.5).

Table 23. Results from the final multivariable logistic regression model used to identify cow-level RF associated with subclinical endometritis

Model		Cases, % (n)	OR	95% CI	P-value
Dependent	**Independent**				
BCS	Cow breed				
	Dairy crossbred	86.4 (309)	5.9	1.4-25.3	0.015
	Indigenous cattle	97.4 (78)	11.2	2.5-50.2	0.002
	Pure dairy breeds	77.2 (79)	Reference		
Gestation length	Twin births				
	Yes	75.0 (4)	0.1	0.0-0.9	0.046
	No	97.0 (462)	Reference		
Milk fever	Stillbirth				
	Occurrence	9.1 (33)	14.3	2.8-74.1	0.001
	Non-occurrence	0.7 (433)	Reference		
Stillbirth	Calf sex				
	Male	10.3 (213)	2.7	1.3-5.6	0.011
	Female	4.3 (253)	Reference		
	Cow parity				
	Primiparous	9.9 (161)	1.9	0.9-4.1	0.061
	Multiparous	5.6 (305)	Reference		
Mastitis	Cow parity				
	Primiparous	49.7 (161)	1.4	0.4-0.9	0.037
	Multiparous	58.7 (305)	Reference		
	Cow breed				
	Dairy crossbreds	51.5 (309)	0.5	0.3-0.9	0.017
	Indigenous cattle	61.5 (78)	0.6	0.4-1.1	0.082
	Dairy pure breeds	65.8 (79)	Reference		
Retained placenta	Stillbirth				
	Occurrence	24.2 (33)	6.3	2.5-16.2	0.001
	Non-occurrence	4.6 (433)	Reference		
	Twin births				
	Yes	50.0 (4)	14.4	1.7-122.1	0.014
	Non	5.6 (462)	Reference		
Subclinical endometritis	Body condition score				
	<3	33.9 (404)	2.2	1.1-4.7	0.036
	≥3	17.7 (62)	Reference		
	Season of calving				
	Rainy	36.7 (237)	2.1	1.3-3.3	0.003
	Dry	26.6 (229)	Reference		
	Mastitis				
	Positive	40.2 (259)	2.5	1.6-3.9	0.001
	Negative	21.3 (207)	Reference		

Dystocia				
Occurrence	37.8 (299)	2.2	1.4-3.6	0.001
Non-occurrence	21.0 (167)	Reference		
Retained placenta				
Occurrence	43.2 (88)	1.8	1.1-3.0	0.027
Non-occurrence	29.1 (378)	Reference		
Stillbirth				
Yes	57.6 (33)	3.3	1.5-7.2	0.002
No	29.8 (433)	Reference		
Cow parity				
Multiparous	37.3 (161)	1.5	0.9-2.3	0.088
Primiparous	28.9 (305)	Reference		

OR = odds ratio, 95%CI = 95% confidence interval for odds ratio.

The cow-level RF directly or indirectly ($p<0.05$) associated with SCLE were used to construct the final path analysis model presented in Figure 20. From the path model, increased cases of SCLE were directly associated with stillbirth (OR = 3.3 times), mastitis (OR = 2.5 times), poor body condition (BCS<3) and dystocia (each, OR = 2.2 times), rainy-season calving (OR = 2.1 times) and retained placenta (OR = 1.8 times). Some indirect association between RF and SCLE was observed. Breed of cow indirectly associated with SCLE cases through poor body condition (BCS<3) and mastitis, while sex of the calf had indirect association through stillbirths. While parity of cow directly had an association with SCLE cases, indirect associations occurred through mastitis and stillbirths. Further, stillbirth indirectly associated with SCLE cases through a case of retained placenta and twin birth also associated with SCLE cases through a retained placenta.

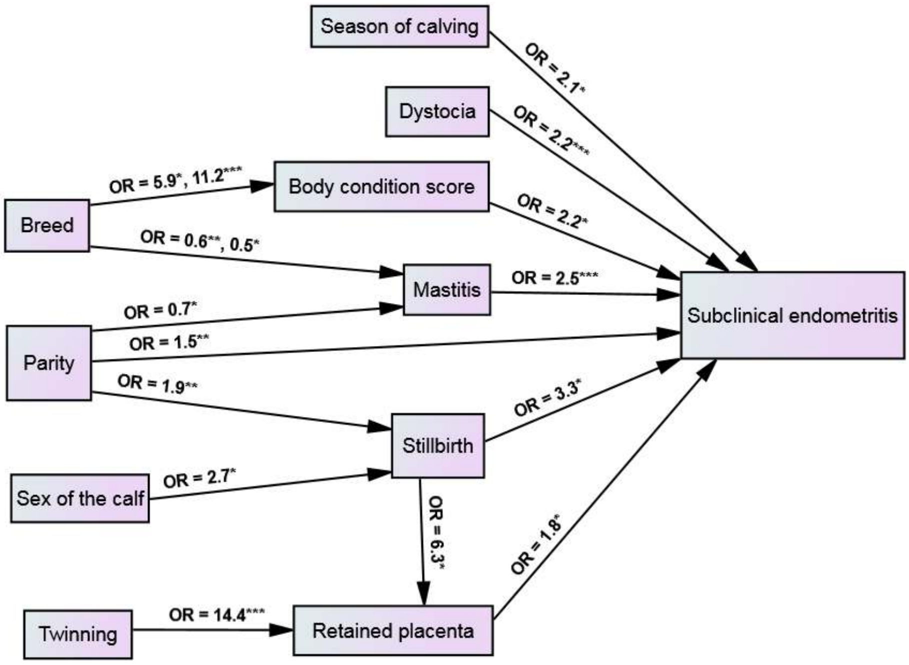


Figure 20. Final path analysis model for cow-level RF associated with subclinical endometritis in smallholder zero-grazed dairy cows, * p<0.05; ***p<0.001. Arrow weight = OR =: Odds ratio. An OR>1 indicates a positive association, and an OR< 1 indicates a negative association.

5.3.3 Herd-level risk factors associated with clinical and subclinical endometritis

In the univariable logistic regression analysis (Table 24), herd-level RF associated (p<0.25) directly with CLE cases were dirty cowshed, absence of housing of cows within the first 30 dpp, not using bedding materials, large herd size, earthen flooring, and absence of calving pen. Therefore, they were offered for building the multivariable model. In contrast, farmer dairying experience was not associated (p≥0.25) directly with CLE and so was excluded for multivariable analysis.

Herd-level RF associated (p<0.25) directly with SCLE in the univariable analysis were dirty cowshed, absence of calving pen, not using bedding materials and earthen floor cowshed. Thus, they were offered to the multivariable logistic regression model. The remaining RF that includes the housing of cows within the first 30 dpp, farmer dairying experience, and herd size were not associated (p≥0.25) directly with SCLE and consequently were not offered to the multivariable logistic regression analysis. There was no evidence of any high correlation among these RF for CLE or SCLE.

Table 24. Descriptive statistics and results from univariable logistic regression analysis of the association between herd-level risk factors and clinical or subclinical endometritis cases diagnosed at38.5±0.7 dpp

Risk factors	Clinical endometritis				Subclinical endometritis			
	Cases, % (n)	OR	95% CI	P-value	Cases, % (n)	OR	95% CI	P-value
Bedding material[1]								
Not using	71.9 (135)	1.3	0.8-2.1	0.242	41.5 (135)	1.6	1.0-2.4	0.044
Using	66.0 (235)	Ref			31.1 (235)	Ref		
Cleanliness of cowshed[1]								
Dirty cowshed	82.4 (290)	24.2	12.4-47.0	0.001	42.4 (290)	9.1	3.8-21.6	0.001
Clean cowshed	16.3 (80)	Ref			7.5 (80)	Ref		
Housing of cows within the first 30 dpp[2]								
Absence	68.9 (363)	5.5	1.1-28.9	0.043	35.3 (363)	3.3	0.4-27.4	0.275
Presence	28.6 (7)	Ref			14.3 (7)	Ref		
Farmer dairying experience								
<8	65.8 (199)	0.8	0.5-1.2	0.310	36.7 (199)	1.2	0.8-1.8	0.428
≥8	70.8 (171)	Ref			32.7 (171)	Ref		
Herd size[2]								
≥3	77.2 (114)	1.9	1.1-3.1	0.013	38.6 (114)	1.3	0.8-1.9	0.315
<3	64.1 (256)	Ref			33.2 (256)	Ref		
Cowshed flooring[1]								
Earthen	73.9 (295)	3.4	2.0-5.8	0.001	36.6 (295)	1.5	0.9-2.6	0.164
Concrete	45.3 (75)	Ref			28.0 (75)	Ref		
Calving pen[1]								
Absence	69.0 (355)	2.5	0.9-7.2	0.078	35.8 (355)	3.6	0.8-16.3	0.094
Presence	46.7 (15)	Ref			13.3 (15)	Ref		

[1]Risk factors associated (p<0.25) with both clinical and subclinical endometritis cases, [2]Risk factors only associated (p<0.25) with clinical endometritis cases. Risk factors with p<0.25 were offered to the multivariable logistic regression model. Ref = Reference; OR = odds ratio; 95% CI = 95% confidence interval for OR.

The risk factors statistically significant ($p<0.05$) from multivariable logistic regression model are presented in Table 25. Farms that had ≤ 3 acres were 2.9 times more likely to have herds of ≤ 3 cows than those with >3 acres. Herds with <3 cow were less likely to have a cowshed with a concrete floor than those with ≥ 3 cows (OR = 0.3). Farmers with <8 years of dairying experience were 1.7 times more likely to manage a herd of ≤ 3 cows than farmers with ≥ 8 years in dairy farming. Herds that had cowshed with earthen floor were 10.6 times higher likely to use bedding materials than those that had cowshed with concrete floor. Hygiene was less frequently observed in herds that had cowshed with an earthen floor than in those that had concrete floor (OR = 0.1). Herds without housing of cows within the first 30 dpp were 0.1 less likely to have clean cowshed than herds that had housing of cows within the first 30 dpp ($p = 0.013$). The final path analysis model from multivariable logistic regression analysis for CLE cases had a good model fit of the data: Hosmer-Lemeshow goodness-of-fit test ($p = 0.561$). In this analysis, bedding materials and calving pen did not have a significant direct effect on the occurrence of CLE ($p>0.05$).

Table 25. Results from the final multivariable logistic regression models for herd-level risk factors associated with clinical endometritis cases

Model: Dependent	Model: Independent	Cases, % (n)	Odds ratio	95% CI	P-value
Herd size	Farm size				
	≤3	76.1 (272)	2.9	1.8-4.8	0.001
	>3	50.0 (98)	Reference		
	Farmer's experience				
	< 8	75.4 (199)	1.7	1.1-2.6	0.030
	≥ 8	62.0 (171)	Reference		
Cowshed flooring	Herd size				
	<3	14.5 (256)	0.3	0.2-0.6	0.001
	≥3	33.3 (114)	Reference		
Bedding materials	Cowshed flooring				
	Earthen	74.2 (295)	10.6	5.8-19.6	0.001
	Concrete	21.3 (75)	Reference		
Cleanliness of cowshed	Cowshed flooring				
	Earthen	18.3 (295)	0.4	0.2-0.7	0.005
	Concrete	34.7 (75)	Reference		
	Housing of cows within the first 30 dpp				
	Absence	20.7 (363)	0.1	0.0-0.6	0.013
	Presence	71.4 (7)	Reference		
Clinical endometritis	Cleanliness of cowshed				
	Dirty cowshed	82.4 (290)	25.1	12.2-51.5	0.001
	Clean cowshed	16.3 (80)	Reference		
	Herd size				
	≥3	77.2 (114)	3.1	1.5-6.6	0.003
	<3	64.1 (256)	Reference		
	Cowshed flooring				
	Earthen	73.9 (295)	6.6	2.9-14.9	0.001
	Concrete	45.3 (75)	Reference		

The results from the multivariable logistic regression model for the interrelationship among risk factors and between risk factors and CLE cases were used to construct the final path model presented in Figure 21. The path model reveals that increased cases of CLE could be directly associated with unhygienic cowshed (OR = 25.1 times), earthen floor cowshed (OR = 6.6 times), and large herd size (OR = 3.1 times). Through herd size, the farm size and farmer experience in dairy farming indirectly associated with CLE cases. Herd size also indirectly associated with CLE cases through cowshed flooring, while cowshed flooring indirectly associated with CLE through the cleanliness of the cowshed. Absence of housing of cows within the first 30 dpp indirectly associated with CLE cases through the cleanliness of the cowshed as well. The Hosmer-Lemeshow goodness-of-fit test suggested that the model fit the data (p = 0.561).

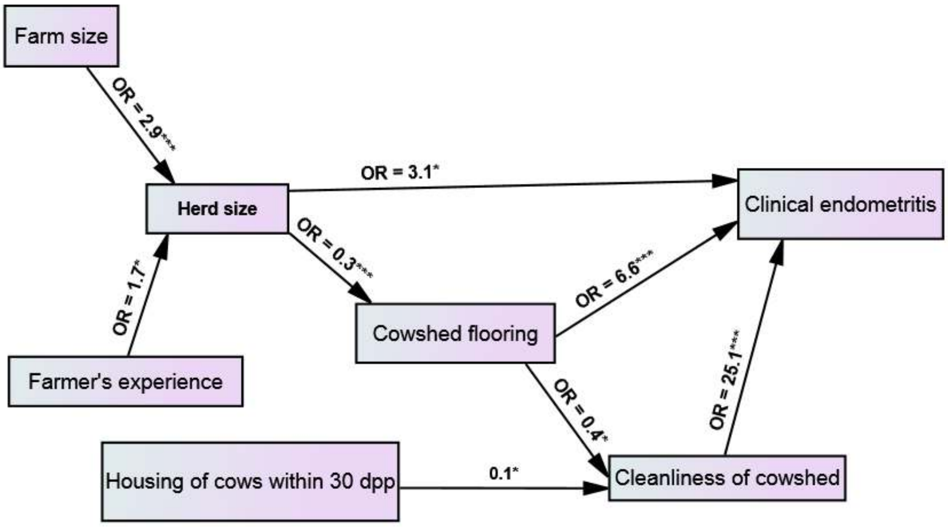


Figure 21. Final path analysis model for herd-level RF associated with clinical endometritis in smallholder zero-grazed dairy farms, *p<0.05, ***p<0.001. Arrow weight = OR = Odds ratio. An OR>1 indicates a positive association, and an OR<1 indicates a negative association.

The final multivariable model of herd-level RF associated directly with SCLE cases had a good model fit for the data: Hosmer-Lemeshow goodness-of-fit test (p = 0.633) (Table 26). Except for cowshed flooring and calving pen (p>0.05), all the variables entered remained significant (p<0.05) and directly associated with increased cases of SCLE. Those include bedding materials and cleanliness of cowshed. Herds that did not use bedding materials were 1.5 more likely to have SCLE cases than herds that did use (p = 0.042). The odds of finding a cow positive for SCLE was higher in herds that had dirty cowshed than in those that had clean cowshed (OR = 8.9).

Table 26. Results from the final multivariable logistic regression models for herd-level risk factors associated with subclinical endometritis cases

Model		**Cases, % (n)**	**Odds ratio**	**95% CI**	**P-value**
Dependent	**Independent**				
Herd size	Farm size				
	≤3	76.1 (272)	2.9	1.8-4.8	0.001
	>3	50.0 (98)	Reference		
	Farmer's experience				
	<8	75.4 (199)	1.7	1.1-2.6	0.030
	≥ 8	62.0 (171)	Reference		
Cowshed flooring	Herd size				
	≤3	14.5 (256)	0.3	0.2-0.6	0.001
	>3	33.3 (114)	Reference		
Bedding materials	Cowshed flooring				
	Earthen	74.2 (295)	10.6	5.8-19.6	0.001
	Concrete	21.3 (75)	Reference		
Cleanliness of cowshed	Cowshed flooring				
	Earthen	18.3 (295)	0.4	0.2-0.7	0.005
	Concrete	34.7 (75)	Reference		
	Housing of cows within the first 30 dpp	20.7 (363)	0.1	0.0-0.6	0.013
	Absence	71.4 (7)	Reference		
	Presence				
Subclinical endometritis	Bedding materials				
	Not using	41.5 (135)	1.5	0.9-2.4	0.042
	Using	31.1 (235)	Reference		
	Cleanliness of cowshed				
	Dirty cowshed	42.4 (290)	8.9	3.8-21.3	0.001
	Clean cowshed	7.5 (80)	Reference		

*95% CI = 95% confidence interval for odds ratio.

The final path model constructed from the risk factors significantly associated with SCLE cases, directly or indirectly, is presented in Figure 22. Directly, SCLE cases increased when cowshed was unhygienic (OR = 8.9 times) and when bedding materials were not used (OR = 1.5 times). Cowshed flooring indirectly associated with SCLE cases through bedding materials and cleanliness of the cowshed, which had a direct association with herd size. Absence of housing of cows within the first 30 dpp indirectly associated with SCLE cases through the cleanliness of the cowshed. Farmers with larger farms and long experience with dairy production seemed to keep larger herd size.

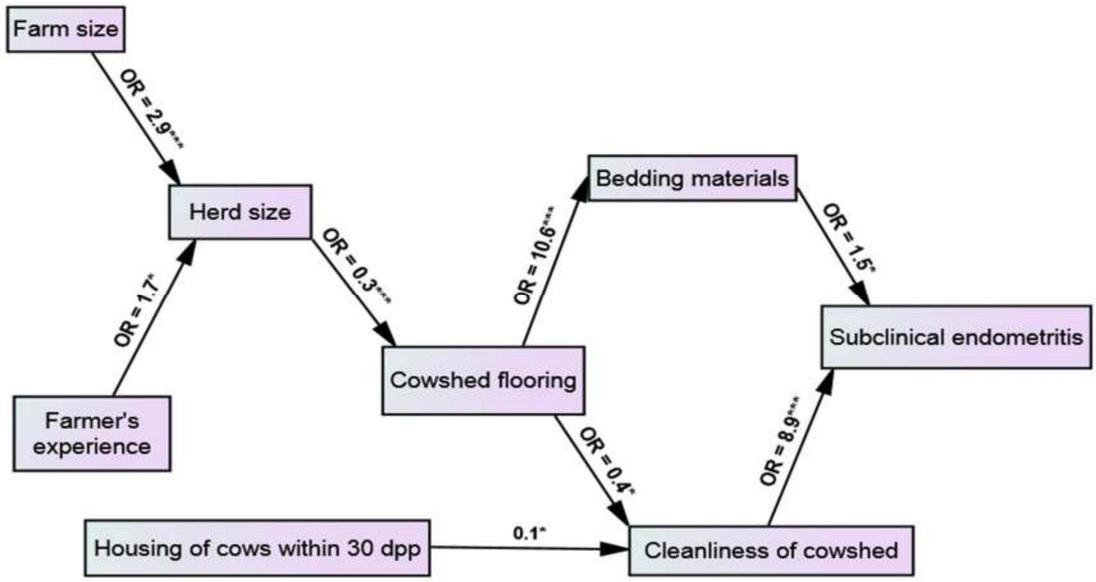


Figure 22. Final path analysis model depicting interrelationship among herd-level RF and their association with subclinical endometritis in smallholder zero-grazed dairy farms, *p<0.05, ***p<0.001. Arrow weight = OR = Odds ratio. An OR>1 indicates a positive association, and an OR<1 indicates a negative association.

5.4 Discussion

This study applied path analysis model constructed from multivariable logistic regression models with backward stepwise variable selection to identify and quantify cow- and herd-levels risk factors for CLE and SCLE cases in zero-grazed dairy cows on smallholder farms in Rwanda. The modelling approach revealed cow- and herd-levels risk factors directly and indirectly associated with CLE and SCLE cases and, therefore, the targets for management interventions on the farms for prevention and control of endometritis.

5.4.1 Risk factors with direct association with endometritis

The study has identified that dystocia is a risk factor for increased odds of CLE and SCLE occurrence. In this study, assisted calving without gloves (89.0%) were associated with more cases of CLE and SCLE compared to when gloves were used (11.0%). This finding reveals that assisted calving is frequent, and by not wearing gloves during calving assistance, farmers often do not observe hygiene practices, which represent a risk for causing physical trauma and bacterial contamination in the female genital reproductive tract. This possibly increased risk of developing CLE and SCLE cases (Potter *et al*., 2010; Prunner *et al*., 2014a). Several authors have reported similar findings in Florida, the United States of America (Vieira-Neto *et al*., 2016), Vietnam (Nguyen-Kien & Hanzen, 2017), Argentina (Giuliodori *et al*., 2017), and Ireland (Kelly *et al*., 2020). Therefore, the current findings would imply that applying good hygienic management practices such as the use of clean, lubricated, gloved hands and observing good hygiene during an assisted calving may help reduce physical trauma and contamination of the genital reproductive tract. This would subsequently minimise cases of CLE and SCLE in the dairy herd (Chapter three and four).

In this study, the retained placenta had a positive association with CLE and SCLE cases, which agrees with previous observations (Chan Lee *et al*., 2018; Nguyen-Kien & Hanzen, 2017). This association has been explained partly as the result of deterioration of the foetal membranes and the delay of lochia expulsion, which become a favourable medium for the growth of various bacteria in the uterine lumen, and necrotic tissue delays uterine involution and the repair of the endometrium (Fesseha, 2020; Mordak & Anthony, 2015). Furthermore, Potter *et al*. (2010) reported that residual tissue encountered in the case of a retained placenta might result in the cervix being held open, and an increase in bacterial contamination of the endometrium appears as a consequence. Another possibility by which the retained placenta increased risks of CLE and SCLE is the nutritional imbalance due to poor feeding practices and low level of feed supplementation reflected in low body condition score observed in the study cows. According to Boro *et al*. (2014) and Islam *et al*. (2013), the nutritional imbalance may compromise immune function especially impaired neutrophil function in retained placenta-affected cows, which decreases the ability of the cows to expel the placenta after calving. Practices that farmers could use to prevent retained placenta and thus reduce the odds of CLE and SCLE cases include ensuring an adequate balanced diet in the transition period (Mordak & Anthony, 2015). In particular, vitamin E and selenium administration during the three weeks of the transition period prior to calving have been shown to be an effective prophylactic strategy for the prevention of retained placenta (Julien *et al*., 1976).

The study further revealed that CLE and SCLE cases were more likely when cows calve in the rainy season. This finding could be due to the management conditions of the sample farms. More than a third of the farms had a cowshed without a roof, where cows were not protected from rain, and as consequence cows calved in a damp and dirty environment. These poor hygienic conditions might have led to an increase in the risk of genital reproductive tract contamination by environmental organisms during or in the post-calving period, thus increased the odds of CLE and SCLE occurrence. The current findings concur with those of Bacha & Regassa (2010) in Ethiopia and Nguyen-Kien & Hanzen (2017) in Vietnam. Therefore, observing hygienic practices during the rainy season would be a strategy to reduce the risk of CLE and SCLE cases.

In the present study, cows in poor body condition (BCS <3) were more likely to be positive for CLE and SCLE cases than cows in good body condition (BCS ≥ 3). This finding demonstrates the role that pre-and/or post-calving negative energy and protein balance likely plays in the occurrence of endometritis (Kelly *et al*., 2020). This result has been observed in other studies as well where cows in poor BCS at calving (Carneiro *et al*., 2014) and in post-calving (Kelly *et al*., 2020) manifest increased odds of CLE and SCLE cases. Moreover, Walsh *et al*. (2011) observed that inadequate nutrition in the transition period could lead to poor BCS, metabolic disorders, and consequently, negative energy and protein balance that trigger suppressed immune function. Therefore, cows with poor BCS in the transition period were more likely to suffer from an increased endometritis case. The poor BCS can be managed with adequate quality feeding of cows in the mid/late lactation period of the previous lactation to attain good BCS at calving and to minimise CLE and SCLE cases.

This study identified mastitis as a significant RF for SCLE, which can partly be linked to the presence of common causes for both SCLE and mastitis, such as an unhygienic environment because cows were mostly crowded together in a dirty and wet zero-grazing unit. In these conditions, the environmental organisms tend to be concentrated if appropriate prevention and control measures for diseases are not in place. The findings concur with those of Adnane *et al*. (2017) that mastitis results from bacterial contamination within the environment. Furthermore, the decrease in immune function predisposes to both mastitis and SCLE (Bacha & Regassa, 2010). These observations suggest that the joint implementation of routine mastitis and endometritis prevention and control practices may help to reduce the risk of SCLE cases in smallholder dairy herds (Chapter three and four).

The more SCLE cases associated with increased cow parity in this study could be attributed to the cumulative exposure over the years of multiparous cows to uterine bacterial contamination from the unhygienic farmers' intervention during calving assistance. Cow parity was positively associated with increased SCLE, possibly due to a gradual decrease in the efficiency of the immune system in the transition period (Sheldon *et al*., 2009; Potter *et al*., 2010). Furthermore, the inadequate nutrition (Sheldon, 2018), reduced uterine elasticity and slower uterine involution than in primiparous cows (Adnane *et al*., 2017) may predispose multiparous cows to SCLE. Similarly, lower immune function in the transition period (Galvão, 2013), high metabolic stress of lactation in the first month (Toni *et al*., 2015) coupled with the common bacterial contamination of the uterus in dirty cowshed might in combination increased susceptibility of multiparous cows to SCLE than primiparous cows (Dolezel *et al*., 2010).

In this study, cows under unhygienic cowshed were more likely to be CLE and SCLE positive cases. This finding may be explained by poor waste drainage system on the study farms that resulted in accumulated manure and urine, which favours the growth of environmental organisms (Azevedo *et al*., 2016), and likely to ascend the reproductive tract of fresh cows to cause uterine disorders (Adnane *et al*., 2017). In agreement with the present study, a significant association between poor hygiene in a cowshed and increased risk of endometritis have also been reported in other studies (Bacha & Regassa, 2010; Moges & Jebar, 2012). In an unhygienic condition, the bacteria that contaminate the genital reproductive tract comes directly from the vagina or the environment via the vagina and also from the cow's skin and faeces (Piersanti & Bromfield, 2019; Potter *et al*., 2010). This is because the cow is heavily soiled with dirt and/or faeces leading to faeces entering the vagina and so ascending bacterial infection of the genital reproductive tract.

The dirty on higher side of a cow is likely related to diarrhoea. When cows have diarrhoea, they can smear dung in some part of their pelvis, which then is dirt in the vulva, udders and legs. The dirty can contain pathogens (Hulsen, 2016). Also, diarrhoea is a health problem which reduces the amount of energy and nutrients intake. Because of this, clean cows contribute to good health and to good production of cows and youngstock.

The study further revealed that cows kept in herds with at least three cows were more likely to have CLE positive cases. This result might be associated with over-crowding in the small, wet and dirty cowshed in the study herds, which with frequently assisted calving, increase cow exposure to bacteria including for CLE in their environment, in concurrence with the observations of Bacha & Regassa (2010) and Moges & Jebar (2012). The current finding may also indicate that individual management of cows was better in smaller herds.

Farms not using bedding materials had higher odds of SCLE cases. This is an unhygienic challenge on smallholder dairy farms with their earthen floor being often very dirty and muddy so a potential source for endometritis-causing bacteria, predominantly the environmental organisms that can ascend female genital tract and endometritis as a result (Giuliodori *et al*., 2017; Kelly *et al*., 2020). It was observed in Ithaca, United States of America (Cheong *et al*., 2011) that the herds using bedding materials had lower herd SCLE cases compared to herds that were not using the bedding materials. The current finding should inform providers of dairy extension and animal health services that awareness and education targeted to managing cow- and herd-levels RFs for endometritis is needed to achieve effective prevention and control of the disease (Chapter four).

The earthen floor was directly and significantly associated with more CLE cases, probably because earthen floors are usually more challenging to keep clean of wet dirt and manure than concrete floors with or without bedding material. These conditions expose cows to heavy soiling and a media of proliferation of microorganisms, which increase the risk of transmission of environmental organisms contaminating the uterus (Moges & Jebar, 2012; Daros *et al*., 2017; Piersanti & Bromfield, 2019).

5.4.2 Risk factors with indirect association with endometritis

The results from this study show that the sex of the calf was indirectly associated with increased cases of CLE and SCLE. This is probably because, male calves increase the odds of having stillbirth than female calves due to disproportion between the size of male calves at birth and the pelvic area of the dam, which causes difficult calving and increases stillbirth parturition cases (Mahnani *et al*., 2017). In an attempt to assist cows in such cases, farmers often apply the do-it-your-self interventions, such as self-stillbirth assistance without seeking help from veterinarians, non-use of gloves and providing unhygienic assistance under muddy cowshed as observed in the study farms. These malpractices are mostly associated with physical trauma and bacterial contamination of the female genital reproductive tract (Adnane *et al*., 2017; Appiah *et al*., 2020; Vieiro-Neto *et al*., 2016), and CLE and SCLE may develop subsequently.

