

Prevalence and Economic Impacts of Retained Placenta and Subclinical Mastitis in Tanzanian Dairy Cows

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Abstract

Background: Three dairy farms were investigated for occurrence of retained placenta and subclinical mastitis, and the economic impacts attributable to the conditions. Reproductive performance data from 1169 calvings of dairy cows for five consecutive years spanning from 2014 to 2018 were used in this study. A total of 167 cows were screened with the California Mastitis Test (CMT), and udder quarters with CMT score of ≥ 3 (scale 1–5) were milk sampled for culture and final bacteriological identification.

Results: Overall prevalences of subclinical mastitis and retained fetal membranes were 35.93% and 10.35%, respectively. The prevalence of bacterial isolates at animal level was 38.92%. Individual bacteria prevalence rates were 66.15% for *Staphylococcus aureus*, 13.85% for *Escherichia coli*, 6.15% for *Bacillus* spp., 6.15% for *Streptococcus agalactiae* and 7.69% for none typed microorganisms. Overall loss from subclinical mastitis was estimated at 10,603,800/= Tanzanian shillings (Tshs) equivalent to USD \$ 4,700 with major losses (81.75%) attributable to the production loss followed by treatment expenditure (18.25%). Average loss attributed to mastitis per quarter was estimated at 49,320/= Tshs (USD \$21.5). Retained placenta resulted in an increase ($P < 0.05$) in the period from parturition to conception (148.7 vs. 96.0 days), number of services per conception (2.9 vs. 1.9) and calving interval (434.7 vs. 374.0 days).

Conclusion: These findings point out to the economic importance of subclinical mastitis and retained fetal membranes in the dairy industry. This calls for improved milking hygiene, proper attention to health of mammary glands, regular screening tests and application of biosecurity measures as intervention strategies to lower the prevalence of subclinical mastitis and minimize the spread of pathogens in dairy farms. Since retained fetal membrane is an important problem causing great economic losses, appropriate control measures have to be instituted including cow comfort, proper immunization and careful nutritional management (mineral supplementation), particularly during the transition period.

Introduction

Reproductive performance is one of the major factors affecting profitability of a dairy herd. As a result reproductive and production disorders of dairy cattle significantly reduce their productivity thereby presenting a great concern to dairy producers across the world [1]. Poor reproductive performance due to post-partum reproductive disorders can reduce the number of born calves and milk production; and increase the cost of nutrition, therapy and artificial insemination. Aberrations of the post-partum period which include retained fetal membranes, cystic ovaries, metritis/endometritis, uterine prolapse, and pyometra are major causes of infertility during this period [2].

Normally fetal membranes drop within a short time post-partum (within 8 hours of parturition). If the membranes are retained up to 12 hrs they are referred to as delayed removal and if retained for more than 24 hours of parturition then the phrase 'Retention of placenta' is used [2, 3]. Retention of fetal membranes is the most common condition occurring in cattle following parturition [4]. The incidence of retained placenta varies from 4.0 to 16.1% and can be much higher in problem herds [5, 6]. Placental retention is usually accompanied by delayed involution of the uterus [7], reduced pregnancy rate [8], increased interval from calving to service, increased number of services per conception [9–11] and reduced milk yield [10, 12]. The condition also delays the postpartum resumption of cyclic ovarian function and prolongs the interval from calving to first ovulation [3, 10, 13, 14]. There are a number of predisposing factors to retention of fetal membranes, including mechanical, nutritional, managerial and infectious factors [5, 14, 15]. Manual removal, administration of intra-uterine and/or systemic antibiotics, injection of oxytocin, PGF₂ α and β 2-receptor blockers are commonly used methods for treatment of fetal membrane retention [2].

Mastitis, an inflammation of the mammary gland, is a highly prevalent disease in dairy cattle worldwide with a major economic impact in the dairy value chain (16–18]. It is the most important and expensive disease in the dairy industry resulting into severe economic losses from milk production quality and quantity. It leads to reduced milk production, veterinary intervention costs, milk discards due to bacterial or antibiotic contamination, premature culling and occasionally deaths [19–21]. Depending on the visibility of effects of inflammation of the mammary gland, which is attributable to the degree of inflammation, mastitis is classified as clinical or subclinical [22]. Clinical mastitis includes gross abnormality in milk, physical abnormalities of udder and abnormality of cow with systemic involvement while subclinical mastitis does not produce visible effects on udder or milk

quality but has important effects on milk composition, mainly an increase in somatic cell counts [23, 24]). While it is easy to detect clinical mastitis (seeing clotted milk), subclinical mastitis can only be demonstrated using various tests such as California Mastitis Test (CMT), Whiteside test (WST), Surf field mastitis test (SFMT), sodium lauryl sulphate test (SLST), Microscopic Somatic Cell Count (MSCC) and Electrical Conductivity (EC) [25–27]. However, CMT has been recognized as a highly sensitive test to detect bovine subclinical mastitis [24, 25].

The occurrence and economic losses associated with bovine reproductive and productive disorders (including mastitis) are well documented in developed countries [16, 28]. Such information is scanty in developing countries, including Tanzania. Therefore, the objective of this study was to determine the occurrence and economic impact of retained fetal membranes and subclinical mastitis among dairy cattle kept in the tropical environment under the semi-intensive production system.

Materials And Methods

Study area

This study was conducted during June to September 2018. Data were collected from three dairy farms, identified as farm A (Kitulo Livestock Multiplication Unit; Kitulo LMU), farm B (Mazimbu dairy farm) and farm C (Magadu dairy farm). Farm A is situated at an altitude of 2630–2820 meters above sea level in Makete district, Njombe region. The farm is in a semi-temperate climate with minimum and maximum temperatures ranging from 4°C to 8°C and from 14.5°C to 18.5°C, respectively. The area receives unimodal rainfall with a range of 1200 to 1600 mm per annum. The rain season starts in October and ends in May and it is followed by a cool dry period up to August. Farms B and C belong to Sokoine University of Agriculture and are located within the same climatic zone in Morogoro municipality, Tanzania. Geographically, the municipality is at an elevation of 500 to 600 m above sea level with a mixture of warm and cool temperatures ranging from 27 to 33.7°C in the dry/warm season and 14.2 to 21.7°C in cool/wet season. The area experiences a sub-humid tropical climate with a bimodal rainfall pattern characterized by two rainfall seasons in a year with a dry season separating the short rains (October to December) and long rains (which fall from March to May/June). There are about 6 months of dryness, the peak being September. The mean annual rainfall is about 870 mm and the total annual evapotranspiration is about 1300 mm.

