Problem-oriented field epidemiological study in dairy production medicine using a causal inference approach

Takeshi Miyama

Problem-oriented field epidemiological study in dairy production medicine using a causal inference approach

（因果推論を用いた酪農生産獣医療における問題指向型実地疫学的研究）

Veterinary Epidemiology

Doctoral Course of Veterinary Medicine

Graduate School of Veterinary Medicine

Rakuno Gakuen University Graduate School

Takeshi Miyama

Supervisor: Professor, Dr. Kohei Makita, Veterinary Epidemiology Unit,

Graduate School of Veterinary Medicine

2019

CONTENTS

ABBREVIATIONS

AI: artificial insemination

AIC: Akaike's Information Criteria

BCS: body condition score

BHB: β-hydroxybutyrate

CI: confidence interval

CK: clinical ketosis

CMT: California Mastitis Test

CNS: coagulase-negative staphylococci

co-formulations: OP-SP co-formulations

DAG: directed acyclic graph

EBATIC: evidence-based acaricide tick control

ECF: East Coast Fever

GLM: generalized linear model

GLMM: generalized linear mixed-effects model

ICC: intraclass correlation coefficient

JICA: Japan International Cooperation Agency

LMM: linear mixed-effects models

NEB: negative energy balance

OP: organophosphate

OR: odds ratio

PCR: polymerase chain reaction

RTC: Research Center for Ticks & TBD Control

SCK: subclinical ketosis

SCM: subclinical mastitis

SE: standard error

SP: synthetic pyrethroid

TBD: tick-borne disease

YDFC: Yamagata Prefecture Dairy Farming Cooperative

GENERAL INTRODUCTION

Field epidemiology in dairy production medicine

Sustainable dairy farming can be achieved by controlling common problems faced by those in the dairy industry, such as farmers, veterinarians, and animal husbandry extension officers. The Sustainable Agriculture Initiative Platform Working Group has developed principles and practices for sustainable dairy farming [99], specifically related to: 1) farming systems (farm site, feed production, milking hygiene, and milk storage), 2) dairy cow care (animal genetics, health planning and bio-security, and animal husbandry), 3) economic sustainability (increasing output, food safety and quality, and market opportunities and access), 4) social sustainability (social and human capital, and local community/economy), and 5) environmental sustainability (soil, water, biodiversity, energy, and waste). A variety of problems affect dairy farms, and epidemiological approaches provide powerful tools for solving those problems.

Epidemiology is the study of the frequency, distribution, and determinants of health and disease in populations [20]. Dairy production medicine addresses the same issues to optimize production through the elimination and control of diseases and the implementation of management practices that promote animal health, welfare, productivity, and farm profitability [46]. By evaluating the condition of a dairy farm epidemiologically, it is possible to identify and investigate important risk factors—and causes—associated with adverse health and disease events [22, 46] and develop appropriate interventions and evaluations. Epidemiological monitoring is thus essential for continued improvement of dairy farm productivity. The collection of baseline data on animal health and productivity is the first step in this process and necessary to establish norms for a particular region [22].

Causal inference in observational data

A randomized control trial is the best way to evaluate the causal effect of factors of interest. The use of randomization in experiments provides a probabilistic basis for balancing known and unknown factors between exposed and un-exposed groups by minimizing the possibility of confounding [19]. However, in epidemiological studies, researchers often use observational data to estimate the effect of an exposure on the outcome of interest. In observational data, the conditions faced by the exposure and non-exposure groups differ, which can introduce bias (confounding) in the observed measures of associations. Our challenge is to identify and control factors that cause confounding in order to obtain valid estimates of causal effects. A factor is considered a confounder if:

1. It is a cause of the outcome,
2. It precedes and is associated with the exposure, and
3. It cannot be determined by the exposure or the outcome [19, 53].

Identifying confounding variables using causal diagrams

Potential confounders that must be controlled can be identified using a causal diagram (directed acyclic graph, DAG) [19, 53, 66]. A causal diagram expresses the cause-and-effect relationship. The causal-ordering assumption is usually based on a known time sequence and/or plausibility considerations. The following guideline can be used to construct a causal diagram [23]:

1. Begin at the left with variables that are predetermined (*e.g.* Z in Fig. 1) and progress to the right;
2. The remaining variables are placed in the diagram in their presumed causal

order (*e.g.* X, exposure; and Y, outcome in Fig. 1).

In Figure 1, the total association between X and Y consists of the following two elements: 1) the genuine causal effect of X on Y (X → Y), and 2) the common dependence of X and Y on Z (Z → X and Z → Y). In such cases, it is often said that the causal effect of X on Y is confounded by Z (*i.e.*, Z is a confounder) [66].

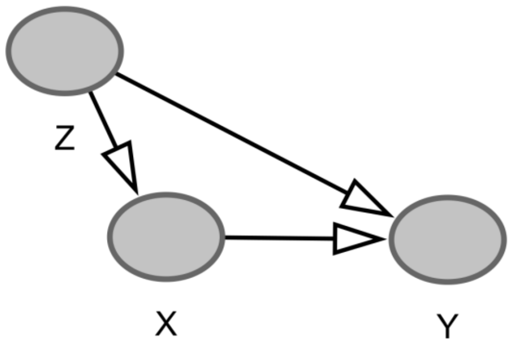


Figure 1. A graph in which the causal effect of X on Y is confounded by Z

A procedure commonly used to identify confounder sets that must be adjusted to estimate the causal effect involves blocking all paths that generate non-causal associations between the causal variable and the outcome variable. This procedure is known as Pearl's back-door criterion, and it was explained by Morgan and Winship as follows [67]:

> *If one or more back-door paths connect the causal variable to the outcome variable, the causal effect is identified by conditioning on a set of variables Z if*
>
> > *Condition 1. All back-door paths between the causal variable and the outcome variable are blocked after conditioning on Z, which will always be the case if each back-door path*
> >
> > > *a) Contains a chain of mediation $A \rightarrow C \rightarrow B$, where the middle variable C is in Z, or*
> > >
> > > *b) Contains a fork of mutual dependence $A \leftarrow C \rightarrow B$, where the middle variable C is in Z, or*
> > >
> > > *c) Contains an inverted fork of mutual causation $A \rightarrow C \leftarrow B$, where the middle variable C and all of C's descendants are not in Z; and*
> >
> > *Condition 2. No variables in Z are descendants of the causal variable that lie on any of the directed paths that begin at the causal variable and reach the outcome variable,*

where *a path* is any sequence of edges pointing in any direction that connects one variable to another, and *a back-door path* is a path between any causally ordered sequence of two variables that begins with a directed edge that points to the first variable [67].

DAGity [100] is an online software (a package of R statistical software is also available) that applies an extension of Pearl's back-door criterion to identify valid conditioning sets of covariates. A causal diagram can be designed depending on the known information and/or the researcher's hypothesis using DAGity, and the minimal sufficient adjustment sets needed to estimate the effect can be identified for further causal estimation.

Change-in-parameter criterion for detecting confounders

Once potential confounders have been identified, the magnitude of a confounder can be determined using the change-in-parameter criterion [19]. The difference between the estimate adjusted by a confounder and the unadjusted crude estimate can be examined to determine how the confounder affects the estimate. If the difference is sufficiently large, the covariate is classified as a potential confounder. Note that failure to determine the difference in estimates using the change-in-estimate criterion with the data at hand this does not mean that the variable is not a confounder [53] in terms of generalizability (if different datasets are used, the magnitude of the confounder's effect might vary). Thus, confounders should be selected based on *a priori* knowledge.

Statistical identification of confounders

Statistical algorithms can be used to identify potentially confounding variables from a regression model based on their statistical significance (*e.g.*, stepwise methods) [19]. However, the use of such statistical approaches to control confounding and estimate causal effects has rapidly lost favour in recent years [19]. The major problem is that it is not possible to distinguish between intervening and other types of extraneous variables [19].

Models (*e.g.*, regression models) are generally built with two broad objectives, one for prediction and the other for understanding the (causal) relationships between one or more predictors and the outcome of interest [24]. Automated selection procedures are suitable for prediction models but not causal estimation models because, as explained above, automated selections cannot differentiate between exposure, confounders, and intervening variables.

Problems in dairy production medicine examined in this study

This doctoral research project addressed problems in dairy production medicine related to sustainable dairy farming. I focused on bovine leptospirosis in Japan and East Coast Fever (ECF) in Uganda. In terms of the principals of sustainable dairy farming, leptospirosis and ECF are important considerations in health planning and ensuring bio-security in dairy cow care. I also addressed milking hygiene/mastitis control and feeding and reproductive management in Uganda, which are important issues affecting farming systems, dairy cow care, and economic sustainability.

Chapter 1 discusses the sero-prevalence and risk factors associated with *Leptospira* Hardjo infection of dairy herds in Yamagata Prefecture, located in south Tohoku, Japan. Leptospirosis is one of the most prevalent zoonoses caused by bacteria of the genus *Leptospira* [3]. The most common causal species of bovine leptospirosis is *Leptospira* of serovar Hardjo, for which cattle serve as the maintenance host [34, 56]. Bovine leptospirosis is economically important due to reproductive losses resulting from abortion, stillbirth, and infertility, as well as increased services per conception and prolonged calving intervals [15, 16]. To date, there have been no risk factor analyses of *L*. Hardjo infection on dairy farms in Japan. Although a previous study did demonstrate the sero-prevalence in Tohoku [48], the sample size was small.

Chapter 2 presents the results of farm-side analyses of tick control practices, tick infestation of dairy cattle, acaricide resistance, and *Theileria parva* infection to control ticks and ECF in Mbarara District, Uganda. ECF is a tick-borne disease of cattle in which the etiological agent is the protozoan parasite, *T. parva*. The parasite is transmitted by *Rhipicephalus appendiculatus* ticks [73]. ECF is problematic due to economic losses resulting from high morbidity and mortality, declining milk production, and costs of tick and disease control measures [33, 57]. In addition, severe acaricide resistance has been reported in Uganda [109]. Inappropriate farm tick control practices can both facilitate and accelerate the development of tick resistance to acaricidal agents.

Chapter 3 describes the results of a study conducted to characterize prevailing milk hygiene practices in addition to the prevalence of and risk factors for subclinical mastitis (SCM) in Mbarara District, Uganda. SCM is difficult to detect due to a lack of clinical signs [29]. The majority of mastitis losses result from subclinical infections with high somatic cell counts, which in turn leads to depressed production [11]. Mastitis is regarded as the most costly disease on dairy farms worldwide [38]. A high prevalence of SCM has been reported in Uganda: 86.2% [2] and 90.0% [10] at the cow-level, in Kampala, the capital of Uganda; and 76.1%, 87.9%, and 60.7% in Kiruhura [98], Kiboga [44], and Jinja Districts [13], respectively. No risk factor analysis of milking hygiene and practices in terms of development of SCM has been conducted in Uganda.

Chapter 4 discusses an investigation of the production status in terms of milk yield, nutrition of dairy cows, and dairy management practices in the Mbarara District of Uganda. The relationship between feeding management and the milk production and nutritional status of dairy cows was evaluated. From the viewpoint of milking physiology, dairy intensification involving a change in breeds and management style can increase the risk of ketosis in dairy herds. Subclinical ketosis (SCK) results in reduced milk production [114] and subsequent economic losses [68]. Exotic-breed cattle exhibiting a higher milk yield are more susceptible to negative energy balance than indigenous breeds and thus require careful feeding management. In peri-urban Kampala, Uganda, the reported prevalence of SCK in dairy cows during early lactation was 18.8% in 2013 and 13.9% in 2014 [17]; however, the prevalence of SCK and feeding management practices in the southwestern region of Uganda have not been reported.