The results from this study also show that cows giving birth to male calves and being primiparous were indirectly at a higher risk of CLE and SCLE through increased risk of stillbirth. This is likely associated with physical trauma, human interventions, and bacterial contamination of the cow's reproductive tract around the time of calving. All these factors predispose the cow to CLE and SCLE cases (Sheldon *et al*., 2009). Indeed, the big size of a male calf as a risk for stillbirth may be mediated by calving assistance and this can be reduced by matching cows to suitable sire for ease calving. Further, primiparous cows had significantly higher odds of a stillbirth, which was probably related to the smaller pelvis of such cows than multiparous cows. These results are consistent with some of the previous studies (Dhakal *et al*., 2013; Mellado *et al*., 2017), which suggest the need for using preventive corrective measures. These should include the use of sexed embryo or sexed semen, selecting sires with low percent stillbirths, or utilizing sire and daughter calving ease information when selecting sires to breed heifers in dairy herds (Diers *et al*., 2020).

Twin births and stillbirth increased the odds indirectly for CLE and SCLE cases through increased risk of retained placenta, which is in agreement with Correa *et al*. (1993), Gonçalves *et al*. (2019), and Potter *et al*. (2010). In management of dairy cows, therefore, it is important to perform pre-calving diagnosis of twin pregnancies (Kirkpatrick, 2011) to inform targeted appropriate nutrition and health management, such as clean calving facilities, for twinning cows (Wakchaure & Ganguly, 2016) for decreasing the risk of CLE and SCLE cases. Furthermore, Julien *et al*. (1976) suggested that vitamin E feeding or administration for two weeks prior to calving would be an effective approach for preventing retained placenta for twin-producing cows.

In the present study, the poor BCS was a risk for CLE and SCLE cases. Cases of poor BCS were more prevalent among the indigenous cattle and dairy crossbreds than among the dairy pure breeds. This finding is suggestive of selective feeding practices for the breeds because Napier grass basal feed with limited supplementation (21.6%) was more frequently a practice for pure dairy breeds (14.1%) than for dairy crossbreds (6.8%) or indigenous cattle breeds (0.8%). Therefore, the selective feeding practices might have contributed to better BCS of pure dairy breeds than crossbreds and indigenous cattle. This explanation concurs with the observations elsewhere with local and crossbreds cows (Lobago *et al*., 2006).

The indirect association between earthen floor cowshed and SCLE cases and between the absence of housing cows within the first 30 dpp and CLE cases are to large part consequences of the poverty level of the sample farmers. Over half (62.4%) of them were resource-poor households by the poverty classification of Rwanda's government (Cho & Kim, 2017). This observation implies that they were unable to afford the capital needed to build a cowshed to specifications of the extension service requiring allowance for the adequate walking area, feeding and water troughs, milking place, holding crush, fodder chopping area, and manure storage (Chapter four). It has been observed in Kenya (Nalunkuuma *et al*., 2015) that constructing a dairy zero-grazing unit needs high initial investment, and many farmers being unable to afford do construct small crowded units without adequate spacing. As a result, cows calved in poor hygienic conditions and crowded together with other animals of the herds in a dirty cowshed. In such environments, cows are highly exposed to environmental microorganisms, and thus, farmers need to maintain good hygiene to reduce exposure (Durst, 2011; Pascottini *et al*., 2020). However, in the present study, no attempt was made to isolate and profile the environmental pathogens prevalent on smallholder farms. This should attract research in future epidemiological studies of endometritis on smallholder zero-grazed dairy cows in Rwanda.

The study was carried out in Gasabo district of Rwanda, and the sample farms were selected through the exponential non-discriminative snowballing technique. This was applied because it was difficult to locate the farms with the set study criteria and the choice of new farm depends on inclusion criteria defined in this study (Babbie, 2013). The weakness of exponential non-discriminative snowball sampling was addressed by making efforts to obtain a large number of cows in the study area. With the knowledge that regular herd data recording is not a practice by smallholder farmers (Opoola *et al*., 2019), recall data was obtained by limiting the recall period to the recent past one month. Data were obtained in an integrated manner using cross-sectional surveys to collect data at cow- and herd- levels. Cows and farms that had missing data were not included in the analysis. Therefore, 12 cows from 10 farms could not be included in the analysis of risk factors associated with endometritis due to lack of SCLE results.

Some cow- and herd- levels risk factors were specific for SCLE cases (mastitis, parity of cow and not using bedding materials) or CLE cases (earthen floor cowshed and large herd size), and some others were common for both SCLE and CLE cases (calving season, dystocia, poor BCS, stillbirth, retained placenta, breed and parity of cow, sex of calf, twin births, farm size, unhygienic cowshed, farmer experience in dairy farming, and absence of housing of cows within their first 30 dpp). The difference of specific risk factors between CLE and SCLE is probably because SCLE is a consequence of dysregulation of the postpartum inflammatory process of uterine endometrium rather than changes in uterine microbiota (Wang *et al*., 2018). This is supported by the findings of Pascottini *et al*. (2020), who reported that the uterine microbiota of cows with SCLE was similar to that of healthy cows, but the uterine microbiome differed in cows with CLE. In their study, Prunner *et al*. (2014b) and Wang *et al*. (2018) concluded that major uterine pathogens are not associated with SCLE. In contrast, CLE is generally a consequence of physical trauma of the female genital reproductive tract and the presence of uterine pathogens from vagina and/or dirty environment. This suggests that application of good hygiene of environment and during calving assistance is a good strategy to prevent CLE cases, and regulation of postpartum uterine inflammation is worthy of pursuit for prevention and treatment of SCLE.

The possibility of information bias could be from nonuniformity in risk factors diagnosis and recording. The SCLE and CLE diagnoses were reliable and uniform across different dairy herds, but the occurrence of other diseases and calving-related information were less reliable and sometimes not even recorded. We attempted to reduce this bias first by explaining to the farmers and animal health service providers the definitions of risk factors that are not obvious. Therefore, some cow-level risk factors data such as days dry, gestation length, cow parity, sex of the calf, season of calving, breeding services, dystocia, retained placenta, twinning, milk fever, ketosis, left displaced abomasum, and status of a calf at birth were collected retrospectively from the animal health service providers' records, farm records or farmer recall through an interview, and direct observations.

Diagnosis of SCLE depends on the proportion of PMNs in endometrial cytology samples. To prevent variability in sampling and interpretation, all the cytology slides were collected by one investigator and were analysed and read by one laboratory technician trained in the endometrial cytological examination and had no information about the CLE results. The presence of mucus in some of the cytology samples resulted in poor adhesion of cells to the slides, resulting in some cytology slides not having sufficient cells to make a diagnosis. Therefore, only 2.5% (n=12) of the samples collected could not be included in the analysis of CLE and SCLE prevalence due to poor quality of endometrial cytology samples.

5.5 Conclusion

The use of the path analysis model enabled the identification and quantification of cow- and herd-levels risk factors that are directly and indirectly associated with CLE and SCLE cases in zero-grazed dairy cows managed on smallholder farms. Cows were at increased risk of CLE and SCLE when in poor BCS and with cases of stillbirth, retained placenta, and dystocia and when calving occurs in the rainy season. Further, dairy herds were at increased risk of CLE and SCLE cases when cowsheds were in unhygienic conditions, farms are large, and farmers have long experience in dairy farming but housing cows within their first 30 dpp reduced the risk. Farmers can effectively prevent and control endometritis among their zero-grazed dairy cows by practicing good hygiene and feeding, implementing on-farm biosecurity measures and seeking professional veterinary services. Extension service has the responsibility of equipping farmers with the set knowledge and skills for on-farm application at the cow- and herd- levels.

CHAPTER SIX

EFFECTS OF ENDOMETRITIS ON REPRODUCTIVE PERFORMANCE OF ZERO-GRAZED DAIRY COWS ON SMALLHOLDER FARMS IN RWANDA

Abstract

Endometritis is a prevalent post-partum uterine infection in dairy cows resulting in suboptimal reproductive performance. This study evaluated the effects of endometritis diagnosed at 38.5 ± 0.7 days postpartum (dpp) on subsequent reproductive performance of dairy cows managed under zero-grazing feeding practice on smallholder farms. Reproductive performance of 436 cows from 345 farms was recorded for 210 dpp. Values for reproductive performance indicators were less ($p<0.05$) in cows determined to be positive compared to negative for endometritis. Cows that tested positive, as compared to negative, for endometritis had longer periods after parturition until initiation of oestrous cycles (median, interquartile range; 85.0, 57.5 –127.0 and 62.6, 49.0–90.0 days, respectively), longer durations before being detected pregnant (95.5, 61.8–145.5 and 63.0, 50.0–83.0 days, respectively), lesser pregnancy rates as a result of the first breeding postpartum (16.5% and 32.7%, respectively), more natural-mating or artificial inseminations per pregnancy (1.3±0.1 and 1.1±0.0, respectively) and more occurrences of anoestrus postpartum (48.4% and 11.7%, respectively). These results provide evidence of a strong association between endometritis and suboptimal fertility performance in zero-grazed cows on smallholder farms in Rwanda. Considering there were 70.2% of cows in the present study were diagnosed with endometritis, this is indicative of a widespread herd health issue, warranting that field veterinary practitioners prioritise endometritis in their herd health service delivery to smallholder dairy farmers for effective disease management and herd sustainability.

6.1 Introduction

Endometritis is a prevalent uterine disease of dairy cows between the periods extending from 21 to 90 days postpartum (dpp). The prevalence can be as great as 89.0% in some herds between 21 and 90 dpp (Denis-Robichaud & Dubuc, 2015; Pascottini *et al*., 2015). The disease results in suboptimal fertility (Mohammed *et al*., 2019). Optimal fertility is an important component of production efficiency (milk produced per cow/day and number of calves born/year) in a dairy herd (Kim & Jeong, 2019). In herd fertility management, the extension service recommends farmers to attain a herd average calving interval of about 12 months (Rukundo *et al*., 2018). Though a 12-month calving interval is a general herd fertility management objective, very few smallholders achieve this. The disruption of reproductive functions is an underlying cause but often unknown to smallholder producers (Chapter three). At cow-level, farmer- perceived prevalence of endometritis was much less (3.2%) than the observed prevalence (67.2%) (Chapter three). This finding indicates that farmers underestimate the disease and are unaware of the prevalence, which is indicative of a large knowledge gap about the disease.

In a dairy herd, cows with disrupted reproductive functions lead to suboptimal fertility performance measures: prolonged days to first oestrus following parturition (Hussein *et al*., 2017), longer periods to first natural mating or artificial insemination (AI) service after parturition (Barrio *et al*., 2015), longer periods of days not-pregnant (Rinaudo *et al*., 2017), and a greater proportion of anoestrus postpartum cows in the herd (Chan Lee *et al*., 2018). In some studies, there have been associations reported between endometritis prevalence and lesser pregnancy rates resulting from the first breeding postpartum (37.3% to 15.6%) (Marques *et al*., 2015) and increased number of times natural mating or AI services per conception (3.9 to 4.7) (Nguyen-Kien *et al*., 2017). The majority of these results, however, are based on studies conducted in commercial dairy systems in the Americas, Asia, and Europe where dairy herds are managed in confinement housing conditions. In contrast, there are few reports regarding the effects of endometritis on the subsequent reproductive performance of cows in smallholder dairy farms.

Rwanda is highly dependent on agricultural income and smallholder dairy herds are an important source of wealth and income from milk, calves and manure sales (IFAD, 2016; RLMP, 2017). Smallholder dairy herds represent 92.0% of the herds and are managed in zero-grazing feeding practice (Mupenzi *et al*., 2019), and they attain suboptimal fertility performance measures (Bishop & Pfeiffer, 2008; Manzi *et al*., 2019; Rukundo *et al*., 2018). There is a large incidence of anoestrous cows following parturition (44.0%), less than optimal conception at first natural mating or AI service (35.0%), a relatively greater number of times cows are bred per conception than is optimal for dairy (3.0 ± 1.3), long interval between times of calving (18.3±4.5 months), and relatively longer period of days-not pregnant than is optimal (298.7±199.0 days). Suboptimal fertility has been explained as resulting from poor herd health and feeding management but without identifying the specific underlying cause (s) (Nishimwe *et al*., 2015). With the large prevalence of endometritis (70.2%) in dairy herds of Rwanda (Chapter three), it is likely that endometritis could be an underlying cause of the prevalent suboptimal fertility performance of cows on smallholder farms. Empirical evidence, however, is lacking in Rwanda on smallholder dairy herds about the association of endometritis with fertility performance subsequent to calving. Such information would inform targeted management interventions for endometritis prevention and control. The objective of the present study was to evaluate the influence of endometritis on the reproductive performance of postpartum dairy cows managed under zero-grazing feeding practices on smallholder farms.

6.2 Materials and methods

6.2.1 Data source

The study was implemented from September 2018 through August 2019 in the Gasabo district of Rwanda. The sample smallholder zero-grazed farms and the study area were described in Section 3.2.

6.2.2 Study design

The study was a prospective observational design, involving 345 dairy farmers recruited through exponential non-discriminative snowball sampling as given in Chapter three. In addition to the inclusion criteria described in Section 3.2., the farmers should have the will to record the reproductive performance data. For each recruited farmer, informed written consent was sought before delving into the study details.

On the first day of the farm visit, general information on farmer and herd management were collected by trained enumerators through direct observation, and interviews using a pre-tested structured questionnaire. Cows were examined for endometritis. Cows were monitored for 210 dpp to record reproductive performance outcomes. Dairy cows (n = 436) within their 21 to 60 dpp were assigned for evaluations in this study. The breed distribution of the sample cows was 66.7% dairy crossbreds, 16.7% indigenous cattle, and 16.5% dairy pure breeds. These cows were maintained in zero-grazing housing units and there was no access to forage for grazing, and the dominant feeding practice was a cut-and-carry system.

6.2.3 Diagnosis of postpartum endometritis

This was as given in Chapter three. Briefly, at every farm on which there were assessments, cows were examined for CLE by ascertaining the vaginal mucus using the Metricheck device (McDougall *et al.*, 2007) and for SCLE by evaluating endometrial cytological samples collected using a cytotape (Pascottini *et al.*, 2015). Cows with vaginal mucus ≥1 were recorded CLE- positive (Williams *et al.*, 2005), whereas cows that had ≥5% PMN in the endometrial cytological samples were recorded SCLE- positive (Wagener *et al.*, 2015).

Further grouping was relative to the uterine health status within 21 to 60 dpp with cows being classified CLE only (vaginal mucus ≥1 and <5% PMN), SCLE only (vaginal mucus−0 and ≥5% PMN), both CLE and SCLE (vaginal mucus ≥1 and ≥5% PMN) or without CLE and SCLE (vaginal mucus−0 and <5% PMN) (Gobikrushanth *et al*., 2016). From these diagnoses, cow status was grouped as positive for endometritis (CLE only, SCLE only, both CLE and SCLE) or negative for endometritis (neither CLE nor SCLE).

6.2.4 Reproductive performance variables

For the cows examined for endometritis, reproductive performance data were recorded using a prospective observational design for 210 dpp without any interventions on herd management practiced by the farmers. In Rwanda, the average days-not pregnant (DNP) in dairy cows is about 298.7±199.0 days (Manzi *et al*., 2019; Rukundo *et al*., 2018). A 210-day pregnancy status, therefore, was considered as an economically important measure of reproductive performance variables. Furthermore, the study was limited to 210 dpp because the effects of endometritis on subsequent reproductive performance in postpartum dairy cows can be effectively evaluated until cows were at 210 dpp, a period during which the reproductive performance is markedly impaired in cows with this disorder (Chan Lee *et al*., 2018; Hussein *et al*., 2017).

Cows were either naturally mated with bulls or were artificial insemination (AI) serviced based on visual oestrous detection conducted daily by the farmer in the early morning (05:00 - 06:00 a.m.) and late in the afternoon (5:00 - 6:00 p.m.) (Chaudhari *et al*., 2017). Farmers used standing-to-be-mounted, bellowing more frequently, restlessness and trailing of other cows, mucus discharge from the vulva, and sniffing and licking the genitalia of other cows as symptoms to detect behavioural oestrus in cows. For natural mating, the breeding bulls or the cows on oestrus were moved from one location to another for natural mating, and bulls or cows were not pre-screened for diseases before being used for natural mating (Chapter four). For AI, there were local technicians who routinely inseminate cows according to the “a.m.-p.m. rule” for AI of dairy cattle whereby a cow observed on oestrus in the morning (05:00-06:00 a.m.) was inseminated the following evening (4:00 - 6:00 p.m.). Furthermore, a cow observed on oestrus in the evening was inseminated the next morning. The reproductive performance data were recorded by the farmer on a form provided by the researchers, and subsequently verified and confirmed by researchers at 10-day intervals. Researchers used a calendar designed for highlighting dates to guide in the verification of reproductive performance data.

The data collected were used to compute fertility performance measures in dairy cows (Ahmadi *et al*., 2018; Drillich *et al*., 2005) (Table 27). Pregnancy status assessments were conducted using transrectal palpation procedures and occurred at a mean of 74.4±0.4 (range: 60 to 90 days) following natural mating or artificial insemination. A cow not detected in oestrus during the first 2 months after calving was classified as being in postpartum anoestrus (Nguyen-Kien *et al*., 2017; Vinita *et al*., 2018).

Table 27. Measures of reproductive performance used in the study

Group of cows	Measures of reproductive performance	Definitions
All cows (serviced and not serviced cows)	DFO (days)	$\text{Date of first oestrous} - \text{date of calving}$
	DFMA (days)	$\text{Date of first breeding service} - \text{date of calving}$
	ANPP (%)	$\frac{\text{Number of cows not detected in oestrus during the first 2 months after calving}}{\text{Total number of cows enrolled}} \times 100$
Only cows naturally mated or AI serviced	DNP (days)	$\text{Date of successful breeding service} - \text{date of calving(only for cows confirmed pregnant)}$
	NSC (number)	$\frac{\text{Number of breeding services for cows confirmed pregnant}}{\text{Total number of cows pregnant}}$
	CRFMA (%)	$\frac{\text{Number of cows pregnant with first breeding services within 210dpp}}{\text{Total number of cows served}} \times 100$
	CRAS (%)	$\frac{\text{Number of cows pregnant within 210 dpp}}{\text{Total number of breeding services}} \times 100$
	CP210 (%)	$\frac{\text{Number of cows pregnant within 210 dpp}}{\text{Total number of cows enrolled}} \times 100$

DFO = days to first oestrus, DFMA = days to first natural mating or AI service, ANPP = anoestrous postpartum, DNP = days-not pregnant (interval calving to conception), NSC = number of services (bull or artificial insemination) per conception, CRFMA = conception rate at first natural mating or AI service, CRAS = conception rate to all services, CP210 = cows pregnant within 210 dpp (study end), dpp = days postpartum.

6.2.5 Statistical analysis

From first to last day of the follow-up survey, data from 30 cows on 25 farms were not included in the study either because of voluntary culling (n = 25) or involuntary culling (n= 5). Any data from these cows, therefore, were not considered for subsequent statistical analysis. Complete data for the reproductive performance variables assessed were based on 436 cows from 345 farms.

Continuous variables were tested for normality using a Shapiro-Wilk normality test, and the appropriate transformation was conducted to fulfil the parametric assumptions (Manikandan, 2010). Data on DFO, DFMA, and DNP were $\log_{10}$ transformed before analysis, but for ease of interpretation, the results are reported in the original scale (Filho *et al*., 2012). The DFO, DFMA, and NSC were compared between endometritis positive and negative cows using Independent-Samples T-Test. The effect of different categories of endometritis (CLE only, SCLE only, both CLE and SCLE, or no CLE and SCLE) on DFO, DFMA, DNP and NSC were initially analysed using the Linear mixed model specifying cow nested within-herd as a random effect. However, the effects of herd and cow were not significant (p>0.05) probably because of the similarities of the management practices in the smallholder dairy farms (Darboe, 2018; Nguhiu-Mwangi *et al*., 2008). Thus, analysis of variance with general linear model (GLM) procedure was used. Differences between means were tested by using Tukey's honestly significant difference test. The model for the analysis of measures of reproductive performance was fitted as:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where Y_{ij} = the observed reproductive performance (DFO, DFMA, DNP, or NSC) of postpartum dairy cows; μ = the overall mean; α_i = the effect of i^{th} endometritis status (i = 4, no CLE and SCLE, CLE only, SCLE only, and both CLE and SCLE); ε_{ij} = random error term.

The DNP at 210 dpp was evaluated using the Kaplan-Meier survival analysis (Time-to-event analyses)(Kaplan & Meier, 1958). Logistic regression analysis (Thrusfield & Christley, 2018) was fitted to determine the odds ratio of cows positive for endometritis failing to conceive at first breeding postpartum; to be anoestrus postpartum and that failed to conceive by 210 dpp using the model:

$$ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 X_1 + \varepsilon$$

where p is the probability of the outcome (failed to conceive at first breeding postpartum; be anoestrus postpartum and failed to conceive by 210 dpp); $\frac{p}{1-p}$: odds ratio of the outcome; β_0=

intercept parameter, β_1 = regression coefficient of endometritis status; X_1 = endometritis status (positive, negative), ε= random error term.

A nonparametric Kruskal-Wallis test was used to evaluate whether different categories of endometritis have effects on ANPP, CRFMA, CRAS, or CP210. Variables where there were overall differences based on findings with the use of the Kruskal-Wallis test were subsequently evaluated using a Mann-Whitney U test to explore pairwise comparisons. All statistical analyses considered mean differences significant at alpha <0.05 and were all performed using Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows package software.

6.3 Results

6.3.1 Characteristics and management on the sample farms

The majority (62.2%) of farmers were within "poor category" per Government of Rwanda poverty classification. Their average (mean ± standard error of the mean) herd size was 2.9±0.1 cows (range of one to five cows and a median of two cows) on less than three acres of farm holding. On average, cows were at 38.5±0.7 dpp. The cows that were evaluated had a median parity of 2.0 and age of 4.9 (±1.8) years. The mean age at first calving was 2.6±0.2 years, with an average calving interval of 1.4±0.2 years. More than half (57.8%) of farmers were educated (farmers with formal school education). Cows were primarily maintained in a zero-grazing unit with concrete floors (79.7%) and a variety of locally available forages were cut and transported daily ("cut-and-carry") to feed cows. Napier grass (*Pennisetum purpureum*) fodder was the basal feed that was fed to most of the cattle (98.0%), followed by banana fodder (76.0%) and *Brachiaria* grass (67.0%). Feeding of the basal feeds was combined with the feeding of forage maize (35.4%), crop residues (32.4%), and agro-industrial by-products (22.0%), and mineral blocks (31.0%). Animal performance records either were not recorded (75.4%) or were incomplete (24.6%).

6.3.2 Effect of endometritis on subsequent reproductive performance

Of the cows examined, there were 2.4 times more cows positive for endometritis (70.2%, 306/436, 95% CI = 65.7 – 74.3) than those negative for endometritis (29.8%, 130/436, 95% CI = 25.7 – 34.3). The distribution of cows that were examined were 38.3% (167/436, 95% CI = 33.9 – 42.9) with CLE only, 2.8% (12/436, 95% CI = 1.6 – 4.8) with SCLE only, and 29.1% (127/436, 95% CI = 25.1 – 33.6) with both CLE and SCLE while 29.8% (130/436, 95% CI = 25.7 – 34.3) had neither CLE nor SCLE.

All the reproductive performance variables considered in this study differed ($p<0.05$) among the different categories of endometritis (Table 28). The days to first oestrus following calving was 23 days more among cows positive for CLE only and 40 days more for cows positive for SCLE only compared with cows not diagnosed with CLE or SCLE. In contrast, days to first natural mating or artificial insemination was about 27, 44, and 25 days more in cows with CLE only, SCLE only, and both CLE and SCLE, respectively, than in those diagnosed as having neither CLE nor SCLE. Cows with CLE only, and both CLE and SCLE required more (natural mating or artificial insemination) services to conceived as compared with cows not diagnosed with these disorders for which there was a lesser number of services for conception. None of the cows diagnosed with SCLE were diagnosed as being pregnant. The conception rate at first natural mating or artificial insemination and cows pregnant within 210 dpp were less in cows with CLE only, and both CLE and SCLE than cows not diagnosed with these disorders ($p<0.05$). The number of service per conception and anoestrus postpartum were greater in cows diagnosed with CLE only compared with cows diagnosed with SCLE only, both CLE and SCLE, and cows that were diagnosed as not having either of these conditions ($p<0.05$).

Table 28. Effects of different categories of endometritis status diagnosed between 21 and 60 days postpartum on reproductive performance measures (n = 436)

Measures of reproductive performance	**Categories of endometritis status**			
	No endometritis (n=130)	**CLE only (n=167)**	**SCLE only (n=12)**	**CLE and SCLE (n=127)**
DFO, Mean days (n)	68.7±2.9 (106)[a]	91.9±4.9 (84)[ab]	108.5±17.7 (8)[b]	91.8±5.3 (77)[ab]
DFMA, Mean days (n)	73.8±3.4 (103)[a]	100.8±4.6 (79)[ab]	117.3±20.9 (7)[b]	98.3±5.2 (71)[ab]
DNP, Mean days (n)	68.8±3.1 (91)[a]	99.9±10.0 (21)[b]		106.8±7.7 (33)[b]
ANPP, % (n)	11.7 (51)[a]	27.1 (118)[b]	2.1 (9)[c]	19.3 (84)[d]
CRFMA, % (n)	32.7 (85)[a]	6.5 (17)[b]		10.0 (26)[c]
CRAS, % (n)	32.2 (91)[a]	7.4 (21)[b]		11.7 (33)[c]
CP210, % (n)	20.9% (91)[a]	4.8 (21)[b]		7.6 (33)[c]
NSC, Mean number (n)	1.1±0.0[a] (100)	1.3±0.1[b] (27)		1.2±0.1[ab] (41)

Different letters within the same row indicate differences ($p<0.05$),
CLE = clinical endometritis, SCLE = subclinical endometritis,
DFO = days to first oestrus,
DFMA = days to first natural mating or AI service,
DNP = days-not pregnant (interval calving to conception),
ANPP = anoestrous postpartum,
CRFMA = conception rate at first natural mating or AI service,
CRAS = conception rate to all services,
CP210 = cows pregnant within 210 dpp (study end), NSC = number of services (bull or artificial insemination) per conception.

Survival analysis results (Figure 23) indicated that cows with CLE only took a median 20 days longer, and cows with both CLE and SCLE took 56 days longer to conceive as compared with cows not diagnosed to have these disorders. The probability of conception by 210 dpp was less in cows with CLE only (hazard ratio = 0.48, 95% CI = 0.31–0.73) and in cows with both CLE and SCLE (hazard ratio = 0.43, 95% CI = 0.29 –0.62) than in cows without CLE and SCLE.