Study Animals

In the study, two types of dairy cattle were involved. Farm A had Friesian cattle, full time grazed on planted temperate pastures species comprising mainly of *Lolium perenne*, *Lolium multiflorum* and *Infolium repens*. Milking cows were supplemented with homemade concentrate composed of maize bran and rice polishings (60–70%), sunflower seed cake or cotton seed cake (25%), mineral supplements (2%) and salt (1%) during milking. The amount of concentrate offered depended on the quantity (kg) of milk produced from each cow. Lactating cows were machinery milked and all animals had ad libitum water supply. The farm practiced both artificial insemination and natural breeding. Animals are routinely vaccinated against common diseases, regularly dewormed and dipped against external parasites. Farms B and C had crossbred dairy cattle which were allowed to graze on natural pastures (*Hypertheca* spp, *Panicum maximum*, *Cenchrus ciliaris* and *Brachiaria* spp) for about 8 hours and fed on hay (*Chloris gayana* and *Pennisetum purpureum*) after returning to the housing pallor in the afternoon and during night hours. Milking cows were supplemented with homemade concentrates. All farms practiced machinery-milking twice daily and used both artificial insemination and natural breeding methods.

Milk Production Data

Trends in milk production for a minimum of 15 days' milk yields before and after the treatment were recorded to gather data on incidence rate and economic losses due to subclinical mastitis. For calculating the economic losses due to the disease, information on average daily milk yield, prevailing price of milk in the location, reduction in milk yield during the illness period, number of days of illness, discarded milk during illness period and veterinary expenses was collected from the sample farms. The loss of milk during the period of mastitis was calculated by the difference between the average milk yield potential of the dairy cows and average milk yield during disease. The milk yield potential of the dairy animals was taken as the average of 14 day's milk yield before the date of occurrence of disease and 14 days after the cure of animals. The quantity of milk that was

reduced due to mastitis was considered as milk loss. The reduction in milk yield was recorded in liters. The cost of mastitis treatment consists of veterinary fees, drug cost and farmer's extra labour cost [29]. However, the farms involved in this study employ their own veterinarian with fixed salary, thus, making it a fixed cost, so we did not have to take the veterinary fees into account in our calculations. Similarly, the extra labour of the farmer was regarded as a fixed cost as this activity is part of the job of the farm employees with fixed salary.

Screening for subclinical mastitis

A cross sectional study was conducted that involved screening a total of 668 mammary quarters from 167 lactating cows. The animals were first examined for presence of mastitis based on final cardinal signs of udder inflammation as previously described by Bachaya et al. [30]. At the time of each examination, information about breed, age, parity and stage of lactation were recorded. Before examination, the udder was thoroughly washed, dried with a clean towel and the teats were sprayed with 70% ethanol. Presence of subclinical mastitis was checked using California Mastitis Test (CMT) protocol as previously described [31]. Briefly, CMT score ranging from 0 to 4 was used, where 0 is negative result (no gel formation), 1 is traceable (possible infection), and 2 or 3 indicates a positive result and 4 has the thickest gel formation. A sample was defined as positive to SCM when one or more quarters with CMT ≥ 2 were detected. Before collecting milk samples from CMT positive quarters, all teats were cleaned with 70% alcohol. After a few millimeters of milk were poured off, 25 mL of milk sample were aseptically collected in a sterile bottle and sent to the Microbiology laboratory at the College of Veterinary Medicine and Biomedical Sciences of Sokoine University of Agriculture (SUA) under cold chain for further analysis.

Microbiological analyses of milk samples

In the laboratory, samples were inoculated onto Nutrient agar, Blood agar and Chapman's agar. The plates were incubated under aerobic conditions at 37 °C for 24 to 48 h before final examination. To be classified as a positive bacterial growth, at least one colony forming unit (CFU) was needed for the following pathogens: *Bacillus* spp., *Streptococcus agalactiae*, *Escherichia coli* and *Staphylococcus aureus*. Positive isolates were initially characterized based on colony morphology, hemolytic characteristics, Gram reaction, catalase production and biochemical tests. Catalase test was performed on Gram (+) isolates for differentiation of *Staphylococcus* and *Streptococcus* sp. Catalase negative colonies were preliminarily identified as *Streptococcus* sp. Coagulase positive colonies were regarded as *S. aureus*. *Bacillus* spp. was confirmed by colony morphology and Gram-staining. Gram (-) isolates were inoculated to Eosin Methylene Blue (EMB) agar and Mac Conkey agar, then incubated at 37 °C for 24 h for identification of *E. coli*. The isolates with a greenish metallic sheen on EMB agar and lactose positive pink colored isolates were preliminarily identified as *E. coli* after IMVIC (Indole, Methyl red, Voges-Proskauer, Citrate) reactions. Samples were considered contaminated if three or more bacterial types were isolated from one milk sample and growth of the mentioned major pathogens was not identified. Isolates recovered CMT positive samples and not among the mentioned major pathogens were considered other mastitis pathogen. The culture was considered negative if no growth occurred after 48 h of incubation.

Animal Reproductive performance data

Reproductive performance data of 1169 calvings of dairy cows for five consecutive years (2014 to 2018) were used in this study. Data were extracted and compiled from records kept for each individual animal record at the respective farms. Important records collected were animal identification number, breeding system, calving date, occurrence of retained placenta, service date, pregnancy diagnosis results/next calving date and treatment costs. Data were entered in Microsoft Excel spread sheet and used to calculate number of services per conception and calving intervals. Cow calving interval was calculated by the difference between the dates of the last calving and the last insemination, for cows with positive pregnancy diagnosis.