Finally, in Chapter 5, the approaches, challenges, and opportunities learned from different epidemiological causality studies conducted in Japan and Uganda are discussed.

Chapter 1. Herd-level risk factors associated with *Leptospira* Hardjo infection in dairy herds in the southern Tohoku, Japan

1.1. Introduction

Leptospirosis is one of the most prevalent zoonoses caused by bacteria of the genus *Leptospira* [3] and it has been reported world-wide in wild and domestic animals [56]. Sixteen genomospecies and >260 serovars of *Leptospira* have been identified [56]. *Leptospira* are shed in the urine of infected animals which contaminates water or soil, and they can survive there for weeks to months [50]. Infection with pathogenic strains of *Leptospira* commonly occurs through direct contact with infected animal urine or indirectly through contaminated water [9, 56]. The bacteria can enter the body through the skin or mucous membranes (eyes, nose or mouth), especially if the skin is broken from a cut or scratch [56]. Leptospirosis may present a wide variety of clinical manifestations in humans [56]. Many human cases are mild, influenza-like illnesses lasting 3-5 days, which are difficult to distinguish from other infectious diseases [28]. The most severe disease form is Weil's syndrome, which is characterized by jaundice, acute renal failure and bleeding [9, 28, 50, 56].

The most common causal species of bovine leptospirosis is *Leptospira* belonging to serovar Hardjo, and cattle are the maintenance host [34, 56]. A notification of cattle which contract the disease by *L*. Hardjo is obligated in Japan. Two types of *L*. Hardjo serovar have been identified: *Leptospira interrogans* serovar Hardjo (type Hardjoprajitno) and *L. borgpetersenii* serovar Hardjo (type Hardjo-bovis) [34, 56]. A high prevalence of serovar Hardjo in dairy herds has been reported world-wide [55, 75, 92]. Although reported cases of *L*. Hardjo in Japan are limited [48, 102], a cross sectional study throughout Japan found that the overall sero-prevalence of *L*. Hardjo was also high [48]. The risk factors for infection with *L*. Hardjo in dairy herds reported in previous studies in several parts of the world included herd size, geographical region, movement of cattle onto and off farms, co-grazing, professional visitors not wearing protective clothing, rearing calves naturally, adult cows in contact with calves, and not cleaning oral drenching equipment [55, 74, 92, 95]. Bovine leptospirosis is economically important due to reproductive losses from abortions, stillbirths and infertility, as well as increased services per conception and prolonged calving intervals [15, 16].

There is no reported case of human leptospirosis caused by *L*. Hardjo in Japan, and the most human cases caused by the other serovars are reported in Kyushu and Okinawa district [70] where a subtropical climate is prevalent. However, in New Zealand, it was the most common serovar in notified human cases between 2011 and 2014, and slaughterhouse workers and livestock farmers were the most frequently reported occupations among the cases [93]. Human leptospirosis cases in Tohoku which is located in the northern part of Japan and has a subarctic climate are rare, and there may be a possibility that leptospirosis cases caused by *L*. Hardjo relating to livestock occupations in Japan are neglected.

Fifteen serovars have been reported in Japan [70]. Although it has not been isolated, and the antibody showed cross-reactions between *L*. Hardjo and *L*. Hebdomadis, the serological detections of *L*. Hardjo have been reported [48, 102]. Epidemiological study of *L*. Hardjo has not been clearly conducted yet in Japan, and further researches are required. Thus, it is important to identify the prevalence and the risk factors for *L*. Hardjo infection on dairy herds to control it in the northern part of Japan on the aspects of both economic impacts on farming productivity and human public health. However, there is no risk factor analysis for *L*. Hardjo infection on dairy farms in Japan so far. The previous study

[48] showed the sero-prevalence in Tohoku, but the sample size was small. Therefore, Yamagata Prefecture Agricultural Mutual Aid Association initiated investigations into the situation including epidemiological analysis. The objectives of this study were to investigate the sero-prevalence and risk factors for *L*. Hardjo infection of dairy herds in Yamagata Prefecture, the southern Tohoku, Japan.

1.2. Materials and methods

1.2.1 Bulk milk sampling and diagnosis with *Leptospira* Harjo

A cross-sectional study was conducted with all of the 109 dairy herds belonging to the biggest dairy association in Yamagata, Yamagata Prefecture Dairy Farming Cooperative (YDFC), which covers the majority of the Okitama region (Yonezawa, Takahata, Nanyo, Kawanishi, Iide, Nagai and Shirataka) and the southern part of the Murayama region (Yamagata, Kaminoyama, Yamanobe and Asahi) (Fig. 2). This study population accounts for 33.6% (109/ 322 farms) of the target population in Yamagata Prefecture [64]. None of the herds in this study were vaccinated against *L*. Hardjo.

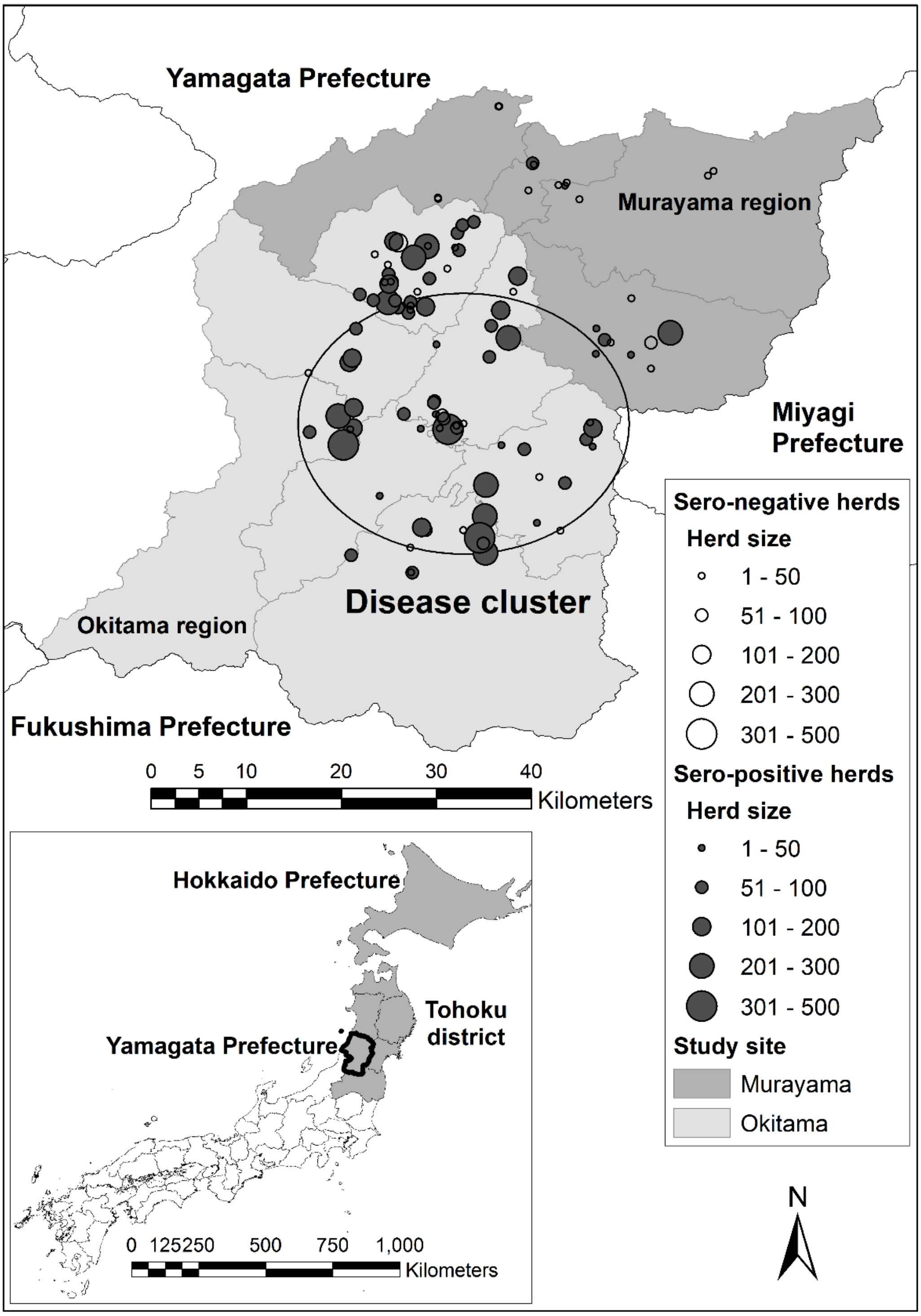


Figure 2. Map showing the geographical locations and the sizes of *Leptospira* Hardjo sero-positive and negative herds in Yamagata Prefecture. A disease cluster was detected in Okitama region.

Bulk tank milk samples were collected to test the herd level sero-prevalence of *L*. Hardjo antibodies. Bulk milk sampling was conducted on 25th March 2014 at the normal time of milk collection. The Linnodee *Leptospira* ELISA Kit™ (Linnodee Animal Care, Ballyclare, Northern Ireland) was used [115] which detects an IgG antibody response to a lipopolysaccharide outer envelope epitope common to both *L. borgpetersenii* serovar Hardjo and *L. interrogans* serovar Hardjo [115]. The test was assumed to have a sensitivity and specificity for individual serum samples of 94.1% and 94.8%, respectively [90].

1.2.2 Questionnaire survey

A questionnaire was designed to investigate the risk factors for herd level sero-positive responses to *L*. Hardjo. Table 1 shows the contents of the questionnaire. The questions were chosen based on the results of similar studies of dairy herds [55, 75, 92, 95]. Open questions were used for the items of the farm information, and closed questions were used for the other items of the questionnaire. The survey was conducted between September 2014 and January 2015 by veterinary medical clinicians who regularly visit these herds, and the herd owners were interviewed. While the survey was conducted, no investigator knew the result of the ELISA test. Pre-testing on a small sample from the study population was conducted and then the questionnaire was revised before applying it to the study population. All the investigators were explained how to conduct the survey before the administration. Data from the survey and the diagnostic tests were digitized using an Excel® spreadsheet (Microsoft® Office® 2013, USA).

Table 1. The contents of the questionnaire regarding *Leptospira* Hardjo infection in dairy herds (n = 109) in southern Tohoku, Japan

Category	Items
Farm information	Location of the farm, herd size, average parity
Herd management	Stall style, conduct of grazing, sources of feed and water, bedding material, calf management (feeding, contact with cows), parturition management (existence of separate calving pen, timing to discard placenta, isolation of neonatal calves), contact with other domestic pet animals
Hygiene management	Frequency of cleaning and disinfecting pens, change of clothing and boots of visitors at farm visits, invasion of rodents and other wild animals, types of vaccination and vermicide used
Disease incidence	Frequency of visits by a veterinarian, frequency of abortion
Movement of cattle	History of introduction of cows within one year/ five years, history of use of common grazing within one year/ five years

1.2.3 Collection of annual milk yield data

To understand the economic impact of leptospirosis on milk yield, annual milk yield data of 109 farms in 2014 were obtained from YDFC.

1.2.4 Statistical analysis

The sensitivity and specificity for the ELISA kit were known as stated above. However, these parameters were calculated at the individual animal level, and cannot be applied to the herd level [94]. Therefore, this study used the apparent prevalence, and the 95% confidence interval (CI) was calculated.

For the univariable risk factor analysis at the herd level, comparisons between the herd level sero-positivity on each factor of the questionnaire were analyzed. Wilcoxon rank sum test was performed for count and non-normally distributed continuous data, student’s *t* test for normally distributed continuous data, Pearson's Chi-squared test with Yates' continuity for binary and categorical data, and Fisher’s exact test for data when at least one cell included expected frequencies < 5.