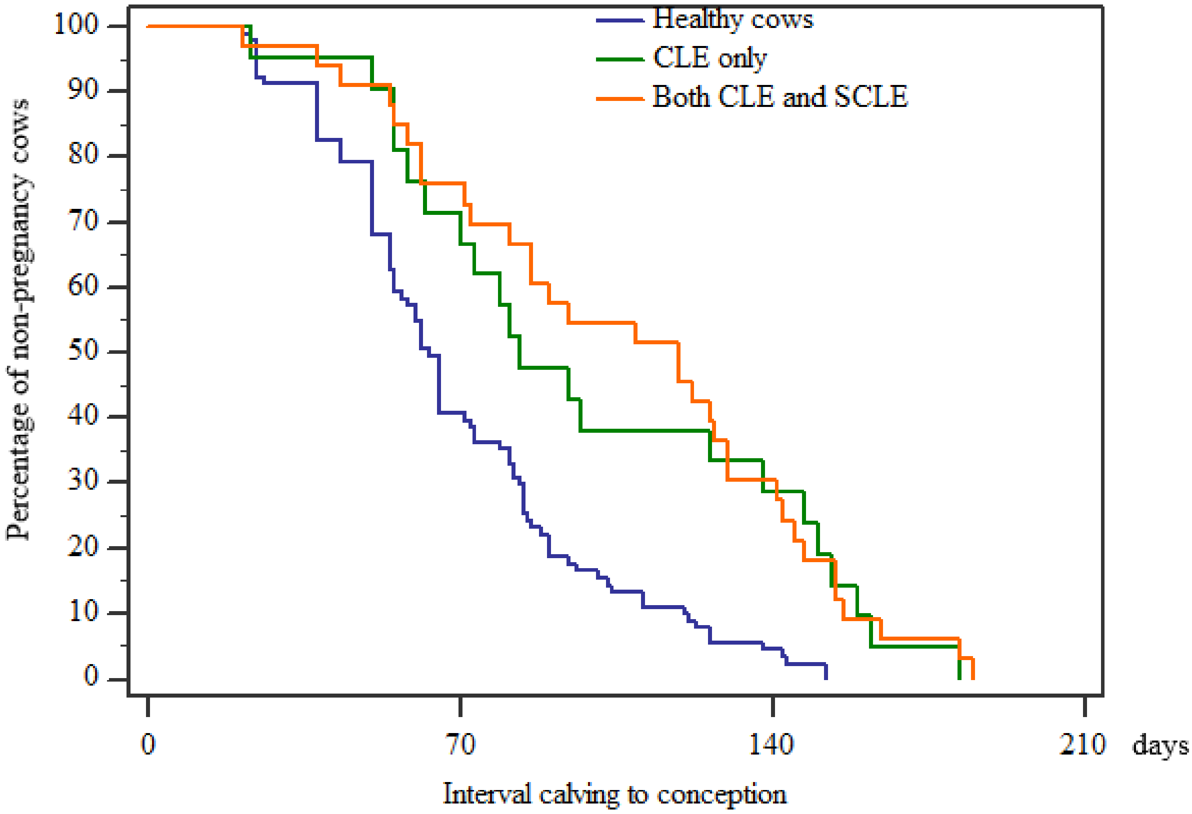


Figure 23. Kaplan-Meier survival curves for time (in days) to conception after parturition until 210 dpp in 436 dairy cows examined 38.5±0.7 dpp and classified as not having endometritis (VMC−0 and <5% PMN, n = 130), or as having clinical endometritis (CLE) only (VMC≥1 and <5% PMN, n = 166), subclinical endometritis (SCLE) only (VMC−0 and ≥5% PMN, n = 13), or both CLE and SCLE (VMC≥1 and ≥5% PMN, n = 127); Median time to conception (95% CI) was 63 days (59 – 67), 83 days (61 – 105), and 119 days (83 – 155) for cows without endometritis, CLE only and both CLE and SCLE, respectively.

The data for subsequent reproductive performance of cows that were endometritis positive and negative are presented in Table 30. For all reproductive variable determinations, results indicated there was a lesser (p<0.001) reproductive performance in endometritis positive cows than those diagnosed as not having endometritis. The DFO and DFMA were longer in cows with endometritis than those not diagnosed with endometritis. The cows not diagnosed with endometritis conceived 33 days earlier than the endometritis positive cows (63 and 96 days, respectively). The cows that were endometritis positive expressed the first behavioural oestrus postpartum on average 22 days later as compared with cows not diagnosed with endometritis. Of the cows that came into oestrus, 94.5% (260/275) were bred; more by mating with bulls (65.4%) compared (p<0.001) to artificial insemination (34.6%). Of these cows, 85.0% (221/260) were naturally mated with bulls or artificially inseminated at their first postpartum oestrus. There were 3.4% (n = 15) of the cows that expressed behavioural oestrus before farmers wanted to breed them; therefore, these cows were not (naturally mated or AI) serviced at the time of the first detected oestrus postpartum.

Table 29. Reproductive performance of cows with and without endometritis at 38.5±0.7 dpp

Measures of reproductive performance	Endometritis positive cows (n=306)			Endometritis negative cows (n=130)			Chi-square test
	n	**Median**	**IQR**	**n**	**Median**	**IQR**	
DFO (days)	169	85.0	57.5–127.0	106	62.6	49.0–90.0	***
DFMA (days)	157	94.0	64.0–137.5	103	65.0	50.0–90.0	***
DNP (days)	54	95.5	61.8–145.5	91	63.0	50.0–83.0	***
NSC (number)	68	1.3	1.0 – 2.0	100	1.1	1.0 -1.2	*
CRFMA (%)	43	16.5		85	32.7		***
CRAS (%)	54	19.1		91	32.2		***
CP 210 (%)	54	12.4		91	20.9		***
ANPP (%)	211	48.4		51	11.7		***

*p<0.05 and ***p<0.001, IQR = interquartile range, DFO = days to first oestrus. DFMA = days to first natural mating or AI service, DNP = days-not pregnant (interval calving to conception), NSC = number of services (bull or artificial insemination) per conception, CRFMA = conception rate at first natural mating or AI service, CRAS = conception rate to all services, CP210 = cows pregnant within 210 dpp (study end), ANPP = anoestrous postpartum.

The conception rate as a result of the first service postpartum differed (p<0.001) between cows positive and negative for endometritis. The endometritis negative cows had a greater CRFMA (32.7%) compared with endometritis positive cows (16.5%). Overall, 33.3% (145/436) of cows in this study were naturally mated or AI serviced and became pregnant during the study period; 12.4% of cows were those diagnosed with endometritis and 20.9% were those diagnosed as not having endometritis (p<0.001). In contrast, 26.4% (n= 115) of cows utilised in this study were naturally mated or AI serviced and diagnosed as being non-pregnant at the end of the study, 2.8% of study cows were those negative for endometritis and 23.6 were those positive for endometritis

There were145 cows that became pregnant with there being 168 natural mated or AI services, of which cows became pregnant with an average of 1.2 NSC. For all services, there was natural mating or AI of 260 cows with there being 283 natural mated or AI services. There were more ($p < 0.05$) (natural mating or AI) services of cows with endometritis that occurred before cows conceived (1.3±0.1) than there were for cows that were not diagnosed with endometritis (1.1±0.0). Consequently, the DNP were more as the number of (natural mating or AI) services increased; 71.5 days when there was one service and 81.0 days when there were two (natural mating or AI) services. The ANPP was much larger (48.4%) among endometritis positive cows compared ($p<0.001$) to cows not diagnosed with endometritis (11.7%). Overall, only 33.3% of the cows were pregnant; 3.4% were observed in oestrus but not (naturally mated or AI) serviced, 26.4% were (naturally mated or AI) serviced but did not conceive, and 36.9% were not detected in oestrus by the end of the study period.

From univariable logistic regression, endometritis positive cows were 3.4 times more likely to be in anoestrus postpartum [95% confidence interval (CI): 2.2 - 5.3; $p= 0.001$], 0.3 times less likely to conceive at first (natural mating or AI) service (95% CI: 0.1 - 0.8; $p= 0.017$), and 0.1 times less likely to conceive by 210 dpp (95% CI: 0.0 - 0.1; $p = 0.001$) compared to cows not diagnosed with endometritis. From survival analysis, cows with endometritis had a 55.0% lesser hazard of pregnancy and 33 days longer median time to conception than cows not diagnosed with endometritis (Figure 24). The vertical axis shows the probability of non-pregnant or proportion of non-pregnant cows, whereas the horizontal axis represents time (days not-pregnant) in days. For example, at time zero (0), the probability of non-pregnancy is 1.0. In other words, at time zero, 100% of the cows were not pregnant.

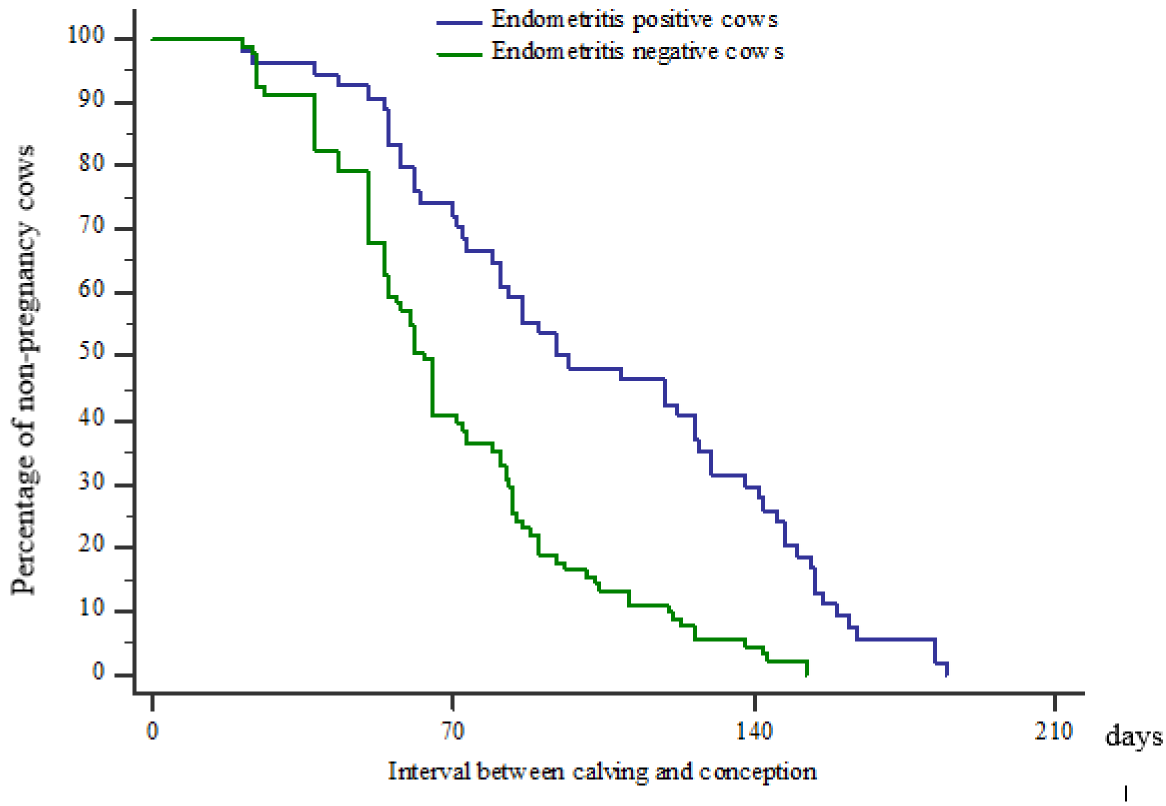


Figure 24. Kaplan-Meier survival curves for the days-not pregnant in endometritis positive and negative cows at 38.5±0.7 dpp; Median time to conception for cows positive and negative for endometritis was 96 days (95% CI = 60 - 128) and 63 days (95% CI = 59 - 67), respectively. Chance of conception by 210 dpp was less ($p<0.001$) for cows with endometritis than for cows without endometritis (hazard ratio (HR) = 0.45, 95% CI = 0.32 - 0.62)

6.4 Discussion

The present study is novel for investigation of the effect of endometritis on subsequent reproductive performance of zero-grazed dairy cows managed on smallholder farms without any intervention on herd management practiced by the farmers. Previous studies on endometritis have been with commercially managed large herds (Chan Lee *et al.*, 2018; Pascottini *et al.*, 2017; Ryan *et al.*, 2020; Tayebwa *et al.*, 2015; Wijayanto *et al.*, 2019). The estimated prevalence of endometritis in smallholder zero-grazed dairy cows indicated there was a general herd fertility health problem. In a sample of ten cows, seven were infected (Chapter three).

The current study revealed that cows diagnosed with CLE only had lower CRFS, lower CRAS, lower CP210, high ANPP rate, and more NSC compared to those with both CLE and SCLE or cows not diagnosed with CLE or SCLE. This is probably due to an additive combined effect of cervicitis, vulvovaginitis, urethritis, and CLE because both conditions are expressed clinically by a mucopurulent or purulent discharge and associated with impaired subsequent reproductive performance (Deguillaume *et al*., 2012; Dubuc *et al*., 2010; Hartmann *et al*., 2015; Solomon *et al*., 2020; Vieira-Neto *et al*., 2016). This observation is supported by the earlier findings (Chapter three) where the high number of false-positive cows (56.3%) was diagnosed by MED. The current findings corroborate with those reported in Canada, whereby Gobikrushanth *et al*. (2016) found that cows that had CLE only were less likely to conceive to the first service compared to SCLE only or cows diagnosed as not having CLE or SCLE.

The cows that were positive for endometritis had a lesser reproductive performance than cows not diagnosed with endometritis, indicating the condition of bacterial infection of the uterus is difficult for farmers to manage to minimise the disease effects. Endometritis infections are associated with delayed uterine involution and disruption of the oestrous cycle patterns following parturition (Chan Lee *et al*., 2018; Okawa *et al*., 2019). Endometritis results from a bacterial infection that persists when there are failures in herd fertility management, probably due to a lack of awareness of the disease among smallholder farmers, extension service, and field veterinary practitioners serving smallholder farmers in Rwanda (Chapter four).

The results of the present study indicate there is a marked association between endometritis and suboptimal fertility performance in cows managed under zero-grazing feeding practice on smallholder farms. In the present study, endometritis positive cows were determined to have about a four times greater incidence of anoestrus postpartum (ANPP) than cows not diagnosed with endometritis. This can be associated with the susceptibility of the cows of the dairy breeds to the disease, particularly when dietary intake is suboptimal. The cows of pure dairy breeds and crossbreds had a greater prevalence of endometritis compared to those of indigenous cattle breeds (Chapter three), and in the sample herds, breed distribution was 66.3% dairy crossbreds, 17.0% dairy pure breeds and 16.7% indigenous cattle. This high susceptibility of dairy breeds to the disease is partly due to the malnutrition, management and environmental conditions to which they are exposed (Singh *et al*., 2019). Napier grass was the basal feed with occasional supplementation with a commercial dairy meal and mineral licks. Offering adequate quality feed is a challenge for smallholder farmers, of which the majority had limited economic flexibility in the households as indicated by their poverty category in the Rwandan poverty index (Cho & Kim, 2017). The farmers managed on average three cows on the land of less than three acres to grow food crops and livestock fodder, which is insufficient for stable fodder supply to the dairy herd.

Inadequate feed resources and suboptimal feeding practices may result in more cows having a negative nutrient balance –energy and protein-which increases susceptibility of cows to endometritis infections (Ahmadi *et al*., 2018; Esposito *et al*., 2014; Hammon *et al*., 2006). Moreover, in their study, Esposito *et al*. (2014) reported that during the transition period, the risk for poor health is higher, as negative energy and protein balance and immune suppression concur, making cows more prone to uterine diseases and metabolic disorders. Also, Calvalho *et al*. (2019) observed that the effects of disease conditions extend beyond the transition period, reduces subsequent reproductive performance by affecting conception rate and embryo survival. A potential mechanism has been proposed that the influences of nutrition on reproduction performance are due to changes in metabolic substrates such as glucose, fatty acids, amino acids, and their products, and circulating concentration of metabolic hormones (Singh *et al*., 2019).

Being unable to ensure high quality of basal and supplementary feeds, smallholders have to wait for relatively longer periods for cows to resume reproductive functions after parturition than what would occur if there were adequate feed resources. Consistent with this observation, Sarder *et al*. (2015) in Bangladesh observed a significant effect of feed quality on the prevalence of reproductive disorders. The prevalence was greater for cows fed feed of sub-optimal quality than those that had feed resources that were of greater quality (15.0% and 9.7%, respectively). This nutritional inadequacy can explain the delay in return to cyclical patterns of ovarian function or disruption of the endocrine function leading to failure of ovulation resulting in ANPP (Ibtisham *et al*., 2018; Tesfaye *et al*., 2015). This lesser reproductive efficiency subsequently leads to a decrease in herd productivity and profitability by increasing the DNP, calving interval, NSC, culling rate, and veterinary cost (Ibtisham *et al*., 2018). Therefore, it is advisable that farmers improve feeding practices in the transition period to improve the fertility performance of their cows.

There was a greater percentage of ANPP cows in the present study than that reported (22.8%) by Tayebwa *et al*., (2015) in Uganda, but lesser than those (67.4%) reported by Chan Lee *et al*. (2018) in Korea, while the percentage is comparable with those in Belgium (42.8%) observed in the study of Pascottini *et al*. (2017). In the present study, the large prevalence of cows that were ANPP can explain the prolonged DFO, CRFMA, and subsequently longer DNP. The results indicate the need to monitor cows in the transition period and improving herd health management and feeding practices. The cows positive for endometritis were mated about a month later compared to cows not diagnosed with endometritis, because of ANPP and had a delayed onset of oestrous cycles postpartum. The DFO for endometritis positive cows in the current study is within the range of 67 to 100 days postpartum (Ahmadi *et al*., 2018; Rinaudo *et al*., 2017) but is nevertheless shorter than the range of 108 to 249 days (Chan Lee *et al*., 2018; Nguyen-Kien *et al*., 2017) for cows positive for endometritis reported previously in different management regimens. The estimated DFO in endometritis positive cows, however, is not within the recommended optimum (≤70 days) for a cow to calve every year (Noakes *et al*., 2001).

The longer DNP observed among cows positive for endometritis is directly associated with more services per conception. On average, those cows positive for endometritis required 1.3 (natural mating or AI) services to get pregnant at 96 dpp compared to cows not diagnosed with endometritis for which there were 1.1 natural mating or AI services for conception to occur by 63 dpp. This delay of 33 days in DNP for endometritis positive cows is equivalent to a period in which 1½ oestrous cycles would have occurred, which negatively affects cow reproduction and production performance. Related results indicated that the median DNP was extended for 9 and 42 days in cows positive for endometritis (Bicalho *et al*., 2016; Ryan *et al*., 2020). Although the estimated DNP is within the range of that reported in previous studies, there cannot be a calf per cow per year when this situation exists, which is obtainable when there are about 85 days of DNP (Noakes *et al*., 2001). Longer DNP is costly to the farmer as it represents reproductive wastage. It is difficult to shorten the DNP in smallholder dairy herds without having high-quality feed resources and regular herd fertility interventions. Conception rate affected DNP because if a cow did not conceive for a service (natural mating or AI), at least one additional oestrous cycle (21 days) had to occur before conception could occur. This observation is supported by a greater CRFMA in cows not diagnosed with endometritis resulting in fewer DNP (32.7% and 63 days, respectively). In contrast, there was a lesser CRFMA in endometritis positive cows resulting in more DNP (16.5% and 96 days, respectively).

In addition, CRFMA is directly related to the number of (natural mating or AI) services per conception (NSC). For this reason, the NSC was greater in endometritis positive compared to cows not diagnosed with endometritis because the cows have a delayed time of conception following parturition for a duration similar to the length of a typical oestrous cycle (i.e., about 21 days). Indeed, more of the endometritis positive than endometritis negative cows required two (natural mating or AI) services for conception to occur. Results of previous studies indicated there were more (natural mating or AI) services per conception for the endometritis positive compared to endometritis negative cows (Chaudhari *et al*., 2017; Hussein *et al*., 2017; Ryan *et al*., 2020). The results from a study by Gilbert (2012) in the U.S.A. indicated the reason for the additional AI services per conception in cows could be due to the inflammation of endometrium, which results in relatively lesser sperm motility in the reproductive tract and quality of the fertilised ovum. Furthermore, there are greater early embryonic losses and a larger proportion of pregnancy losses as a consequence of endometritis (Gilbert, 2012).

Another possible explanation for the lesser conception rate at first (natural mating or AI) service could be that most cows were naturally mated with bulls or artificially inseminated at their first postpartum oestrus. This could have resulted in a relatively lesser conception rate compared to when there was (natural mating or AI) service at subsequent oestrous periods postpartum. This is probably because endometritis infections are more likely prevalent at the time of the initial as compared with subsequent oestrous periods postpartum. The situation likely reflects the lack of awareness of the disease among farmers, extension service, and field veterinary practitioners. To manage this, extension service personnel, including field veterinary practitioners, need to include endometritis in their herd health programs that they deliver to smallholder farmers. The current findings are supported by results from previous studies conducted in Kenya (Gitonga, 2010) and in Belgium (Pascottini *et al.*, 2017) indicating cows with uterine infections at the time of (natural mating or AI) service are unlikely to conceive. A potential mechanism has been proposed that the effects of uterine infections on impairment of the reproduction are due to detrimental actions on sperm functions, increased rates of sperm phagocytosis, failures in fertilization processes, disruptions of embryonic implantation and lack of placental development. These effects could lead to reduced conception rates in cows positive for endometritis compared with cows not diagnosed with endometritis (Sheldon & Owens, 2017).

In the current study, only 20.9% of cows not diagnosed with endometritis were pregnant at 210 dpp, however, 12.4% of cows positive for endometritis were pregnant at 210 dpp. This corroborates the findings in Korea (Chan Lee *et al.*, 2018), where there was a greater percentage of cows pregnant at 200 dpp that were diagnosed to not have endometritis (35.5%) compared to those that were endometritis positive (26.3%). This finding is indicative of the extent of the long lasting-effects of endometritis on the number of days cows remain not pregnant after calving before conception occurs.

6.5 Conclusion

The results provide evidence of a strong association between endometritis and suboptimal fertility performance in cows under zero-grazing management on smallholder farms of Rwanda. Compared to negative cows, the positive endometritis cases had longer days to first oestrus, days to first natural mating or AI service, and days-not pregnant, lesser pregnancy rates at first service and all services and cows pregnant within 210 dpp, more natural-mating or AI per pregnancy and more occurrences of anoestrus postpartum. This warrants prioritisation by the field veterinary practitioners in their herd health service delivery to smallholder dairy farmers for effective management of the disease for improved reproductive performance of the dairy cows.

CHAPTER SEVEN

INFLUENCE OF ENDOMETRITIS ON MILK YIELD OF ZERO-GRAZED DAIRY COWS ON SMALLHOLDER FARMS IN RWANDA

Abstract

Endometritis being a post-partum uterine infection in dairy cows is likely with substantial production loss through a reduction in milk yield, discarded milk during treatment and withdrawal period, and increased cost of veterinary treatment. This study quantified the influence of endometritis on milk yield of zero-grazed dairy cows managed on smallholder farms in Rwanda. The study enrolled a total of 461 cows within their 21 to 60 days postpartum to examine for clinical endometritis (CLE) and subclinical endometritis (SCLE). A cow was considered having endometritis if it was positive for at least one test (CLE or SCLE), otherwise was negative. The milk yield data were collected prospectively from endometritis positive and negative cows for 30-day post-endometritis diagnosis. Compared to cows negative for endometritis, the positive endometritis cows were 2.4 times more (29.7% vs. 70.3%). On average, the daily milk yield of endometritis positive cows was 15.3% lower ($p<0.05$) compared to endometritis negative cows (7.5 ± 0.2 vs. 8.9 ± 0.3 litres), representing a reduction of 1.4±0.2 litres of milk/cow/day. Of the CLE positive cows, 33.4% (104/311) were treated using different veterinary drugs, which resulted in 23.5% more discarded milk compared ($p<0.05$) to untreated CLE positive cows. Discarded milk was higher ($p<0.05$) among cows treated with oxytetracycline (65.9±4.4 litres) compared to cows treated with procaine penicillin G and dihydrostreptomycin (35.5±2.7 litres). The percentage of total milk loss was much higher (45.6%) among CLE positive cows that received treatment compared to the untreated cows (16.3%). These results demonstrate a strong association between milk yield loss and endometritis. Therefore, timely diagnosis and treatment of the disease are recommended using conventional veterinary drugs that have zero withholding time for milk to reduce the milk yield loss and associated economic loss, estimated at US$ 154 in a lactation period.

7.1 Introduction

Endometritis is a uterine disease of dairy cows occurring between 21st and 90th days postpartum (dpp) (Pascottini *et al*., 2017). The disease may be clinical endometritis (CLE) often characterised by vaginal purulent or mucopurulent contents (Eslami *et al*., 2015) and/or subclinical endometritis (SCLE) characterised by the presence of ≥5% of polymorphonuclear inflammatory (PMN) cells in endometrial cytology sample (Pothmann *et al*., 2015). Endometritis is commonly associated with decreased milk yield and discarded milk, impaired reproductive performance, increased culling rates, additional costs for drugs, and veterinary services (McDougall *et al*., 2011; Sharma *et al*., 2019). These are production losses representing the loss of milk supply, a stream of incomes, and other livelihood benefits of dairy to producers (Juan Piñeiro, 2016; Satish & Purohit, 2019).

The prevalence of endometritis in dairy cows can vary widely, from as low as 3.6% observed in Uganda (Tayebwa *et al*., 2015) to as high as 89.0% observed in Canadian dairy herds (Denis-Robichaud & Dubuc, 2015). The large variation in prevalence suggests that some farms experience substantial production loss from the disease, depending on the management of the disease (Lima, 2018). Yet estimates of the prevalence and associated losses are scarce in smallholder dairy farms that derive a livelihood from dairying. This knowledge gap impedes informed decision-making on effective management practices for endometritis in the dairy herds. Moreover, loss in milk arising from a decrease in the yields and discarded milk during treatment and withholding times are a direct component of production loss (Sharma *et al*., 2019), yet accurate estimates of the loss are rare, particularly for smallholder dairy herds. Discarded milk is milk produced but not used for human consumption during treatment and withdrawal periods for milk because of the risk of veterinary drugs (VD) residuals and pathogen contamination (Gibson, 2012; Mahnani *et al*., 2015). Several statistical methods have been used to evaluate the decrease in milk yield from diseases (Fourichon *et al*., 1999; Hadrich *et al*., 2018; Lyons *et al*., 2015). The common method compares milk yield from diseased cows with healthy cows (Angara & Elfadil, 2014; Can *et al*., 2016; Fourichon *et al*., 1999). Some few studies of the effect of endometritis on milk yield decrease in commercial dairy herds, for instance in India (Sharma *et al*., 2019), New Zealand (McDougall *et al*., 2011), and Colorado in the United States of America (Juan Piñeiro, 2016) have estimated milk yield decrease of between 0.5 and 2.4 litres/cow/day in cows positive for endometritis compared to cows negative for endometritis. However, the extent of production loss in smallholder dairy herds due to endometritis disease remains lacking.

Endometritis positive cows require treatment of the infection to return to a normal uterine cyclicity (Satish & Purohit, 2019). Previous research evaluating the effect of treatment of uterine postpartum diseases has found that treated cows had an increased milk production over their entire lactation period compared to untreated cows (Farney *et al*., 2013; Shock *et al*., 2018). Similarly, in their study, Carpenter *et al*. (2014) reported that cows treated with non-steroidal anti-inflammatory drugs increased whole-lactation milk and protein yields by 6.0 to 9.0%. This demonstrates that treatment of endometritis positive cows has a greater importance on herd productivity and thus increases farmer's income. Conventional therapy and phytotherapy are used for the treatment of endometritis (Mandhwani *et al*., 2017; Satish & Purohit, 2019). The former is commonly and extensively used with different VD. These VD include antimicrobials (Ceftiofur hydrochloride, oxytetracycline, procaine penicillin G and dihydrostreptomycin, gentamicin, tetracycline hydrochloride and cephapirin benzathine) (Bartolome *et al*., 2014; Kasimanickam *et al*., 2005; Manimaran *et al*., 2019; Okawa *et al*., 2017; Pierre, 2010; Satish & Purohit, 2019), non-steroidal anti-inflammatory drug (Phenylbutazone) (Tek *et al*., 2010); and hormones (Prostaglandins) (Ahmadi *et al*., 2018; Makki *et al*., 2017;). These VD are used systemically or locally into the uterus alone or in combination of two or three (Satish & Purohit, 2019; Sood *et al*., 2002). In the latter, different phytotherapeutic agents used include *Tinospora cordifolia, Withania somnifera, Curcuma longa, Ocimum sanctum, Allium sativum,* and *Azadirachta indica* (Bakare *et al*., 2020; Mandhwani *et al*., 2017). According to Bakare *et al*. (2020) and Sharma *et al*. (2018b), the use of herbal plants has many advantages such as no microbial resistance, simple modes of preparation and easy administration to animals, low treatment cost, no withdrawal periods for milk, and no residual effect in treated cows.

In contrast, in conventional therapy due to VD residues in the milk, which may present health hazards to consumers (Beyene, 2016), the milk must not be traded and not consumed during the treatment and withdrawal periods for milk (Geary *et al*., 2012; Gibson, 2012). However, despite the advances in endometritis treatment, there is no study in the literature documenting losses in discarded milk due to the treatment of endometritis in dairy cows. In addition, it is rare to find the evaluation of the effect of endometritis on total milk yield loss (combined milk yield decreased and discarded). Thus, the economic importance of endometritis in dairy herds has received little attention from research, more so in smallholder dairy systems where indiscriminate use of conventional therapy is more likely (Galvão, 2011; LeBlanc, 2008). The general principle of therapy of endometritis is to halt and reverse inflammatory changes that impair fertility. Practically, treatments aim to reduce the load of pathogenic bacteria and enhance the processes of uterine defence and repair (LeBlanc *et al*., 2002).