Statistical analysis

Data collected in this study were compiled and cleaned in Microsoft excel® and then imported into SPSS statistical package version 17 for analysis. Estimation of the prevalence of commonly isolated pathogens in dairy farms was determined using standard formulae i.e. the number of positive animals/samples divided by the total number of animals/samples examined. Descriptive statistics were computed to determine frequencies of detection of the conditions. Figures were generated in Microsoft excel®. The quantification of economic impacts due to subclinical mastitis was done using the equation according to Sinha et al. [32]: Loss per animal = $\{(MiPm + Ct) + (V - Vd)\}$. Where D is average duration of the infection; Mi is milk loss per

animal per day; Pm is price of milk per liter; Ct is cost of treatment and prevention per animal per day; V is market value of adult animal; and Vd is market value of affected animal.

Results

One hundred and sixty seven lactating dairy cows (93 Friesian and 74 crossbred) were examined for presence of mastitis in the study. Out of these, 65 (38.92%) were found to be affected with either clinical ($n = 5$; 2.99%) or sub-clinical mastitis ($n = 60$; 35.93%) based on clinical examination of the udder and CMT results. Out of the 668 quarters examined, normal, clinical and sub-clinical abnormalities were detected in 453 (67.81%), 5 (0.75%) and 210 (31.44%), respectively. The prevalence of subclinical mastitis in cows at Kitulo, Magadu and Mazimbu farms was 41.94%, 32.08% and 42.86%, respectively; and there was no significant difference in the prevalence between the three farms (Fig. 1). Most of subclinical mastitis cases ($n = 60$) were observed affecting four quarters (45.24%) than three (34.29%), two (16.19%) and one (4.29%) quarter (Table 1). The overall prevalence of subclinical mastitis based on bacterial isolation at farm level was 38.92% ($n = 65$) and bacteria that were isolated included *Bacillus* spp. (6.15%), *Streptococcus agalactiae* (6.15%), *Escherichia coli* (13.85%), *Staphylococcus aureus* (66.15%) and none typed microorganisms (7.69%) (Table 2).

Table 1
Distribution of subclinical mastitis in cow quarters in the three herds

Farm	Number of cows affected with subclinical mastitis				Total number of quarters affected
	One quarter	Two quarters	Three quarters	Four quarters	
KITULO	5	20	45	74	144
MAZIMBU	3	10	18	10	41
MAGADU	1	4	9	11	25
TOTAL	9 (4.29%)	34 (16.19%)	72 (34.29%)	95 (45.24%)	210 (100%)

Table 2
Frequency of bacterial isolates from cases of mastitis in three dairy farms

Bacterial isolates	Number of isolates				% isolates
	Kitulo	Magadu	Mazimbu	Total	
<i>Bacillus</i> spp.	3	0	1	4	6.15
<i>Escherichia coli</i>	5	2	2	9	13.85
<i>Staphylococcus aureus</i>	24	7	12	43	66.15
<i>Streptococcus agalactiae</i>	3	0	1	4	6.15
Others	4	0	1	5	7.69
Total	39	9	17	65	100.00

Table 3 presents the component wise economic losses due to occurrence of mastitis in farms. The overall loss from subclinical mastitis in studied farms during the study period was estimated at 10,603,800/=. Major losses were attributable to the production loss in milk occurring during treatment (81.75%) followed by treatment expenditure (18.25%). A higher loss was estimated in Kitulo farm due to presence of large number of cow mammary quarters (70.22%; $n = 146$) affected by the disease than Mazimbu (17.28%; $n = 43$) and Magadu (12.51%; $n = 26$) dairy herds. The average loss attributed to mastitis per quarter was estimated at 49,320/= Tanzanian shillings which was equivalent to 21.45 US Dollar.

Table 3
Economic loss due to mastitis in cows

Farm	No cows (quarters) affected	Average loss of milk/ quarter / day (liter)	Average price of milk per liter	Production loss during treatment period (milk discarded/day X milk price X 7 days of treatment)	Average days under treatment	Treatment expenditure / day /quarter (Tshs)	Treatment costs (3 days in treatment X drug cost X No. of quarters)	Grand total loss (Production loss + treatment expenditure)
Kitulo	39 (146)	6	1,2000/=	6,132,000/=	3	3000/=	1,314,000/=	7,446,000/=
Mazimbu	17 (43)	4	1,200/=	1,444,800/=	3	3000/=	387,000/=	1,831,800/=
Magadu	9(26)	5	1,200/=	1,092,000/=	3	3000/=	234,000/=	1,326,000/=
Overall	65 (215)	5	1,133/=	8,668,800/=	3	3000/=	1,935,000/=	10,603,800/=

The overall incidence of retained placenta among the 1169 calvings was 10.35%, and ranged from 4.0 to 16.4% among three dairy herds for the year 2014 to 2018 (Fig. 2). Lower retained placenta occurrences were recorded in in Mazimbu and Magadu herds while highest occurrence was observed Kitulo herd (Fig. 3). The retention of fetal membrane greatly influenced the reproductive performance of animals (Table 4). Open days was longer ($P < 0.05$) in cows exhibiting retained placenta as compared to normal cows by about 53 days. Calving interval was longer ($P < 0.05$) in cows exhibiting retained placenta as compared to normal cows by about 61 days. Number of service per conception for cows exhibiting retained placenta was higher ($P < 0.05$) as compared to normal cows (2.9 vs. 1.7).

Table 4
Association of various reproductive parameters with retained placenta

Farm	Number of cows		Average number of days open		Average number of service per conception		Average calving interval (days)	
	Normal	RFM	Normal	RFM	Normal	RFM	Normal	RFM
KITULO	596	80	90 ± 5.6	150 ± 1.5	1.8 ± 0.2	3.0 ± 1.8	372 ± 9.6	435 ± 11.8
MAZIMBU	296	21	102 ± 5.7	156 ± 11.5	2.1 ± 0.7	2.7 ± 0.5	381 ± 15.8	437 ± 12.3
MAGADU	156	20	96 ± 14.6	140 ± 4.5	1.7 ± 0.5	2.9 ± 0.6	369 ± 14.6	432 ± 8.9
TOTAL	1048	121	96 ± 8.6	148.7 ± 5.8	1.9 ± 0.5	2.9 ± 1.0	374 ± 13.3	434.7 ± 11.2

Discussion

Mastitis is a multi-etiological disease of the mammary gland characterized mainly by reduction in milk production and considered an economically important disease in the dairy subsector in developed and developing nations. The results from this study show a high prevalence of subclinical mastitis; a finding that was comparable to what was previously reported both in the country [33–35] and elsewhere [36–39]. The overall high prevalence could be attributed to the invisible and silent nature of subclinical mastitis which is usually given little attention by farms when it comes to treatment, and the lack of mastitis control program which has been proved to decrease the prevalence of mastitis in developed countries [40]. However, the prevalence of subclinical mastitis reported in this study is lower than those reported in Uganda [41], Rwanda [31, 42, 43], Kenya [44], Ethiopia [39] and Vietnam [45]. It has been pointed out by previous authors that subclinical mastitis is a complex disease and discrepancies in the

reported incidences in different studies could be attributed to differences in animal breeds, management systems, parity, teat morphology, and milking hygiene [46, 47].