The variables with a *p*-value < 0.2 [24] in univariable analyses were investigated further for collinearity, and variables whose correlation coefficient were < 0.9 with any of the other variables, and variables that had biological plausibility were fed into a multivariable model as explanatory variables. For the multivariable analysis, a logistic regression was performed using a generalized linear model (GLM) with binomial errors choosing the serological test results as the outcome variable. Step-wise model simplification was performed checking with a likelihood ratio test. Confounding was tested by monitoring the change of logit of a factor of interest by removing a suspected confounder from the model

[19]. Interaction terms among all the variables in the simplest model [24] were tested. To evaluate a model, goodness-of-fit tests by Hosmer-Lemeshow test and Akaike's Information Criteria (AIC) were used. All the statistics were performed using statistical software R version 3.1.0 [81].

To investigate for spatial clustering of *L*. Hardjo sero-positivity, spatial scan statistics were performed using SaTScan™ version 9.4.4 [52]. For the analysis, a purely spatial analysis for scanning the clusters with high rates using the Bernoulli model was used, and the default settings were used for the other features. The locations of participating farms were provided by YDFC.

For the economic impact, annual milk yield per cow, as total milk yield delivered from a farm in 2014 divided by the number of adult cows in the farm, was compared between sero-positive and negative herds using Wilcoxon Rank Sum test.

1.3. Results

1.3.1 Prevalence

Seventy-one of the 109 sampled herds were positive, and the herd-level apparent prevalence was 65.1% (95% CI: 56.2-74.1%).

1.3.2 Univariable analysis

The size of sero-positive herds (mean: 60.4, median: 39, range: 4 - 500) were significantly larger than that of sero-negative herds (mean: 30.1, median: 27, range: 4 - 160, $p < 0.001$). The average herd size of adult cows of the studied farms was 50.0, and was representative of dairy herds in Japan (49.1

animals/farm), but not Yamagata Prefecture (the target population, 30.3 animals/farm) [64]. Table 2 shows the results for binary and categorical variables. A significantly negative correlation of the presence of cats at the farm premises and sero-prevalence was identified ($p = 0.005$). A recent history of the introduction of cattle and/or use of common grazing was positively associated with sero-prevalence ($p < 0.001$). Herds with cattle introduced from or returned from Hokkaido had significantly higher sero-positivity than those without ($p < 0.001$), while this relationship was reversed for herds in Tohoku district ($p < 0.001$).

There was no variable whose correlation coefficient was > 0.9 with any of the other variables. The variable indicating the introduction from and/or the use of common grazing in Tohoku district was eliminated from the multivariable model, as the farms which did this had lower sero-prevalence than those that did not, while all the farms which did not introduce cattle from Tohoku introduced animals from Hokkaido.

Table 2. Univariable analysis results for binary and categorical data ($p < 0.2$) associated with sero-positivity against *Leptospira* Hardjo

Variables	Positive herds	Negative herds	Prevalence (%)	Statistics	*p*-value
Stall style				Fisher's Exact Test	0.09
Free stall	11	1	91.7		
Free barn	1	1	50.0		
Tie stall	55	34	61.8		
Bedding material					
Straw				Chi-squared test	0.08
Yes	18	16	52.9	$x^2 = 3.08$, df = 1	
No	48	18	75.0		
Sawdust				Chi-squared test	0.19
Yes	9	9	50.0	$x^2 = 1.71$, df = 1	
No	57	25	69.5		
Presence of cats				Chi-squared test	0.005
Yes	14	18	43.8	$x^2 = 7.84$, df = 1	
No	57	20	74.0		
Frequency of vets visits (time/week)				Chi-squared test	0.08
Twice or more	28	8	77.8	$x^2 = 3.1$, df = 1	
Once or fewer	39	28	58.2		
Introduction of cows/use of common grazing within 5 years				Fisher's Exact Test	< 0.001
Yes	67	26	72.0		
No	0	10	0		
Introduced/returned from					
Hokkaido				Chi-squared test	
Yes	57	7	89.1	$x^2 = 23.6$, df = 1	< 0.001
No	8	15	34.8		
Tohoku				Chi-squared test	
Yes	14	16	46.7	$x^2 = 18.5$, df = 1	< 0.001
No	51	5	91.1		

1.3.3 Multivariable analysis

Two factors: the herd size and the place of introduction and/or common grazing remained in the simplest model (see Table 3). The larger herd size (slope = 0.08, standard error (SE) = 0.03; odds ratio (OR) = 1.08, 95% CI: 1.03 – 1.15, $p = 0.004$) and the introduction of cattle from and/or use of common grazing in Hokkaido (difference of logit = 2.84, SE = 0.71; OR = 17.13, 95% CI: 4.69 – 79.23, $p < 0.001$) were identified as risk factors for *L.* Hardjo. The removal of herd size from the final model changed the logit of introduction from/use of common grazing in Hokkaido by 4.3% ((2.84097-2.7257)/ 2.7257, data not shown in the table), thus no strong confounding was found between the two factors. Also, no significant interaction was found between the two factors. However, the number of adult cows was significantly larger in the farms introducing cattle from/use of common grazing in Hokkaido (mean: 66.6, median: 36, range: 4 - 500) than those do not (mean: 33.3, median: 28, range: 12 - 140, $p = 0.04$).

The variable of presence of cat which was significantly associated with sero-positivity of *L.* Hardjo in the univariable analysis did not remain in the final model. Herds with cats (mean: 30.4, median: 28, range: 4 - 100) were significantly smaller than those without (mean: 58.3, median: 38.5, range: 4 – 500, $p < 0.01$), and three models were compared for goodness-of-fit further: (1) the simplest model, (2) a model including the variables in the simplest model plus presence of cats, and (3) a model including place of introduction and presence of cats as explanatory variables. Model 2 had the lowest AIC although the variable of cat presence was statistically not significant (Table 3). Presence of cat was a significant factor in the model 3. All the three models passed Hosmer-Lemeshow tests ($p > 0.05$).

Table 3. Multivariable analysis results of herd-level risk factors associated with *Leptospira* Hardjo infection in dairy herds in the southern Tohoku, Japan

Factors	Coefficient	SE	Odds ratio	95% confidence interval	*p*-value	AIC	*p*-value of Hosmer-Lemeshow test
1. Simplest model							
Reference	3.20	1.03	0.04	0.00 – 0.25	<0.01	64.7	0.91
Herd size	0.08	0.03	1.08	1.03 – 1.15	<0.01		
Introduced/returned from Hokkaido	2.84	0.71	17.13	4.69 – 79.23	<0.01		
2. Model including Herd size, place of introduction and presence of cats as explanatory variables							
Reference	-2.51	1.11	0.08	0.01 – 0.59	<0.05	64.0	0.90
Herd size	0.07	0.03	1.08	1.02 – 1.14	<0.05		
Introduced/returned from Hokkaido	2.76	0.73	15.79	4.16 – 76.73	<0.01		
Presence of cats	-1.12	0.69	0.33	0.08 – 1.26	0.10		
3. Model including place of introduction and presence of cats as explanatory variables							
Reference	0.02	0.52	1.02	0.36 – 2.85	0.97	73.5	0.96
Introduced/returned from Hokkaido	2.66	0.63	14.35	4.39 – 54.05	<0.01		
Presence of cats	-1.57	0.63	0.21	0.06 – 0.70	<0.05		

SE: standard error, AIC: Akaike's Information Criteria

1.3.4 Spatial analysis

The spatial distribution of *L*. Hardjo sero-positive and sero-negative herds is shown in Figure 2. The spatial scan statistic detected a most likely cluster (relative risk = 1.87, log likelihood ratio = 9.93, radius = 13.70 km, $p < 0.01$) in the south part of the study area (Fig. 2). No significant secondary cluster was detected.

The proportion of herds with cattle introduced/returned from Hokkaido in the Okitama region (81.7% (58/ 71)), where the disease cluster was detected, was higher than that in Murayama region (37.5% (6/ 16), $p < 0.001$). The herd size in Okitama (median: 35 adult cows) was larger than that in Murayama (median: 18 adult cows, $p = 0.002$). The farm density (number of sample farms/area) and cow density (number of adult cows at sample farms/area) in Okitama was 0.05/ km^2 (87 farms/1757.7 km^2) and 2.7/ km^2 (4698 adult cows/1757.7 km^2) respectively, whilst in Murayama the densities were 0.02/ km^2 (22 farms/880.5 km^2) and 0.8/ km^2 (706 adult cows/880.5 km^2) respectively.

1.3.5 Impact on milk production

The annual milk yield per cow was not significantly different between sero-positive (mean 8,853.7 litre) and negative herds (mean 8,071.2 litre, $p = 0.11$).

1.4. Discussion

In this study, the herd level sero-prevalence of *L*. Hardjo in 109 herds belonging to the largest dairy association in Yamagata Prefecture, Japan was determined by ELISA. A previous study of only 10 herds in Yamagata reported a high herd level sero-prevalence of *L*. Hardjo (70%) using the same ELISA kit [48]. In this study a more representative status for the region was identified because all 109 farms in the largest dairy association were sampled, indicating a slightly lower apparent prevalence of 65.1%. Moreover, it was confirmed that, geographically, *L*. Hardjo is prevalent throughout Yamagata.

This was the first risk factor analysis for *L*. Hardjo infection in Japanese dairy herds. We identified two risk factors from risk factor analysis which were large herd size and history of cattle staying in Hokkaido. A third factor of farm/cow density was determined from spatial analysis. In most of the previous studies, large herd size was a common risk factor for both beef and dairy herds [55, 75, 89]. Several studies have proposed that this may be due to a greater chance of susceptible animals coming into contact with urine from infected animals, because cows are kept at higher within-farm density, and *L*. Hardjo may persist longer in larger intensive herds [36, 55, 75, 89]. In fact, herd size is a common risk factor for a wide variety of animal diseases [89]. The herd size of the study population was relatively larger than that of the target population because the study population included an intensive dairy farming area in Yamagata. The herd size was close to that of whole Japan and was representative of dairy herds in Japan.

In the risk factor analysis we found a remarkable change of logit for the factor of large herd size when the factor of history of cattle staying in Hokkaido was removed from the model which suggested a strong association between the two variables. Hokkaido is the largest dairy industry region in Japan and it plays a role in distributing heifers to dairy farmers in all of the other parts of the country. Particularly large scale dairy farmers are introducing heifers from, and/or sending them to common grazing in Hokkaido because it is so difficult for them to find an alternative way. We confirmed the findings of a previous study [48] which showed high sero-prevalence of *L*. Hardjo in dairy cattle in Hokkaido. Therefore, vaccination of susceptible animals against *L*. Hardjo and/or improvement of hygiene practices are highly recommended for farmers introducing cattle from Hokkaido, not only in Yamagata, but also in other prefectures.

Although the presence of cats at a farm did not remain in the simplest model, it was a significant factor in the univariate analysis. Further analysis showed that the model including herd size, cattle introduction from Hokkaido and presence of cats had the best prediction among the three models compared, though the factor, presence of cat was not significant. Larger farms were less likely to have a cat, and this is biologically plausible because larger farms may introduce higher biosecurity and do not have domestic animals other than cattle in the farms. Having cats may be an effective preventative option because they reduce the number of rodents by catching them. Rodent infection with *L*. Hardjo is sporadic because they are not the maintenance host [36]. However, they have a high probability of contact with both cattle urine and feed, which may facilitate the long term maintenance of the pathogen in a farm.

This study found a disease cluster in the south of the study area in the Okitama region, where cow and dairy farm densities are high. Higher prevalence was associated with regions characterized as livestock production area in Mexico [96], and herd management practices such as larger herd size [89, 90], beef cattle compared to dairy [62], grazing acres, proportion of wet land grazed and stock bull [89], and longer period of housing cattle during winter, which was relevant to close contact between cows [55] in Ireland.