Rwanda is not any different regarding studies of the effect of endometritis on milk yield of dairy cows. The country has about 92.0% of the smallholder dairy herds confined in zero-grazing housing units, and milk production of dairy cows is typically suboptimal (Hirwa *et al*., 2017; Manzi *et al*., 2020; Rukundo *et al*., 2018). The suboptimal milk production is estimated at 3.6 litres/day for indigenous cattle, 5.5 litres/day for crossbred, and 8.6 litres/day for exotic breeds against an average of 5, 14 and 21 litres, respectively. This low productivity of the national herd resulted in the low per capita milk consumption in urban and rural areas estimated at 63.0 litres/person/year, which is below the 220 litres of FAO recommendation (FAO, 2013). The suboptimal production has been explained as resulting from poor management of dairy herds but without identifying the underlying specific cause (s) involved. With the high prevalence of endometritis (70.2%) observed in dairy herds (Chapter three), it is likely that endometritis could be an underlying cause of the prevalent suboptimal production performance of dairy cows. However, in Rwanda to date, empirical evidence remains not presented for the influence of endometritis on milk yield. Such evidence would be valuable to inform targeted management interventions for endometritis prevention and control on dairy herds. This may ameliorate the milk production so that milk consumption per capita requirements can be met. This study quantified the effects of endometritis on milk yield of zero-grazed dairy cows managed under existing smallholder farming conditions in Rwanda.

7.2 Materials and methods

7.2.1 Study area

Data were collected from zero-grazed cows on smallholder dairy farms. All the farms were located in the Gasabo district of Rwanda, described in Section 3.2.

7.2.2 Study design

Data collection was prospective in the observational study for eight months running from September 2018 to April 2019. A total of 366 smallholder zero-grazing dairy farms were selected through exponential non-discriminative snowball sampling as given in Section 3.2. In addition to the inclusion criteria described in Section 3.2, the farmers should have the will to record the milk yield within 30 days post-endometritis diagnosis and authorising that the veterinarian treating the herd be contacted to verify VD being used for the treatment of cows positive for CLE. For each recruited farmer, an explanation of the objectives of the study was presented, and informed written consent was sought prior to starting data collection. Four hundred sixty-one (461) cows within their 21 to 60 dpp at sampling were enrolled, and they comprised 66.4% dairy crossbreds, 16.9% dairy pure breeds, and16.7% indigenous cattle.

7.2.3 Data collection

Endometritis diagnosis

This was as given in Chapter three. In brief, on each farm visited, cows were examined for CLE using vaginal mucus with the aid of a MED (McDougall *et al*., 2007) and for SCLE using endometrial cytological sample collected with CYT (Pascottini *et al*., 2015). Cows with vaginal mucus ≥1 were recorded as positive for CLE (Williams *et al*., 2005), whereas cows that had ≥5% PMN in the endometrial sample were recorded positive for SCLE. In this study, a cow was considered endometritis positive if positive for at least one test (CLE or SCLE); otherwise was endometritis negative.

Further grouping was by relative to uterine health status within 21 - 60 dpp with cows grouped as suffered from CLE only (vaginal mucus ≥1 and <5% PMN), SCLE only (vaginal mucus-0 and ≥5% PMN), both CLE and SCLE (vaginal mucus ≥1 and ≥5% PMN) or cows without CLE and SCLE (vaginal mucus-0 and <5% PMN) (Gobikrushanth *et al*., 2016). The data on daily milk yield, VD used in the treatment of CLE positive cows and socio-economic characteristics of the farmers were obtained from direct observations and interviews with the farmers and/or veterinarians treating the herd guided by a pre-tested structured questionnaire.

Milk yield recording

Under existing farm management conditions, milk yield data were collected from endometritis positive cows as well as negative cows followed-up for 30 days post-endometritis diagnosis. In herds visited, pre-milking palpation to stimulate milk let down by allowing calves to suckle prior to milking was a common practice. Milking was practiced manually and routinely twice daily [morning (06:00 a.m.) and evening (5:00 p.m.)]. Daily milk yield was collected in a plastic bucket and measured with a jug calibrated in 0.25 litres intervals, corresponding to farmer practice when selling milk litres/day. Morning and evening yields were added together to obtain the daily milk yield /cow. Milk yield was, therefore, recorded on a daily basis by the farmer on a record form provided by the researchers, and subsequently verified and confirmed by researchers at 10-day intervals. In the present study, the milk yield recorded was the milk offtake, because calves were allowed to suckle their dams. Lactation milk yield (L/year) was computed from projection of daily milk yield and lactation length (Hirwa *et al*., 2017).

Veterinary drugs used in the treatment

At farmers' request for veterinary service, local field veterinarians treated 33.4% (104/311, of which 11.9%: 37/311 were CLE only and 21.5%: 67/311 were cows that had both CLE and SCLE only) of CLE positive cows without knowledge of the result of the endometrial cytology examination, using VD available and commonly used in veterinary practice to treat bacterial infections. Therefore, the CLE positive cows were categorised into two groups: (1)

treated cows, and (2) untreated cows. The information on VD used (Table 30) was collected from treated CLE positive cows. The VD administration frequency expressed in days and types of VD used were recorded from farmers' records and crosschecked with field veterinarians' records. The withdrawal period for milk expressed in days was recorded from labelling materials such as outer wrapper carton/bottle or leaflets insert accompanying the VD (Figure 25). Milk recorded for the duration of /CLE treatment and withdrawal periods for milk was assumed discarded. This is to avoid the risk of VD residuals in the milk supplied to consumers and processors, and pathogen contamination (Geary *et al*., 2012; Mahnani *et al*., 2015).

Table 30. Frequency of administration and withdrawal periods for milk of veterinary drugs used in the treatment of CLE positive cows (n=104)

Veterinary drugs classification	**Veterinary drugs as an active substance**	**Drug administration frequency (days)**	**Milk withdrawal periods (days)**
Antimicrobials	Oxytetracycline	3	5
	Procaine penicillin G and dihydrostreptomycin	3	3
	Tetracycline hydrochloride	2	4
Non-steroidal anti-inflammatory	Phenylbutazone	3	4
Anti-pyretic	Novalgin	2	7

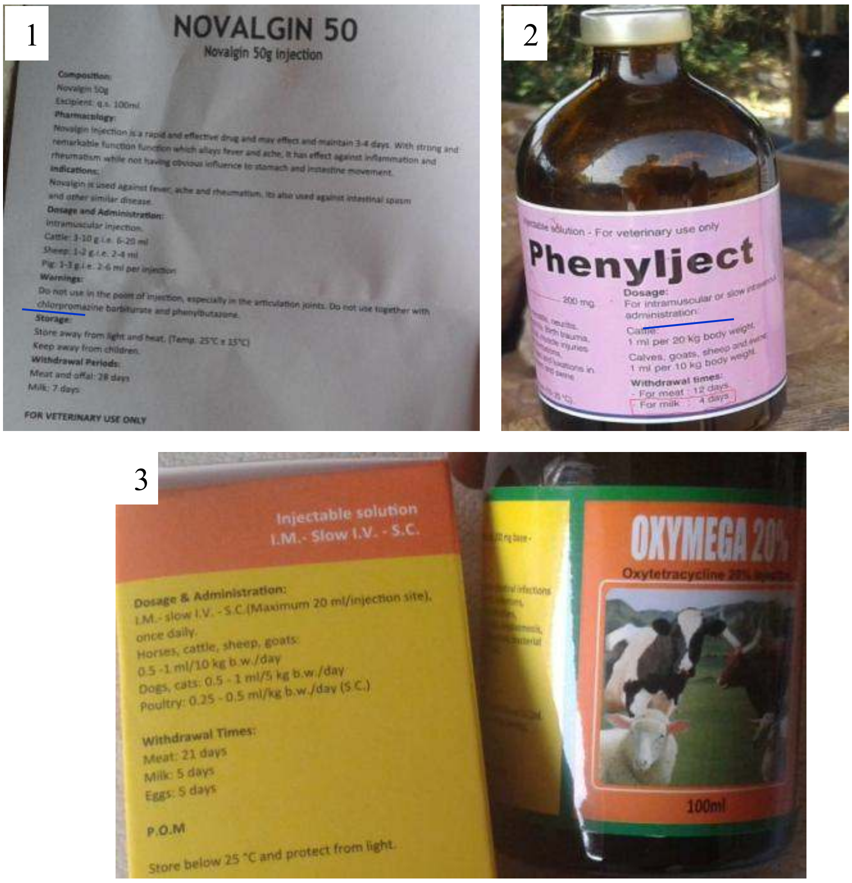


Figure 25. Some of the sources of information about the withdrawal periods for milk. (1) On leaflet insert accompanying the veterinary drug and on an outer wrapper bottle (2) or carton (3).

Clinical endometritis cases were treated using single-drug therapy or combined drug therapy (Table 31). A combination oxytetracycline, procaine penicillin G and dihydrostreptomycin and novalgin, a combination of oxytetracycline, procaine penicillin G and dihydrostreptomycin, and phenylbutazone, a combination of procaine penicillin G and dihydrostreptomycin, and phenylbutazone and a combination of procaine penicillin G and dihydrostreptomycin, and tetracycline hydrochloride were used to treat CLE positive cases. In this study, in case of two or more VD were used in combination for treating CLE positive cows, the one which had the longest withdrawal period for milk represented the group of VD used during that treatment (Edmondson, 2014). In contrast, in some cases, procaine penicillin G and dihydrostreptomycin was used alone in treating CLE positive cases. Therefore, VDs were categorised into five groups: (i) novalgin, (ii) oxytetracycline, (iii) phenylbutazone, (iv) tetracycline hydrochloride, and (v) procaine penicillin G and dihydrostreptomycin.

Table 31. Combination of veterinary drugs used in the treatment of dairy cows positive for clinical endometritis (n = 104)

Combination of VD	Veterinary drugs (VD)	VD representing the combination
Combination of three veterinary drugs	Oxytetracycline, procaine penicillin G and dihydrostreptomycin, and novalgin	Novalgin
	Oxytetracycline, procaine penicillin G and dihydrostreptomycin, and phenylbutazone	Oxytetracycline
Combination of two veterinary drugs	Procaine penicillin G and dihydrostreptomycin, and phenylbutazone	Phenylbutazone
	Procaine penicillin G and dihydrostreptomycin, and tetracycline hydrochloride	Tetracycline hydrochloride
Veterinary drug used alone	Procaine penicillin G and dihydrostreptomycin	Procaine penicillin G and dihydrostreptomycin

7.2.4 Statistical analysis

Five cows from four farms were excluded from statistical analysis because they left the study for different reasons including culling (n=1), diseases (n=2), and injury (n=2). Thus, complete data for milk yield analysis was available from 461 cows from 366 farms.

Descriptive statistics were used to compute the prevalence of endometritis. In this study, total milk yield loss for the recorded 30-day milk yield was computed from the drop in milk yield and discarded milk due to treatment of CLE positive cows. Milk reduction before treatment of endometritis was calculated as the difference between the average milk yield from endometritis negative and positive cows, whereas the discarded milk was the daily milk yield discarded during treatment period and withholding times for milk. The parameters considered in the computation of milk yield loss and their definitions are shown in Table 33. The definitions of the parameters were obtained from literature (Angara & Elfadil, 2014; Can *et al*., 2016; Fourrichon *et al*., 1999; Mahnani *et al*., 2015; Sharma *et al*., 2019).

Table 32. Computation of milk yield loss in the study

Group	Parameters	Unit	Definition
All cows	Daily milk yield (DMY)	Litres/cow/day	$\frac{\text{Total 30 day MY}}{30}$
	Decrease in DMY	Litres/cow/day	Average DMY for health cow – Average DMY for positiv
	Total decrease in 30-day MY	Litres/cow/30days	Decrease in DMY $\times$ 30
	MY decrease	%	$\frac{\text{Total decrease in 30 day MY}}{\text{Total 30 day MY of healthy cow}} \times 100$
All treated cows	Period of discarding milk	Days	VD administration frequency + milk withdrawal period per VD used
	Discarded MY	Litres/cow/day	Average DMY from treated cow
	Total discarded MY	Litres/cow	Average DMY from treated cow $\times$ period of discarding milk
	Discarded MY	%	$\frac{\text{Total discarded milk}}{\text{Total 30 day MY from treated cow}} \times 100$
Total MY loss		Litres/cow/30days	Total decrease in 30 day MY + Total discarded MY
		%	$\frac{\text{Total MY loss in 30 day MY}}{\text{Total 30 day MY}} \times 100$
Cost of MY loss per cow per lactation length		US$	Total loss milk litres/cow/day × lactation length (255 days) × farm gate milk price (US$ 0.2 /litre of milk)

* MY = milk yield, DMY = daily milk yield, % = percent, US$ = United States dollar, *Lactation milk yield was predicted from milk yield/cow/day and lactation length.

Independent samples T-Test was used to compare daily milk yield between endometritis negative and positive cows, and between treated and untreated cows. The milk yield data were subjected to analysis of variance with general linear model procedure to determine the mean differences in milk yield among different categories of endometritis, VD used, and healthy, treated and untreated cows. Means separation was with Tukey's honestly significant difference test in a model specified as:

$$Y_{ijkl} = \mu + E_i + V_j + T_k + \varepsilon_{ijkl}$$

Where Y_{ijk} = the recorded 30-day milk yield, discarded milk yield or total milk yield losses; μ = the overall mean; E_i = the effect of i^{th}endometritis status ($i = 4$, cows with neither CLE nor SCLE, CLE only, SCLE only, and both CLE and SCLE), V_j = the effect of j^{th}veterinary drugs used ($k = 5$, oxytetracycline, procaine penicillin G and dihydrostreptomycin, tetracycline hydrochloride, phenylbutazone, novalgin) ;T_k= the effect of k^{th} treatment ($k = 3$, healthy, treated and untreated), ε_{ijkl} = random error term. The effect of days postpartum on milk yield was specified in the model as a covariate, but the effect was not significant ($p>0.05$), probably because the sample cows were in the early lactation stage (21 to 60 dpp) and the follow-up period was within the early lactation phase. All analyses were performed using SPSS software for windows (version 22.0), and the level of significance was set at alpha <0.05.

3.1 Results

3.1.1 Farm characteristics

This was as given in Section 6.3. Briefly, in the sample farms, farmers were feeding their cattle locally available feed resources, with Napier grass (*Pennisetum purpureum*) fodder as the basal feed (98.0%) together with banana fodder (76.0%) and *Brachiaria* grass (67.0%). These basal feeds were supplemented with forage maize (35.4%), crop residues (32.4%), and occasionally fed with commercial dairy meals (22.0%) and mineral blocks (31.0%). Feeding was inadequate in energy and protein as 86.6% (399/461) of the cows were scored poor body condition score (BCS<3) and a few (13.4%; 62/461) were scored good body condition (BCS≥3). The daily milk yield per cow was estimated at 7.9±0.2 litres (minimum-maximum: 1.5 – 18.0, Median: 7.5), and lactation milk yield (litres/lactation length of 255 days) was 2014.5 litres per cow.

3.1.2 Effect of endometritis on milk yield

The cows positive for endometritis were 70.3% (324/461, 95% CI = 65.9 -74.3) of which CLE was more, 67.5% (311/461, 95% CI = 63.1 –71.6) than SCLE, 32.5% (150/461, 95% CI = 28.4 – 36.9). Those negative for endometritis were 29.7% (137/461, 95% CI = 25.7

– 34.0). Of the cows examined based on their uterine health statuses, 38.0 % (175/461, 95% CI = 33.7 – 42.3) had CLE only, 3.0% (14/461, 95% CI = 1.8 – 5.0) SCLE only and 29.3% (135/461, 95% CI = 25.3 – 33.6) both CLE and SCLE while 29.7% (137/461, 95% CI = 25.7 – 34.0) were not diagnosed with CLE or SCLE.

Results in Table 33 show that the mean milk yield was lower ($p<0.05$) among endometritis positive cows (7.5±0.2 litres/cow/day) than endometritis negative cows (8.9±0.3 litres/cow/day). Therefore, a case of endometritis had milk yield reduced in 30-day by 40.7±5.0 litres/cow representing a reduction of 1.4±0.2 litres/cow/day (15.3%) when compared to milk yield of endometritis negative cow. The decrease in milk yield was higher in treated cows (18.1%) compared to untreated cows (14.0%). The total milk yield loss was much higher (45.6%) among CLE positive cows that were treated than among cows that were not treated (16.3%). The large component of milk yield loss among treated cows was discarded milk (23.5%) and reduction in milk yield (18.1%). The mean total milk yield loss was higher among treated cows (3.3±0.2 litres/cow/day) than among untreated cows (1.6±0.2 litres/cow/day). In the current study, the overall daily milk yield loss due to endometritis was 2.5±0.2 litres/cow during 30 days after the diagnosis of endometritis. At the farm gate price of US$ 0.2 per litre of milk for a daily loss of 2.5 litres of milk in 255 days of lactation, farmers incur US$ 154 loss worth of revenue.

Table 33. Comparison of milk yield between endometritis positive and negative cows

Milk yield parameters	Endometritis negative cows (n = 137)	Endometritis positive cows		
		Untreated (n = 220)	Treated (n = 104)	Overall (n = 324)
Average MY, litres/cow/day, mean±SEM	8.9±0.3[b]	7.7±0.2[a]	7.3±0.3[a]	7.5±0.2
Recorded 30-day MY, litres/cow, mean±SEM	267.3±9.6[b]	229.8±6.3[a]	218.9±8.0[a]	226.3±5.0
Decreased MY in recorded 30-day MY, litres/cow, mean±SEM (% decrease in MY)		37.5±6.3 (14.0)	48.4±8.0 (18.1)	40.9±5.0 (15.3)
Discarded MY in recorded 30-day MY, litres/cow, mean± SEM (% discarded MY)			51.4±2.2 (23.5)	51.4±2.2 (23.5)
Total MY loss, litres/cow (% milk yield loss)		37.5±6.3 (16.3)	99.8±6.1 (45.6)	57.5±4.9 (25.4)

Means with different letters in superscript within a row differ ($p<0.05$), MY = milk yield.

Table 34 presents the differences in milk yield observed in healthy cows, cows with CLE only, SCLE only, and cows positive for both CLE and SCLE. The mean daily milk yield differs ($p<0.05$) among the different categories of endometritis. It was lower among cows positive for CLE only (7.6±0.2 litres/cow), SCLE only (7.6±0.7 litres/cow) and both CLE and SCLE (7.5±0.3 litres/cow) than among healthy cows (8.9±0.3 litres/cow). The decrease in MY was 1.4±0.3 litres/cow/day for cows positive for both CLE and SCLE, 1.3±0.2 litres/cow/day for cows with CLE only, and 1.3±0.7 litres/cow/day for cows having SCLE only. Mean milk yield loss (litres/cow/day) was3.1±0.3 for cows having both CLE and SCLE, 3.0±0.2 for CLE only, and 1.3±0.7 for SCLE only.

Table 34. Effect of different categories of endometritis diagnosed at 21-60 dpp on milk yield

Milk yield parameters	Different categories of endometritis (no. of cows)			
	Healthy (n=137)	CLE only(n=175)	SCLE only (n=14)	Both CLE and SCLE (n=135)
Average MY, litres/cow/day, mean±SEM	8.9±0.3[b]	7.6±0.2[a]	7.6±0.2[a]	7.5±0.3[a]
Recorded 30-day MY litres/cow, mean ± SEM	267.3±9.6	227.1±6.8	229.3±20.4	224.9±7.9

Decreased MY in recorded 30-day MY, litres/cow, mean±SEM (% decrease in MY)	39.9±6.8 (15.0)	37.7±20.4 (14.1)	42.4±7.9 (15.9)
Discarded MY in recorded 30-day MY, litres/cow, mean±SEM (% discarded MY)	51.0±3.6 (22.5)		51.7±2.7 (22.9)
Total MY loss, litres/cow (percent milk yield loss)	90.9±6.8^{a} (40.0)	37.7±20.4^{b} (16.4)	94.1±7.7^{a} (41.8)

Means with different letters in superscript within a row differ (p<0.05).

3.1.3 Veterinary drugs used in the treatment and estimated milk yield loss

Mean milk yield litres/cow/day recorded in 30-day was 8.9±0.4 for pure dairy breeds, 8.3±0.2 for crossbreeds and 5.4±0.2 for indigenous cattle breeds (p<0.05). The mean decrease in milk yield was higher in pure dairy breeds (2.5 litres/cow/day) compared to dairy crossbreds (1.1 litres/cow/day) and indigenous cattle breeds (0.6 litres/cow/day) (p<0.05).

Milk yield discarded due to the treatment of CLE positive cows is presented in Table 35. The veterinarians had treated 33.4% (104/311) of CLE positive cows on 84 farms with tetracycline hydrochloride (29.8%), oxytetracycline (24.0%), procaine penicillin G and dihydrostreptomycin (17.3%), phenylbutazone (16.3%) and novalgin (12.5%). The treatment was thus more frequently based on antimicrobials (71.2%) relative to anti-inflammatory drugs (16.3%) or antipyretic drugs (12.5%). The mean withdrawal period for milk was 4.6±0.7 days, with a range of 3.0 to 7.0 days and a median of 4.0 days. The mean days of VD administration frequency was 2.6±0.5 (median = 3) days, with a range of 2.0 to 3.0 days. Therefore, the mean period of discarding milk (days of treatment plus milk withdrawal days) was 7.2±0.1 days with a range of 6.0 to 9.0 days and a median of 7.0 days. During the period of discarding milk, the estimated mean milk yield discarded was 51.4±2.2 litres/ cow with a median of 51.5 litres/cow. This represents a discarded milk of 7.3±0.3 litres/cow/day. Total discarded litres MY/cow/day differed significantly (p<0.001) among different VD used in the treatment of CLE positive cows. The highest percent of discarded milk was found among cows treated with novalgin (29.9%) and the lowest was found among cows treated with tetracycline hydrochloride (19.8%).

Table 35. Mean with standard errors of milk yield discarded due to treatment of clinical endometritis positive cows (n=104)

Veterinary drugs used in the treatment	Cows (n)	Total period of discarding milk	Milk yield parameters		
			Average MY*, (litres/cow/day)	Total 30-day MY, (litres/cow)	Total discarded MY, litres/cow, (%)
Oxytetracycline	25	8	8.2 ± 0.5^{b}	247.5 ± 16.3^{b}	65.9 ± 4.4^{c} (26.6)
Procaine penicillin G and dihydrostreptomycin	18	6	5.9 ± 0.5^{a}	177.5 ± 13.6^{a}	35.5 ± 2.7^{a} (20.0)
Tetracycline hydrochloride	31	6	7.3 ± 0.5^{ab}	219.2 ± 14.2^{ab}	43.8 ± 2.8^{ab} (19.8)
Phenylbutazone	17	7	8.2 ± 0.5^{b}	245.3 ± 16.1^{b}	57.2 ± 3.8^{bc} (23.3)
Novalgin	13	9	6.2 ± 0.9^{a}	186.6 ± 29.7^{a}	55.9 ± 8.9^{bc} (29.9)

Means with different letters in superscript within a column differ ($p<0.05$), % = percent discarded milk, *average milk yield represents discarded milk yield

7.4 Discussion

This study pioneered an estimation of the effect of endometritis disease on milk yield and milk discarded during treatment and milk withdrawal periods among zero-grazed dairy cows under existing smallholder farming conditions in Rwanda. The main novelty of this study is that is conducted in smallholder farms in Africa, thus highlighting a different scenario from that seen in modern high-yielding dairy cows. The disease effects on milk yield were measured for 30-day post-endometritis diagnosis among cows that were within their 21-60 dpp at diagnosis.

The study estimated milk yield as a decrease and volume and percent of the total (decrease and discarded). The decrease was significant by up to 1.4±0.2 litres/cow/day, representing 15.3% relative to healthy cows. The decrease is substantial to warrant impetus to prevent and control endometritis in smallholder herds. The present estimate (1.4±0.2 litres/cow/day) is higher than the previously reported milk yield decrease in New Zealand (1.0 litre/cow/day) (McDougall *et al*., 2011) and 0.5 litres/cow/day reported by Burke *et al*. (2010).

However, the estimates in daily decrease in milk yield is lower than 2.4 litres/cow/day obtained in Colorado commercial farms (Juan Piñeiro, 2016) and 1.9 litres/cow/day in India (Sharma *et al*., 2019). The percent reduction in MY found in this study (15.3%) is much higher than 5.5% estimated in New Zealand dairy cows (McDougall *et al*., 2011) and lower than (25.9%) reported in India (Sharma *et al*., 2019).

The decrease in milk yield during endometritis infection is likely through reduced energy and protein intake due to the decline in feed intake (Bell & Roberts, 2007; Wittrock *et al*., 2011), compromised welfare (Gilbert, 2016), and a prolonged period of endometrium inflammation (Sharma *et al*., 2019). An association between reduction in dry matter intake and uterine disorders has been observed in previous studies. For instance, Bell & Roberts (2007) in the United Kingdom reported that cows with uterine infections had a reduced daily feed intake and in turn decreased the energy available for galactopoiesis leading to reduced milk yield compared to the healthy cows. Other studies have also shown that daily milk yield and feed intake are reduced in cows positive for uterine disease as compared to negative ones (Bareille *et al*., 2003; Deluyker *et al*., 1991; Hammon *et al*., 2006; Huzzey *et al*., 2007; Sharma *et al*., 2016; Wittrock *et al*., 2011). These observations imply that prevention and control measures for endometritis in the transition period and timely diagnosis of the disease at the early stage are essential MIs to mitigate the disease effects on the milk yield.

In contrast, some other studies have reported no reduction in milk yield in endometritis positive cows (Dubuc *et al*., 2011; Gobikrushanth *et al*., 2016). The discrepancy with the present study could be explained by differences in endometritis definition criteria among studies, method and different time frames of comparing milk yield between diseased and healthy cows, and prevalence of endometritis. For instance, Gobikrushanth *et al*. (2016) in Canada, examined all cows in a commercial dairy farm on 25.0±1.0 dpp, for the presence of vaginal mucus using the vaginoscopy and %PMN in an endometrial cytological sample collected using the cytobrush. The authors considered the cows with vaginal mucus ≥2 as cases of CLE and ≥8% PMN as cases of SCLE. The follow-up period was ten days, and CLE positive cows were not treated unlike in the present study. The prevalence was 35.7% for CLE only, 11.9% for SCLE only, and 23.8% for both CLE and SCLE (Gobikrushanth *et al*., 2016). However, in the current study, all cows were examined on 38.5±0.7 dpp for CLE using MED with higher sensitivity diagnose of CLE than the vaginoscopy used by Gobikrushanth *et al*. (2016).

The findings of the current study have shown that total milk yield loss in recorded 30-day post-endometritis diagnosis was different among categories of endometritis. It was lower among cows positive for SCLE only compared to cows positive for CLE only and cows that had both CLE and SCLE. This could be related to the study condition: cows with SCLE only were not treated because local field veterinarians treated CLE endometritis positive cows without knowledge of endometrial cytology results.

This study was a pioneer estimation of milk yield discarded and total milk yield loss due to the treatment of endometritis because the literature search did not yield similar studies. In the present study, veterinarians treated endometritis using a large variety of combinations of veterinary drugs (VD) available and commonly used in veterinary practice for the treatment of bacterial infections. The most used VD was antimicrobials (71.2%) more than any other drugs, anti-inflammatory drugs (16.3%) or antipyretic drugs (12.5%). The use of these VD is associated with discarding of milk during treatment and milk withdrawal periods. The work of Kumar *et al*. (2019) corroborates the present findings that antimicrobial agents and anti-inflammatory are a good choice for the treatment of endometritis despite prescribed withdrawal times for milk and meat associated with their use. In addition, conventional veterinary medicine for treating endometritis in dairy cows represents an important source of environmental pollution (contamination of water and soil) due to intensive agri- and aquaculture production (Arnold *et al*., 2013; Bártíková *et al*., 2016). This may result in phytotoxicity because veterinary drugs accumulate easily and persist in the deeper soils and thus, they can be taken up by crop plants from manure-amended soils (Wei *et al*., 2016). This indicates the need to apply alternative and phytoremediation strategies to prevent veterinary drugs from entering into terrestrial and aquatic environments, and mitigate the contaminated land (Hasan *et al*., 2019; Wei *et al*., 2016; Yan *et al*., 2020). Therefore, the adoption of phytotherapy in the management and treatment of endometritis in dairy cows may represent one of the potential alternative innovations to avoid plants' toxicity (Sharma *et al*., 2018). Thus, timely diagnosis of endometritis and appropriate therapeutic measures are necessary to ameliorate the reduction in milk production and associated production and economic loss.