The bacterial pathogens isolated in the present study were dominated by *Staphylococcus aureus* (Table 2). The prevalence rate of *S. aureus* (66.15%) in the current study agrees with previous findings reported in Tanzania [34, 35, 48], Rwanda [31], Algeria [49], Italy [50] and Brazil [51]. However, the prevalence of *S. aureus* in mastitic cows reported here is higher than 25.7% previously reported in Tanzania [52], 40% in Morocco [53], 24.1–48.75% in Ethiopia [39, 54] and 30.6% in Kenya [55]. The high rate of isolation of *S. aureus* may be attributed to the fact that *S. aureus* easily localizes the skin of the udder, and has the capacity to penetrate into the tissue, producing deep seated foci protected by a tissue barrier [56]. This high frequency of staphylococcal mastitis is considered to be due to poor milking hygiene and lack of proper attention to the health of the mammary gland in general. Thus, hygiene at milking is of paramount importance in control of these infections because they are spread during the milking process causing intramammary infections.

The findings of the current study have shown a low prevalence (13.85%) of *E. coli*, the coliform environmental bacteria associated with wet and muddy conditions. These findings corroborate with those previously reported in Tanzania [57] and Rwanda [31]. *E. coli* have been reported to be of more importance in clinical mastitis than subclinical mastitis, despite the environmental factors [58]. This confirms that the prevalence of coliform bacteria in this study would be low, as few samples were of clinical mastitis. Although environmental *Streptococcus agalactiae* (6.5%) were ranked third in the current study, the microorganism have been reported as the most prominent bacteria in cow mastitis in some countries [26, 45]. The prevalence rate of *S. agalactiae* reported in the current study agrees with previous findings observed elsewhere [35, 59]. *Bacillus* spp., were present in 6.15% of cases in the current work. *Bacillus* spp. has also been identified as important pathogens in both CM and SCM in previous investigations [60, 61]. Nonetheless, these results contradict other studies showing that mastitis caused by *Bacillus* spp. is rare in dairy cows [36, 62]. The presence of *Bacillus* spp. could be explained by the poor hygienic conditions of milkers as the bacteria are widely distributed in dairy environment, including on teat skin, milkers' skin and gloves, and farm floors, which represent reservoirs of bacteria associated with intramammary infections [61, 63].

The estimated financial milk losses per farm associated with the presence of subclinical mastitis in this study ranged from 1,326,000/ to 7,446,000/= Tanzanian shillings (577.00–3,239.00 US Dollar) while the average loss attributed to mastitis per quarter was estimated at 49,320/= Tshs (21.45 US Dollar). These estimated losses are in agreement with previous studies [20, 64, 65]. Subclinical mastitis is an invisible problem that can cause financial losses to producers through the reduction of milk production, lowered milk quality, discarded milk, veterinary fees, drug costs and extra labor to farmer [16, 17, 66, 67]. Estimation of the financial impact observed in this study could be used to advocate for the implementation of prevention methods that reduce the impact of subclinical mastitis in Tanzanian dairies.

The incidence of retained fetal membranes observed in the present study ranged from 4.0 to 16.4% (average of 10.35%). This finding is in agreement with other studies conducted in the country [68] and in other countries [2, 6, 8, 69]. Some other authors have however reported higher incidences than the observation made in the current study [10, 14, 70]. Nevertheless, significantly lower incidences of RFM have also been reported in Bangladesh crossbred and local cattle [71] and Ethiopian dairy cattle [72, 73]. The differences in the incidences of RFM reported by different authors could be attributable to various factors, such as age, breed, heredity, environment, hormonal status, nutrition, and immunity.

Higher incidences of retained fetal membranes cause considerable economic losses to the farm [74]. Since the direct costs associated with RFM are difficult to derive because of a multitude of influences [75, economic losses are normally measured through establishing indirect costs to farmers resulting from periparturient disease, decreased milk production, extension of calving interval, reduced pregnancy rates, and increased risk of culling [76]. In this study, the mean number of services per conception in normal control group was 1.9 ± 0.5 , whereas the corresponding value for the retained fetal membrane group was 2.9 ± 1.0 , which differed significantly ($P < 0.05$), and corroborated with findings reported elsewhere [8, 10, 11, 14,]. Open days was longer ($P < 0.05$) in cows exhibiting retained placenta as compared to normal cows by about 507 days (Table 4). This is because more than 50% of animals that calved normally had days open within the three months post-partum, while more than 70% of cows with retained placenta conceived after 150 days. This finding concurs with the results reported by other researchers [8, 10]. Calving interval was significantly longer ($P < 0.05$) in cows exhibiting retained placenta as compared to normal cows by about 60

days. A similar trend has been reported by other authors [3, 14, 10]. Retention of placenta is associated with secondary uterine infections, which may be related to probability of subsequent conception [77]. Cows with retained fetal membranes had longer intervals from calving to first service and to conception and required more services per conception and lower pregnancy rate and conception to first service.