In terms of the public health impact, in New Zealand, although almost all dairy farmers vaccinate their livestock against leptospirosis [25], the number of notified human cases caused by *L*. Hardjo is the largest among leptospiral serovars [93], suggesting infected cattle and/or dairy products may be the largest source of infection. However, human cases of *L*. Hardjo infection have never been reported in Japan. This is probably because conducting a serotype specific *L*. Hardjo diagnostic test is not common for human leptospirosis in Japan. The most human cases are reported in the southern part of Japan, which has a subtropical climate, and only two leptospirosis cases in farmers in Yamagata Prefecture were reported between 2012 and 2014 [91], and the serotype specific *L*. Hardjo diagnostic tests were not conducted [91]. However, considering high prevalent areas of *L*. Hardjo in dairy cattle like Yamagata Prefecture, human leptospirosis caused by *L*. Hardjo may be underreported especially among farmers and those working in livestock industries.

In our study, there was no significant relationship between herd level milk yield and sero-prevalence of *L*. Hardjo, and this was consistent with a previous report [76]. However, leptospirosis may pose economic impact due to degraded reproductive performance. One previous study reported a significant negative relationship between *L*. Hardjo herd-level sero-prevalence and reproductive performance [76]. Future studies should investigate this, as it is particularly important for understanding the magnitude of the economic losses from this disease.

In conclusion, this study revealed that *L.* Hardjo is prevalent among large herds, particularly in the aggregated dairy production area in Yamagata Prefecture. The main source of disease introduction was purchase of cattle from and/or use of common grazing in Hokkaido. An investigation into the epidemiology and economic burden in dairy herds in other parts of the country is urgently required to understand the potential public health impact as well as economic losses in the dairy industry.

A coordinated control program for *L.* Hardjo in dairy herds should include vaccination and improvement of biosecurity and rodents control. Communication to farmers about the risk of infection should be started.

1.5. Abstract of Chapter 1

A cross-sectional study was designed to generate information on the herd level prevalence and the risk factors for *Leptospira* serovar Hardjo in Yamagata, the southern Tohoku, Japan. Bulk tank milk samples from 109 dairy herds were used to test the herd level sero-prevalence of *L.* Hardjo using a commercial ELISA kit, which detects both *L. interrogans* serovar Hardjo and *L. borgpetersenii* serovar Hardjo. A questionnaire survey was conducted at the sampled farms, and univariable and multivariable analyses were performed. Spatial clustering of *L.* Hardjo at the herd level was examined using spatial scan statistics. Seventy-one herds were found to be positive for *L.* Hardjo, and the apparent herd prevalence was 65.1% (95% CI: 56.2-74.1%). The risk factors for sero-positivity were larger herd size ($p = 0.004$) and cows with a history of staying in Hokkaido ($p < 0.001$). The spatial scan statistic detected a most likely cluster (relative risk = 1.87, log likelihood ratio = 9.93, radius = 13.70 km, $p < 0.01$) in the southern part of the

study area where there are large herd sizes and farm density is high. This study revealed that *L.* Hardjo is prevalent throughout Yamagata, and large scale herd owners introducing cows from Hokkaido in particular should be aware of the risk of infection.

Chapter 2. Effect of chemical tick control practices on tick infestation and *Theileria parva* infection in an intensive dairy production region of Uganda

2.1. Introduction

East Coast fever (ECF) is a tick-borne disease (TBD) of cattle whose etiological agent is the protozoan parasite *Theileria parva*. The parasite is transmitted by the tick *Rhipicephalus appendiculatus*, which feeds and drops from infected cattle during the preceding stage of its lifecycle [73]. *R. appendiculatus* feeds on animals at the larval, nymph, and adult stages. ECF is a problem due to the economic losses resulting from high morbidity and mortality, milk production loss, and the cost of controlling the ticks and the disease [33, 57]. In order to control ECF on dairy farms, several methods have been developed to control the vector. One major method is a chemical control by use of acaricides. However, severe acaricide resistance has been reported in Uganda [109] as well as the rest of the world [1]. Acaricide resistance is a natural response to selection pressure [85], and inappropriate farm tick control practices may facilitate and accelerate tick resistance development. Abbas et al. (2014) noted that acaricide application practices are the most important factors that influence the pace at which resistance develops. Consistent use of the same acaricide class on a farm for a long period of time is amongst the leading drivers of selection for resistance [40].

In Uganda, a high proportion of acaricide-resistant ticks has been reported [109]: 90% (27/30) of *Rhipicephalus* tick populations tested are resistant to the synthetic pyrethroid (SP) class; resistance has also been observed for organophosphate (OP, 13.3%), amitraz (12.9%) and OP-SP co-formulations (co-formulations, 43.4%). Farmers in western Uganda complain about acaricide failure due to acaricide resistance [109]. It has been reported that in the southwestern region of Uganda, all the farms use chemical acaricides for tick control, and inappropriate dilutions, higher frequency of use, malpractice in acaricide rotation, and use of substandard equipment for spraying are observed (Vudriko et al., 2018). Without proper knowledge of acaricide resistance, farmers' tick control practices may have even worsened over time. To overcome this situation in Uganda, an integrated qualitative intervention approach, evidence-based acaricide tick control model (EBATIC model) (Vudriko et al., 2018), has been developed. Within this framework, acaricide rotation from either SP, OP, or co-formulations to amitraz; and amitraz to SP, OP, or co-formulations is recommended. The present study focused on the farm-side evaluation of tick control practices based on the EBATIC model and risk factors associated with tick infestation of dairy cattle, acaricide resistance, and *T. parva* infection to provide quantitative evidence to prioritize effective countermeasures that are suitable for the intensive dairy production areas in southwestern Uganda.

2.2. Materials and methods

2.2.1. Study area, design, farm selection

A cross-sectional study was conducted from October 2016 to May 2017 among dairy cattle herds in Mbarara District, located in southwestern Uganda (Fig. 3). Thirty farms were selected by purposive sampling by the district principal veterinary officer to represent the area based on the following criteria: (a) herd size: 5 farms with small herds (< 10 adult cows per herd, including both milking and dry cows), 20 with medium herds (between 10 and 40 adults per herd), and 5 with large herds (> 40 adults per herd); (b) herd management type: 5 to 10 zero-grazing farms, and 20 to 25 grazing farms; (c) accessibility: farms accessible by the project team using a vehicle for regular visits; (d) distribution of farms: 2 to 5 farms per sub-county and 5 to 6 farms per milk collection center, to facilitate diffusion of techniques; and (e) commitment to continue participating in the Japan International Cooperation Agency (JICA) Safe Milk Promotion Project, which aims to improve dairy productivity in Mbarara District.

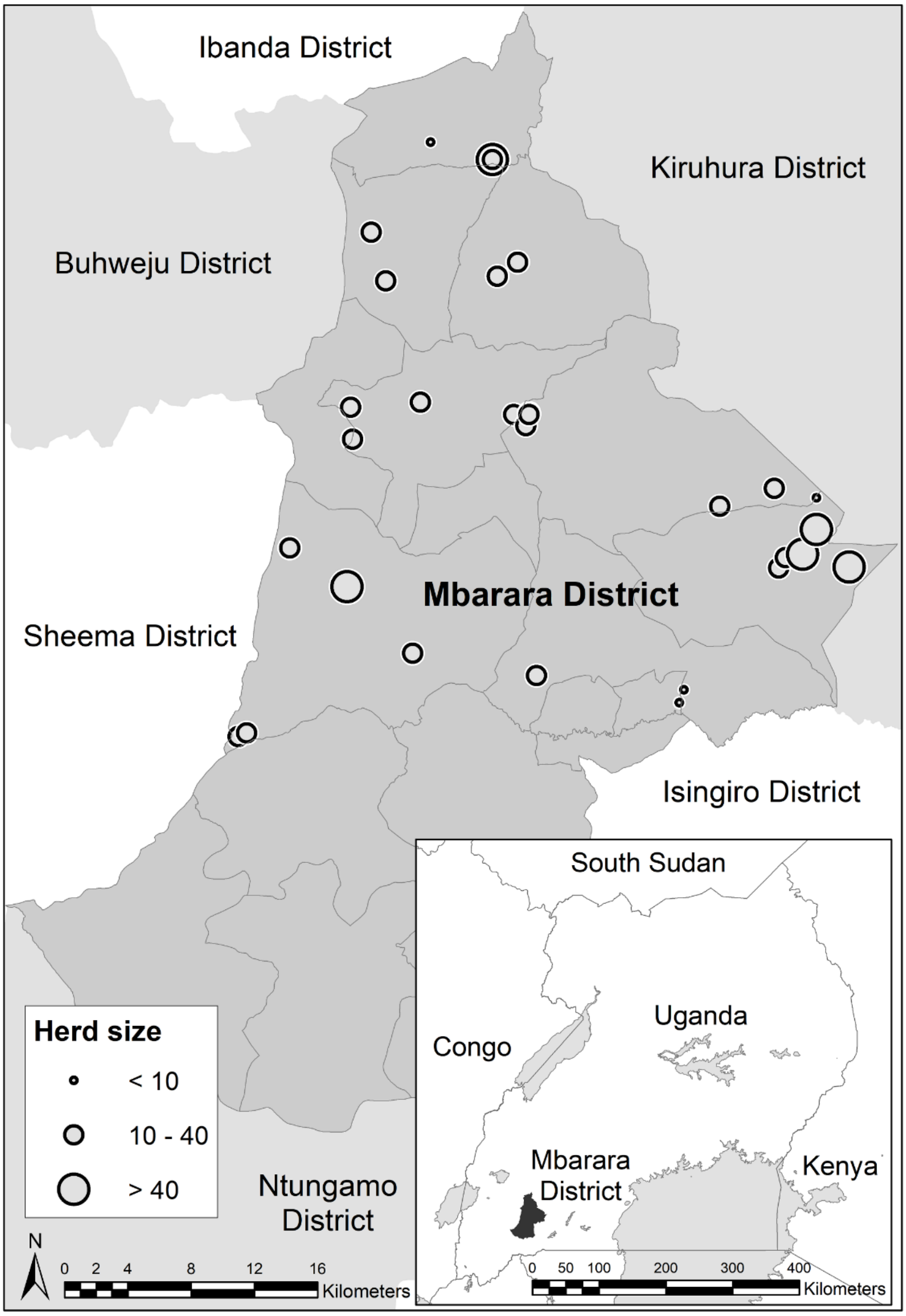


Figure 3. Map showing the geographical locations and the sizes of the study herds in Mbarara, Uganda

2.2.2. Data collection

Questionnaire survey

The presence and quality of herd-level tick control infrastructure, acaricide use, production systems, and herd size were studied between July and August, the dry season, in 2017. The presence and quality of tick control infrastructure and equipment such as farm fences, cattle crushes, clean water sources, bucket pumps for spraying, stores for acaricides, measuring cylinders, and mixing vessels for acaricides (0: absent, 1: poor, 2: fair, 3: good, 4: very good) were assessed and recorded on a check list during the survey. The quality was assessed by referring to the recommended infrastructure and equipment criteria prepared under the JICA EBATIC project (Vudriko et al., 2018). Information on acaricide class, duration in months and frequency used, and who applies and supervises acaricide application were collected for the current (acaricide in-use at the time of data collection), immediately previous, and past acaricide use through interviews using a structured questionnaire. Acaricide rotation was considered incorrect if the change of acaricide was within the same class of acaricide, changing from co-formulated acaricide to constituent mono-formulations, or not being sure of the brand name of the acaricide used before changing to the current acaricide in use.

