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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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particularly for smallholder dairy herds. Discarded milk is milk produced but not used for human consumption during treatment and withdrawal period because of the risk of antibiotic residuals and pathogen contamination (Mahnani, Sadeghi-Sefidmazgi & Cabrera, 2015). Several statistical methods have been used to evaluate the decrease in MY from diseases (Hadrich, Wolf, Lombard & Dolak, 2018; Lyons et al., 2015). The common method compares MY from diseased cows with healthy cows (Elnour Angara & Mohammed Elfadil, 2012; Can et al., 2016). Some few studies of the effect of endometritis on MY 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 MY decrease of between 0.6 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 (Kumar & Purohit, 2019). Conventional therapy and phytotherapy are used for the treatment of endometritis (Mandhwani, Bhardwaz, Kumar, Shivhare & Aich, 2017). The former is commonly and extensively used with different veterinary drugs (VD). These VD include antimicrobials (Ceftiofur hydrochloride, oxytetracycline, procaine penicillin G and dihydrostreptomycin, gentamicin, tetracycline hydrochloride and cephapirin) (Bartolome, Khalloub, de la Sota, Drillich & Melendez, 2014; Kasimanickam et al., 2005; Okawa et al., 2017; Pierre, 2010, Kumar & Purohit, 2019, Manimaran et al., 2019), non-steroidal anti-inflammatory drug (Phenylbutazone) (Tek et al., 2010); and hormones (Prostaglandins) (Ahmadi, Mogheiseh, Mirzaei, Nazifi & Fallah, 2018; Makki, Ahmadi, Gheisari & Nazifi, 2017). In the latter, different phytotherapeutic agents used include *Tinosporacordifolia, Withaniasomnifera, Curcuma longa, Ocimum sanctum, Allium sativum,* and *Azadirachtaindica* (Mandhwani et al., 2017; Bakare et al., 2020). According to Sharma, Srivastava, Kumar and Singh (2018) and Bakare et al. (2020), the use of herbal plants has many advantages such as no microbial resistance, simple modes of preparation and administration to animals, low treatment cost, 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 period (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 MY loss. 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 antibiotics 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 defense and repair (LeBlanc et al., 2002).

Rwanda is not any different regarding studies of the effect of endometritis on MY 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 Claire et al., 2017; MINAGRI, 2015). This suboptimal production has been explained as resulting from poor management but without identifying the underlying specific cause (s) involved. It is possible that zero-grazed dairy cows on smallholder farms could be at higher risk of endometritis infections. However, in Rwanda to date, empirical evidence remains not presented for the presence of endometritis and its influence on MY. This study quantified the effects of endometritis on MY of zero-grazed dairy cows managed under existing smallholder farming conditions in Rwanda.

2. Materials and methods

2.1. Data source

Data were collected from zero-grazed cows on smallholder dairy farms. All the farms were located in Gasabo District of Rwanda (1°52′S, 30°06′E), 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 (Shapiro, Gebru & Solomon Desta, 2017,). The total cattle population in the study area is about 12 414 comprising of indigenous cattle (29.0%), dairy crossbred (52.0%), and pure dairy breeds (19.0%) (NISR, 2018).

Data collection was prospective in observational study for 8 months running from September 2018 to April 2019. A total of 366 smallholder zero-grazing dairy farms were selected through a snowball sampling. Identification and access to the initial farms were facilitated by sector animal resources officers who provided at least two farms in the neighbourhood which the researcher visited. The farms had to fit defined study criteria: (i) having at least one cow within 21 and 60 DIM and not appeared sick, (ii) willingness of the farmer to record the MY within 30 days post-endometritis diagnosis, (iii) farm owner authorizing that the veterinarian treating the herd be contacted to verify VD being used for the treatment of endometritis and (iv) physical accessibility to the farm during the study period. An incentive to enrolled farms was free cow screening for mastitis and brucellosis and prescription for treatment whenever a positive case was detected.

For each recruited farmer, an explanation of the objectives of the study was presented and written informed consent was sought prior to starting data collection. Four hundred sixty-one (461) cows within their 21 to 60 DIM at sampling were enrolled and they comprised 16.7% indigenous cattle, 66.4% dairy crossbreds, and 16.9% dairy pure breeds.

The sample farms had an average herd size of 3.1 ± 1.5 cows with a range of 1 to5 cows and a median of 2 cows. Cows sampled on each farm ranged from 1 to 4 per farm with a median of 1 and the average DIM at the sampling was 38.5 ± 14.7 (median 35.0 DIM). 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).

All procedures performed involving animals were in accordance with the ethical standards of Rwanda Agriculture and Animal Resources Development Board (Ref: N^{o}. 01.11/928/018/PK/HQ of 17/05/2018). Written informed consent was obtained from all farmers participating in the study.