Abbreviations

°C: Degrees Celsius; CMT: California mastitis test; FAO; Food and Agriculture Organization of the United Nations; KTL: Kitulo Livestock Multiplication Unit; MGD; Magadu farm; MZB; Mazimbu farm; RFM; retained foetal membrane; SCM; Subclinical mastitis; SPSS; Statistical Package for the Social Sciences; SUA; Sokoine University of Agriculture; TSHS: Tanzanian shillings; USD \$: United States Dollar;

Declarations

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CONFLICTS OF INTEREST

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

AUTHORS' CONTRIBUTIONS

IPK conceptualized the study, performed data collection, laboratory and statistical analysis, interpreted the results, and prepared the manuscript. **EVGK** assisted with refining the study design, laboratory analysis and implementation of the study and critically reviewed the manuscript. All authors read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analysed during this study are included in this published article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical clearance to conduct this research was obtained from the ethical committee of College of Veterinary Medicine and Biomedical Sciences of Sokoine University of Agriculture. Verbal consent was obtained from the farm managers to collect dairy cattle information and biological samples.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

Calderón A, Rodríguez V, Máttar S, Arrieta G. Leptospirosis in pigs, dogs, rodents, humans, and water in an area of the Colombian tropics. *Trop Anim Health Prod.* 2014;46(2):427–32.

References

1. Yusuf JJ. A review on retention of placenta in dairy cattles. *Inter J Vet Sci.* 2016; 5(4): 200-207.
2. Tucho TT, Ahmed WM. Economic and Reproductive Impacts of Retained Placenta in Dairy Cows. *J Reprod Infertil* 2017; 8 (1): 18-27.
3. Swiefy AS. Effect of retained placenta on postpartum reproduction performance of Frisian cows. *Egypt J Anim Prod.* 2003; 40: 111-121.
4. Sharma M, Bhat Y, Sharma N, Rawat S. Effect of parity of animal and season of the year on the rate of retention of placenta in Dairy cattle. *Int J Curr Microbiol Appl Sci.* 2017; 6(12): 3103-3108.
5. LeBlanc S, Dufield T, Leslie K, Bateman K, Keefe G, Walton J, Johnson W. The effect of treatment of clinical endometritis on reproductive performance in dairy cows. *J Dairy Sci.* 2002; 85: 2237-2249.
6. Islam MH, Sarder MJU, Rahman M, Kader MA and Islam MA.) Incidence of Retained Placenta in Relationwith Breed, Age, Parity and Body Condition Score of Dairy cows. *Int J Nat Sci.* 2012; 2(1): 15-20
7. Patel RV, Parmar SC. Retention of fetal membranes and its clinical perspective in bovines. *S J Agric Vet Sci.* 2016; 3(2): 111-116.
8. Shiferaw Y, Tenhagen BA, Bekana M, Kassa T. Reproductive disorders of crossbred dairy cows in the central highlands of Ethiopia and their effect on reproductive performance. *Trop Anim Health Prod.* 2005; 37(5): 427-441.
9. Fourichon C, Seegers H, Malher X. Effect of disease on reproduction in the dairy cow: a meta-analysis. *Theriogenology.* 2000; 53:1729–1759
10. Gafaar HMA, Shamiah SM, Shitta AA, Ganah HAB.) Factors affecting retention of placenta and its influence on postpartum reproductive performance and milk production in Friesian cows. *Slovak J Anim Sci.* 2010; 43(1): 6-12.
11. Rokde K, Kumar S, Mahour SS, Nema SP, Aich R, Bhardwaz A. Influence of Retained Fetal Membranes on Reproductive Health of Crossbred Cows. *Indian J Vet Sci Biotech.* 2017; 12(3): 27-29.
12. Bar D, Ezra E. Effects of common calving diseases on milk production in high yielding dairy cows. *Israel J Vet Med.* 2005; 60:106–111.
13. Opsomer G, Grohn YT, Hertl J, Coryn M, Deluyker H, de Kruif A. Risk factors for post-partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology.* 2000; 53: 841–857.
14. Han IK, Kim IH..Risk factors for retained placenta and the effect of retained placenta on the occurrence of postpartum diseases and subsequent reproductive performance in dairy cows. *J. Vet. Sci.* 2005; 6(1): 53-59.
15. Gröhn YT, Rajala-Schultz PJ Epidemiology of reproductive performance in dairy cows. *Anim Reprod Sci.* 2000; 60(61):605–614
16. Halasa T, Huijps K, Østerås O, Hogeveen H. Economic effects of bovine mastitis and mastitis management: A review. *Vet Q.* 2007; 29:18–31. doi: 10.1080/01652176.2007.9695224
17. Petrovski KR, Trajcev M, Buneski G. A review of the factors affecting the costs of bovine mastitis. *J S Afr Vet Assoc.* 2006; 77:52–60. doi: 10.4102/jsava.v77i2.344.
18. Biffa D, Debela E, Beyene F. Prevalence and risk factors of mastitis in lactating dairy cows in Southern Ethiopia. *Int. J. Appl. Res. Med.* 2005; 3: 189-198.