Tick infestation and acaricide resistance

From 10 to 15 adult dairy cattle were randomly selected and were driven to the cattle crush where they were assessed for the presence or absence of visible adult ticks on one half (side) of the body. The level of tick infestation was graded as very low, low, high, and severe the criteria developed for this study: very low, no visible adult ticks on half the body; low, 0-5 mean number of adult ticks on half the body; high, 5-20 mean number of adult ticks on half the body; and severe, 20< mean number of adult ticks on half the body. Visibly engorged female ticks were hand-picked from their various attachment sites. The ticks were transferred into aerated sample bottles and transported to the Research Center for Ticks & TBD Control (RTC) laboratory at Makerere University, and their species were identified using a published manual [111]. Acaricide efficacy tests were conducted for farms where engorged female ticks were available (n = 13). We adopted the method proposed for insecticide resistance testing by World Health Organization [112] following the method described in a previous study [109]. The manufacturers' recommended concentration was considered as the diagnostic/discriminating dose for all chemicals.

T. parva positivity

Sample size was calculated based on estimating prevalence in the study population [20], setting a confidence level of 95%, a precision of the estimate of 5%, and an *a priori* estimate of the prevalence of 56.2% [43], which required 378 cattle. As a result, a total of 420 blood samples from calves to adults of exotic- and cross-breed dairy cattle at the study farms were collected. Each blood sample collected was applied on an FTA Classic Card (GE Healthcare) in accordance with the manufacturer's instructions. *T. parva* diagnosis via FTA card-NaOH-based polymerase chain reaction (PCR) [51, 101, 103, 116] was performed for the collected blood samples. A farm that had at least one *T. parva*-positive cattle was classified as a positive farm.

2.2.3. Statistical analysis

Descriptive statistics

Response counts and proportions were calculated for categorical variables of the data collected. The mean herd size, as well as the median and range were calculated. The overall prevalence of *T. parva* was calculated at the individual- and farm-levels using the PCR results above.

Effect of the quality of farm tick control infrastructure/equipment on the level of tick infestation at the herd level

To evaluate the relationships between tick control infrastructure/equipment and tick infestation on dairy cattle, univariable analyses were performed first at the herd-level using proportional odds models for tick infestation, an ordinal response variable. Then second, using the factors whose p-values were < 0.2 from the univariable analyses above and in the next section, a causal diagram was built using DAGitty software [100] to understand the causal relationships between those factors and to detect potential confounders. To estimate the total effects of tick control infrastructure/equipment on tick infestation, multivariable analyses were performed by setting each factor with a p-value < 0.2 from the univariable analyses as an exposure variable and potential confounders detected by the causal diagram.

Effect of the quality of farm tick control infrastructure/equipment, and the frequency and duration of acaricide use on *T. parva* positivity at the individual cow level

To evaluate the effects of the presence/quality of tick control infrastructure/equipment and the frequency and duration of acaricide use on *T. parva* positivity, univariable analyses were performed using generalized mixed-effects models (GLMMs) with binomial errors, selecting *T. parva* positivity as the binary response variable, and each presence/quality of tick control infrastructure/equipment and acaricide use as the exposure variable. The reason why the effects of farm level chemical tick control were measured at the individual cow level was that the age effect on *T. parva* positivity was thought to be important enough to separate it from the herd level effects. Multivariable analyses were performed to estimate the total effects of tick control infrastructure and acaricide use on *T. parva* positivity using GLMMs with binomial errors using the same procedure as in the previous section: setting each factor with a p-value < 0.2 from the univariable analyses as an exposure variable, setting variables detected by the causal diagram as confounders, and setting *T. parva* positivity as the response variable. An integrated prediction GLMM for *T. parva* positivity was also created including all the factors with p-values < 0.2 from the above univariable analyses in this section and the potential confounders detected by the causal diagram. Then, model simplification using likelihood ratio tests was performed by a backward stepwise method to obtain the final model.

Power calculation

To ensure sufficient power for multivariable analyses, power calculations were performed using the "pwr" package in the statistical software R [14] with a confidence level at 95%, large effect size (0.35) [14], and intraclass correlation coefficient (ICC) from each analysis for samples from the same herd.

2.3. Results

2.3.1. Descriptive statistics

Characteristics of farming and tick control

The mean herd size of the adult (primiparous and multiparous) cows of the study population was 35.5 (median: 28.5, range: 4-250). Most of the study herds applied an open-grazing system (93.3%, 28/30), and the rest of them, zero-grazing. The proportion having good quality farm fencing was 80.0% (24/30). That of having a cattle crush, 50% (15/30), a clean water source for acaricide dilution, 40% (12/30), and a bucket pump for spraying acaricide, 83.4% (25/30). Twenty farms out of 30 (66.7%) kept the acaricide in good storage conditions as recommended by the manufacturer's guide. The proportions of farms that used good quality measuring cylinders (well calibrated and clean) and mixing vessels for acaricide (well calibrated and clean) were 90% (27/30) and 66.7% (20/30), respectively.

The most frequently used acaricide at the time of the survey was co-formulations (40.0%, 12/30), followed by amidine (36.7%, 11/30), SP (10.0%, 3/30), and OP (10.0%, 3/30). An acaricide that was being used by one of the farms was not known because the acaricide class was not written on the product used at the farm, and thus could not be classified. The mean duration for which one acaricide was used on the farm was 8.6 months (median: 3, range: 1-60). Sixty percent of the farms (18/30) applied the acaricide twice a week, while the rest of them (40%, 12/30) applied it once a week. All farms used the acaricides at a higher frequency than the manufacturers' recommendations: fortnight and/or monthly acaricide application during the dry season and weekly application during the rainy season (Vudriko et al., 2018). Incorrect acaricide rotation was observed in 43.3% (13/30) of the farms.

Tick infestation and acaricide resistance

High tick infestation was observed at 30% of the farms (9/30). Tick acaricide efficacy tests were conducted for the farms where engorged female ticks were collected (n = 13). All the tick populations tested from all the 13 farms were resistant to SP (13/13). In addition, 38.5% (5/13) of the tick populations tested were resistant to OP, 69.2% (9/13) were resistant to co-formulations, while 84.6% (11/13) were resistant to amidines. Thirty-eight percent (4/13) of the tick populations tested from all the farms were resistant to all four classes of acaricide, while 23.1% (3/13) and 38.5% (5/13) of the tick populations tested were resistant to 3 and 2 classes of acaricide, respectively. Overall, 92.3% (12/13) of the farms had multi-acaricide resistance. *R. appendiculatus*, a vector of ECF, was the dominant tick species in 61.5% (8/13) of the farms, whereas *Rhipicephalus (Boophilus) decoloratus*, a vector of babesiosis and anaplasmosis, was dominant in 38.5% (5/13).

T. parva prevalence

The overall prevalence of *T. parva* infection at the farm-level was 83.3% (25/30, 95% confidence interval (CI): 64.5-93.7), and that at the individual animal-level was 45.2% (190/420, 95%CI: 40.4-50.1). The mean age of the sampled cattle was 6.4 months (median: 4, range: 1-120). *T. parva* positivity significantly increased over cattle age (odds ratio (OR): 1.05, 95%CI: 1.01-1.09, p = 0.024).

2.3.2. Effect of farm tick control infrastructure on tick infestation at the herd level

Figure 4 shows a causal diagram including three main hypothetical associations: i) the quality of tick control infrastructure/equipment influences the appropriateness of acaricide use and thus the level of tick infestation on the farm (FAO, 1983; Vudriko et al., 2018); ii) the appropriateness of acaricide use influences the level of tick infestation [1, 40, 109] as well as the probability of *T. parva* infection [47]; and iii) the level of tick infestation influences the probability of *T. parva* infection [30, 47]. For the herd-level analysis, the herd size and grazing system were considered as confounders, whereas for individual-level analysis, the age of cattle was considered. A multivariable model found that a good quality cattle crush was a preventive factor for tick infestation at the herd-level, meaning that farms that had good quality cattle crushes had lower tick infestation on the body surfaces of cattle (OR: 0.32, 95%CI: 0.15-0.63, p = 0.001, n = 30, Table 4). This analysis had 78.1% power to detect a large effect size for a model including 3 explanatory variables.

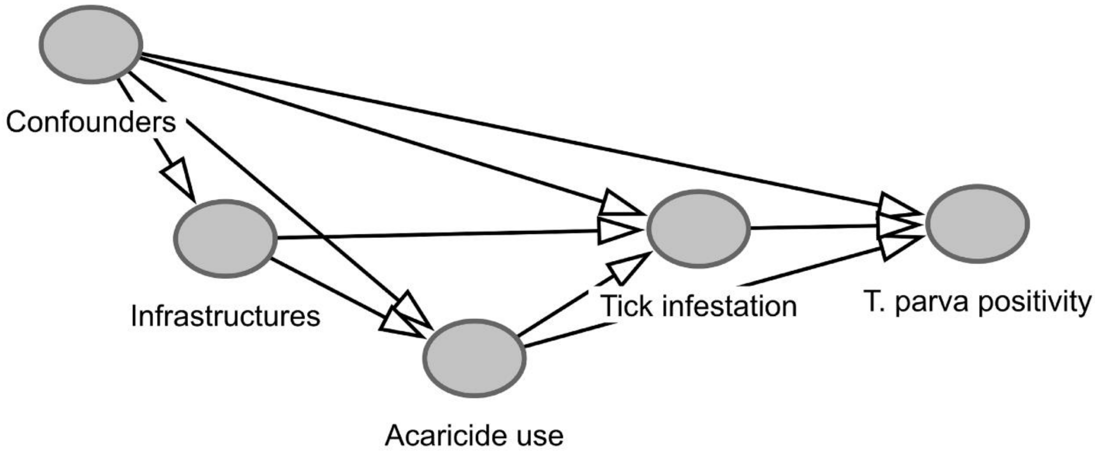


Figure 4. Causal diagram of the relationships between tick control infrastructure, acaricide use, tick infestation, and *Theileria parva* infection

Table 4. Effect of tick control infrastructure on tick infestation at the herd-level: multivariable risk factor analysis results for factors whose *p*-values were less than 0.2 from the univariable analysis

	OR	95%CI	*p*-value
Cattle crush	0.32	0.15-0.63	0.001
Herd size	1.01	1.00-1.03	0.099
Grazing	0.76	0.06-9.71	0.827

OR: odds ratio, CI: confidence interval

Ordinal variables were used for tick infestation (1: very low, 2: low, 3: high, 4: severe) and the quality of cattle crush (0: absent, 1: poor, 2: fair, 3: good, 4: very good). Herd size was the total number of milking and dry cows. A binomial variable was used for grazing (0: zero-grazing, 1: open-grazing).

2.3.3. Effect of the quality of farm tick control infrastructure/equipment, and the frequency and duration of acaricide use on *T. parva* positivity at the individual cow level

To estimate the total effect of the quality of tick control infrastructure/equipment on *T. parva* infection with variables whose *p*-values were < 0.2 from the univariable analyses, four models were built to assess the effects of the qualities of acaricide storage, acaricide measuring cylinders, acaricide mixing vessels, and farm fencing on *T. parva* positivity at the individual-level (Table 5). As a result, two preventive factors for *T. parva* infection were found: the presence of secure acaricide storage (OR: 0.36, 95%CI: 0.17-0.76, p = 0.008, n for herd = 30, n for cattle = 420) and good quality measuring cylinders for acaricide dilution (OR: 0.32, 95%CI: 0.11-0.93, p =0.036, n for herd = 30, n for cattle = 420). These models with ρ of 0.5 had 94.1% power.