2.2. Data collection

2.2.1. Endometritis detection

On each farm visited, cows were examined for clinical endometritis (CLE) using vaginal mucus character (VMC) with the aid of a Metricheck device (McDougall, Macaulay & Compton, 2007) and for subclinical endometritis (SCLE) using endometrial cytological sample collected with Cytotape (Pascottini, Dini, Hostens, Ducatelle & Opsomer, 2015). Cows with VMC ≥1 were recorded as positive (Williams et al., 2005). Based on previously published work (De Boer et al., 2014; Madoz et al., 2013; Melcher, Prunner & Drillich, 2014; Rinaudo, Bernardi & Marini, 2017) PMN thresholds to define SCLE positive cows vary according to the timing of evaluation during the postpartum period. The threshold values of PMN for predicting SCLE positive case are 8% PMN for 21 to 33 DIM; 6% PMN for 34 to 47 DIM; and 4% PMN for 48 to 60 DIM. However, the PMN threshold for the overall period (21 to 62 DIM) is ≥5%

when the applied method counts 300 cells per endometrial cytology slide (Madoz et al., 2013; Melcher et al., 2014; Okawa et al., 2017; Chan Lee et al., 2018).

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 DIM with cows grouped as suffered from CLE only (VMC ≥1 and < 5% PMN), SCLE only (VMC-0 and ≥5% PMN), both CLE and SCLE (VMC ≥1 and ≥5% PMN) or cows without CLE and SCLE (VMC-0 and <5% PMN) (Gobikrushanth, Salehi, Ambrose & Colazo, 2016).

The data on daily MY, VD used in the treatment and socioeconomic characteristics of the farmers were obtained from observations and direct interviews with the farmers guided by a pre-tested structured questionnaire.

2.2.2. Milk yield recording

Under existing farm management conditions, MY 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 and evening). Daily MY 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 MY/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 MY recorded was the milk offtake, because calves were allowed to suckle their dams.

2.2.3. Veterinary drugs used in the treatment

At farmers' request for veterinary service, local field veterinarians treated 33.4% (104/313) of CLE positive cows without knowledge of the result of the endometrial cytology examination, using VD commonly used to treat infections. Therefore, the CLE positive cows were categorized into two groups: (1) treated cows, and (2) untreated cows. The information on VD used 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. Withdrawal period for milk expressed in days was recorded from labeling materials such as outer wrapper carton/bottle or leaflets insert accompanying the VD (Table 1).

Milk recorded for the duration of VD treatment and for withdrawal period 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).

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). Therefore, VDs were categorized into four groups: (i) dipyrone, (ii) oxytetracycline, (iii) phenylbutazone, and (iv) tetracycline hydrochloride (Table 2). The procaine penicillin G and dihydrostreptomycin was the VD used alone for CLE treatment.

Table 1
Frequency of administration and withdrawal period for milk of veterinary drugs used in treatment.

Veterinary drugs classification	Veterinary drugs as active substance	Drug administration frequency (days)	Milk withdrawal period (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	Dipyrone	2	7

2.3. 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 MY analysis was available from 461 cows from 366 farms.

Descriptive statistics were used to compute the prevalence of endometritis. In this study, total MY loss for the recorded 30-day MY was computed from the drop in MY and discarded milk due to treatment (Table 3).

Independent samples T-Test was used to compare daily MY between endometritis negative and positive cows, and between treated and untreated cows. The MY data were subjected to a one-way analysis of variance (ANOVA) using the general linear model (GLM) for mean differences in MY among different categories of endometritis, VD used, and healthy treated and untreated cows. Means separation was with Tukey's Honest Significant Difference test in a model specified as:

$$Y_{ijk} = \mu + E_i + V_j + \varepsilon_{ijk}$$

Where Y_{ijk} = the recorded 30-day yield, discarded milk yield or total MY; μ = the overall mean; E_i = the effect of i^{th}endometritis status ($i = 4$, Healthy, CLE only, SCLE only, and both CLE and SCLE), the effect of j^{th}veterinary drugs used ($k = 5$, oxytetracycline, procaine penicillin G and dihydrostreptomycin, tetracycline hydrochloride, phenylbutazone, dipyrone); ε_{ijk} = random error term. The effect of DIM on MY was specified in the model as covariate but the effect was not significant ($p>0.05$), probably because the sample cows were in early lactation stage (21 to 60 DIM) 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. Results

3.1. Effect of endometritis disease on milk yield

The cows positive for endometritis were 70.3% (324/461) of which CLE were more, 67.5% (311/461) than SCLE, 32.5% (150/461). Those negative for endometritis were 29.7% (137/461). Of the cows examined, 38.0% (175/461) had CLE only, 3.0% (14/461) SCLE only and 29.3% (135/461) both CLE and SCLE while 29.7% (137/461) were healthy.