19. Gröhn YT, González RN, Wilson DJ, Hertl JA, Bennett G, Schulte H, Schukken YH. Effect of pathogen-specific clinical mastitis on herd life in two New York State dairy herds. *Prev. Vet. Med.* 2005;71:105-125
20. Wolfová M, Stípková M, Wolf J. Incidence and economics of clinical mastitis in five Holstein herds in the Czech Republic. *Prev Vet Med.* 2006; 77(1-2):48-64
21. Dürr JW, Cue RI, Monardes HG, Moro-Méndez J, Wade KM. Milk losses associated with somatic cell counts per breed, parity and stage of lactation in Canadian dairy cattle. *Liv Sci.* 2008;117: 225- 232
22. Motaung T, Petrovski K, Petzer I, Thekiso O, Tsilo T. Importance of bovine mastitis in Africa. *Anim Health Res Rev.* 2017; 18(1): 58–69. doi: <https://doi.org/10.1017/S1466252317000032>.
23. Karimuribo ED, Fitzpatrick JL, Swai ES, Bell C, Bryant MJ, Ogden NH, Kambarage DM, French N P. Prevalence of subclinical mastitis and associated risk factors in smallholder dairy cows in Tanzania. *Vet Rec.* 2008; 163(1): 16–21.
24. Iraguha B, Hamudikuwanda H, Mushonga B, Kandiwa E, Mpatwenumugabo JP. Comparison of cow-side diagnostic tests for subclinical mastitis of dairy cows in Musanze district, Rwanda. *J S Afr Vet Assoc.* 2017; 88: 1464
25. Sharma N, Pandey V, Sudan NA. Comparison of some indirect screening tests for detection of subclinical mastitis in dairy cows. *Bulg J. Vet. Med.* 2010; 13 (2): 98-103.
26. Hegde R, Isloor S, Nithin PK, Shome BR, Rathnamma D, Suryanarayana VVS, Yatiraj S, Prasad R, Krishnaveni C, Sundareshan N, Akhila DS, Gomes AR, Hegde NR. Incidence of sub-clinical mastitis and prevalence of major mastitis pathogens in organized farms and unorganized sectors. *Indian J Microbiol.* 2013; 53(3): 315–320.
27. Hoque MN, Das ZC, Talukder AK, Alam MS, Abu NM, Aminoor RA. Different screening tests and milk somatic cell count for the prevalence of subclinical bovine mastitis in Bangladesh. *Trop Anim Health Prod.* 2015; 47:79–86.
28. Viguier C, Arora S, Gilmartin N, Welbeck N, O’Kennedy R. Mastitis detection: current trends and future perspectives. *Trends Biotechnol.* 2009; 27(8): 486–493
29. Ózsvári L, György K, Illés BC, Bíró O. Quantification of economic losses caused by mastitis on a large-scale Holstein-Friesian dairy farm (in Hungarian). *Hung Vet J.* 2003; 125 (5): 273-279.
30. Bachaya HA, Raza MA, Murtaza S, Akbar IUR. Subclinical bovine mastitis in Muzaffar Garh district of Punjab (Pakistan). *J Anim Plant Sci.* 2011; 21(2): 16–19.
31. Mpatwenumugabo JP, Bebora LC, Gitao GC, Mobegi VA, Iraguha B, Kamana O, Shumbusho B, Mestorino N. Prevalence of Subclinical Mastitis and Distribution of Pathogens in Dairy Farms of Rubavu and Nyabihu Districts, Rwanda. *J Vet Med.* 2017; 8. <https://doi.org/10.1155/2017/8456713>
32. Sinha MK, Thombare NN, Mondal B. Subclinical Mastitis in Dairy Animals: Incidence, Economics, and Predisposing Factors. *Sci World J.* 2014; 2014.523984. <http://dx.doi.org/10.1155/2014/523984>
33. Kivaria FM, Noordhuizen JPTM, Kapaga AM. Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania. *Trop Anim Health Prod.* 2004; 36: 581–592.
34. Mdegela RH, Kusiluka LJM, Kapaga AM, Karimuribo ED, Turuka FM, Bundala A, Kivaria FM, Kabula B, Manjurano A, Loken T, Kambarage DM. Prevalence and determinants of mastitis and milk borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in eastern Tanzania. *J Vet Med B.* 2004; 51: 123–128
35. Mdegela RH, Ryoba R, Karimuribo ED, Phiri EJ, Løken T, Reksen O, Mtengeti E, Urio NA. Prevalence of clinical and subclinical mastitis and quality of milk in smallholder dairy farms in Tanzania. *J S Afr Vet Assoc.* 2009; 80 (3): 163–168.
36. Sori H, Zerihun A, Abdicho S. Dairy cattle mastitis in and around Sebeta, Ethiopia. *Int. J. Appl. Res. Vet. Med.* 2005; 3: 332–338.
37. Hashemi M, Kafi M, Safdarian M. The prevalence of clinical and subclinical mastitis in dairy cows in the central region of Fars province, south of Iran. *Iran J Vet Res.* 2011; 12: 236–241.
38. Abebe R, Hatiya H, Abera M, Megersa B, Asmare K. Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Vet. Res.* 2016; 12: 270. doi: 10.1186/s12917-016-0905-3.
39. Zeryehun T, Abera G. Prevalence and Bacterial Isolates of Mastitis in Dairy Farms in Selected Districts of Eastern Harrarghe Zone, Eastern Ethiopia. *J Vet Med.* 2017: 6498618 <https://doi.org/10.1155/2017/6498618>