Table 5. Effect of tick control infrastructure on *Theileria parva* positivity at the individual-level: multivariable risk factor analysis results for factors whose *p*-values were less than 0.2 from the univariable analysis

	OR	95%CI	*p*-value
Effect of acaricide storage management			
Fixed effect variables			
Acaricide storage	0.36	0.17-0.76	0.008
Herd size	1.00	0.98-1.02	0.938
Grazing	2.54	0.09-70.70	0.583
Age (months)	1.05	1.01-1.09	0.015
Random effect variable	Variance	Standard deviation	ICC
Herd	3.39	1.84	0.51
Effect of acaricide measuring cylinder quality			
Fixed effect variables			
Measuring cylinder for acaricide	0.32	0.11-0.93	0.036
Herd size	1.00	0.98-1.02	0.799
Grazing	4.86	0.16-149.61	0.366
Age (months)	1.04	1.01-1.08	0.023
Random effect variable	Variance	Standard deviation	ICC
Herd	3.96	1.99	0.55
Effect of acaricide mixing vessel quality			
Fixed effect variables			
Acaricide mixing vessel	0.69	0.37-1.29	0.247
Herd size	1.00	0.98-1.02	0.932
Grazing	6.79	0.19-245.26	0.296
Age (months)	1.04	1.01-1.09	0.026
Random effect variable	Variance	Standard deviation	ICC
Herd	4.45	2.11	0.58

Table 5 (continued)

	OR	95%CI	*p*-value
Effect of farm fence quality			
Fixed effect variables			
Farm fence	0.58	0.28-1.22	0.152
Herd size	1.00	0.98-1.02	0.819
Grazing	8.01	0.26-242.20	0.232
Age (months)	1.05	1.01-1.09	0.022
Random effect variable	Variance	Standard deviation	ICC
Herd	4.08	2.02	0.55

OR: odds ratio, CI: confidence interval, ICC: intraclass correlation coefficient
Binomial variables were used for *Theileria parva* positivity (0: negative, 1: positive) and grazing (0: zero-grazing, 1: open-grazing). Ordinal variables were used for the quality of farm tick control infrastructure: farm fencing, acaricide storage, measuring cylinder, and mixing vessel for acaricides (0: absent, 1: poor, 2: fair, 3: good, 4: very good). Herd size was the total number of milking and dry cows.

To estimate the total effect of the frequency and duration of acaricide use on *T. parva* infection, three models were built for the duration of acaricide use, and the frequency of use for immediately previous and past acaricide use (Table 6). Of these factors, two were found to be risk factors for *T. parva* infection: a longer period of the same acaricide use (OR: 1.06, 95%CI: 1.01-1.10, p =0.012, n for herd = 28, n for cattle = 337), and a higher frequency (twice a week compared to once a week) of immediately previous acaricide use (OR: 11.70, 95%CI: 1.95-70.13, p =0.007, n for herd = 26, n for cattle = 326). The model for the effect of acaricide duration had 85.7% power.

Table 6. Effect of frequency and duration of acaricide use on *Theileria parva* positivity at the individual-level: multivariable risk factor analysis results for factors whose *p*-values were less than 0.2 from the univariable analysis

	OR	95%CI	*p*-value
Effect of duration of acaricide use			
Fixed effect variables			
Duration of acaricide use (months)	1.06	1.01-1.10	0.012
Acaricide storage	0.53	0.26-1.09	0.083
Measuring cylinder for acaricide	0.39	0.14-1.10	0.075
Acaricide mixing vessel	1.38	0.75-2.52	0.297
Farm fence	0.59	0.32-1.08	0.085
Herd size	1.05	1.00-1.10	0.062
Grazing	1.19	0.07-19.69	0.906
Age (months)	1.06	1.02-1.10	0.004
Random effect variable	Variance	Standard deviation	ICC
Herd	1.98	1.41	0.38
Effect of frequency of immediate previous acaricide use			
Fixed effect variables			
Frequency of immediate previous acaricide use	11.70	1.95-70.13	0.007
Acaricide storage	0.25	0.11-0.57	0.001
Measuring cylinder for acaricide	0.60	0.21-1.69	0.335
Acaricide mixing vessel	2.35	1.13-4.88	0.022
Farm fence	0.67	0.35-1.28	0.228
Herd size	1.02	0.97-1.08	0.378
Grazing	1.21	0.08-19.15	0.892
Age (months)	1.10	0.97-1.24	0.125
Random effect variable	Variance	Standard deviation	ICC
Herd	1.80	1.34	0.35

Table 6 (continued)

	OR	95%CI	*p*-value
Effect of frequency of second previous acaricide use			
Fixed effect variables			
Frequency of second previous acaricide use	1.77	0.13-24.26	0.670
Acaricide storage	0.43	0.17-1.11	0.080
Measuring cylinder for acaricide	0.46	0.14-1.49	0.195
Acaricide mixing vessel	1.54	0.63-3.73	0.341
Farm fence	0.65	0.28-1.53	0.327
Herd size	1.02	0.96-1.09	0.430
Grazing	2.09	0.03-165.00	0.741
Age (months)	1.06	0.97-1.16	0.168
Random effect variable	Variance	Standard deviation	ICC
Herd	2.55	1.60	0.44

OR: odds ratio, CI: confidence interval, ICC: intraclass correlation coefficient
Binomial variables were used for *T. parva* positivity (0: negative, 1: positive), frequency of acaricide used (0: once a week, 1: twice a week), and grazing (0: zero-grazing, 1: open-grazing). Ordinal variables were used for the quality of tick control infrastructure: farm fencing, acaricide storage, measuring cylinder, and mixing vessel for acaricides (0: absent, 1: poor, 2: fair, 3: good, 4: very good). Herd size was the total number of milking and dry cows.

A final integrated prediction model for *T. parva* infection using the predictor variables of quality of tick control infrastructure/equipment and the frequency and duration of acaricide use was created (Table 7). The final model included significant factors: frequency of immediately previous acaricide use (OR: 35.62, 95%CI: 2.71-468.85, p =0.007), frequency of past acaricide use (OR: 0.07, 95%CI: 0.00-0.92, p =0.043), the quality of acaricide storage (OR: 0.38, 95%CI: 0.21-0.68, p = 0.001), and farm fencing (OR: 0.46, 95%CI: 0.23-0.91, p =0.027, n for herd = 22, n for cattle = 277). This analysis had 86.5% power.

Table 7. Final integrated prediction model for *Theileria parva* positivity at the individual-level

	OR	95%CI	*p*-value
Final integrated prediction model			
Fixed effect variables			
Frequency of immediate previous acaricide use	35.62	2.71-468.85	0.007
Frequency of second previous acaricide use	0.07	0.00-0.92	0.043
Acaricide storage	0.38	0.21-0.68	0.001
Farm fence	0.46	0.23-0.91	0.027
Herd size	1.01	0.97-1.06	0.568
Grazing	1.31	0.03-51.14	0.885
Age (months)	1.07	0.99-1.16	0.109
Random effect variable	Variance	Standard deviation	ICC
Herd	1.46	1.21	0.31

OR: odds ratio, CI: confidence interval, ICC: intraclass correlation coefficient
Binomial variables were used for *T. parva* positivity (0: negative, 1: positive), frequency of acaricide used (0: once a week, 1: twice a week), and grazing (0: zero-grazing, 1: open-grazing). Ordinal variables were used for the quality of tick control infrastructure/equipment: farm fencing and acaricide storage (0: absent, 1: poor, 2: fair, 3: good, 4: very good). Herd size was the total number of milking and dry cows.

2.4. Discussion

Tick-borne diseases, particularly ECF, create a huge economic burden to the dairy industry in southwestern Uganda, and in this study, the majority of farms (>66.7%, 20/30) had infrastructure and equipment for chemical tick control of an acceptable quality, except for a clean water source. Fences, cattle crushes, bucket pumps, acaricide storage conditions, measuring cylinders, and mixing vessels are reasonably cheap and accessible for many farmers. In addition, they may be easily replaced with good quality options at relatively small cost, although this might not be feasible for some smallholder farms. However, improving water source quality requires a high expenditure, because the quality of the water source depends on geographical and climatic conditions such as distance from clean river or a town, altitude, or annual rainfall. The proportion of farms having appropriate infrastructure/equipment for chemical tick control in our study was mostly consistent with a previous study in southwestern Uganda, but the proportion of farms having access to clean water was lower in our study (92.1% in the previous study in southwestern Uganda) (Vudriko et al., 2018). This difference could be from variations in geographical conditions.

The only ticks identified were *R. appendiculatus* and *R. (B.) decoloratus* in this study. This was the same result as a previous study in southwestern Uganda where intensive dairy farming has been conducted, while in the northwestern region, a greater diversity of tick species was observed (Vudriko et al., 2018). The limited tick species might be due to the high frequency of acaricide use (Vudriko et al., 2018).

The top-two acaricide classes used at the farms currently were co-formulations (40%, 12/30) and amidines (36.7%, 11/30). This result is consistent with the previous report (Vudriko et al., 2018). The lower use of SPs maybe due to increased resistance in *R. appendiculatus* ticks in southwestern Uganda (Vudriko et al., 2017). As shown in this study, all the tick samples were resistant to SPs. In this situation, farmers may wish to shift from using SP to using co-formulations and/or amidines. However, in this study high proportions of resistant ticks to co-formulations (69.2%, 9/13) and Amitraz (84.6%, 11/13) were detected from the farms where engorged female tick samples were collected. All the farms used acaricides at a higher frequency than the manufacturers' recommendations, which is once per fortnight in the dry season (Vudriko et al., 2018). They instead applied twice a week at 60% (18/30) and once a week at 40% (12/30) of the farms. In nearly half of the farms (43.3%, 13/30), incorrect acaricide rotation was observed. The high exposure of ticks to acaricides and incorrect acaricide rotation might be associated with the high tick resistance observed in this study population.

Good cattle crush quality was a preventive factor for tick infestation from the risk factor analysis. A crush is an infrastructure for restraining cattle when the farmers are spraying cattle with acaricide. The quality of a crush contributes to proper acaricide application to the whole-body surface of the cow. A previous study also reported that inappropriate acaricide spraying or apparatus were risk factors for acaricide resistance [40].

The risk factor analysis also revealed that a good quality acaricide measuring cylinder was a preventive factor for *T. parva* infection. Improper acaricide dilution at lower concentrations than the manufacturers' recommendations may accelerate the development of tick acaricide resistance. Moreover, a higher concentration may increase the health risk of toxification in farmers. In this study, we could not estimate the effect of acaricide use and/or the condition of tick control infrastructure on acaricide resistance due to the limited sample size. The sample size was calculated to estimate the prevalence of *T. parva* infection. The effects of the acaricide use on *T. parva* infection revealed by this study may include the mediated effect of acaricide resistance (because acaricide use influences resistance followed by tick infestation and *T. parva* infection). In order to estimate the direct effect of acaricide use on resistance, further studies including adequate sample sizes should be designed. The use of the same acaricide for a long period of time and at high frequency were the main risk factors from this study. The longer that farmers use the same acaricide, the higher the risk of tick resistance development [1], which may result in high tick infestation followed by high *T. parva* infection rates and ECF cases. On the other hand, too short a period of time using the same class of acaricide will increase the frequency of acaricide change, which also can facilitate multiple drug resistance in ticks. The recursive association between higher-frequency of acaricide use and *T. parva* infection (*T. parva* infection → higher-frequency of acaricide use) may also exist. Well-coordinated tick chemical control guidance by a regional veterinary authority would be key to target tick acaricide resistance, as resistant ticks can be spread between farms.

In our study, older cattle had higher *T. parva* prevalence. Similar age-dependence in *T. parva* prevalence targeting all age groups has been observed in South Sudan [58]; however, no significant relationship [47] and even a negative relationship [41] have also been reported. This variation might be due to several factors including immunity and disease control programs, but in southwestern Uganda, the positive relationship between age and *T. parva* prevalence in young cows may be due to constant infection on the farms. Overall prevalence also varies among studies [41, 43]; however, background age structure, tick species and population, and/or breeds are different, and no integrated information is available.