In the current study, treatment of the disease with oxytetracycline was associated with more milk discarded (65.9±4.4 litres/cow) compared to treatment with procaine penicillin G and dihydrostreptomycin (35.5±2.7 litres/cow). This demonstrates that treatment with VD requiring long withdrawal period for milk and drug administration frequency resulted in a higher quantity of milk discarded. This has an overall bearing on the magnitude of total milk yield loss that was observed in the current study. The treatment influenced the milk yield loss positively. For instance, the highest total milk yield loss occurred when positive cows were treated (3.3±0.2 litres/cow/day) compared to the untreated cows (1.6±0.2 litres/cow/day). A possible explanation for this could be that the most used VD in the treatment had a long withdrawal period for milk (between three to seven days). For farmers, use of VD with zero withdrawal time or use of non-antibiotic based treatments for the disease (Makki *et al*., 2017; Sharma *et al*., 2018b; Tison *et al*., 2016) would be attractive for food and nutrition security, sustaining stream of incomes and stable livelihood base derived from milk (Migose *et al*., 2018). Thus, efficacy should be the first concern, and so the selection of the VD should always be a medical option.

In their study, Makki *et al*. (2017) in Iran and Tison *et al*. (2016) in Canada used cephapirin benzathine and prostaglandin F2 alpha for the treatment of CLE. Similarly, herbal plants, for instance, *Tinospora cordifolia* (Kumar *et al*., 2004; Saha & Ghosh, 2012), *Withania somnifera* (Rahi *et al*., 2013); *Curcuma longa* (Kumar, 2016), *Ocimum sanctum* (Mandhwani *et al*., 2017), *Allium sativum* (Alagar *et al*., 2018), and *Azadirachta indica* (Bakare *et al*., 2020; Mandhwani *et al*., 2017) have been used for the treatment of endometritis in dairy cows. These conventional VD and herbal medicinal plants having zero milk withdrawal time and better clinical cure, with cheaper treatment costs, and not associated with the emergence of resistant bacteria are readily available worldwide (Kasimanickam *et al*., 2005; Sharma *et al*., 2018) and have been used in commercial dairy farms of developed countries with no applications in sub-Sahara African countries, specifically in Rwanda. This could be explained by a lack of awareness by the extension service and animal health service providers on the use and importance of those VD and herbal medicine in the treatment of endometritis in dairy cows. This indicates that with diligent monitoring of fresh cows, combine with early diagnosis, timely and effective management intervention, the health, productivity, and economic impacts of endometritis can be reduced.

In this study, the estimated average decrease in milk yield was higher in pure dairy breeds (2.5 litres/cow/day) than in dairy crossbreds (1.1 litres/cow/day) and indigenous cattle breeds (0.6 litres/cow/day). This suggests that pure dairy cattle breeds are more sensitive to the effects of the disease, likely through reduced energy intake when dry matter intake is suppressed during the period of disease infections (Huzzey *et al*., 2007; Wittrock *et al*., 2011). This high susceptibility of pure dairy breeds could also be linked to the management and environmental conditions to which they were exposed in the study area. With pure dairy breeds and other breeds, applying prevention and control measures for endometritis in the transition period and timely diagnosis of the disease or cow at high risk are good strategies to mitigate the effects of endometritis on milk yield and reduce the need for veterinary drugs.

7.5 Conclusion

The results of this study show significant milk yield loss from endometritis, which represents substantial production and economic loss estimated at US$ 154 in a lactation. Treatment with VD requiring a long withdrawal period results in large milk yield loss as discarded milk. The volume of milk losses during the period of discarding milk and the decrease in milk yield that result from endometritis was 7.3 ± 0.3 and 1.4 ± 0.2 litres/cow/day, respectively, in smallholder zero-grazed. Therefore, the authors recommend a timely diagnosis of endometritis and treatment using VD that have zero withholding times for milk to minimise milk yield loss and associated production and economic loss. The interventions need to involve the animal health service providers to promote the use of VD with zero milk withdrawal periods in the treatment of endometritis. Further research is highly recommended to identify plant species with the potential for ethnoveterinary medicine use in the treatment of endometritis. The herbal medicine while preventing and controlling endometritis would also reduce the use of antimicrobials due to increasing development of antimicrobial resistance, and assure safer milk and meat from dairy cows.

CHAPTER EIGHT

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General discussion

This section focuses on the research issues addressed, methodological approaches and the findings.

8.1.1 Research issues addressed

Extensive literature search on endometritis studies revealed that research attention is biased towards industrial commercial dairy herds with cows managed and fed in stalls. Literature on endometritis was limited from smallholder farms that manage cows in near similar housing and feeding conditions, described as smallholder zero-grazed dairy production. In Rwanda, smallholder zero-grazing dairy production is a prioritised development intervention for rural farming households, but reproductive and productive performance of the zero-grazed dairy cows is suboptimal (Rukundo *et al*., 2018). A large number of dairy cows are served several times but remain unproductive, which results in production and economic losses to the farmers as such cows have reduced potential to produce own heifer replacement needed to sustain the herd (Nishimwe *et al*., 2015). Yet this dairy farming system holds the majority (92.0%) of the cattle population and supplies the bulk of the milk for domestic market (IFAD, 2016). The smallholder dairy zero-grazing are characterised by prevalent muddy conditions, low hygienic standards, weak and inconsistent herd health management plan, and a high-risk exposure to bacterial disease (Ndahetuye *et al*., 2019). These conditions present risks for prevalent endometritis infections (Dutta *et al*., 2021; Sheldon & Owens, 2017).

Persistent suboptimal reproductive and productive performance of zero-grazed dairy cows has a strong association with underlying prevalent endometritis, a uterine disease that interrupts cow reproductive cycles as a clinical or subclinical condition between day 21 and 90 of the postpartum. This study, therefore, tested the hypothesis that suboptimal fertility and production performance of zero-grazed dairy cows on smallholder farms in Rwanda results from prevalent CLE and SCLE cases and multiple RF at the cow- and herd- levels are involved.

The specific objectives were to determine: farmer perceived and observed endometritis prevalence; farmer perceived effective MIs for endometritis prevention and control; risk factors for endometritis; and the influence of endometritis on milk yield and reproductive performance.

These objectives answered the research questions:

(i) What is the perceived and observed prevalence of endometritis among zero-grazed cows in Gasabo district?

(ii) What are the management interventions that farmers consider most effective for endometritis prevention and control on smallholder zero-grazed dairy farms in Gasabo district?

(iii) What are the risk factors associated with the prevalence of CLE and SCLE in smallholder zero-grazed cows in Gasabo district?

(iv) Are the number of services per conception, conception rate at first service, conception rate to all services, days- not pregnant, days to first oestrus, days to first natural mating or artificial insemination service, cows pregnant within 210 dpp, and anoestrus postpartum rate significantly different between endometritis positive and negative cows in Gasabo district?

(v) What is the volume of milk losses during the period of discarding milk and the decrease in milk yield that results from endometritis infection in smallholder zero-grazed cows in Gasabo district?

The study outputs provided empirical evidence relevant to informing MIs for endometritis prevention and control in order to improve herd fertility and productivity for food, nutrition and income security of smallholder farmers.

8.1.2 Methodological approaches

The study was carried out in Gasabo district of Rwanda because of relatively higher concentration of smallholder dairy zero-grazing farms here than in the other districts and the farm (MINAGRI, 2013). The sample farms were selected through exponential non-discriminative snowballing technique, therefore not random representatives. Exponential non-discriminative snowball sampling was applied because it was difficult to locate the farms with the set study criteria and the choice of new farm depends on the inclusion criteria (Babbie, 2013). The weakness of exponential non-discriminative snowballing sampling was addressed by making efforts to obtain a maximum number of cows in Gasabo district. The areas not covered were those considered as urban where livestock rearing is not allowed because the agricultural land are reserved for residential, commercial, social and economic infrastructures (RLMUA, 2017). The sampled farms conditions represent the available herds in the study area and the national herd (MINAGRI, 2013), and even smallholder zero-grazing dairy production in other East African Countries (Chawala *et al*., 2019; Duguma & Janssens, 2016; Klass, 2018; Odero-Waitituh, 2017). The results, therefore, have some relevance to smallholder dairy zero-grazing that is promoted for poverty reduction and for enhancing food, nutrition and income security of rural farming households.

Data collection and measurements

With the knowledge that smallholder farmers do not do regular recording of herd data (Opoola *et al*., 2019), recall data was obtained by limiting the recall period to recent past one month and one year. Data were obtained in an integrated manner using cross-sectional surveys at cow- and herd- levels (Chapters three, four, and five), prospective observational surveys to collect fertility performance (Chapter six) and lactation milk yield (Chapter seven) under existing smallholder farming conditions in Rwanda. Quality of the data was improved with laboratory analyses and regular monitoring of milk yield for 30 days post-endometritis diagnosis and fertility performance up to 210 dpp. Further, pre-designed recording forms were provided to the farmers for data collection. Regular farm visits at ten-day intervals improved quality of data collected by helping farmers to keep milk yield records and track the dates of breeding services, return to oestrus and predict the calving date.

In contrast to most other recent studies on CLE and SCLE, this study was performed on smallholder zero-grazed dairy farms under existing management practices. Endometrial cytology samples collected with CYT were subjected to laboratory analysis to obtain cow- and herd-level prevalence of SCLE, whereas the vaginal discharge retrieved using MED was assessed directly at cow-level to estimate the cow- and herd-levels prevalence of CLE (Chapter three). Data on the farmer’s perception of endometritis prevalence was obtained by asking farmers in reference to key clinical symptoms of endometritis show many endometritis positive cows there were in the herd on the day of the visit. The best-worst scaling choice method used to gather the farmer’s opinion on the effectiveness of different MIs for endometritis prevention and control under field conditions (Chapter four) was easy to implement as farmers were presented with only a few cases to select from the extremes (best and worst) MIs. Data on mastitis from milk samples were from CMT tests while blood samples for estimating brucellosis prevalence (Chapter five) were subjected to laboratory analysis. Follow-up data on fertility performance and milk yield were collected from endometritis positive and negative cows (Chapters six and seven).

Statistical procedures

Data analysis employed different analytical methods depending on the research question and the nature of data collected. This was to enable in-depth data mining for better understanding and reporting of endometritis condition among zero-grazed dairy cows on smallholder farms.

Categorical data were cross-tabulated to generate frequencies and differences in prevalence and association between factors tested with Chi-square test statistics for CLE and SCLE, herd characteristics, and diagnostic performance of CYT and MED. The correlation between vaginal mucus and %PMN was tested using Spearman rank's correlation. The prevalence of endometritis detected by farmers and researchers was compared using Wilcoxon's signed-rank test, and their agreement was tested using kappa statistic (Chapter three).

The effectiveness of different MIs for endometritis prevention and control under field conditions in Rwanda was qualitatively assessed by BWS method on a standardised score on a scale from −1.0 (indicates that the MI was chosen as least effective more often than as most effective) to +1.0 (indicates that the MI was chosen as most effective more often than as least effective) (Chapter four).

Path analysis model and multiple logistic regression in backward stepwise selection method were fitted to model direct and indirect relationships among risk factors at cow- and herd-levels, and between risk factors and endometritis (CLE or SCLE) (Chapter five). The path analysis model approach was used because it holds the advantage of measuring the magnitudes of direct and indirect influences on the dependent variables over conventional statistical techniques (Rougoor *et al*., 1997).

Logistic regression analysis was fitted to determine the probability of endometritis positive cows failing to conceive at first service, to experience anoestrus postpartum and to fail to conceive by 210 dpp. It was also used to assess the effect of predictor variables (cow- level variables, herd-level variables, and endometritis status) on anoestrus postpartum. Days-not pregnant (interval calving to conception) at 210 dpp between endometritis positive and negative cows was evaluated using the Kaplan-Meier survival analysis (time-to-event analysis) (Chapter six).

The Independent-Samples T-Test was used to compare daily milk yield and reproductive performance between endometritis positive and negative cows. Analysis of variance with general linear model procedure was used to estimate the mean differences in milk yield and reproductive performance among the different categories of endometritis (Chapters six and seven). It was also used to estimate the mean differences in milk yield among veterinary drugs used, healthy, treated, and untreated cows (Chapter seven).

Linear regression models were used to assess the relationship between predictor variables (cow- and herd-variables and endometritis status) and the response variable (days not-pregnant or milk yield). For this analysis, days not-pregnant and anoestrus postpartum were considered as overall indicators of reproductive efficiency status in dairy cows (Pinedo & De Vries, 2010; Smith *et al*., 2017; Toni *et al*., 2015).

8.1.3 Prevalence of endometritis

The farmer perceived prevalence of endometritis reflected gross underestimation of the disease when compared to observed prevalence (3.2% vs. 67.2%). This was attributable to low farmer awareness of the disease in their zero-grazed cows as less than half (41.6%) could recognize some of endometritis clinical signs, including repeat breeding (43.5%), abortion (24.7%), purulent or mucopurulent vaginal discharge (18.8%) and white or whitish-yellow mucopurulent vaginal discharge (13.0%) (Chapter three). This revealed knowledge gaps about endometritis by farmers and farmers most likely become aware of the signs of the disease when it has already impaired the reproductive performance of the dairy cows through disturbances of establishment and maintenance of pregnancy (Molina-Coto & Lucy, 2018). To bridge this knowledge gap: Firstly, there is a need to increase awareness and training among farmers on early and timely diagnosis of endometritis and cow at high risk of endometritis, and secondly, farmers are advised to adopt and implement management interventions for endometritis prevention and control in their dairy herds.

At the cow- level, the farmer perceived prevalence and researchers observed prevalence had a poor agreement ($k = 0.03$, $p<0.05$). In their studies, Bell (2006) and Leach *et al*. (2010) have suggested a possibility that farmers are distracted from noticing cows by others tasks such as delivery of milk at the milk collection centre, agricultural activities and social activities. The authors reported that in a situation where farmers and researchers examined the same cows simultaneously, the agreement was closer. Extension service and veterinary service delivery to farmers may also be failing to diagnose endometritis cases on smallholder farms probably due to the absence of an extension programme and control strategy targeting endometritis in Rwandan dairy farming, which then limits farmer awareness and implementing targeted management interventions. This could explain the high prevalence of endometritis, with a sample of ten cows having seven (67.2%) positive for CLE on at least two-thirds of the farms (68.1%) and three cows (31.8%) positive for SCLE on at least a third of the farms (34.9%)

(Chapter three). The large variation of endometritis prevalence at cow- and herd- levels strongly reflects the presence of multiple risk factors at play at the cow- and herd- levels (Chapter five). So it is necessary that farmers implement integrated MIs to prevent and control the disease in their herds (Chapter four).

In this study, MED had relatively high sensitivity than CYT (90.5% vs. 42.8%) but low specificity (43.7% vs. 90.8%), suggesting that the combination of MED and CYT could optimise detection of endometritis (Chapter three), enable timely implementation of MIs (Chapter four) and improved reproductive efficiency (Chapter six) while minimising milk yield losses (Chapter seven). The results corroborate previous studies (Molina-Coto & Lucey, 2018; Rana *et al*., 2020).

Of the cows that were negative for SCLE, more than half (56.3%) were also positive for CLE (Chapter three), demonstrating a large proportion of false-positive for CLE exists (Westermann *et al*., 2010). Further supportive evidence of this are poor agreement and weak correlation obtained between the proportion of PMN and vaginal mucus score (Chapter three) indicating that the presence of mucopurulent or purulent discharge in the vagina might not be representative for endometritis. A similar observation was reported in several previous studies (Bicalho *et al*., 2016; Deguillaume *et al*., 2012; Dubuc *et al*., 2010; Gobikrushanth *et al*., 2016; Pascottini, 2016; Westermann *et al*., 2010). The condition has been explained by the fact that vaginitis, cervicitis, urethritis and endometritis are all expressed clinically by mucopurulent or purulent vaginal discharge. In the current study, it was not possible to evaluate the contribution of each condition (vaginitis, cervicitis, urethritis or endometritis) to the production of retrieved vaginal discharge, but it could be considered in further research.

Purulent or mucopurulent vaginal discharge of false-positive cows (56.3%) could be linked to do-it-your-self interventions by farmers, such as self-calving assistance without seeking the help from animal health service providers, non-use of gloves and providing unhygienic assistance during calving as observed in the study farms (Chapters three and five). These practices are mostly associated with physical trauma and contamination of the female reproductive tract especially vulva, urethra, vagina, cervix and body of the uterus (Adnane *et al*., 2017; Hartmann *et al*., 2015; Vieira-Neto *et al*., 2016), and endometritis may develop subsequently (Chapter five).

The observations indicate that to classify cows as positive or negative for endometritis, a reliable complementary confirmatory test is necessary. In the same way, Pascottini (2016) and Westermann *et al*. (2010) reported that the diagnosis of endometritis requires endometrial cytology test for a conclusive diagnosis. That is why in the current study, MED and CYT were used for CLE and SCLE diagnosis, respectively (Chapter three). Furthermore, in their study, Dubuc *et al*. (2010) concluded that SCLE and CLE might represent different reproductive tract conditions and cows positive for endometritis should be classified in three different uterine health statuses: CLE only, SCLE only, or both CLE and SCLE (Chapters six and seven).

8.1.4 Perception of farmers about endometritis prevention and control measures

The high prevalence of endometritis (Chapter three) and associated effects on reproductive performance (Chapter six) and milk yield (Chapter seven)demonstrate the need for targeted management practices in the transition period to prevent and control cow uterine infections (Tayebwa *et al*., 2015). The extensions services have to empower farmers to effectively implement MIs and evaluate their effectiveness. Using a subset of the sample farmers (41.6%) with knowledge of clinical signs of endometritis, this study identified candidate MIs among a sample (n=20) that smallholders considered highly effective (60.0%) in endometritis prevention and control (Chapter four). These were avoiding equipment-sharing with neighbouring farms (MI 02), consulting ANHS providers about the treatment of endometritis positive cases (MI 07), washing the hands and udder before each milking (MI 20), keeping cows in a clean and dry shed (MI 10), using gloves during calving assistance (MI 17), selecting sires based on calving ease (MI 14), disinfecting equipments used in calving assistance before and after use (MI 09), consulting ANHS providers about the treatment and prevention of diseases (MI 06), avoiding sharing or hiring a breeding bull (MI 05), using sexed semen during AI service (MI 19), avoiding off-farm bedding materials and maintain adequate bedding materials per cow (MI 04), and avoiding housing fresh cow with diseased cows or those with chronic diseases such as mastitis (MI 03). This demonstrates that BWS choice method informed appropriate MIs that when effectively implemented would improve animal health and performance and herd profitability.

The MIs considered effective relate to implementation of biosecurity measures though previous studies (Kuster *et al*., 2015; Shortall *et al*., 2017) have shown that perception of the effectiveness of biosecurity measures does not automatically lead to their implementation because some measures may be impractical to implement. Similarly, the adoption and applications of MIs perceived most effective remain limited on the dairy farms under the study area conditions, probably associated with:

(i) Low awareness of endometritis by the farmers. In the sample farmers, endometritis received less attention compared to commonly known dairy cow diseases (Chapters three and four). Therefore, the knowledge gaps may lead to do-it-your-self practices

(Chapters three, four and five). These practices may lead to endometritis occurrence (Eticha & Janssens, 2016; Ruano & Aguayo, 2017).

(ii) Low literacy because over a third (33.8%) of the sample farmers were without formal school education. This low literacy may be a threat on the implementation of MIs. This finding is supported by previous researchers who found that low literacy of farmers was a limiting factor of the effectiveness of management practices for improved reproductive performance of dairy cows in smallholder farms (Ali *et al*., 2015).

(iii) Lack of an extension programme and control strategy targeting endometritis in Rwandan dairy farming. Cows were mostly crowded together and maintained in dirty and wet areas. Therefore, poor hygienic standards of dairy environment favour the proliferation and transmission of uterine disease-causing organisms (Bicalho *et al*., 2012; Wagener *et al*., 2015). This requires hygienic practices to be adhered to in order to lower the concentration of environmental bacteria and consequently reduce the high prevalence of endometritis (Chapter three).

(iv) Poverty level: The sample farmers were resource-poor families because over half (63.0%) were in the category of poor by poverty classification of the Government of Rwanda (Cho & Kim, 2017) and, therefore, unable to implement some MIs that require high investment.

For reducing the prevalence of endometritis in smallholder herds, implementing MIs considered most effective will necessitate the involvement of different stakeholders in the dairy subsector because it involves capital investment and infrastructure that farmers may not be willing to incur as the majority (63.0%) were resource-poor farmers (Chapters four and five). Furthermore, endometritis is a multifactorial disease (Chapter five), so no one MI is universally effective, MIs application in combination is necessary on smallholder zero-grazed dairy farms (Omore *et al*., 1999).

Some management interventions that farmers perceived most effective for endometritis prevention and control (MI 02, 03, 04, and 10) are related to herd-level RF identified to be associated with CLE and SCLE cases (bedding materials; cleanliness of cowshed and housing of cows within the first 30 dpp). In contrast, some others (MI 06, 09, 14, 17, 19 and 20) are linked to cow-level RF associated with CLE and SCLE cases (dystocia, mastitis, retained placenta and stillbirth) (Chapters four and five). These findings would suggest that adoption and implementation of MIs may help decrease endometritis cases for improved production and reproductive performance of zero-grazed dairy cows managed on smallholder farms.

8.1.5 Risk factors for endometritis in zero-grazed dairy cows

Previous studies have investigated the risk factors for only one form of endometritis (CLE or SCLE) in postpartum dairy cows managed on commercial farms (Chan Lee *et al*., 2018; Giuliodori *et al*., 2017; Kadivar *et al*., 2013; Pascottini *et al*., 2017). In all those studies, RF were evaluated using the conventional regression analysis, which only assumes direct associations between individual risk factor and endometritis. This study chose the use of the path analysis model, specifying hypothesised interrelationships among variables, with direct and indirect causal associations (Rougoor *et al*., 1997), unlike conventional regression analyses (Correa *et al*., 1993). This way, this present study is a pioneer in demonstrating the use of path analysis model in identifying and quantifying cow- and herd-levels risk factors associated with CLE and SCLE diagnosed in cows managed under existing smallholder farming conditions (Chapter five). Knowledge of individual cow- and herd- levels risk factors associated with CLE and SCLE was essential in providing management practices that help reduce substantial economic losses from endometritis in the dairy herds.

The prevalence and severity of the disease are related to management conditions of the herd and to RFs specific to each cow (Chapter five), indicating that even in the same herd some cows may be more susceptible to developing and sustaining endometritis than other cows. This has a practical application: prevention and before treating cows positive for endometritis, the most important RFs must be identified and treatment should be adapted specifically to each cow according to the examination of cow- and herd-levels risk factors identified (Adnane *et al*., 2017; Daros *et al*., 2017; Fesseha, 2020). This may result in better management of endometritis and thus reduce the associated influence on subsequent fertility performance and milk yield (Chapters six and seven).

Some cow- and herd-level RF were specific for CLE (earthen floor cowshed and large herd size) or SCLE (mastitis, parity of the cow, and not using bedding materials) while some others were common for both CLE and SCLE (calving season, dystocia, poor body condition score, stillbirth, retained placenta, breed of the cow, sex of the calf, twin birth, farm size, unhygienic cowshed, farmer's experience in dairying, and absence of housing of cows within their first 30 days postpartum) (Chapter five), but primarily, their interactive relationships with other risk factors turn CLE and SCLE into diseases that are multifactorial (Adnane *et al*., 2017; Appiah *et al*., 2020). The difference of specific risk factors between CLE and SCLE is probably because SCLE is a consequence of dysregulation of the postpartum inflammatory process of uterine endometrium rather than changes in uterine microbiota (Wang *et al*., 2018). This is supported by the findings of Pascottini *et al*. (2020), they reported that the uterine microbiota of cows with SCLE was similar to healthy cows, but the uterine microbiome differed in cows with CLE. In their study, Prunner *et al*. (2014b) and Wang *et al*. (2018) concluded that major uterine pathogens are not associated with SCLE case. This difference also supports the weak agreement ($k = 0.10$, $p<0.05$) between CLE and SCLE diagnostic criteria (Chapter three). This implies that in some instances, the two methods (CYT and MED) diagnose different manifestations of female genital tract disease (Dubuc *et al*., 2010; Kelly *et al*., 2020). Although CLE and SCLE may represent different diseases of reproductive tract both had detrimental effects on the subsequent fertility performance (Chapter six) and milk yield (Chapter seven).

The risk factors for CLE and SCLE occur as a complex because a cow that developed one risk factor was at increased risk for other risk factors (Chapter five). Similarly, a herd that had one risk factor was at increased risk for other risk factors. These findings suggest that prevention and control of one risk factor might decrease the prevalence of the other related risk factors both directly and indirectly. This implies that cow that develops one risk factor should be observed for the other risk factors as well so as to allow for early detection and management decisions taken as early as possible. These represent potential interventions that were not previously identified.

Findings from Chapter five showed that there were multiple risk factor associated with CLE and SCLE cases. These risks may represent an alternative to treatment by including them in endometritis prevention and control plan to master at least the most important risk factor to reduce the prevalence and effect of the disease on reproductive performance (Chapter six) and milk yield (Chapter seven) in dairy cows on smallholder farms. This corroborates the findings of Bohlen & Widener (2019) that creating a sterile environment for calving and expecting a sterile uterus post-parturition is unrealistic. Instead, minimizing stressors that may allow bacterial contamination to thrive is critical (Sheldon *et al*., 2020). Therefore, smallholder dairy farmers should work together with their animal health service providers for the implementation of MIs, identification of cows at high risk for endometritis and treatment of endometritis positive cases as early as possible (Abdullah *et al*., 2015; Gilbert, 2016).

The study also analysed the effect of cow- and herd-levels risk factors and endometritis status on milk yield (Chapter seven), days not-pregnant and anoestrus postpartum (Chapter six). This analysis was hypothesised to give the insights on which one weighs more on reduced milk yield, longer days not-pregnant or greater percentage of anoestrus postpartum cows. This knowledge is relevant in extension service and smallholder farmers in making informed decisions on selecting corrective MIs targeting high-risk factors to improve herd health, profitability and sustainability. The findings showed that in a decreasing magnitude, the milk yield was negatively and significantly influenced by cow breed (standardised regression coefficient (β)±Standard error: −2.6±0.4, p<0.001), endometritis (β = −1.1±0.3, p<0.001), poor body condition (β = −1.0±0.4, p<0.05) and mastitis positive case (β = −0.8±0.3, p<0.05). This is consistent with the previous observations (Bisrat & Nigussie, 2016; Bobbo *et al*., 2017; McDougall *et al*., 2011; Singh *et al*., 2015). Standardised regression coefficient indicates the effect in the response variable associated with a one-unit change in the predictor variable. These findings point to the importance of crossbreeding low milk potential yielding breeds with the high potential yielding breeds, adequate feeding, and controlling mastitis and maintaining housing hygiene in the dairy herd. Implementing these interventions offers the additional advantage of improving milk quantity (Mekonnen *et al*., 2020; Pandey & Voskuil, 2011; Roschinsky *et al*., 2014; Sheldon *et al*., 2020).

Further observations revealed influence of endometritis and anoestrus postpartum on days- not pregnant (β = 21.3, SE = 5.8, p<0.001; 64.1, SE = 5.1, p<0.001, respectively) while endometritis only influenced anoestrus postpartum (β = 1.3, SE = 0.2, p<0.001). The findings indicate a strong association between endometritis and the suboptimal reproductive performance of dairy cows (Chapter six). This can be associated with inadequate and poor feeding practices largely observed in the study farms (Chapters six and seven) and weak animal health program on the farms (Chapters four and five). To manage this, dairy farmers are advised to implement MIs for endometritis prevention and control and improve feeding practices for improved reproductive performance towards smallholder dairy herd profitability and sustainability.

8.1.6 Influence of endometritis on reproductive performance of zero-grazed dairy cows

The results demonstrated that cows positive for endometritis suffer suppressed fertility performance relative to cows negative for endometritis for all measures of fertility (Chapter six). The findings are in agreement with previous studies (Bidne *et al*., 2018; Chaudhari *et al*., 2017; Ryan *et al*., 2020) and represent associated loss that farmers incure from endometritis infections. The cows remain unproductive over long period of repeated serving and risk culling and limiting the generation of heifer replacement in the herd (Buckley *et al*., 2014; Nishimwe *et al*., 2015). In their study, Melendez & Risco (2005) identified uterine infection after calving as costly to producers due to impairment of reproductive performance. Furthermore, Gröhn *et al*. (2003), Pascottini *et al*. (2015), and Sharma *et al*. (2018a) observed that the reproductive status of a cow in the postpartum period is the single most important factor influencing culling decisions on dairy farms and that uterine infection contributes to the high rates of involuntary culling, resulting in reduced profitability of the dairy herds. Preventing and controlling endometritis is thus important, which extension and veterinary services need to prioritise in their herd health programme for effective disease management and herd sustainability.