Therefore, the SCLE prevalence was 23.8% (50/210) from 21 to 33 DIM, 18.8% (18/96) from 34 to 47 DIM, 26.5% (41/155) from 48 to 60

Table 2
Combination of veterinary drugs used in the treatment.

Combination of VD	Veterinary drugs (VD)	VD representing the combination
Combination of three veterinary drugs	Oxytetracycline, procaine penicillin G and dihydrostreptomycin, and dipyrone	Dipyrone
	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

Table 3
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 daily MY	Litres/cow/day	DMY for health cow − DMY for positive cow
	Total decrease in 30-day MY	Litres/cow/30days	Decrease in daily MY × 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 admnistration frequency + milk withdrawal period per VD used
	Discarded MY	Litres/cow/day	DMY from treated cow
	Total discarded MY	Litres/cow	DMY from treated cow × 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 $\frac{\text{Total loss in 30 day MY}}{\text{Total 30 day MY}} \times 100$
Cost of milk loss per cow per lactation length		US$	Total loss milk litres/cow/day × lactation length (255 days) × farm gate milk price (0.2 US$/liter of milk)

*VD = veterinary drugs, MY = milk yield, DMY = daily milk yield.

DIM, and an overall prevalence of 31.0% (143/461) for the full period from 21 to 60 DIM.

Results in Table 4 show that the mean MY 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 MY 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 MY of healthy cows. The decrease in MY was higher in treated cows (18.1%) compared to untreated cows (14.0%). The total MY 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 MY loss among treated cows was discarded milk (23.5%) and reduction in MY (18.1%). The mean total MY 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 MY 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 0.2 US$ per liter of milk for a daily loss of 2.5 litres of milk in 255 days of lactation, farmers incur 154US$ loss worth of revenue.

Table 5 presents the differences in MY observed in healthy cows, cows with CLE only, SCLE only, and cows positive for both CLE and SCLE. The mean daily MY differ (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 MY 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 4
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 milk 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.

3.2. Veterinary drugs used in the treatment and estimated milk yield loss

Mean MY 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 (p<0.05). Mean decrease in MY 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 6. 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

Table 5
Effect of different categories of endometritis diagnosed at 21–60 DIM 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^{b}	227.1 ± 6.8^{a}	229.3 ± 20.4^{a}	224.9 ± 7.9^{a}
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 (40.0)	37.7 ± 20.4 (16.4)	94.1 ± 7.7 (41.8)

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

Table 6
Mean with standard errors of milk yield discarded due to treatment of clinical endometritis positive cows ($n = 104$).

Veterinary drugs used in the treatment	Cases treated (n)	MY parameters		
		Average MY, (litres/cow/day)	Total 30-day MY, (litres/cow)	Discarded MY,litres/cow, (%)
Oxytetracycline	25	8.2 ± 0.5^{b}	247.5 ± 16.3^{b}	65.9 ± 4.4^{c} (26.6)
PPD	18	5.9 ± 0.5^{a}	177.5 ± 13.6^{a}	35.5 ± 2.7^{a} (20.0)
Tetracycline hydrochloride	31	7.3 ± 0.5^{ab}	219.2 ± 14.2^{ab}	43.8 ± 2.8^{ab} (19.8)
Phenylbutazone	17	8.2 ± 0.5^{b}	245.3 ± 16.1^{b}	57.2 ± 3.8^{bc} (23.3)
Dipyrone	13	6.2 ± 0.9^{a}	186.6 ± 29.7^{a}	55.9 ± 8.9^{bc} (29.9)

PPD = Procaine penicillin G and dihydrostreptomycin, Means with different letters in superscript within a column differ ($p<0.05$),% = percent discarded milk.

(17.3%), phenylbutazone (16.3%) and dipyrone (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 period of discarding milk (days of treatment plus 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 MY 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 milk was higher among cows treated with oxytetracycline (65.9 ± 4.4 litres/cow) compared to cows treated with procaine penicillin G and dihydrostreptomycin (35.5 ± 2.7 litres/cow). The percent discarded milk was much higher among cows treated with dipyrone (29.9%) compared to those treated with tetracycline hydrochloride (19.8%).