In conclusion, in this study we quantitatively found that the farm tick control practices and infrastructure reported in the EBATIC model were feasible and valuable, and that the factors included in the model were significantly associated with tick infestation and *T. parva* infection. In the current situation with intensive dairy farming areas in southwestern Uganda, tick control infrastructure, especially a cattle crush for restraining cattle, acaricide measuring cylinders, and acaricide mixing vessels, and the frequency and duration of the same acaricide use with effective acaricides, should be given higher priority in order to effectively manage ticks and *T. parva* infections. Tick resistance to acaricides is a very severe problem for dairy cattle management in the area. A lack of effective acaricide types is often discussed among farmers without discussing improper acaricide use practices and farm infrastructure, which are associated with tick infestation and *T. parva* infection as revealed by this survey. Teaching the right knowledge and practices to dairy farmers and to technical staff can help overcome the severe challenge of acaricide resistance.

2.5. Abstract of Chapter 2

Chemical tick control is a major means of preventing East Coast fever (ECF), especially in sub-Saharan Africa. However, in southwestern Uganda, improper tick control practices and severe acaricide resistance have been reported. The objectives of this study were to determine the risk factors associated with tick

infestation in dairy cattle and *Theileria parva* infection, and to generate evidence for the prioritization of effective countermeasures for tick control. A cross-sectional study was conducted in 30 farms in Mbarara District, and information on tick control practices and tick infestation were collected. Tick samples were collected from 13 farms to test tick acaricide efficacy. A total of 420 blood samples from calves to adults of exotic- and cross-breed dairy cattle were collected, and *T. parva* diagnosis via polymerase chain reaction was performed. All the tick populations tested were resistant to synthetic pyrethroid (13/13). Resistance to organophosphate was 38.5% (5/13); co-formulations, 69.2% (9/13); and amidines, 84.6% (11/13). The overall prevalence of *T. parva* infection at the individual-level was 45.2% (190/420, 95% confidence interval (CI): 40.4-50.1), and that at the farm-level was 83.3% (25/30, 95%CI: 64.5-93.7). A good quality cattle crush was a preventive factor for tick infestation (odds ratio (OR): 0.32, 95%CI: 0.15-0.63, p = 0.001). Well-managed acaricide storage was also a preventive factor (OR: 0.36, 95%CI: 0.17-0.76, p = 0.008), and a good quality measuring cylinder for acaricide was a preventive factor (OR: 0.32, 95%CI: 0.11-0.93, p =0.036) for *T. parva* infection. A longer period of acaricide use of the same brand was a risk factor (OR: 1.06, 95%CI: 1.01-1.10, p =0.012), and a higher frequency (twice a week) of acaricide use rather than once a week was a risk factor (OR: 11.70, 95%CI: 1.95-70.13, p =0.007) for *T. parva* infection. In current dairy farms in Mbarara District, these risk factors should be given high priority for ECF control in order to effectively manage ticks and *T. parva* infections. Teaching proper practices to dairy farmers and to technical staff should be used to overcome the severe challenge of acaricide resistance.

Chapter 3. Prevalence of subclinical mastitis and its association with milking practices in an intensive dairy production region of Uganda

3.1. Introduction

Mastitis is one of the most prevalent production diseases in dairy herds worldwide; however, information on the economic burden of this disease is limited for developing countries [29]. Clinical mastitis, which is characterized by the changes in milk composition and appearance, decreased milk yield, and clinical signs of inflammation, is easily detected, while subclinical mastitis (SCM) is difficult to detect due to the limited signs [29]. The majority of mastitis losses are from subclinical infections, which result in depressed production [11], and mastitis is regarded as the most costly disease on dairy farms worldwide [38].

Dairy production in Uganda has recently shown steady annual growth [31]. The total population in Uganda showed a rapid increase from 24.2 million in 2002 to 34.6 million in 2014 [104]. This population growth and the increase of annual per capita milk consumption [113], as well as a sharp increase of dairy exports from USD 299,032 in 2000 to USD 79,021,937 in 2017 [106], are pushing the dairy industry into an important position for economy of the country.

A high prevalence of SCM was reported in Uganda: at the cow-level, 86.2% [2], and 90% [10] in Kampala, the capital of Uganda; and 76.1%, 87.9%, and 60.7% in Kiruhura [98], Kiboga [44], and Jinja Districts [13], respectively. These studies also showed the high prevalence of contagious mastitis due to contagious bacteria such as *Staphylococcus aureus* and *Corynebacterium bovis* [2, 10, 13, 44]. A few studies reported poor milking practices in Uganda [13, 44]. Risk factors for SCM reported in Uganda included lactation stage, breed [2], herd size, zero- grazing [10], and parity [2, 10]. Very few study in Africa where cows are still milked by hand reported the relationship between milking practices and SCM [42]. No risk factor analysis has been conducted on the role in SCM of milking hygiene and practices in Uganda. Mbarara District located in southwestern Uganda is one of the most intensive and important dairy production areas in Uganda [8]; however, no studies on the prevalence of SCM have been conducted in this region. The objectives of this study were to describe the prevailing milk hygiene practices, as well as the prevalence of, and risk factors for SCM in Mbarara.

3.2. Materials and Methods

3.2.1. Study design and farm selection

This study was conducted among dairy cattle herds in Mbarara District, Uganda (Fig. 3). A cross-sectional study was carried out as a baseline survey for a three-year project, Japan International Cooperation Agency (JICA) Safe Milk Promotion in Mbarara (Safe Milk) Project between October 2016 and May 2017. Thirty farms in Mbarara District were selected by purposive sampling by the district principal veterinary officer to represent the area, based on the following criteria: (a) herd size: 5 farms with small herds (< 10 adult cows per herd, including both milking and dry cows), 20 with medium herds (between 10 and 40 adults per herd), and 5 with large herds (> 40 adults per herd); (b) herd management type: 5 to 10 zero-grazing farms, and 20 to 25 grazing farms; (c) accessibility: farms accessible by the project team using a vehicle for regular visits; (d) distribution of farms: 2 to 5 farms per sub-county and 5 to 6 farms per milk collection center, to facilitate diffusion of techniques; and (e) commitment to continue participation in the Safe Milk Project. According to the District Veterinary Office in Mbarara, the current total cattle population and the number of dairy farms in Mbarara District are estimated to be 185,680 and 10,200, respectively (personal communication).

The sample size at the cow-level was calculated based on a comparison of two proportions with the confidence level at 95% and the power at 80%: one proportion as prevalence of SCM in the exposed group and another as that of non-exposed. The mean of the prevalences was set as 75%, which was about an average of previous studies [2, 10, 13, 44, 98], and detection of a risk factor was aimed at 20% difference. The sample size was adjusted for multivariable analysis [20] by increasing 20% considering medium level confounders, and was further adjusted for a clustering effect assuming the intraclass correlation coefficient of 0.1 [20]. The number of animals per cluster of 20 (the average number of cows per farm, personal communication with the district veterinary office) was used for the calculation. Consequently, the required sample size was calculated as 504 cows (252 cows per arm).

All milking cows present in the farms at the visits were included in the study, except at the largest farm, where only the first 50 cows in the milking order were evaluated.

3.2.2. Data collection

Teat-end scoring

Teat-end scoring was conducted just before the California Mastitis Test (CMT) was performed. Teat-ends were scored according to the method described by Hulsen [39] using a scale of 1-4: 1, no observable callus ring; 2, a smooth callus ring around the teat orifice; 3, a rough callus ring; 4 a very rough callus ring. The highest quarter-level teat-end score within a cow was defined as the cow-level score.

California Mastitis Test (CMT)

CMT was performed to detect mastitis just after the individual milking in the afternoon. For farms that did not milk cows in afternoons, CMT was performed at farm visits. The results were classified as negative, trace, or score 1, 2, or 3, depending on the amount of gel formed [86]. A quarter was defined as CMT positive if it had a score 1 or above, and a cow was defined as CMT positive if it had at least one CMT-positive quarter. CMT-positive cows/quarters with and without clinical signs of inflammation were defined as clinical and SCM, respectively. Milk samples from quarters with CMT scores of 2 or higher only were collected just after CMT for microbiological tests.

Milking practice

A herd-level checklist was used to evaluate the milking practice and the level of hygiene during afternoon milking. For farms that did not milk cows in afternoons, milking practice information was requested from the owners/managers. The contents of the checklist included cow and milk equipment hygiene, cow comfort (if the cows showed stepping, kicking or restive behavior when being milked before, at the beginning and end of milking), milkers’ hand hygiene (wearing gloves; rinsing, disinfecting, and drying hands before touching teats), fore-milking by stripping the teat of the first 4 to 5 squeezes of milk, calf suckling before milking, observation to find abnormality (e.g., clots or flecks) in the milk, pre-milking dipping and wiping of the teats and teat tips, whether wiping and drying of the teats was performed by using one towel per cow, good hand-milking technique (holding a teat in the palm and removing the milk by squeezing the teat with all five fingers [18, 42]), and post-milking dipping with a proper concentration of the chemical and > 75% teat coverage. The cow hygiene was classified into two levels, good or not, referring udder hygiene score chart [87]. The original hygiene score [87] had four levels (score 1 to 4), but in the study population where grazing system is predominant, the majority of cows are free of dirt, thus the score was simplified as good (the original was score 1) and not good (> score 1). The milking cows' comfort was checked for three different timing (before, beginning of and end of milking) to understand cows' behavior when they are close to workers (signal of stress from human [39]), their teats were touched by workers (signal of teat or udder problem already with), and the milk flow was low (signal of over milking [79]).

Collection of farm and cow information

The farm-level information collected included herd size and conduct of grazing, and the cow-level information, breed, age, parity, body condition score (BCS) [32], and the calving date for cows that had delivered within roughly the past 3 months from the farm visits.

3.2.3. Microbiological testing

The microbiological testing of milk samples followed the method recommended by the National Mastitis Council of the United States of America [71]. Immediately upon collection, milk samples were stored in an ice-cooler box with cold packs, then transferred to the laboratory at Mbarara District Veterinary Office and kept at 4°C in a refrigerator. Within 24 hours after collection, a 10-μL aliquot of each sample was inoculated on a 5% sheep blood agar plate using an inoculating loop; the plates were then incubated aerobically at 37°C for 24 to 48 hours. The diagnostic tests were performed according to the method mentioned above [71]. Milk samples that yielded more than two different microbe species on a plate were considered as contaminated, unless *S. aureus* was present, in which case *S. aureus* was defined as the dominant pathogen.

3.2.4. Statistical analysis

Descriptive statistics

For numeric variables, the mean, median, and range were calculated, and for categorical variables, response counts and proportions. The overall and average herd-level prevalence of SCM were calculated. Pathogens that are known to cause mastitis, tend to live on the cow's udder and teat skin, and are transferred cow/teat to cow/teat during milking were defined as contagious [11], and the microbes isolated by plating were classified into two groups of pathogens: contagious or environmental. Since it is difficult to determine whether coagulase-negative staphylococci (CNS) behave as contagious or environmental pathogens [80], separate analyses were performed treating CNS as either contagious or environmental pathogens. The proportion of contagious mastitis out of total mastitis cases from which microbes were isolated was calculated.

Causal diagram

In order to understand the relationships between milking practices and mastitis, and to identify confounders and/or intervening variables of the relationships in multivariable analyses, a causal diagram [19] was built, including factors with the *p*-values less than 0.2 in the univariable analyses, as well as other potential confounders on a priori basis, using the DAGitty software [100].