40. Hillerton JE, Bramley AJ, Staker RT, McKinnon CH. Patterns of intramammary infection and clinical mastitis over a 5-year period in a closely monitored herd applying mastitis control measures. *J Dairy Res.* 1995; 62:39–50.
41. Abrahmsén M, Persson Y, Kanyima B, Båge R. Prevalence of subclinical mastitis in dairy farms in urban and periurban areas of Kampala, Uganda. *Trop Anim Health Prod.* 2014; 46(1), 99–105.
42. Iraguha B, Hamudikuwanda H, Mushonga B. Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda. *J S Afr Vet Assoc.* 2015; 86(1): 275–6.
43. Ndahetuye JB, Persson Y, Nyman A, Tukei M, Ongol MP, Båge R. Aetiology and prevalence of subclinical mastitis in dairy herds in peri-urban areas of Kigali in Rwanda. *Trop Anim Health Prod.* 2019; 51:2037–2044
44. Mureithi DK, Njuguna MN. "Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika Sub County, Kenya," *Liv Res Rural Dev.* 2016; 28(2): 2016.
45. Östensson K, Lam V, Sjögren N, Wredle E. Prevalence of subclinical mastitis and isolated udder pathogens in dairy cows in Southern Vietnam. *Trop Anim Health Prod.* 2013; 45(4): 979–986.
46. Radostits OM, Gay KW, Hinchcliff CC, Constable PD. Mastitis. *Veterinary Medicine, A Text Book of Disease of Cattle, Sheep, Pigs, Goats, and Horses.* 10th edit Bailliere Tindall, UK; 2007. p. 674–762.
47. Almwaw G, Zerihun A, Asfaw Y. Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia. *Trop. Anim. Health Prod.* 2008; 40: 427-432.
48. Mdegela RH, Karimuribo ED, Kusiluka LJM, Kabula B, Manjurano A, Kapaga AM, Kambarage DM. Mastitis in smallholder dairy and pastoral cattle herds in the urban and peri-urban areas of the Dodoma Municipality in the central Tanzania. *Liv Res Rural Dev.* 2005; 17(11): 2005.
49. Saidi R, Khelef D, Kaidi R. Subclinical mastitis in cattle in Algeria: Frequency of occurrence and bacteriological isolates *J S Afr Vet Assoc.* 2013; 84(1): 84:E1-5.
50. Moroni P, Pisoni G, Antonini M, Villa R, Boettcher P, Carli S. Antimicrobial drug susceptibility of *Staphylococcus aureus* from subclinical bovine mastitis in Italy. *J Dairy Sci.* 2006; 89: 2973–2976. <http://dx.doi.org/10.3168/jds>.
51. Dittmann KK, Chaul LT, Lee SHI, Corassin CH, de Oliveira CAF, De Martinis ECP, Alves VF, Gram L, Oxaran V. *Staphylococcus aureus* in Some Brazilian Dairy Industries: Changes of Contamination and Diversity. *Front Microbiol.* 2017; 8: 2049. doi: 10.3389/fmicb.2017.02049
52. Kashoma IP, Lalata EP, Maiga CJ, Mtemela BO, Medardus JJ. Prevalence and antimicrobial susceptibility profiles of *Staphylococcus aureus* from cow's milk, nasal and environmental swabs in selected dairy farms in Morogoro, Tanzania. *Tanz Vet J.* 2015; 30(2): 61- 75.
53. Bendahou A, Lebbadi M, Ennane L, Essadqui FZ, Abid M. Characterization of *Staphylococcus* species isolated from raw milk and milk products (lben and jben) in North Morocco. *J Infect Dev Ctries.* 2008; 2: 218–225.
54. Daka D, Solomon G, Yihdego D. Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. *Ann Clin Microbiol Antimicrob.* 2012; 11:26-37.
55. Shitandi A, Sternesjö Å. Prevalence of multidrug resistant *Staphylococcus aureus* in milk from large and small-scale producers in Kenya. *J Dairy Sci.* 2004; 87:4145–4149.
56. Ranjan R, Gupta MK, Singh S, Kumar S. Current trend of drug sensitivity in bovine mastitis. *Vet World.* 2010; 3(1):17-20
57. Kivaria FM, Noordhuizen JPTM, Nielen M, Interpretation of California mastitis test scores using *Staphylococcus aureus* culture results for screening of subclinical mastitis in low yielding smallholder dairy cows in the Dar es Salaam region of Tanzania," *Prev Vet Med.* 2007; 78(3-4); 274–285.
58. Hogan J, Smith KL. Coliform mastitis. *Vet Res.* 2003; 34(5): 507–519,
59. Persson Y, Nyman AKJ, Grönlund-Andersson U. Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden, *Acta Vet Scand.* 2011; 53:36. doi:10.1186/1751-0147-53-36
60. Nieminen T, Rintaluoma N, Andersson M, Taimisto AM, Ali-Vehmas T, Seppälä A, Priha O, Salkinoja-Salonen M. Toxinogenic *Bacillus pumilus* and *Bacillus licheniformis* from mastitic milk. *Vet. Microbiol.* 2007; 124:329–339.

61. Amer S, Gálvez FLA, Fukuda Y, Tada C, Jimenez IL, Valle WFM, Nakai Y. Prevalence and etiology of mastitis in dairy cattle in El Oro Province, Ecuador. *J Vet Med Sci.* 2018; 80(6):861-868.
62. Abera, M., Elias, B., Aragaw, K., Denberga, Y., Amenu, K and Sheferaw D. 2012. Major causes of mastitis and associated risk factors in smallholder dairy cows in Shashemene, southern Ethiopia. *Afri J. Agri. Res.*, 7: 3513–18.
63. De Visscher A, Supré K, Haesebrouck F, Zadoks RN, Piessens V, Van Coillie E, Piepers S, De Vliegher S. Further evidence for the existence of environmental and host-associated species of coagulase-negative staphylococci in dairy cattle. *Vet. Microbiol.* 2014; 172: 466–474.
64. Cha E, Bar D, Hertl JA, Tauer LW, Bennett G, González RN, Schukken YH, Welcome FL, Gröhn, YT. The cost and management of different types of clinical mastitis in dairy cows estimated by dynamic programming. *J. Dairy Sci.* 2011; 94: 4476-4487.
65. Romero J, Benavides E, Meza C Assessing Financial Impacts of Subclinical Mastitis on Colombian Dairy Farms. *Front. Vet. Sci.* 2018; 5:273. doi: 10.3389/fvets.2018.00273
66. Impact of Mastitis in Small Scale Dairy Production Systems, Animal Production and Health Working Paper. 2014.
67. Asfaw M, Negash A. Review on Impact of Bovine Mastitis in Dairy Production. *Adv Biol Res.* 2017; 11 (3): 126-131.DOI: 10.5829/idosi.abr.2017.126.131
68. Swai ES, Bryant MJ, Karimuribo ED, French NP, Ogden NH, Fitzpatrick JL, Kambarage DM. A cross sectional study of reproductive performance of smallholder dairy cows in coastal Tanzania. *Trop Anim Health Prod.* 2005; 37: 513-525.
69. Beredu Y, Biruk A. Reproductive Disorders in Dairy Cattle; Retrospective Study in Asella Town, Central Ethiopia. *Dairy Vet Sci J.* 2019; 9(4): 555767. DOI: 10.19080/JDVS.2019.09.555767.
70. Muhairwa AP, Barnabas E, Nonga HEA. retrospective study of production and reproductive conditions in crossbred dairy cows attended at Sokoine University of Agriculture Animal Hospital, Tanzania. *Tanz Vet J.* 2014; 29 (1) 2014.
71. Islam MH, Sarder MJU, Jahan SS, Rahman M, Zahan M, Kader MA, Mozaffor Hossain KM Retained placenta of dairy cows associated with managemental factors in Rajshahi, Bangladesh, *Vet. World.* 2013; 6(4):180- 184, doi: 10.5455/vetworld.2013.180-184
72. Hadush A, Abdella A, Regassa F. Major prepartum and postpartum reproductive problems of dairy cattle in Central Ethiopia. *Vet Med Anim Health.* 2013; 5: 118-123.
73. Getenet A, Berhanu M, Desie S. Major postpartum problems of dairy cows managed in small and medium scale production systems in Wolaita Sodo, Ethiopia. *Afri J Agric Res.* 2014; 9: 2775-2780
74. Joosten I, Stelwagen I, Dijkhuizen FA. Economic and Reproductive consequences of retained placenta in dairy cattle. *Vet Record.* 1988; 123: 53-57.
75. Cooper RL. Retained foetal membranes in cattle: The knowns and unknowns. *Cattle Prac.* 2014; 22:17-25
76. Peter AT Bovine placenta: a review on morphology, components and defects from terminology and clinical perspectives. *Theriogenology.* 2013;. 80(7): 693 – 705.
77. Grunert E. Etiology and Pathogenesis of Retained Bovine PlacentaThe post-partum cow. *Current Therapy in Theriogenology.* WB Saunders; 1986. p.237-242.