8.1.7 Influence of endometritis on milk yield

Endometritis infection was associated with a substantial production loss from milk yield, discarded milk during treatment and withdrawal periods, and additional incurred cost of veterinary treatment (Overton & Fetrow, 2008; Sharma *et al*., 2019). The endometritis positive cows produced less milk (7.5±0.2 litres/cow/day) compared to negative one (8.9 ± 0.3 litres/cow/day) or loss of 2.5±0.2 litres of milk/cow/day to the farmer. At the farm gate price of US$ 0.2 per litre of milk for a daily loss of 2.5 litres of milk in 255 days of lactation, farmers incur US$ 154 loss worth of revenue.

Cows with poor body condition (BCS<3) at 21-60 dpp had greater odds of CLE and SCLE cases than cows with good body condition (BCS ≥3) (Chapter five), which is in agreement with some of the previous studies (Kelly *et al*., 2020; Souissi & Bouraoui, 2019). Poor body condition reflects inadequate energy and protein feeding, which can be linked to five issues on the sample study farms:

(i) Small farm sizes (2.8±0.1 acres) in which food crops and fodder compete for land use and this limits sufficient fodder production;

(ii) Improper processing of green forages like no chopping of forages and no wilting contributing probably to low dry matter intake;

(iii) Low level of supplementation of Napier grass with a commercial dairy meal and mineral licks, and limited access to water;

(iv) Use of forage (Napier grass) with low quality, however, is often insufficient to provide nutrients to meet cows' maintenance and support milk production requirements (Kashongwe *et al*., 2017);

(v) Very low use of crop residues probably because feeding these materials to animals competes with conservation agriculture where crop residues are valuable materials for mulch for crop production and making green manure (Turmel *et al*., 2014).

These observations demonstrate that adequate feeding practice and feed resources are challenges for smallholder farmers, requiring that appropriate nutritional management in the transition period should be provided for successful production and reproduction performance (Chapters six and seven).

At farmers' request for veterinary service, local field veterinarians treated 33.4% of cows positive for CLE regardless of endometrial cytology results using VD available and commonly used in veterinary practice for the treatment of bacterial infections. A large proportion of cows were treated using tetracycline hydrochloride (29.8%), oxytetracycline (24.0%), procaine penicillin G and dihydrostreptomycin (17.3%), phenylbutazone (16.3%) and novalgin (12.5%) groups (Chapter seven). These VD have been criticised for being associated with high milk disposal, microbial resistant and inconsistent recovery rate (Gilbert, 2016). The proportional milk yield losses were 45.6% in treated cows and 16.3% in untreated cows, which is substantial and warrants the use of alternative therapeutic management that have no residual effect in the treated cows. These alternative include phytotherapy agents (Nikhade *et al*., 2019; Sharma *et al*., 2018b), hormone therapy (Ahmadi *et al*., 2018) and VD with zero-withdrawal periods for milk (Tison *et al*., 2016). These management options have been used in commercial dairy farms of developed countries with no applications in sub-Saharan African countries, specifically in Rwanda. This could be explained by a lack of awareness by the animal health service providers on the use and importance of these alternatives in the treatment of endometritis. Consequently, there is a need for interventions targeting animal health service providers regarding the importance, utilisation, and economic benefits of VD having zero milk withdrawal period and herbal medicine with the potential to treat endometritis to enable them efficiently treat endometritis positive cases.

8.2 Conclusions

(i) The prevalence of endometritis is highly prevalent in dairy cows (70.2%) but is grossly underestimated by farmers (3.2%) and large knowledge gap about endometritis is apparent in the field among farmers and practicing veterinarians probably due to the absence of an extension programme and control strategy targeting endometritis in Rwandan dairy farming.

(ii) The management interventions that farmers consider are most effective in the prevention and control of endometritis disease in the dairy herds are on-farm biosecurity and hygiene, seeking veterinary services for disease treatment, and selecting sires for ease of calving.

(iii) Some risk factors were specific for SCLE cases (mastitis, parity of cow and not using bedding materials) or CLE cases (earthen floor cowshed and large herd size), and some others were common for both SCLE and CLE cases (calving season, dystocia, poor body condition score, stillbirth, retained placenta, breed and parity of cow, sex of calf, twin births, farm size, unhygienic cowshed, farmer experience in dairy farming, and absence of housing of cows within their first 30 dpp) in smallholder zero-grazed dairy cows.

(iv) Compared to negative cows, the positive endometritis cases had longer days to first oestrus, days to first natural mating or artificial insemination service, and days-not pregnant, lesser pregnancy rates at first service and all services and cows pregnant within 210 days postpartum, more natural-mating or artificial insemination per pregnancy and more occurrences of anoestrus postpartum.

(v) The volume of milk losses during the period of discarding milk and the decrease in milk yield that result from endometritis infection was 7.3±0.3 and 1.4±0.2 litres/cow/day, respectively, in smallholder zero-grazed dairy cows.

8.3 Recommendations

(i) Extension services need to increase awareness and training among smallholder dairy farmers about the detection and management of endometritis in their cows.

(ii) Prioritise the farmer perceived most effective management interventions for endometritis prevention and control in the extension messages to effectively improve uterine health in the postpartum cows

(iii) Extension services should prioritise endometritis in their herd health service delivery to smallholder dairy farmers for effective disease management and improved reproductive performance of dairy cows towards herd sustainability.

(iv) Effective management practices of risk factors with direct and indirect influences on CLE and SCLE cases should be a priority in extension education and services to enable smallholder farmers effectively manage them to prevent and control endometritis among their zero-grazed dairy cows.

(v) Adopt early and timely diagnosis of endometritis and treatment using conventional veterinary drugs having zero withholding times for milk or phytotherapeutic agents to reduce the milk yield loss and associated economic loss.

8.4 Areas for further research

Areas that need be prioritised for further studies are:

(i) Efficacy and cost-effectiveness of the management interventions that farmers consider effective for endometritis prevention and control for use on smallholder dairy farms.

(ii) Factors influencing the adoption and implementation of the MIs on the dairy herds.

(iii) Profiling plant species in the country with potential as ethnoveterinary medicine to treat endometritis.

(iv) Epidemiological studies of endometritis by isolating and profiling the environmental pathogens prevalent on smallholder farms.

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APPENDICES

APPENDIX I. Questionnaire used for data collection

Dear Sir/Madam,

I am Pascal Nyabinwa, a PhD student at Egerton University-Njoro Campus (Kenya). I am conducting research on prevalence of endometritis and associated influence on performance of smallholder zero-grazed dairy cows in Gasabo district of Rwanda. The information provided will be used solely for the academic purpose(s) and will remain confidential. The interview will take approximately one hour (1h) and your participation is cornerstone to the success of this study. We are kindly asking for your consent to participate in the study.

Farmer consent obtained Yes [____] No [____] Name of participant / ______/,

Date (dd/mm/yy) / ___/___/____/, Signature / ________________/,

We thank you in advance for your time and appreciate your thoughtful answers to our questions.

SECTION A. General information

Farm No. /____ ____ ____/

Date (dd/mm/yy) /____/____/ ___ /

Name of enumerator name /____________________________ / Enumerator Code / __ __ /

Tel. of enumerator /___ ___ ___ ___ ___ ___ ___ ___ ___ ___ /

Supervised by (Name and Surname) /____ /

SECTION B: Information on farmer and farm management

B.1. Information on farmer

1. Respondent's name [_____________________________________]
2. Tel. Number /___ ___ ___ ___ ___ ___ ___ ___ ___ ___ /
3. Gender of the respondent [____] 1 = Male 2 = Female
4. Age of the respondent [____ ____] years
5. Household size (persons) [____ ____]
6. Category in national wealth ranking system (Ubudehe) **[______]**
7. Education level [__] 1 = No schooling 2 = Adult literacy education 3 = Primary school 4 = Seco ndary school ('O'Level) 5 = University 6 = Other (specify) [___________________]
8. Dairy farming experience in years [___ ___]
9. Farmer's location (i) Village [_________________] (ii) Cell [________________]

   D.. Sector [__________________](iv) GPS coordinates: S_________________

   E________________

B.2. Information on farm management and record keeping

10. Population (number) of cattle kept on the farm by genotypes

1 = Indigenous cattle / __ __ /, 2 = Dairy crossbreds / _ _ /, 3 Dairy pure breeds / __ __/

11. What total land size do you farm now in acres? [____ ____ ___]

12. Herd records keeping / ______ / 1 = Complete, 2 = Incomplete, 3 = Not practiced

If 1 or 2, please fill the following table

Kinds of written records	**Kept** 1 = Yes 2 = No	**How kept** 1 = Record book 2 = Loose papers 3 = Computer4 = other (specify)
Inventory	/ _______ /	/ _______ /
Health	/ _______ /	/ _______ /
Milk production	/ _______ /	/ _______ /
Breeding	/ _______ /	/ _______ /
Sales and purchases	/ _______ /	/ _______ /
Births and deaths	/ _______ /	/ _______ /

13. Breeding service used on the farm /___ / 1 = Artificial Insemination Service, 2 = Bull Service, 3 = Both

14. Breeding service cost (US$), Artificial insemination service / __ /, Bull service / __ / (US$1 = Rwf 920)

15. The price of farm gate milk (US$) / __ __ __ /

16. Source of animal health services / ___ / 1 = Veterinary, 2 = Community-based animal health workers, 3 = Local traditional herbalists

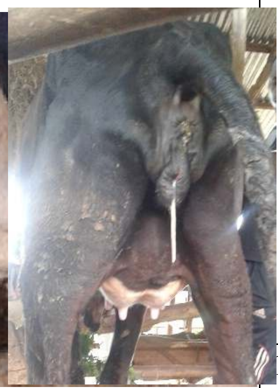
17. Do you cut-and-carry fodder and crop residues to your animals? / _____ / 1 = Yes, 2 = No

If Yes, indicate which feeds are offered and whether they are from on or off-farm.

Type of feeds	**Use** 1 = Yes 2 = No	**Feed sources** 1 = On-farm 2 = Off farm
Napier grass	[________]	[________]
Brachiaria grass	[________]	[________]
Other cultivated grass	[________]	[________]
Roadside grasses	[________]	[________]
Forage maize	[________]	[________]
Forage sorghum	[________]	[________]
Banana fodder	[________]	[________]
Tree fodders	[________]	[________]
Crop residues	[________]	[________]
Agro-industrial by-products	[________]	[________]
Mineral block for dairy cows	[________]	[________]

SECTION C: Management interventions for endometritis prevention and control

C.1. Farmer perception of endometritis signs in cows in a herd

Endometritis is a postpartum disease characterised by an inflammation of the uterine endometrium 21-90 days postpartum. According to its signs:**(i)** white or whitish-yellow mucopurulent vaginal discharge comes out when the animal lies down, urinates or defecates and   abortion.	**18.** Did you observe such signs in cows in a herd for the past one year? 1 = yes, 2 = no If **Yes**, what is the frequency of observation of such cases in the herd? - Mucopurulent or purulent - White or whitish yellow mucopurulent - Repeat breeding - Abortion	/____/ /___ ____/ /___ ____/ /___ ____/ /___ ____/
	19. Now (on the day of the visit), is there endometritis case in your herd? 1 = Yes 2 = No	/_________ /
	20. If **Yes**, how many cows with endometritis signs [(i) and/or (ii)] do you have in the herd?	/_____ ______/

If there is no observation in Question no. 18, please skip Question no. 21 and go to Question n

C.2. Farmer's opinion on the effectiveness of management intervention for endometritis prevention and control among zero-grazed dairy cows on smallholder farms

21. For each best-worst scaling choice card (BWSCC), please pick the most effective and the least effective management interventions (MIs) for endometritis prevention and control on dairy farms

BWSCC-1

MIs codes	Management interventions	Most effective	Least effective
7	Consult ANHS provider about the treatment of endometritis positive cases	☐	☐
12	Maintain clean transition cow housing	☐	☐
1	Avoid equipment-sharing between cows into the farm	☐	☐
5	Avoid sharing or hiring breeding bull	☐	☐
20	Wash the hands and udder before each milking	☐	☐

BWSCC-2

MIs codes	Management interventions	Most effective	Least effective
16	Remove fetal membranes immediately after passing	☐	☐
9	Disinfect equipment used in calving assistance before and after use	☐	☐
8	Cull of persistently endometritis positive cows	☐	☐
13	Maintain regular contact with ANHS provider for advisory support on endometritis prevention in dairy farm	☐	☐
3	Avoid housing fresh cows with diseased cows or those with chronic illnesses such as mastitis	☐	☐

BWSCC-3

MIs codes	Management interventions	Most effective	Least effective
2	Avoid equipment-sharing with neighbouring farms	☐	☐
19	Use a sexed semen during artificial insemination service	☐	☐
6	Consult ANHS provider about the treatment and prevention of mastitis and metabolic diseases	☐	☐
15	Select sires based on low percent stillbirths by referring to sires catalogue	☐	☐
10	Keep cows in a clean and dry shed	☐	☐

BWSCC-4

MIs codes	Management interventions	Most effective	Least effective
18	Use an artificial insemination service	☐	☐
4	Avoid off-farm bedding materials and maintain adequate bedding materials per cow	☐	☐
14	Select sires based on calving ease by referring to dairy sires catalogue	☐	☐
11	Maintain adequate feeding per cow	☐	☐
17	Use gloves during calving assistance	☐	☐

BWSCC-5

MIs codes	Management interventions	Most effective	Least effective
1	Avoid equipment-sharing between cows into the farm	☐	☐
13	Maintain regular contact with ANHS provider for advisory support on endometritis prevention in dairy farm	☐	☐
19	Use a sexed semen during artificial insemination service	☐	☐
11	Maintain adequate feeding per cow	☐	☐
16	Remove foetal membranes immediately after passing	☐	☐

BWSCC-6

MIs codes	Management interventions	Most effective	Least effective
10	Keep cows in a clean and dry shed	☐	☐
8	Cull of persistently endometritis positive cows	☐	☐
20	Wash the hands and udder before each milking	☐	☐
17	Use gloves during calving assistance	☐	☐
3	Avoid housing fresh cows with diseased cows or those with chronic illnesses such as mastitis	☐	☐

BWSCC-7

MIs codes	Management interventions	Most effective	Least effective
18	Use an artificial insemination service	☐	☐
2	Avoid equipment-sharing with neighbouring farms	☐	☐
5	Avoid sharing or hiring breeding bull	☐	☐
9	Disinfect equipment used in calving assistance before and after use	☐	☐
15	Select sires based on low percent stillbirths by referring to sires catalogue	☐	☐

BWSCC-8

MIs codes	Management interventions	Most effective	Least effective
4	Avoid off-farm bedding materials and maintain adequate bedding materials per cow	☐	☐
12	Maintain clean transition cow housing	☐	☐
6	Consult ANHS provider about the treatment and prevention of mastitis and metabolic diseases	☐	☐
7	Consult ANHS provider about the treatment of endometritis positive case	☐	☐
14	Select sires based on calving ease by referring to dairy sires catalogue	☐	☐

BWSCC-9

MIs codes	Management interventions	Most effective	Least effective
13	Maintain regular contact with ANHS provider for advisory support on endometritis prevention in dairy farm	☐	☐
10	Keep cows in a clean and dry shed	☐	☐
12	Maintain clean transition cow housing	☐	☐
18	Use an artificial insemination service	☐	☐
6	Consult ANHS provider about the treatment and prevention of mastitis and metabolic diseases	☐	☐

BWSCC-10

MIs codes	Management interventions	Most effective	Least effective
8	Cull of persistently endometritis positive cows	☐	☐
11	Maintain adequate feeding per cow	☐	☐
7	Consult ANHS provider about the treatment of endometritis positive case	☐	☐
15	Select sires based on low percent stillbirths by referring to sires catalogue	☐	☐
17	Use gloves during calving assistance	☐	☐

BWSCC-11

MIs codes	Management interventions	Most effective	Least effective
4	Avoid off-farm bedding materials and maintain adequate bedding materials per cow	☐	☐
20	Wash the hands and udder before each milking	☐	☐
16	Remove foetal membranes immediately after passing	☐	☐
2	Avoid equipment-sharing with neighbouring farms	☐	☐
1	Avoid equipment-sharing between cows into the farm	☐	☐

BWSCC-12

MIs codes	Management interventions	Most effective	Least effective
19	Use a sexed semen during artificial insemination service	☐	☐
9	Disinfect equipment used in calving assistance before and after use	☐	☐
14	Select sires based on calving ease by referring to dairy sires catalogue	☐	☐
3	Avoid housing fresh cows with diseased cows or those with chronic illnesses such as mastitis	☐	☐
5	Avoid sharing or hiring breeding bull	☐	☐

BWSCC-13

MIs codes	Management interventions	Most effective	Least effective
12	Maintain clean transition cow housing	☐	☐
11	Maintain adequate feeding per cow	☐	☐
2	Avoid equipment-sharing with neighbouring farms	☐	☐
3	Avoid housing fresh cows with diseased cows or those with chronic illnesses such as mastitis	☐	☐
15	Select sires based on low percent stillbirths by referring to sires catalogue	☐	☐

BWSCC-14

MIs codes	Management interventions	Most effective	Least effective
6	Consult ANHS provider about the treatment and prevention of mastitis and metabolic diseases	☐	☐
9	Disinfect equipment used in calving assistance before and after use	☐	☐
17	Use gloves during calving assistance	☐	☐
1	Avoid equipment-sharing between cows into the farm	☐	☐
14	Select sires based on calving ease by referring to dairy sires catalogue	☐	☐

BWSCC-15

MIs codes	Management interventions	Most effective	Least effective
13	Maintain regular contact with ANHS provider for advisory support on endometritis prevention in dairy farm	☐	☐
5	Avoid sharing or hiring breeding bull	☐	☐
4	Avoid off-farm bedding materials and maintain adequate bedding materials per cow	☐	☐
8	Cull of persistently endometritis positive cows	☐	☐
19	Use a sexed semen during artificial insemination service	☐	☐

BWSCC-16

MIs codes	Management interventions	Most effective	Least effective
10	Keep cows in a clean and dry shed	☐	☐
16	Remove foetal membranes immediately after passing	☐	☐
7	Consult ANHS provider about the treatment of endometritis positive case	☐	☐
18	Use an artificial insemination service	☐	☐
20	Wash the hands and udder before each milking	☐	☐

* For the purposes of the study, MI was defined as an action that reduces or targets the risk factors for endometritis in the dairy herds. The effectiveness of MI was the extent to which the MI prevents or controls endometritis-causing agents on-farm.

SECTION D. Risk factors associated with endometritis

D.1. Cow–level variables

22. Cow breed and parity (number of calving) of cows within 20-60dpp?

Cow number	Cow breed	Parity
1.	/ __________ /	/ __________ /
2.	/ __________ /	/ __________ /
3.	/ __________ /	/ __________ /
4.	/ __________ /	/ __________ /
5.	/ __________ /	/ __________ /
6.	/ __________ /	/ __________ /

Cow breed

1 = Indigenous cattle

2 = Dairy crossbreds

3 = Pure dairy breed

Parity of cow

1 = Primiparous

2 = Multiparous

23. Please indicate the following status for each cow within 20-60 days postpartum

Cow no. / Variables	Cow1	Cow2	Cow3	Cow4	Cow5	Cow 6
Days postpartum at sampling	[___]	[___]	[___]	[___]	[___]	[___]
Cow age (Years)	[___]	[___]	[___]	[___]	[___]	[___]
Dry period length (days)	[___]	[___]	[___]	[___]	[___]	[___]
Season of calving (month)	[___]	[___]	[___]	[___]	[___]	[___]
Pregnancy length (days or month)	[___]	[___]	[___]	[___]	[___]	[___]
Breeding service used 1 = AI, 2 = Bull	[___]	[___]	[___]	[___]	[___]	[___]
Retained foetal membrane 1 = occurrence 2 = non-occurrence	[___]	[___]	[___]	[___]	[___]	[___]
Ketosis 1 = occurrence 2 = non-occurrence	[___]	[___]	[___]	[___]	[___]	[___]
Left displaced abomasum 1 = occurrence 2 = non-occurrence	[___]	[___]	[___]	[___]	[___]	[___]
Milk fever 1 = occurrence 2 = non-occurrence	[___]	[___]	[___]	[___]	[___]	[___]

Calf sex 1 = Female 2 = Male	[___]	[___]	[___]	[___]	[___]	[___]
Status of calves at birth 1 = Alive 2 = Stillbirth	[___]	[___]	[___]	[___]	[___]	[___]
Dystocia 1 = occurrence 2 = non-occurrence	[___]	[___]	[___]	[___]	[___]	[___]
If dystocia occurrence, calving was assisted 1 = Yes 2 = No	[___]	[___]	[___]	[___]	[___]	[___]
Assisted by 1=Farmer 2=Veterinary	[___]	[___]	[___]	[___]	[___]	[___]
Manual intervention during assistance 1 = Yes 2 = No	[___]	[___]	[___]	[___]	[___]	[___]
Gloved-hand during assistance 1 = Yes 2 = No	[___]	[___]	[___]	[___]	[___]	[___]
Multiple birth 1 = Singleton 2 = Twins	[___]	[___]	[___]	[___]	[___]	[___]
Body condition score at sampling	[___]	[___]	[___]	[___]	[___]	[___]

Body condition score chart in dairy cattle (5-point scale)

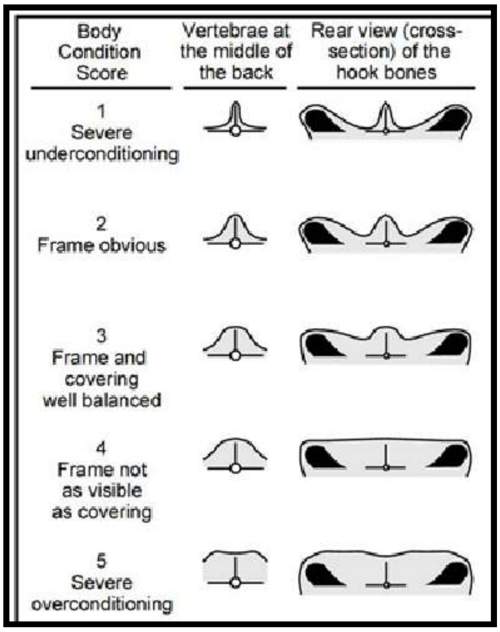
1= Severe under conditioning (Emaciation = Thin cow)

2 = Frame obvious

3 = Frame and covering well balanced

4 = Frame not as visible as covering

5= Severe over conditioning (Obesity = Fat cow)



Source: Adapted from Edmonson *et al*. (1989).

D.2. Herd–level variables

24. According to the following figure, please observe and record the cleanliness of the cow

Figure showing five anatomical sites for cow and their cleanliness scores

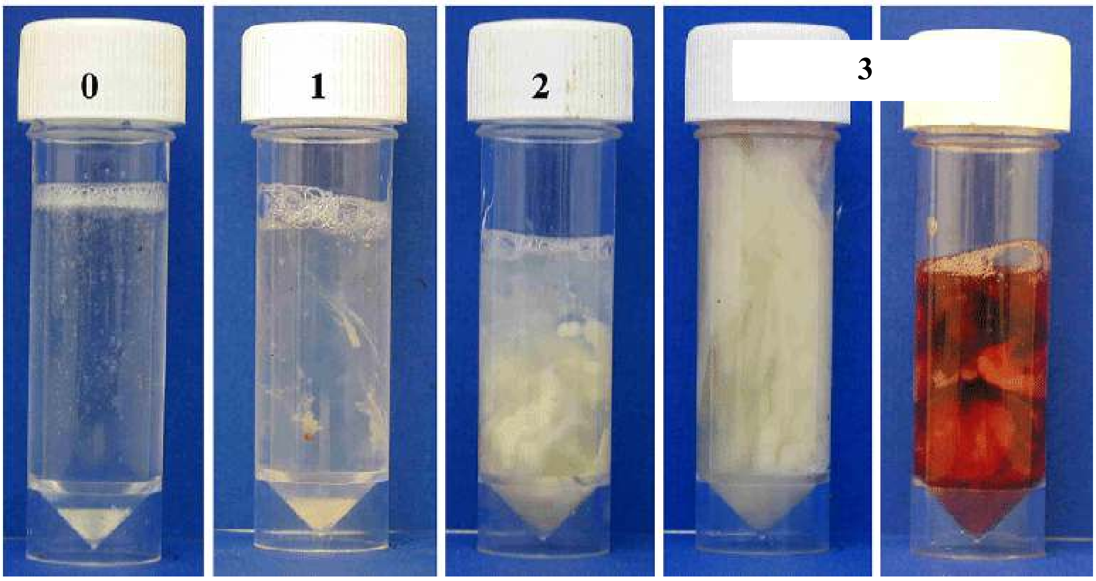
Cow no.	**Cow Cleanliness Scores (CCS)** 1 = Very clean, no dirt or faeces 2 = Moderate soiled with dirt and/ or faeces 3 = Heavily soiled with dirt and/ or faeces						**Cleanliness of cowshed** 1 = clean (CCS = 5) 2 = dirty (CCS > 5)
	Pelvis including the upper part of the tail (**A**)	Flanks including the lower part of the tail (**B**)	Udder (**C**)	Lower hind legs (**D**)	Ventral aspect of the abdomen including knee (**E**)	Overall whole-cow score	
1	[________]	[______]	[__]	[__]	[______]	[_____]	[_____]
2	[________]	[______]	[__]	[__]	[______]	[_____]	[_____]
3	[________]	[_____]	[__]	[__]	[______]	[_____]	[_____]
4	[________]	[_____]	[__]	[__]	[______]	[_____]	[_____]
5	[________]	[_____]	[__]	[__]	[______]	[_____]	[_____]
6	[________]	[_____]	[__]	[__]	[______]	[_____]	[_____]

25. From observation of the herd, indicate the following information:

Herd's information	**Response**
Type of cowshed 1 = With a roof 2 = Without a roof	[________________]
Housing floor types 1 = Concrete 2 = Wooden 3 = Earthen	[________________]
Calving pen 1= Presence 2 = Absence	[________________]
Housing of cows within the first30 dpp 1 = Presence, 2 = Absence	[________________]
Where cows are kept 1 = Individual cowshed 2 = Communal cowshed	[________________]
Do you use bedding materials in cowshed? 1 = Yes, 2 = No	[_______________]
If Yes, indicate which bedding materials are used 1 = Sand, 2 = natural green grasses, 3 = corn, 4 = wood shaving, 5 = leftover or waste feeds from feeding troughs, 6 = hay, 7 = Sawdust, 8 = straw	[___ ____ ____ ___]
Frequency of removing any soiled or damp bedding before adding fresh bedding materials 1= Daily basis, 2 = Once per week, 3 = Twice per week, 4 = Once per month , 5 = Twice per month, 6 = Other (specify)	[________________]
Do you give water to your cows? 1 = Yes, 2 = No	[________________]
What is the source of this water? 1 = Tap, 2 = Rain water, 3 = Shallow wells, 4 = River, 5 = Pond	[_______________]
Hygiene in cowshed (Ref to answers of Question no. 23) 1 = clean , 2 = dirty	[______]

SECTION E: Diagnosis of endometritis, mastitis and brucellosis

26. According to the clinical endometritis-scoring scheme, please observe and record the character of the vaginal mucus retrieved.



Vaginal mucus character score

0 = No discharge or clear or translucent mucus

1 = Clear mucus with flecks of purulent material

2 =vaginal discharge containing ≤50% white mucopurulent material

3 = ≥50% vaginal discharge containing purulent material, usually white or yellow, but occasionally sanguineous

Cow number	Vaginal mucus score
1	/________________/
2	/________________/
3	/________________/
4	/________________/
5	/________________/
6	/________________/

27. Microscopic evaluation of endometrial cytological samples

Cow no.	Glass slide code	Cells count		PMN to – endometrial cells ratio
		Endometrial cells (number)	Polymorphonuclear inflammatory cells (PMN) (number)	
1	[_____]	[___________]	[___________]	[__________]
2	[_____]	[___________]	[___________]	[__________]
3	[___ __]	[___________]	[___________]	[__________]
4	[_____]	[___________]	[___________]	[__________]
5	[_____]	[___________]	[___________]	[__________]
6	[_____]	[___________]	[___________]	[__________]

28. Mastitis screening

Give the score according to descriptions of observed CMT reactions.