4. Discussion

This study pioneered an estimation of the effect of endometritis disease on milk yield (MY) and milk discarded during treatment and withdrawal period among zero-grazed dairy cows on smallholder farms in Rwanda. The disease effects on milk yield were measured for 30-day post-endometritis diagnosis among cows that were within their 21–60 DIM at diagnosis. The study estimated MY 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 is higher than the previously reported MY decrease in New Zealand (1.0 liter/cow/day) (McDougall et al., 2011) and 0.5 liter/cow/day reported by (Burke et al., 2010). However, the estimates in daily decrease in MY 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 MY during endometritis infection is likely through reduced energy and protein intake due to the decline in feed intake (Bell & Roberts, 2007; Wittrock, Proudfoot, Weary & Keyserlingk, 2011); compromised welfare (Gilbert, 2016); lower milk fat and protein composition (Bell & Roberts, 2007; McDougall et al., 2011), and prolonged period of endometrium inflammation (Sharma et al., 2018). An association between reduction in dry matter intake and uterine disorders has been observed in previous studies. For instance, Bell and 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 shown also that daily MY and feed intake are reduced in cows positive for uterine disease as compared to negative ones (Bareille, Beaudeau, Billon, Robert & Faverdin, 2003; Deluyker, Gay, Weaver & Azari, 1991; Hammon, Evjen, Dhiman, Goff & Walters, 2006; Huzzey, Veira, Weary & Von Keyserlingk, 2007; 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 management intervention to mitigate the disease effects on the MY.

In contrast, some other studies have reported no reduction in MY in endometritis positive cows (Dubuc, Duffield, Leslie, Walton & LeBlanc, 2011; Gobikrushanth et al., 2016). The discrepancy with the present study could partly be explained by differences in endometritis definition criteria among studies, method and different time frames of comparing MY 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±1 DIM, for the presence of vaginal mucus using the vaginoscopy and% polymorphonuclear cells (PMN) in an endometrial cytological sample collected using the cytobrush. The authors considered the cows with vaginal mucus character (VMC) ≥2 as cases of CLE and ≥8%polymorphonuclear cells (PMN) as cases of SCLE. The follow-up period was 10 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 ± 14.7 DIM for CLE using Metricheck device with higher sensitivity diagnose of CLE than the vaginoscopy used by Gobikrushanth et al. (2016).

This study was a pioneer estimation of MY discarded and total MY 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) commonly used in veterinary practice for the treatment of infections. The most used VD was antimicrobials (71.2%) more than any other drug, anti-inflammatory drugs (16.3%) or antipyretic drugs (12.5%). The use of these VD is associated with discarding of milk during treatment and withdrawal periods. The work of Kumar & Purohit, 2019 corroborates the present findings that antimicrobial agents and anti-inflammatory are a good choice for the treatment of endometritis despite prescribed withdrawal time for milk and meat. However, 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 and drug administration frequency resulted in a higher quantity of milk discarded. This has an overall bearing on the magnitude of total MY loss that was observed in the current study. For instance, the highest total MY 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 (3–7 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., 2018; Tison, Bouchard, DesCôteaux & Lefebvre, 2017) would be attractive for food and nutrition security, sustaining stream of incomes and stable livelihood base derived from milk (Migose, Bebe, Boer & Oosting, 2018). Thus, efficacy should be the first concern, and so the selection of the VD should always be a medical option.

For instance, Makki et al. (2017) in Iran and Tison et al. (2017) in Canada used cephapirin benzathine and prostaglandin F2 alpha for the treatment of CLE. Similarly, herbal plants, for instance *Tinospora cordifolia* (Kumar, Srivastava, Rawat, Yadav & Kumar, 2004), *Withania somnifera* (Kumar, 2016), and *Allium savitum* (Rahi, 2011) 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, and not associated with the emergence of resistant bacteria 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 those VD and herbal medicine in the treatment of endometritis in dairy cows.

The findings of the current study have shown that the total MY loss in recorded 30-day post-endometritis diagnosis was different among categories of endometritis. It was lower among cows positive for SCLE only (37.7 ± 20.4 litres/cow) compared to cows positive for CLE only (90.9 ± 6.8 litres/cow) and cows that had both CLE and SCLE (94.1 ± 7.7 litres/cow). This could be related to the study condition in which cows with SCLE only were not treated because field veterinarians treated CLE endometritis positive cows without knowledge of endometrial cytology results.

In this study, the estimated average decrease in MY was higher in pure dairy cattle breeds (2.5 litres/cow/day) than in dairy crossbreds (1.1 litres/cow/day) and indigenous cattle (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). With pure dairy cattle breeds and other breeds, prevention and control measures for endometritis in the transition period and timely diagnosis of the disease at the early stage are essential management interventions to mitigate the disease effects on the MY.

5. Conclusion

The results of this study show significant MY loss from endometritis, which represents substantial production and economic loss estimated at 154US$ in a lactation. Treatment with VD requiring a long withdrawal period result in large MY loss as discarded milk. Therefore, the authors recommend a timely diagnosis of endometritis and treatment using VD that have zero withholding time for milk to minimize MY loss and associated production and economic loss. The interventions need to involve the animal health service providers to promote use of VD with zero milk withdrawal period in the treatment of endometritis. Further research is highly recommended to identify plant species with the potential for ethno-veterinary medicine use in treatment of endometritis. The herbal medicine while preventing and controlling endometritis would also reduce the use of antimicrobials, check on increasing development of antimicrobial resistance and assure safer milk and meat from dairy cows.

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