Figures

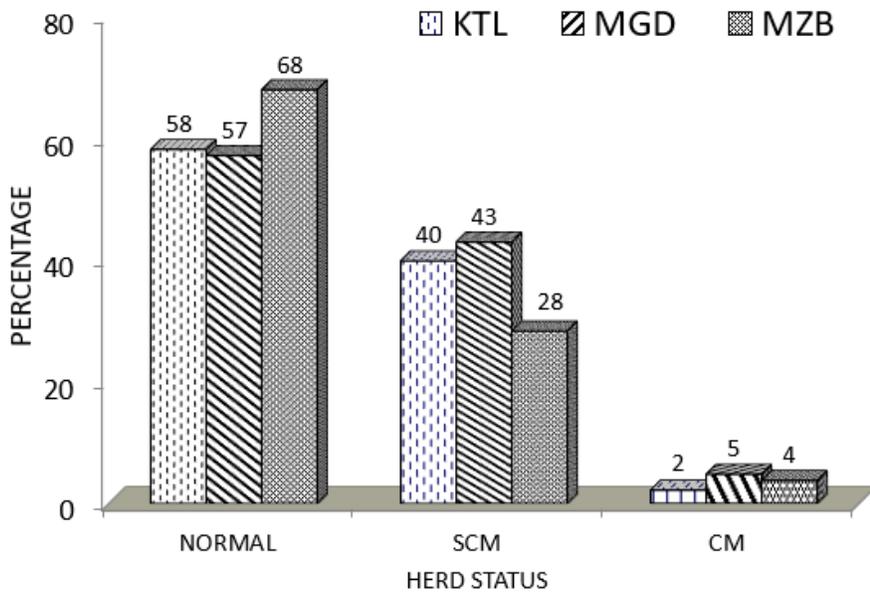


Figure 1

The status of study animals in relation to prevalence of mastitis in study farms

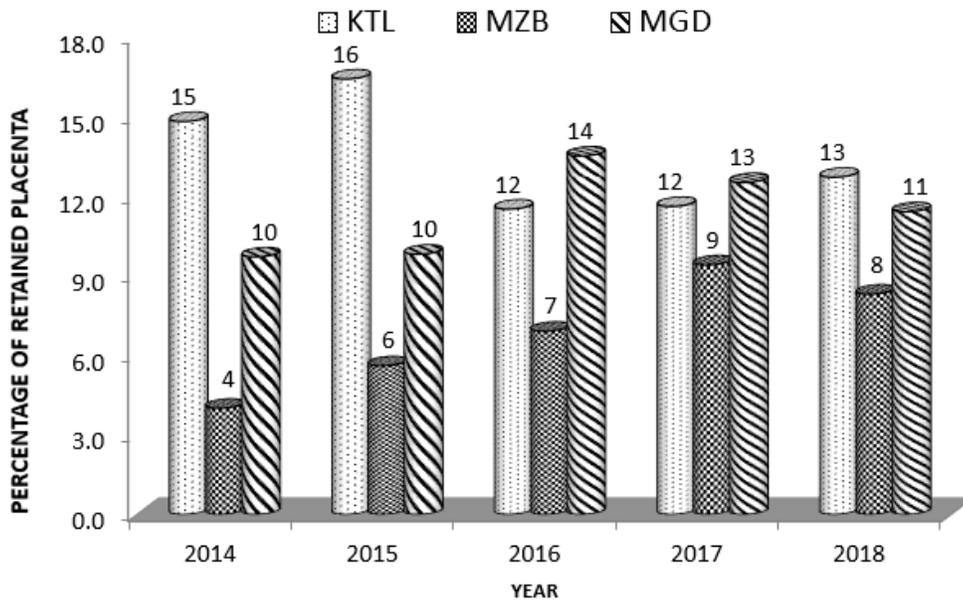


Figure 2

Incidence of retained placenta in 1169 calvings on three dairy farms for year spanning from 2014 - 2018

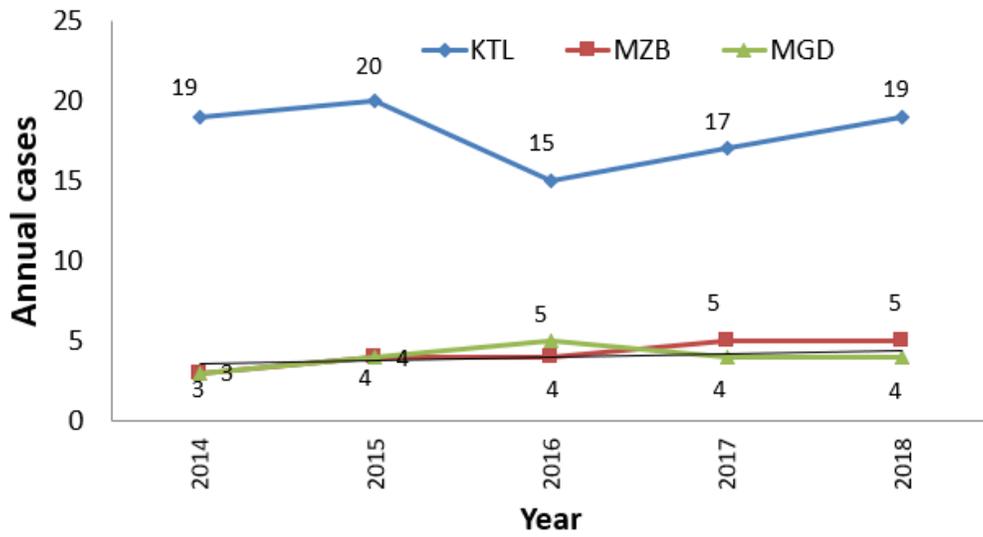


Figure 3

Annual trends of retained placenta observed in the study farms in a five years period (2014 to 2018).