Score value	Descriptions of reaction
1 =	The mixture remains unchanged and homogenous
2 =	Slight thickening of the mixture, tends to disappear with paddle movement
3 =	Distinct thickening of the mixture, but no tendency to form a gel with paddle movement
4 =	Distinct gel formation
5 =	Strong gel formation, which tends to adhere to paddle. Forms distinct central peak

Cow no.	California mastitis test scores				Cow status 1 = Positive 2 = Negative
	Quarter 1	Quarter 2	Quarter 3	Quarter 4	
1	[____]	[____]	[____]	[____]	[____]
2	[____]	[____]	[____]	[____]	[____]
3	[____]	[____]	[____]	[____]	[____]
4	[____]	[____]	[____]	[____]	[____]
5	[____]	[____]	[____]	[____]	[____]
6	[____]	[____]	[____]	[____]	[____]

29. Brucellosis screening

Cow no.	**Tube code**	**Cow status:** 1 = Positive**,** 2 = Negative
1	[___ ___ __]	[_____]
2	[___ ___ __]	[_____]
3	[___ ___ __]	[_____]
4	[___ ___ __]	[_____]
5	[___ ___ __]	[_____]
6	[___ ___ __]	[_____]

SECTION F. Fertility performance of cows after diagnosis of endometritis

30. Please record the fertility performance of sampled cows. Period: 5 months post-endometritis diagnosis.

Cow no.	Current calving date (DD/MM/YY)	First oestrus detection date (DD/MM/YY)	First breeding service Date (DD/MM/YY)	Breeding services used 1 = AI 2 = Bull	Date of return on heat (DD/MM/YY)	Number of services per conception	Pregnant 1 = Yes 2 = No
1	[___/____/___ _]	[___/____/ ____]	[___/____/___ _]	[_____]	[___/____/ ____]	[_____]	[_____]
2	[___/____/___ _]	[___/____/ ____]	[___/____/___ _]	[_____]	[___/____/ ____]	[_____]	[_____]
3	[___/____/___ _]	[___/____/ ____]	[___/____/___ _]	[_____]	[___/____/ ____]	[_____]	[_____]
4	[___/____/___ _]	[___/____/ ____]	[___/____/___ _]	[_____]	[___/____/ ____]	[_____]	[_____]
5	[___/____/___ _]	[___/____/ ____]	[___/____/___ _]	[_____]	[___/____/ ____]	[_____]	[_____]
6	[___/____/___ _]	[___/____/ ____]	[___/____/___ _]	[_____]	[___/____/ ____]	[_____]	[_____]

SECTION G. Treatment and milk yield of the sampled cows

31. Please record the treatment drugs, frequency of drugs used and withdrawal period for milk

Veterinary drugs

1 = Oxytetracycline
2 = Cephapirin (Metricure®)
3 = Procaine penicillin G and dihydrostreptomycin
4 = Ceftiofor hydrochloride
5 = Tetracycline hydrochloride
6= Phenylbutazone
7 = Calcium and energy Supplements
8 = Novalgin
9 = Oxytocin
10 = Prostaglandin F2 Alpha
11 = other (specify)

Drug administration frequency

1 = Once per treatment
2 = Twice per treatment
3 = Thrice per treatment
4 = Other(specify)

Cow number	Veterinary drugs used	Drug administration frequency (days)	Withdrawal periods for milk (days)
1	/_______________/	/_______________/	/_______________/
2	/_______________/	/_______________/	/_______________/
3	/_______________/	/_______________/	/_______________/
4	/_______________/	/_______________/	/_______________/
5	/_______________/	/_______________/	/_______________/
6	/_______________/	/_______________/	/_______________/

32. Please record milk yield of the sampled cows for 30 days post-endometritis diagnosis

Cow no.	**Milking times**	**Milk production records (litres)**														
		Day 1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
	Morning	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]
	Evening	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]
	Morning	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]
	Evening	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]
	Morning	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]
	Evening	[____]	[____]	[____]	[____]	[____]	[____]	[__	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[_

Cow no.	Milking times	Milk production records (litres)														
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	Morning	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
	Evening	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
	Morning	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
	Morning	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
	Evening	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
	Evening	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
	Morning	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]

	Evening	[____]	[____]	[____]	[____]	[____]	[____]	[___]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]
	Morning	[____]	[____]	[____]	[____]	[____]	[____]	[___]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]
	Evening	[____]	[____]	[____]	[____]	[____]	[____]	[___]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]
	Morning	[____]	[____]	[____]	[____]	[____]	[____]	[___]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]
	Evening	[____]	[____]	[____]	[____]	[____]	[____]	[___]	[____]	[____]	[____]	[____]	[____]	[____]	[____]	[____]

Thank you for your participation.

APPENDIX II. Form for confidentiality agreement for research team members during data collection

The objective of this research is to assess prevalence of endometritis and associated influence on performance of smallholder zero-grazed dairy cows in Gasabo district of Rwanda. This will be carried out through direct observation, interviews with dairy farmers, and diseases diagnosis. The study is led by Mr. Pascal Nyabinwa from Egerton University-Njoro Campus, Kenya. The information and experiences from the dairy farmers in interviews, direct observation and status of cows after disease screening are confidential and must not to be shared with anyone outside of the research team. The identities of study participants and the location of participants must not be shared with anyone outside of the research team.

I have read the above text and agree to confidentiality.

SN	Name	Telephone and email	Signature

APPENDIX III. Endometrial cytological slides evaluation procedures

Differential Quik stain kit (Modified Giemsa) solutions in screw bottles:
(1) Solution A Fixative
(Methanol: fixative solution)
(2) Solution C Xanthene dye *(Eosin: stain solution)*
(3) Solution B Blue/Azure dye
(Methylene blue: counter stain)

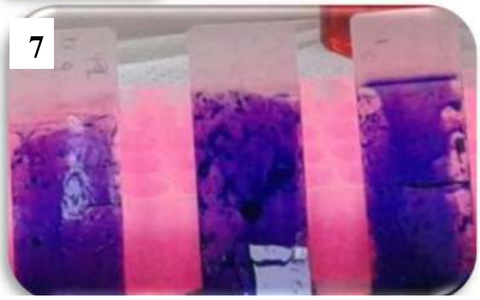
The small pots that contain staining solution: 1 = Solution A, 2 = Solution C, 3 = Solution B

Gently dip the air-dried slide into staining pot one (***Fixing solution A***) for one second and remove. Repeat-dipping the slide a total of five times, each time lasting one second. Drain excess fluid onto the paper towel.

Dip slide into pot two (***Solution C***) for one second and remove. Repeat-dipping the air-dried slide a total of five times, each time lasting one second. Drain excess onto the paper towel.

Dip slide into pot three (***Solution B Blue***) for one second and remove. Repeat-dipping the air-dried slide a total of five times, each time lasting one second. Drain excess onto the paper towel.

Rinse the slide under gently running tap water until the water runs clear.



Rest the slide vertically and allow it to air-dry. Microscopic examination was performed when the entire slide was dried.

Once the slide was completely air-dried, three drops of Eukitt mounting medium were placed on it, and a clean coverslip was placed on the slide. Next, microscopic evaluationof PMN and endometrial cells under microscope mounted with a camera at:
****Lower power (x10 objective***) to check overall cellularity and to find areas within the smear that contain a monolayer of well preserved and adequately stained cells, and
****High power (x40 objective)*** to properly count individual cell (endometrial cell and PMN) within the monolayer.

APPENDIX IV. Diversity of feeding practices in smallholder zero-grazed dairy farms (n = 370) in Gasabo district, Rwanda

Feed preference	Type of feeds	Frequency	Percentage (%)	Source of feeds (%)	
				On-farm	Off-farm
Basal feeds	Napier grass	361	97.6	82.4	17.6
	Brachiaria grasses	249	67.3	60.7	39.3
	Other cultivated grass	199	53.8	46.3	53.7
	Roadside grasses	189	51.1	47.8	52.2
	Banana fodder	280	75.7	91.3	8.7
	Tree fodder	43	11.6	78.0	22.0
Forage supplement	Forage maize	131	35.4	85.8	14.2
	Forage sorghum	77	20.8	76.8	23.2
	Crop residues	120	32.4	90.8	9.2
Concentrates and mineral supplement	Commercial dairy meal	80	21.6	0.0	100.0
	Mineral lick	115	31.1	0.0	100.0

APPENDIX V. Practice of herd records keeping in smallholder dairy farms (n = 370) in Gasabo district, Rwanda

Variables	Frequency	Percentage (%)
Practice of record keeping		
Incomplete records	91	24.7
Not practicing	279	75.3
Types of records kept		
Animal health	10	2.7
Milk production	37	10.0
Breeding practice	21	5.7
Sales and purchases	7	1.9
Birth and deaths	10	2.7
Livestock inventory	6	1.6
How kept		
Loose paper	17	18.7
Record book	74	81.3

APPENDIX VI. Housing of cows, feeding and drainage system in the study area

Cow in cowshed with bedding materials **(A)** and without bedding materials **(B)**

Cow kept in dirty cowshed **(C)**

Cow kept in a clean cowshed **(D)**

A cow kept in concrete-floored **(E)** and cows housed in earthen-floored (**F** and **G**)

A dairy farmer carries fodder on his head **(H)**.

Cow feeding cut and unchopped Napier grass **(I)**. Cows are reared using family residential compounds.

Poor waste drainage system on the study farms that resulted in accumulated manure and urine **(J)**.

APPENDIX VII. Water sources and watering frequency of dairy cows in smallholder zero-grazed farms in Gasabo district, Rwanda

Variables	**Level**	**Frequency**	**Percentage**
Source of water	Tap water	292	78.9
	Shallow well	42	11.4
	Lake	36	9.7
Frequency of watering	Once per day	100	27.0
	Twice a day	225	60.8
	Ad libitum	45	12.2

APPENDIX VIII. Influence of endometritis on reproductive performance of zero-grazed dairy cows (ANOVA tables)

Influence on days to first oestrus

Source	SS	DF	Msq	F	P-value
Model	32361.9	3	10787.3	6.0	0.001
Error	486208.0	271	1794.1		
Corrected total	518569.9	274			

Influence on days-not pregnant (interval calving to conception)

Source	SS	DF	Msq	F	P-value
Model	41108.8	2	20554.4	15.1	0.001
Error	193313.4	142	1361.4		
Corrected total	234422.1	144			

Influence on days to first natural mating or AI service

Source	SS	DF	Msq	F	P-value
Model	42411.4	3	14137.1	8.7	0.001
Error	422330.1	256	1649.7		
Corrected total	464741.5	259			

Effects of different categories of endometritis status on days-not pregnant

Source	SS	DF	Msq	F	P-value
Model	42878.5	2	21439.3	16.4	0.001
Error	185419.3	142	1305.8		
Corrected total	228297.3	144			

APPENDIX IX. Influence of endometritis on milk yield in zero-grazed dairy cows (ANOVA tables)

Milk yield discarded due to treatment of clinical endometritis positive cows

Source	SS	DF	Msq	F	P-value
Model	12494.1	4	3123.5	8.3	0.001
Error	37360.8	99	377.4		
Corrected total	49854.9	103			

Daily milk yield among healthy, treated and untreated cows

Source	SS	DF	Msq	F	P-value
Model	188.8	2	94.4	8.9	0.001
Error	4805.4	458	10.5		
Corrected total	4994.2	460			

Effect of different categories of endometritis status at 21-60 days postpartum on milk yield

Source	SS	DF	Msq	F	P-value
Model	162145.1	3	54048.4	5.7	0.001
Error	4332607.0	457	9480.5		
Corrected total	4494752.1	460			

Effect of days postpartum on milk yield

Source	SS	DF	Msq	F	P-value
Model	437.1	39	11.2	1.0	0.416
Error	4557.1	421	10.8		
Corrected total	4994.2	460			

APPENDIX X. Best-Worst Scaling choice method design for comparison choice cards that displays five management interventions per choice card

Version	BWS choice cards	Management interventions (codes)				
1	1	7	12	1	5	20
1	2	16	9	8	13	3
1	3	2	19	6	15	10
1	4	18	4	14	11	17
2	5	1	13	19	11	16
2	6	10	8	20	17	3
2	7	18	2	5	9	15
2	8	4	12	6	7	14
3	9	13	10	12	18	6
3	10	8	11	7	15	17
3	11	4	20	16	2	1
3	12	19	9	14	3	5
4	13	12	11	2	3	15
4	14	6	9	17	1	14
4	15	13	5	4	8	19
4	16	10	16	7	18	20

APPENDIX XI. Final model for outcome variable DNP, parameter β, standard error, and probability for predictor variables

Predictor variables	**Standardised coefficient (β)**	**S.E.**	**P-value**
Intercept	190.5	30.6	0.001***
BCS (<3 vs. ≥3)	−1.7	6.1	0.779^{NS}
Mastitis (positive vs. negative)	−4.6	4.4	0.298^{NS}
Parity (primiparous vs. multiparous)	0.8	4.9	0.866^{NS}
Cow age (<5 vs. ≥5)	2.5	5.8	0.670^{NS}
Retained placenta (occurrence vs. non-occurrence	12.5	9.3	0.182^{NS}
Endometritis (positive vs. negative)	21.3	5.8	0.001***
Season of calving (rainy vs. dry)	−3.4	5.9	0.562^{NS}
Dystocia (occurrence vs. non-occurrence)	2.4	4.7	0.614^{NS}
Cow breed (improved vs. indigenous)	1.2	4.9	0.806^{NS}
Daily milk yield (high vs. low)	−0.1	0.7	0.938^{NS}
Anoestrus postpartum (yes vs. no)	64.1	5.1	0.001***
Stillbirth (Occurrence vs. non-occurrence)	6.4	7.9	0.422^{NS}

^{NS} not significant (p>0.05), ***p<0.001, SE = Standard Error

APPENDIX XII. Effect of significant variables on days-not pregnant

Predictor variables	**Days-not pregnant**, Mean±SEM	P-value
Endometritis		***
Positive	104.1±5.0	
Negative	69.5±3.9	
Anoestrus postpartum		***
Yes	134.1±4.2	
No	64.0±2.5	

***p<0.001.

Model		Cases, % (n)	Univariable logistic regression analysis			Multivariable logistic regression analysis			Goodness-of-fit test (Hosmer and Lemeshow Test)		
Dependent	**Independent**		**OR**	**95% CI**	**P value**	**OR**	**95% CI**	**P value**	**Chi-square**	**df**	**Sig.**
Stillbirth	Calf sex*										
	Male	10.3 (213)	2.5	1.2-5.4	0.012*	2.6	1.2-5.6	0.011	0.021	2	0.989
	Female	4.3 (253)	Ref								
	Parity*										
	Primiparous	9.9 (161)	1.9	0.9-3.8	0.081*	1.9	0.9-4.1	0.041			
	Multiparous	5.6 (305)	Ref								
	Twin birth										
	Yes	25.0 (4)	4.5	0.5-44.3	0.200*						
	No	6.9 (462)	Ref								
	BCS*										
	≥3	8.1 (62)	1.2	0.4-3.2	0.746						
	<3	6.9 (404)	Ref								
	Dystocia*										
	Occurrence	7.0 (299)	0.9	0.5-2.0	0.948						
	Non-occurrence	7.2 (167)	Ref								
Cleanliness of cowshed	Housing cows within their first 30dpp*										
	Absence	20.7 (363)	0.1	0.0-0.5	0.008*	0.1	0.0-0.6	0.013	0.015	1	0.902
	Presence	71.4 (7)	Ref								
	Cowshed flooring*										
	Earthen	18.3 (295)	0.4	0.2-0.7	0.003*	0.4	0.2-0.8	0.005			
	Concrete	34.7 (75)									
	Calving pen*										
	Absence	20.3 (355)	0.2	0.1-0.6	0.005*						
	Presence	53.3 (15)	Ref								
	Bedding materials*										
	Not using	18.5 (135)	0.7	0.4-1.3	0.273						
	Using	23.4 (235)	Ref								
	Dairying experience*										
	≥8	20.5 (171)	1.1	0.8-1.9	0.617						
	<8		Ref								

APPENDIX XIII. Example of interrelationship among hypothesised risk factors

	22.6 (199)

*Variables subjected to multivariable logistic regression analysis

APPENDIX XIV. Correlation matrix of variable included in the final multivariable logistic regression for clinical endometritis at herd-level

Variable	Bedding materials	Cleanliness of cowshed	Housing of cows within the first 30 dpp	Herd size	Cowshed flooring types	Calving pen
Bedding materials	1					
Cleanliness of cowshed	0.06	1				
Housing of cows within the first 30 dpp	−0.10	0.17**	1			
Herd size	0.37**	0.02	−0.04	1		
Cowshed flooring types	−0.44**	0.16**	0.08	−0.22**	1	
Calving pen	−0.10	0.16**	0.58**	−0.07	0.10	1

*Variables subjected to multivariable logistic regression analysis

**p<0.01

*There is no evidence of any correlation among hypothesised risk factors

APPENDIX XV. Authorization for conducting research in Gasabo district, Rwanda

Gasabo, 19/04/2018
Ref.No: 623/07.0103/2018

REPUBLIC OF RWANDA

CITY OF KIGALI
GASABO DISTRICT
Website : www.gasabo.gov.rw
Email : info@gasabo.gov.rw
P O BOX 7066 KIGALI

Dr. NYABINWA Pascal
TEL: 0785324631
KIGALI

RE: Response to your request.

Dear Pascal,

Reference is made to your letter of 05th April, 2018, requesting for conducting a PhD research in Gasabo District;

Due to your expertise in animal sciences; we therefore give you permission to conduct your research by assessing prevalence of endometritis and associated influence on prevalence of smallholder zero-grazed dairy cows in Gasabo District.

Sincerely,

MBERABAHIZI R. Chretien
V/M
DES AFFAIRES ECONOMIQUES

AKARERE KA GASABO

for.

RWAMULANGWA Stephen
Mayor of Gasabo District

CC:
-His Worship the Mayor of City of Kigali;
-Executive Secretary of Sector (All).
KIGALI

Website: www.gasabo.gov.rw, E-mail: info@gasabo.gov.rw, P.O Box 7066 Kigali

APPENDIX XVI. Ethical clearance for conducting research in Gasabo district, Rwanda

REPUBLIC OF RWANDA
RWANDA AGRICULTURE AND ANIMAL RESOURCES DEVELOPMENT BOARD
Office of the Director General
KIGALI-RWANDA

Kigali, on..17....../.....05.../2018
Ref: N°01.11/...928.../018/PK/HQ

Mr. Pascal Nyabinwa
Tel: +250 785324631
E-mail: nyabpass@gmail.com
KIGALI

Dear Nyabinwa,

Re: Ethical Clearance for your research « ***Assessing Prevalence of Endometritis and Associated Influence on Performance of Smallholder Zero-Grazed Dairy Cows in Rwanda*** **».**

Reference is made to your letter of 17th April, 2018 requesting for Ethical Clearance regarding the above subject matter;

I would like to inform you that your request for the ethical clearance for the PhD project entitled ***"Assessing Prevalence of Endometritis and Associated influence on performance of Smallholder Zero-Grazed Dairy Cows in Rwanda"*** has been approved.

You are recommended to start data collection and to follow the approved protocol during the period of your research. Any amendment should be communicated to the RAB for review and approval. You are requested to submit the report to the RAB upon completion of the research.

I look forward to a successful implementation of the Project, and wish you good luck in these endeavors.

Yours sincerely,

Patrick Karangwa (PhD)
Director General

P.O.BOX: 5016 KIGALI *Website:www.rab.gov.rw*

APPENDIX XVII. Authorization of conducting laboratory work in the School of Veterinary Medicine, University of Rwanda.

UNIVERSITY OF RWANDA

COLLEGE OF AGRICULTURE, ANIMAL SCIENCES AND VETERINARY MEDICINE (CAVM)

OFFICE OF THE PRINCIPAL

Busogo, ..05.../.10../2018
REF : CAVM/...366.../18

To
The Director General
Rwanda Agriculture Board
Kigali

Dear Director General,

Re: Response to your letter

I hereby acknowledge receipt of your letter RAB DG Ref: No 01.11/1169/018/PK/HQ requesting for authorization to Dr. Pascal NYABINWA to conduct the laboratories work of his PhD Research Project in the School of Veterinary Medicine (SVM) Labs.

I am delighted to inform you that the College does not have objection to your request which is supported by the recent signed MoU between MINAGRI and the HLIs operating in Rwanda. However, you have to bring your own consumables.

We are recommending the researcher to liaise with the Head of Campus who is also the Dean of the School of Veterinary Medicine (Dr. Martin NTAWUBIZI: Tel: 0785839926 and e-mail martin.ntawubizi@gmailcom) for facilitation.

Sincerely,

Dr. Laetitia NYINAWAMWIZA
Principal, UR-CAVM
Cc :

- Head of Nyagatare Campus
- Director of Operations, **Nyagatare Campus**
- Dr. Pascal NYABINWA, **RAB-Kigali**

EMAIL: principal.cavm@ur.ac.rw P.O. Box: 210 Musanze, Rwanda WEBSITE: www.ur.ac.rw

APPENDIX XVIII. Abstract of Published Paper on Objective One of this Thesis

Tropical Animal Health and Production
https://doi.org/10.1007/s11250-020-02337-z

REGULAR ARTICLES

Estimating prevalence of endometritis in smallholder zero-grazed dairy cows in Rwanda

Pascal Nyabinwa[1,2] · Olivier Basole Kashongwe[1] · Jean Paul Habimana[3] · Claire d'Andre Hirwa[2] · Bockline Omedo Bebe[1]

Received: 19 August 2019 / Accepted: 17 June 2020

Abstract

Endometritis is a postpartum uterine disease of cows that interrupts reproductive cycles resulting in suboptimal fertility, reduced performance and profitability of the dairy herd. The objective of the study was to estimate the perceived and observed prevalence of endometritis among zero-grazed dairy cows in smallholder farms in Rwanda. A snowball sampling method was applied in cross-sectional survey to obtain data from 370 farms on 466 cows within their 21–60 days postpartum (dpp). The survey, conducted from September 2018 to March 2019, simultaneously examined cows using the Metricheck Device (MED) to determine the presence and type of vaginal mucus (VMC) based on a score scale of 0 to 3. Cows scoring VMC ≥ 1 were recorded as clinical endometritis (CLE)–positive. Cytotape (CYT) was used to determine the percentages of polymorphonuclear cells (PMN) in endometrial cytology sample. Cows with $\geq 5\%$ PMN were recorded subclinical endometritis (SCLE)–positive, whereas cows with VMC-0 and $< 5\%$ PMN were considered healthy cows. At cow-level, endometritis prevalence was 70.2% with 67.2% CLE and 31.8% SCLE while at the herd-level, prevalence was 71.1% with 68.1% CLE and 34.4% SCLE. The differences between the diagnostic performance of the MED and CYT were significant ($p < 0.001$). Perceived prevalence by farmers was much lower (3.2%) and without agreement with the observed prevalence (kappa = −0.02, $p > 0.05$). The highly observed prevalence and farmer underestimation of endometritis prevalence indicate knowledge gaps about endometritis. The extension service therefore needs to increase awareness and education among smallholder farmers about detection and management of endometritis.

Keywords Crossbreds · Cytotape · Dairy cows · Exotic breeds · Metricheck · Rwanda

Introduction

Endometritis is a prevalent disease in postpartum cows that may occur in the form of clinical endometritis (CLE) and/or subclinical endometritis (SCLE). The disease can lead to substantial economic losses in a dairy herd (Dubuc et al. 2010). The CLE is characterized by presence of mucopurulent or purulent uterine discharge detectable in the vagina 21–90 days postpartum (dpp) (Potter et al. 2010; Tayebwa et al. 2015). The CLE may be diagnosed using the Metricheck Device (McDougall et al. 2007); vaginoscopy (Leutert et al. 2012) or ultrasound (Barlund et al. 2008). In contrast, the SCLE is characterized by abnormal presence of the proportion of polymorphonuclear cells (PMN) in endometrial cytology samples collected between 21 and 90 dpp (Kasimanickam et al. 2005; Pascottini et al. 2017). The diagnostic techniques of SCLE include cytotape (Pascottini et al. 2015), cytobrush (Madoz et al. 2014) or low-volume uterine lavage (de Boer et al. 2014).

Both CLE and SCLE have been diagnosed separately between 21 and 90 ddp (Potter et al. 2010; Pascottini et al. 2017), and in most SCLE studies, CLE was used as an exclusion criterion for cows enrolled in the study (de Boer et al. 2014; Kasimanickam et al. 2005). Few studies have considered diagnosing both CLE and SCLE simultaneously, yet this would provide better accuracy in identifying cows with endometrium

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[3] School of Animal Sciences and Veterinary Medicine, University of Rwanda, P. O. Box 57, Nyagatare, Rwanda

Published online: 25 June 2020

APPENDIX XIX. Abstract of Published Paper on Objective Two of this Thesis

Nyabinwa *et al. BMC Veterinary Research* (2020) 16:175
https://doi.org/10.1186/s12917-020-02368-6

BMC Veterinary Research

RESEARCH ARTICLE Open Access

Perception of farmers about endometritis prevention and control measures for zero-grazed dairy cows on smallholder farms in Rwanda

Pascal Nyabinwa[1,2*], Olivier Basole Kashongwe[2], Claire d'Andre Hirwa[1] and Bockline Omedo Bebe[2]

Abstract

Background: Endometritis is a prevalent uterine disease in postpartum cows. The disease reduces fertility performance and milk yield, and subsequently, productivity and profitability of dairy farms. The reduction in performance is associated with considerable economic losses on dairy farms. Smallholder farmers are likely to incur considerable economic losses from the disease where they lack knowledge of effective prevention and control measures for the disease. This study used farmer's perspectives to determine the effectiveness of different management interventions (MIs) for endometritis prevention and control on smallholder farms in Rwanda practicing dairy zero-grazing. The best-worst scaling (BWS) choice method was applied that relied on past 1 year recall data obtained from 154 farmers. These farmers were identified through snowball sampling in a cross-sectional study.

Results: Of the 20 MIs evaluated, 12 scored highly for effectiveness. The top four most effective are: avoiding sharing equipment with neighbouring farms (45.5%), consulting animal health service provider about disease treatment (31.8%), keeping cows in a clean and dry shed (26.7%), and selecting sires based on calving ease (26.6%). The MIs considered least effective were: maintaining clean transition cow housing (35.1%), removal of fetal membrane immediately after passing (33.1%), disinfecting the equipment used in calving assistance before and after use (32.5%), and selecting sires with low percent stillbirths (29.2%).

Conclusion: This study has demonstrated the application of BWS object case method in understanding the MIs that farmers consider are most effective in the prevention and control of endometritis disease in the dairy herds. The MIs are on-farm biosecurity and hygiene, seeking veterinary services for disease treatment and selecting sires for ease of calving. These MIs should be considered for prioritization in extension services and research to continuously improve and enhance their practical application on smallholder dairy farms.

Keywords: Herd health, Best-worst scaling choice, Extension messages, Management interventions, smallholder farmers

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APPENDIX XXII. Abstract of published Paper on Objective Three of this Thesis

Preventive Veterinary Medicine 188 (2021) 105252

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Risk factors associated with endometritis in zero-grazed dairy cows on smallholder farms in Rwanda

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ABSTRACT

Clinical endometritis (CLE) and subclinical endometritis (SCLE) manifesting at the cow- and herd-levels has been associated with multiple risk factors (RFs), but hardly are RFs with direct influences separated from those with mediated indirect influences. This study identified and quantified the direct and indirect associations of cow- and herd-levels RFs with CLE and SCLE cases observed among 466 zero-grazed dairy cows that were in their 21–60 days postpartum (dpp). The cases were observed in a cross-sectional survey of smallholder farms (n = 370) in Rwanda. The direct and indirect associations were constructed with odds ratio (OR) derived from multiple logistic regression modelling. The cow-level RFs that had direct positive association with CLE and SCLE were the season of calving (OR: 5.0, 2.1), dystocia (OR: 1.9, 2.2), poor body condition score (OR: 4.1, 2.2), stillbirth (OR: 3.5, 3.3), and retained placenta (OR: 1.4, 1.8) while mastitis (OR: 2.5) and parity (OR: 1.5) had a direct positive association with SCLE. Breed and parity of cow, sex of calf, and twin births had indirect positive association with both CLE and SCLE cases. At the herd-level, unhygienic cowshed (OR: 25.1, 8.9) had direct positive association with both CLE and SCLE cases. In contrast, earthen floor cowshed (OR: 6.6) and large herd size (OR: 3.1) had direct positive association with CLE and not using bedding materials (OR: 1.5) had direct positive association with SCLE. Herd-level RFs that showed indirect positive association with both CLE and SCLE cases were farm size (OR: 2.9) and farmer's experience in dairying (OR: 1.7) while housing cows within the first 30 dpp (OR: 0.1) showed indirect negative association. These results show which RFs have strong direct and indirect influences on CLE and SCLE cases at the cow- and herd-levels. Effective management of those RFs should be a priority in extension education and services to enable smallholder farmers effectively manage them to prevent and control endometritis among their zero-grazed dairy cows.

1. Introduction

Endometritis is an important postpartum uterine disease of economic importance in dairy cows. The disease disrupts cows' fertility performance and reduces dairy herd productivity and profitability (Chaudhari et al., 2017; Sharma et al., 2019). The disease may manifest as clinical endometritis (CLE) and/or subclinical endometritis (SCLE) between 21st and 90th days postpartum (dpp) period (Okawa et al., 2017; Kelly et al., 2020). The CLE is characterized by presence of mucopurulent or purulent uterine discharge detectable in the vagina between 21 and 90 dpp (Potter et al., 2010; Tayebwa et al., 2015). In contrast, the SCLE is characterized by abnormal presence of the proportion of polymorphonuclear inflammatory cells (≥ 5%) in endometrial cytology samples collected in the period 21–90 dpp (Kasimanickam et al. 2005; Pascottini et al. 2017). In diagnosis of the endometritis disease, Okawa et al. (2017) was successful with diagnosis of the disease between 21–60 dpp, whereas Kelly et al. (2020) did diagnose the disease between 25 and 86 dpp. These are evidences that CLE and SCLE cases can be effectively diagnosed between 21 and 90 dpp period.

The prevalence of CLE and SCLE show large variation at the cow and herd levels as well as in smallholder and large dairy herds. At the cow-level, the SCLE prevalence has varied from 6.7 to 89.0 % for cows examined between 25 and 86 dpp (Denis-Robichaud and Dubuc, 2015; Kelly et al., 2020), and CLE prevalence has ranged from 3.6 to 69.8 % for cows examined between 33 and 60 dpp (McDougall et al., 2007; Tayebwa et al., 2015). At the herd-level, the SCLE prevalence has varied

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Effects of endometritis on reproductive performance of zero-grazed dairy cows on smallholder farms in Rwanda

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A R T I C L E I N F O

Keywords:
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Days-open
Fertility performance
Conception rate

A B S T R A C T

Endometritis is a prevalent post-partum uterine infection in dairy cows resulting in suboptimal reproductive performance. The hypothesis was that endometritis status is associated with suboptimal reproductive performance of postpartum dairy cows managed under zero-grazing feeding practice on smallholder farms. In this study, there was evaluation of effects of endometritis diagnosed at 38.5 ± 14.7 days postpartum (dpp) on subsequent reproductive performance. Reproductive performance of 436 cows from 345 farms was recorded for 210 dpp. Values for reproductive performance indicators were less ($P < 0.05$) in cows determined to be positive compared to negative for endometritis. Cows that tested positive, as compared to negative, for endometritis had longer periods after parturition until initiation of oestrous cycles (median, interquartile range; 85.0, 57.5–127.0 and 62.6, 49.0–90.0 days, respectively), longer durations before being detected pregnant (95.5, 61.8–145.5 and 63.0, 50.0–83.0 days, respectively), lesser pregnancy rates as a result of the first breeding postpartum (16.5% and 32.7%, respectively), more natural-mating or artificial inseminations per pregnancy (1.3 ± 0.1 and 1.1 ± 0.0, respectively) and more occurrences of anoestrus postpartum (48.4% and 11.7%, respectively). These results provide evidence of a strong association between endometritis and suboptimal fertility performance in zero-grazed cows on smallholder farms in Rwanda. Considering there were 70.2% of cows in the present study were diagnosed with endometritis, this is indicative of a widespread herd health issue, warranting that field veterinary practitioners prioritise endometritis in their herd health service delivery to smallholder dairy farmers for effective disease management and herd sustainability.

1. Introduction

Endometritis is a prevalent uterine disease of dairy cows between the periods extending from 21 to 90 days postpartum (dpp). The prevalence can be as great as 89.0% in some herds between 21 and 90 dpp (Denis-Robichaud and Dubuc, 2015; Pascottini et al., 2015). The disease results in suboptimal fertility (Mohammed et al., 2019). Optimal fertility is an important component of production efficiency in a dairy herd (Kim and Jeong, 2019). In herd fertility management, the extension service recommends farmers to attain a herd average calving interval of about 12 months (Rukundo et al., 2018). Though a 12-month calving interval is a general herd fertility

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Influence of endometritis on milk yield of zero-grazed dairy cows on smallholder farms in Rwanda

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ABSTRACT

Endometritis being a post-partum uterine infection in dairy cows is likely with substantial production loss through reduction in milk yield (MY), discarded milk during treatment and withdrawal period, and increased cost of veterinary treatment. This study quantified the influence of endometritis on MY of zero-grazed dairy cows managed on smallholder farms in Rwanda. The study enrolled a total of 461 cows within their 21 to 60 days in milk to examine for clinical endometritis (CLE) and subclinical endometritis (SCLE). A cow was considered having endometritis if it was positive for at least one test (CLE or SCLE), otherwise was negative. The MY data were collected prospectively from endometritis positive and negative cows for 30-day post-endometritis diagnosis. Compared to cows negative for endometritis, the positive endometritis cows were 2.4 times more (29.7 vs. 70.3%) with daily MY 15.3% lower (7.5 ± 0.2 vs. 8.9 ± 0.3 litres; $p<0.05$), representing a reduction of 1.4 ± 0.2 litres of milk/cow/day. Of the CLE positive cows, 33.4% (104/311) were treated using different veterinary drugs, which resulted in 23.5% more discarded milk compared ($p<0.05$) to untreated positive cows. Discarded milk was higher ($p<0.05$) among cows treated with oxytetracycline (65.9 ± 4.4 litres) compared to cows treated with procaine penicillin G and dihydrostreptomycin (35.5 ± 2.7 litres). The percentage of total milk loss was much higher (45.6%) among CLE positive cows that received treatment compared to the untreated cows (16.3%). These results demonstrate a strong association between MY loss and endometritis. A timely diagnosis and treatment of the disease is recommended using conventional veterinary drugs that have zero withholding time for milk to reduce the MY loss and associated economic loss, estimated at 154 US$ in a lactation.

1. Introduction

Endometritis is a uterine disease of dairy cows occurring from 21 days in milk (DIM) (Pascottini, Hostens, Sys, Vercauteren & Opsomer, 2017). The disease may be clinical endometritis (CLE) often characterized by vaginal purulent or mucopurulent contents (Eslami, Bolourchi, Seifi, Asadi & Akbari, 2015) or subclinical endometritis (SCLE) characterized by the presence of ≥5% of polymorphonuclear cells in endometrial cytology sample (Pothmann et al., 2015). Endometritis is commonly associated with decreased milk yield (MY) and discarded milk, impaired reproductive performance, increased culling rates, additional costs for drugs, and veterinary services. These are production losses representing the loss of milk supply, a stream of incomes, and other livelihood benefits of dairy to producers (Juan Piñeiro, 2016; Kumar & Purohit, 2019).

The prevalence of endometritis in dairy cows can vary widely, from as low as 3.6% observed in Uganda (Tayebwa, Bigirwa, Byaruhanga & Kasozi, 2015) to as high as 89.0% observed in Canadian dairy herds (Denis-Robichaud & Dubuc, 2015). The large variation in prevalence suggests that some farms experience substantial production loss from the disease, depending on the management of the disease (Lima, 2018). Yet estimates of the prevalence and associated losses are scarce in smallholder dairy farms that derive a livelihood from dairying. This knowledge gap impedes informed decision-making on effective management practices for endometritis in the dairy herds. Moreover, loss in milk arising from a decrease in the yields and discarded milk after treatment are a direct component of production loss (Sharma, Madhumeet, Kumar & Dogra, 2019), yet accurate estimates of the loss are rare,

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APPENDIX XXIVIII. Poster Presentation on Objective One of this Thesis at 13th Egerton University International Virtual Conference (24th -26th November 2020)

Prevalence of Endometritis in Smallholder Zero-Grazed Dairy Cows in Rwanda

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Introduction

Endometritis is a postpartum uterine disease of cows occurring between 21st and 90th days postpartum (dpp). The disease may occur in the form of clinical endometritis (CLE) and/or subclinical endometritis (SCLE) and interrupts reproductive cycles resulting in suboptimal fertility, reduced performance and profitability of the dairy herd. The prevalence of endometritis in dairy cows can be as great as 89.0% in some herds between 21 and 90 dpp. However, farmers are likely to underestimate the disease in their dairy herds because they have limited knowledge and skills to diagnose the disease. In contrast, literature on endometritis was limited from smallholder farms that manage cows under zero-grazing conditions.

In Rwanda, smallholder zero-grazing dairy farming holds the majority (92.0%) of the cattle population and is a prioritised development intervention for rural farming households, but reproductive and productive performance of the zero-grazed dairy cows is suboptimal. This system is characterised by prevalent muddy conditions, low hygienic standards, weak and inconsistent herd health management plan, and a high risk exposure to bacterial disease. These conditions present risks for prevalent endometritis infections. The objective of the study was to estimate the perceived and observed prevalence of endometritis among zero-grazed dairy cows in smallholder farms in Rwanda.

Materials and Methods

- Data were collected in a cross sectional study conducted in 370 smallholder dairy farms recruited through a snowball sampling method. All the farms were located in Gasabo District, Rwanda.
- Four hundred sixty-six (466) dairy cows within 21-60 dpp at sampling were enrolled.
- The breed distribution of the sample cows was 66.3% dairy crossbreds, 17.0% dairy pure breeds and 16.7% indigenous cattle.
- An explanation of clinical signs of endometritis was presented to farmers to help them to estimate the number of cows positive for endometritis recognized in their herds in the past one year and on the day of the herd visit.
- CLE was diagnosed at 38.5±0.7 dpp using Metricheck Device (MED) by evaluating the presence and type of vaginal mucus (VM) and scored on a 0 to 3 scale (Figure 1), whereas SCLE was diagnosed using Cytotape (CYT) by analyzing endometrial cytology samples to evaluate the proportion of polymorphonuclear inflammatory cells (PMN) (Figure 2).

Figure 1: Top and bottom left: The VMC was retrieved with MED, top middle: VMC-1, bottom middle and right: VMC-3.

Figure 2: Top left: Endometrial cytology sampling, Top middle: Rolling CYT over a microscopic slide, Top right: Slides were stained with Differential Quik Stain Kit, Bottom left: Evaluation of PMN and endometrial cells (ENDOC) under microscope mounted with a camera, Bottom middle: Evaluation at x100 magnification, Bottom right: Evaluation at x400 magnification.

- Cows that had VMC score of ≥1 were recorded CLE positive , otherwise recorded CLE negative. Similarly , a herd was considered positive for CLE if had at least one cow positive for CLE.
- Cows that had ≥5% PMNs were considered as SCLE positive, otherwise considered SCLE negative. A herd was considered positive for SCLE if had at least one cow positive for SCLE.
- Statistical analysis was carried out using SPSS 22.0. Diagnostic performance tests setting screening test with MED and confirmatory test with CYT (Table 1) were used to compute sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), observed and overall prevalence.

Table 1. Contingency table of association between Metricheck Device and Cytotape

Diagnosis of endometritis		Metricheck Device	
		Positive	Negative
Cytotape	Positive	a	b
	Negative	c	d

$Se = \frac{a}{a+c} \times 100$ $Sp = \frac{d}{b+d} \times 100$ $PPV = \frac{a}{a+b} \times 100$ $NPV = \frac{d}{d+c} \times 100$

- The observed and perceived prevalence was compared using Wilcoxon signed-rank test (Z) and their agreement was tested using kappa statistic (k). The level of significance was set at Alpha < 0.05.

Findings

- This study revealed that less than half of farmers (41.6%) could recognize some of clinical signs of endometritis in cows in their herds for the past one year. The most commonly recognised clinical sign of endometritis was repeat breeding (43.5%) followed by abortion (24.7%), mucopurulent or purulent discharge visible at the time of oestrus (18.8%), and white or whitish-yellow mucopurulent vaginal discharge (13.0%) though they could not solely attribute them to endometritis.
- The overall prevalence of endometritis at cow-level was 70.2% with higher CLE (67.2%) than SCLE (31.8%) (Table 2).

Table 2. Observed prevalence of endometritis in smallholder zero-grazed cows at 38.5 ± 0.7 dpp

Prevalence of endometritis		CLE, % (n)	SCLE, % (n)	Overall, % (n)	Chi-square test
Overall	Herd-level	68.1 (252)	34.9 (129)	71.1 (263)	***
	Cow-level	67.2 (313)	31.8 (148)	70.2 (327)	***
Cow breed	Indigenous	19.2 (60)	19.6 (29)	19.6 (64)	NS
	Crossbreds	60.7 (190)	58.1 (86)	60.9 (199)	***
	Pure	20.1 (63)	22.3 (33)	19.6 (64)	***

NS = not significant (p>0.05); ***p<0.001, CLE = clinical endometritis, SCLE = subclinical endometritis

- At cow-level, farmer perceived prevalence of endometritis (3.2%) was much lower than the observed prevalence (67.2%) and had poor agreement (k =0.03, p<0.05) (Table 3).

Table 3. Relationship between the prevalence of endometritis recorded by a researcher and the prevalence of endometritis reported by the farmer.

Endometritis	Cow-level, % (n)	Herd-level, % (n)
Prevalence reported by farmer	3.2 (15)	4.1 (15)
Prevalence recorded by researcher	67.2 (313)	68.1 (252)
Kappa statistic	*0.03**	*0.02[NS]*
Wilcoxon signed-rank test	*-17.5****	*-15.3****

[NS] not significant (p >0.05); *p<0.05, ***p<0.001

- The differences between the diagnostic performance of the MED and CYT were significant (p<0.001) (Table 4).

Table 4. Diagnostic performance of the Metricheck Device and Cytotape

Diagnostic Technique	Diagnostic performance criteria			
	Se	Sp	PPV	NPV
Cytotape	42.8	90.8	90.5	43.7
Metricheck Device	90.5	43.7	42.8	90.8
Chi-square test	***	***	***	***

***p<0.001, Se = sensitivity, Sp = specificity, PPV = positive predictive value, NPV = negative predictive value,

Conclusion

- Endometritis is a prevalent disease and a general herd health problem.
- The high-observed prevalence and farmer underestimation of endometritis prevalence indicate knowledge gaps about endometritis. The extension services, therefore, need to increase awareness and education among smallholder farmers about the detection and management of endometritis.

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APPENDIX XXVV. Poster Presentation on Objective Two of this Thesis at 13th Egerton University International Virtual Conference (24th -26th November 2020)

Farmer's Opinions on the Effectiveness of Management Interventions for Endometritis in Smallholder Dairy Farms in Rwanda

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Introduction

Endometritis is a prevalent uterine disease in postpartum cows. The disease reduces fertility performance and milk yield, and subsequently, productivity and profitability of dairy farms. The reduction in performance is associated with considerable economic losses on dairy farms. Smallholder farmers are likely to incur considerable economic losses from the disease where they lack knowledge of effective management interventions (MINTs) for the disease. This study used farmer's opinions to determine the effectiveness of different MINTs for endometritis prevention and control on smallholder farms in Rwanda practicing dairy zero-grazing.

Methodology

- The best-worst scaling (BWS) choice method was applied that relied on past 1 year recall data obtained from 154 farmers in Gasabo district of Rwanda.
- Farmers were identified through snowball sampling in a cross-sectional study.
- The BWS object case was applied to 20 MINTs considered important in the prevention and control of endometritis, based on the literature review (Table 1).

Table 1. List of 20 management interventions examined in the study

Management interventions (MINTs)	MINTs codes
Avoid equipment-sharing between cows within the farm	01
Avoid equipment-sharing with neighbouring farms	02
Avoid housing fresh cows with diseased cows or those with chronic illnesses such as mastitis	03
Avoid off-farm bedding materials and maintain adequate bedding materials per cow	04
Avoid sharing or hiring a breeding bull	05
Consult animal health service (ANHS) provider about the treatment and prevention of diseases such as mastitis and metabolic diseases	06
Consult ANHS provider about the treatment of endometritis positive cases	07
Cull of persistently endometritis positive cows	08
Disinfect equipments of calving assistance before and after use	09
Keep cows in a clean and dry shed	10
Maintain adequate feeding per cow	11
Maintain a clean transition cow housing	12
Maintain regular contact with ANHS providers for advisory support on endometritis prevention in dairy farm	13
Select sires based on calving ease	14
Select sires based on low percent stillbirths	15
Remove fetal membranes immediately after passing	16
Use gloves during calving assistance	17
Use artificial insemination service as a breeding method	18
Use sexed semen during artificial insemination service	19
Wash the hands and udder before each milking	20

- The check-list for BWS choice was designed in Sawtooth Software (Version 8).
- Each dairy farmer had to respond to a total of 16 choice cards of five MIs each.
- For each choice card, the farmer was asked to choose first the most effective and then the least effective MINTs for endometritis prevention and control.
- The effectiveness of different MINTs for endometritis prevention and control was qualitatively assessed by BWS method on a standardized score on a scale from -1.0 (indicates that the MINT was chosen as least effective more often than as most effective) to +1.0 (indicates that the MINT was chosen as most effective more often than as least effective).
- All statistical analyses were performed in IBM SPSS Statistics 22.0 for Windows.

Results

- Of all MINTs examined, 60.0% (n=12) were identified as most effective whereas 40.0% (n=8) as least effective for endometritis prevention and control on smallholder farms.
- The standardized scores were zero-centred and represent the computed effectiveness scores assigned to each MINT (Figure 1). The y-axis represents the effectiveness score of the MINTs.
- The MINTs located above 0 on the y-axis were chosen as most effective; whereas those with scores over 0 on the x-axis were chosen as least effective.

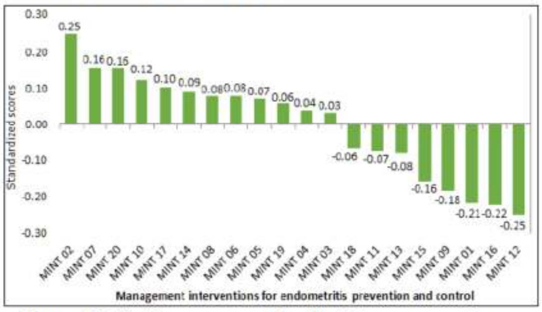

Figure 1. Effectiveness scores for the 20 management interventions for endometritis prevention and control

Conclusion

- BWS choice method informed appropriate MINTs that when effectively implemented would improve animal health and performance and herd profitability.
- Most effective MINTs should be considered for prioritization in extension services and research to continuously improve and enhance their practical application on smallholder dairy farms.

Acknowledgements

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APPENDIX XXVI. Cover Page of Practice Brief Nº 001: Preventing and controlling endometritis on smallholder dairy farms in Rwanda

Practice Brief Nº 001 for Extension Service use in Rwanda

Preventing and controlling endometritis on smallholder dairy farms in Rwanda

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Background

Dairy production is a major livelihood strategy for poor households in Rwanda and contributes 28.0% to the agricultural Gross Domestic Product (GDP) and 4.0% to the national GDP (NISR, 2018). Among the smallholder dairy farms, those practicing zero-grazing hold the majority (92.0%) of the cattle population and supply the bulk of the domestic milk market demand (IFAD, 2016). However, the supply has not satisfied the local demand. The per capita milk consumption of 63.0 litres is still far below the 220 litres recommended by the Food and Agriculture Organization of the United Nations (FAO, 2013). The low per capita milk consumption is to a large extent due to low productivity of the national herd, and this is attributable to suboptimal fertility performance of zero-grazed cows on smallholder farms (Rukundo *et al*., 2018). This suboptimal performance has been explained as resulting from poor management practices but without identifying the underlying specific cause(s) involved.

One likely area of management failure could be in managing uterine health, which is essential for success of breeding services. Cow uterine that is not healthy disrupts the uterine homeostasis and results in conception failures (Galvão *et al*., 2010). The zero-grazed cows on smallholder farms could be at risk of prevalent endometritis disease, a uterine disease in dairy cows occurring between the 21st and 90th days postpartum (dpp). To date, empirical evidence is yet to be presented in Rwanda to support the presence (or absence) of endometritis disease. The disease condition may occur in the form of clinical endometritis (CLE) and/or subclinical endometritis (SCLE). In positive cows, endometritis is associated with a substantial reduction in milk yield, discarded milk during treatment and withdrawal period and increased cost of veterinary treatment. In smallholder herds of Rwanda, these production losses are yet linked to endometritis disease, though could be a gross underestimation.

Good knowledge of the management interventions (MIs) effective for prevention and control of endometritis could be disseminated to farmers for consequent improvement of animal welfare, productivity and profitability of their dairy herds (Renault *et al*., 2017). In extension service delivery, farmers could be the missing link in implementing and evaluating the effectiveness of MIs (Renault *et al*., 2017). Their perception of which MIs are effective for endometritis prevention and control would be informative for managing endometritis disease in the dairy herds.

A study of prevalence of endometritis and associated influence on milk yield and control measures was implemented for zero-grazed dairy cows on smallholder farms in Gasabo district, Rwanda. Endometritis was diagnosed on 466 cows within their 21-60 dpp from 370 farms using the Metricheck Device (MED) to determine the presence and type of vaginal mucus (VMC) based on a score scale of 0 to 3. Cows scoring VMC≥1 were recorded CLE positive (Williams *et al*., 2005). Cytotape (CYT) was used to collect endometrial cytology samples in which the percentage of polymorphonuclear inflammatory cells (%PMN) was determined (Pascottini *et al*., 2015). Cows with ≥5% PMNs were recorded SCLE positive, whereas cows with VMC-0 and <5% PMNs were considered as healthy (Figure 1).

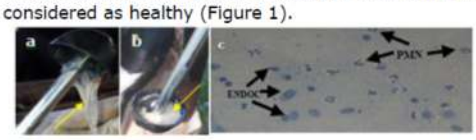

Figure 1. (**a**) VMC-2, (**b**) VMC-3 and (**c**) Microscopic evaluation of endometrial cytology slides at x400 magnification. *PMN: Polymorphonuclear inflammatory cells, *ENDOC: Endometrial cells.

Key messages

- Endometritis is a prevalent disease and a general herd health problem with positive cases in a sample of ten cows being seven (67.2%) for CLE and three (31.8%) for SCLE.
- Early detection and treatment of endometritis is critical in management of the disease.
- Identifying at-risk cows more likely to develop endometritis and infected cows and treating them is a good disease management practice.
- MED is a useful tool and cow-side diagnostic test to diagnose CLE.
- CYT is a good technique to diagnose SCLE but it is not a cow-side diagnostic test.
- Adequate feeding practices can reduce the associated risk factors for endometritis.
- Cows with endometritis can reduce milk yield by 1.4 litres/day and further 7.3 litres//day discarded milk due to treatment. This loss can be ameliorated using veterinary drugs with zero milk withholding period and non-antibiotic based treatments.
- Animal health service (ANHS) providers need include endometritis disease in their herd health programs that they deliver to smallholders for effective management of the disease

Policy recommendations

- Put in place strategic plan for endometritis prevention and control for smallholder dairy farmers
- Promote use of the alternative therapeutics management of endometritis and regulate the use of conventional veterinary drugs in postpartum dairy cows
- Invest in building technical (ANHS) and infrastructural (diagnostic) capacities for endometritis prevention and control in smallholder zero-grazed dairy farms.

APPENDIX XXVII. Cover Page of Practice Brief Nº 002: Endometritis in smallholder zero- grazed dairy cows in Rwanda: Risks and consequences on cow fertility performance

Practice Brief Nº 002 for Extension Service use in Rwanda

Endometritis in smallholder zero-grazed dairy cows in Rwanda: risks and consequences on cow fertility performance

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Background

Endometritis is a prevalent postpartum uterine disease of economic importance in dairy cows. The disease may manifest as clinical endometritis (CLE) and/or subclinical endometritis (SCLE) between period 21st and 90th days postpartum (dpp) (Nyabinwa *et al*., 2020a; Kelly *et al*., 2020). Both CLE and SCLE are related causes of infertility and subfertility in dairy cows, not only during the present infections but also after resolution of clinical signs of the disease (Plontzke *et al*., 2010). These reduce dairy herd productivity and profitability, thus contributing to suboptimal fertility performance and production efficiency in a dairy herd. The disruption of reproductive functions due to endometritis is an underlying cause for suboptimal fertility performance and suboptimal production efficiency in a dairy herd but is often unknown to farmers (Nyabinwa *et al*., 2020a, c, d).

Literature presents evidences of large variable prevalence at the cow-level for SCLE cases between 6.7% and 89.0% and for CLE cases between 3.6% and 69.8% (Denis-Robichaud and Dubuc, 2015). The variability in prevalence is also evident at the herd-level for SCLE being between 4.8% and 64.1% and for CLE being between 4.0% and 87.0% (Ryan *et al*., 2020). The large variation in the prevalence of CLE and SCLE at the cow- and herd-levels could be reflecting involvement of multiple risk factors (RFs), may be some with direct influences and others mediated indirect influences. In literature, the cow-level RFs reported include cow parity, body condition score, cow breed, twins, breeding services, retained placenta, dystocia, gestational length, days dry, left displaced abomasum, sex of the calf, calving season, stillbirth, brucellosis, mastitis, milk fever, and ketosis (Adnane *et al*., 2017; Boudelal *et al*., 2020). At the herd-level, the literature reports RFs that include hygiene conditions, herd size and farm size (Moges and Jebar, 2012). These RFs areapply in the smallholder herds as well and could be predisposing dairy cows to CLE and SCLE, yet have received little research attention.

In Rwanda, smallholder dairy farming accounts for 92.0% of the national dairy herd, predominantly managed under zero-grazing systems and characterised by suboptimal fertility performance and milk production (Rukundo *et al*., 2018; Manzi *et al*., 2019). A recent study of endometritis on smallholder herds in Rwanda (Nyabinwa *et al*., 2020a) shows that prevalence of endometritis is as high as 71.1% at herd-level and 70.2% at cow-level, with CLE reaching 68.1% at herd-level and 67.2% at cow-level. In comparison, SCLE is 34.4% at herd-level and 31.8% at cow-level. This suggests that there are great opportunities to manage the disease with a good understanding of the RFs involved.

Despite the high prevalence of endometritis in smallholder farms in Rwanda, present herd fertility interventions ignore targeting CLE and SCLE because empirical evidence is lacking for the presence of RFs involved and as to which ones of them pose high risks for CLE or SCLE. Furthermore, it is likely that endometritis could be an underlying cause of the prevalent suboptimal fertility performance subsequent to calving (Bishop and Pfeiffer, 2008; Rukundo *et al*., 2018) but empirical evidence is lacking. Such information would inform endometritis prevention and control plan (Nyabinwa *et al*., 2020b).

To address better understanding the RFs associated with CLE and SCLE in smallholder dairy herds, a study was designed that applied a path analysis model in estimating the RFs associated with CLE and SCLE cases in smallholder zero-grazed dairy cows in Rwanda. The study tested the hypothesis that endometritis status at 38.5±0.7 dpp is associated with direct and indirect RFs influences and that CLE and SCLE cases have a strong association with suboptimal reproductive performance subsequent to calving.

Key messages

- Endometritis has a strong association with cow suboptimal fertility performance
- Cows positive for endometritis have poorer reproductive performance compared to those free of endometritis
- CLE and SCLE represent different disease conditions of the reproductive tract with different effects on post calving fertility performance of cows
- Farmers can manage CLE and SCLE incidences with improved feeding of cows in their transition period and improved housing hygiene
- Good hygienic management practices include the use of clean, lubricated, gloved hands during an assisted calving
- Good hygiene practices may help reduce physical trauma and contamination of the genital reproductive tract to minimise exposure to CLE and SCLE cases
- Identification of high-risk cows in the transition period when early will inform early interventions and subsequently reduce economic losses from endometritis in the dairy herds

Policy recommendations

- Farmers, extension service, and animal health service providers all need increased awareness and education about endometritis disease
- Endometritis detection, diagnosis, prevention and control has to be prioritised in extension service and in the herd health service delivery to dairy farmers
- Offering adequate balanced feeding and housing hygiene to cows in the transition period is an effective management of herd health for endometritis disease