Effects of milking practices, cow's calmness, and teat-end score on SCM

In risk factor analysis, first, univariable analyses for SCM were performed at the cow-level for farm and cow attributes, milking practices, and teat-end scores using generalized linear mixed-effects models (GLMMs) with binomial errors, selecting SCM positivity as the binary response variable, and setting herd as the random effect. Cows for which values were missing were excluded from the univariable analyses. Second, multivariable GLMMs were built for each milking practice variable whose *p*-values were < 0.2 in the univariable analysis with potential confounders defined by the causal web as explanatory variables to estimate the total effect of each milking practice [19]. Integrated prediction GLMMs for SCM were also built with all the milking practice variables whose *p*-values were < 0.2 in the univariable analyses and potential confounders and the interaction terms. The variables with multi-collinearity and less biological plausibility were excluded from the models. Backward stepwise model simplifications were performed to identify the risk and/or preventive factors. In the multivariable analysis, two level multiple imputation [12] was used for the missing data using MICE package in the R statistical software. In order to evaluate the effect of clustering at the herd level, intraclass correlation coefficients (ICCs) were calculated [21].

Relationships between milking hygiene management and infection with contagious or environmental pathogens in SCM

In addition to the risk factor analyses, the relationships between milking practices, and between the level of cow hygiene and infection with contagious or environmental pathogens were analyzed using the data from quarters that yielded microbes. Univariable analyses were performed by selecting the isolation of contagious microbes (the rest of the isolates indicate environmental microbes) as response variable in GLMMs, with the two classifications of contagious pathogens: including CNS or not. Quarters from which two microbes were isolated were classified as contagious category if at least one contagious pathogen was detected. Milking practices that may facilitate infection with contagious pathogens, and the level of cow hygiene that could be a cause of environmental mastitis, were selected as explanatory variables. All these statistical analyses were performed using the R statistical software, version 3.6.0 [82].

3.2.5. Ethical consideration

This study was performed as the baseline survey of JICA Safe Milk Promotion in Mbarara Project, based on a bilateral agreement between JICA and Mbarara District Local Government. Animals in this study were properly handled using crush during sampling to avoid their stress and injury. Informed consent was obtained from all farms included in this study.

3.3. Results

3.3.1. Descriptive epidemiology results

Herd-level variable

Mean herd size of adult cattle for the 30 participating farms was 35.5 cattle (median: 28.5; range: 4-250). Herds consisted of 4 small, 21 medium, and 5 large farms, which almost matched the farm selection criteria. Twenty-eight (93.3%) of 30 farms employed grazing systems; the other 2 farms practiced zero-grazing. Milking hygiene and practices surveys were conducted for 29 farms; the exception was a farm that performed milking only in the early morning. On the majority of the studied farms, cows were clean (89.7%, 26/29), and calm before (89.7%, 26/29), at the beginning (89.7%, 26/29), and at the end (82.8%, 24/29) of milking. Table 8 shows the milking hygiene practices observed in the studied farms. Milkers' hand and teat hygiene management levels were low. The proper hand-milking technique (specifically, not finger milking) was employed at 17.2% (5/29) of the farms. The average SCM prevalence at cow-level was 71.8% (median: 82.0%, range: 0.0-100%), while at quarter-level, 40.6% (median: 42.3%, range: 0.0-84.7%).

Table 8. Milking hygiene practices in the 29 studied dairy farms in Mbarara, Uganda

Variables	Number of farms	Percentage
Mastitic cows are milked last (NA = 1)	1	3.6
Milkers' hands		
Wearing gloves	0	0
Hands are disinfected	5	17.2
Presence of buckets to rinse and disinfect hands	6	20.7
Hands are dry before touching teats (NA = 1)	2	7.1
Forestrip 4-5 times	4	13.8
Calf suckling before milking	18	62.1
Try to detect abnormal milk	3	10.3
Apply proper hand-milking technique	5	17.2
Apply pre-dipping	0	0
Wipe and dry teat completely with towel before milking	7	24.1
Wipe teat-end before milking	5	17.2
Use of one towel per cow	5	17.2
Start milking 60-90 sec after the first teat stimulation	14	48.3
Finish milking within 5 min after first teat stimulation (NA = 2)	17	63.0
Apply post-dipping (NA = 1)	3	10.7
Teat coverage rate of >75% in post-dipping (NA = 1)	2	7.1

NA: not available, because the checklist conductor failed to observe.

Cow-level variables

Average of parity, daily milk yield (L/day), and BCS were 2.7 (median: 2, range: 1-11, *n* = 293); 9.5 (median: 8, range: 1-32, *n* = 503); and 3.0 (median: 3, range: 1.75-4, *n* = 571), respectively. The majority of farms (184/214, 86%) kept exotic breeds such as Holstein Friesian, while 14% (30/214) kept cross breeds between indigenous Ankole and exotic breeds. The 30 herds evaluated in the present study comprised a total of 608 cows, of which three (0.5%, 95% confidence interval (CI): 0.1-1.4) cows had clinical mastitis, and 417 (68.6%, 95%CI: 64.9-72.2) had SCM in at least one quarter.

Quarter-level variables

A total of 2411 quarters in 608 cows were checked for mastitis, and the prevalence of clinical mastitis was 0.2% (6/2411, 95%CI: 0.0-0.4) and that of SCM was 39.2% (946/2411, 95%CI: 37.3-41.2). The remaining 21 quarters were blind, dry, or missing teats. In the teat-end scoring, the majority (1,679/2062, 81.4%) was normal (score 1), 342 (16.6%) were smooth (score 2), and 41 (2.0%) were rough (score 3). Very rough teat-ends (score 4) were not observed. Microbiological tests were performed for 576 quarter milk samples that exhibited CMT scores of 2 or more. Of these 576 samples, 289 (50.2%) yielded microbes (Table 9). Contagious bacteria other than CNS consisted of *C. bovis*, *S. aureus*, and *S. agalactiae*, and the remaining isolates were classified as environmental microbes. The proportions of contagious mastitis quarters among those from which microbes were isolated were 75.8% (219/289, 95%CI: 70.3-80.5) and 55.7% (161/289, 95%CI: 49.8-61.5) depending on whether CNS were classified as contagious or non-contagious bacteria, respectively.

Table 9. Microbiological test results from 576 quarter milk samples of which California Mastitis Test scores were 2 or more in studied dairy farms in Mbarara, Uganda

Pathogen isolated	Number of samples	Percentage
Corynebacterium bovis	88	15.3
Staphylococcus aureus	65	11.3
Coagulase-negative staphylococci	56	9.7
Other streptococci (unidentified)	29	5.0
Trueperella pyogenes	24	4.2
Streptococcus agalactiae	5	0.9
Coliforms	4	0.7
Prototheca species	1	0.2
Yeast	1	0.2
Others (unidentified)	10	1.7
No growth	278	48.3
Two different species[a)]	6	1.0
Contaminated[b)]	9	1.6

[a)]Pairs of the listed microbes

[b)]Milk samples with more than two different species isolated on a plate were considered contaminated, unless one of the species was *S. aureus,* in which case *S. aureus* was defined as the dominant pathogen.

3.3.2. Effect of milking practices, cow's calmness, and teat-end score on SCM

Causal relationships for SCM with milking practices, and calmness at end of milking and teat score were hypothesized as Figure 5. Breed [2] and parity [2, 10] have association with SCM on the basis of a priori, so does milk yield in a biological sense. Thus they were regarded as the confounders. The variable of the cow's calmness at the beginning of milking (signal of teat or udder problem already with) was excluded from the causal diagram; although the *p*-value was < 0.2 at the univariable analysis, as this factor was presumed to be the effect (rather than the cause) of mastitis.

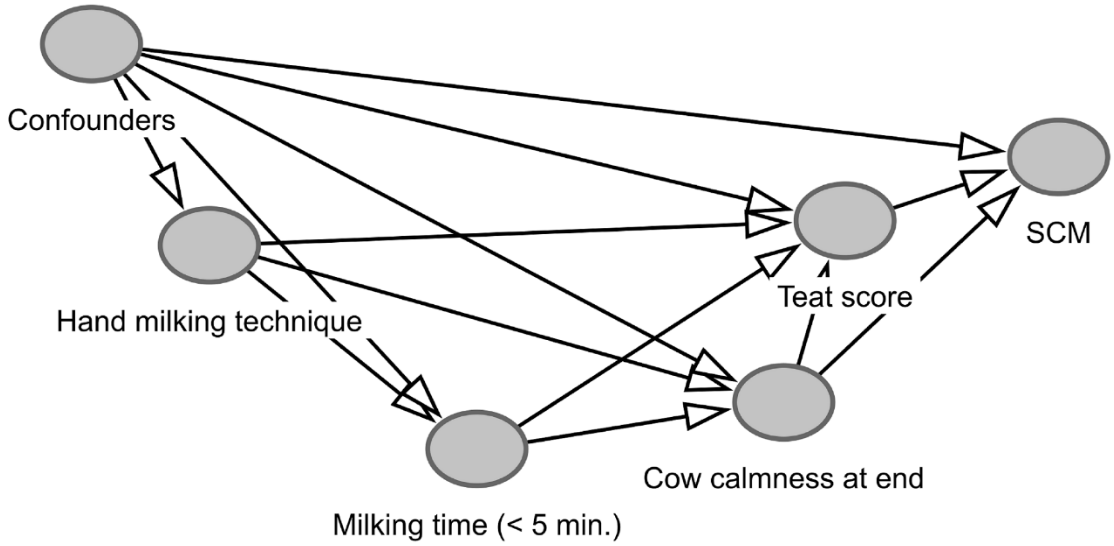


Figure 5. Causal diagram showing relationships between subclinical mastitis (SCM) and milking practices. The variables of milking practices with p< 0.2 at univariable analyses, and the observed variables associated with the milking practices and SCM on the basis of a priori were included in the diagram.

To estimate total effect of milking practices on SCM, three multivariable GLMMs: a) effect of cows calm at end of the milking and b) milking time, and c) teat-end score, were built at the cow-level with the confounders following the causal diagram above. From model a), calmness of cows at the end of milking was a preventive factor for CMT positivity (odds ratio (OR): 0.20, 95%CI: 0.05-0.79, p = 0.021, Table 10 (a)). From model c), higher teat-end score was a risk factor for CMT positivity (OR: 1.75, 95%CI: 1.14-2.68, p = 0.011, Table 10 (c)). Milking time did not have a significant effect on SCM (p = 0.203, Table 10 (b)). The final integrated prediction model included two significant predictors: loss of cow's calmness at end of milking (OR: 0.17, 95%CI: 0.05-0.64, p = 0.009) and bad teat end score (OR: 1.75, 95%CI: 1.14-2.68, p = 0.011, Table 10 (d)). No collinearity with a correlation coefficient > 0.9 was found among variables in the models. No interaction terms between explanatory variables were remained in the final prediction model.

Table 10. Multivariable risk factor analysis results for calmness, milking-practice, teat-end-score, and integrated effects on California Mastitis Test positivity

Variables	Odds Ratio	95%CI	*p*-value
a) Effect of calmness at end of the milking			
Fixed effect variables			
Cow's calmness at end of the milking	0.20	0.05-0.79	0.021
Milking time (< 5 min.)	0.53	0.14-1.96	0.331
Hand-milking technique	0.61	0.15-2.49	0.489
Parity	1.10	0.93-1.30	0.233
Milk yield	0.97	0.92-1.03	0.346
Breed	1.20	0.40-3.55	0.730
Random effect variables	Variance	Standard deviation	ICC
Herd	1.00	1.00	0.23
b) Effect of milking time			
Fixed effect variables			
Milking time (< 5 min.)	0.41	0.10-1.63	0.203
Hand-milking technique	0.55	0.12-2.51	0.433
Parity	1.10	0.93-1.30	0.253
Milk yield	0.98	0.92-1.04	0.462
Breed	1.16	0.28-4.80	0.820
Random effect variables	Variance	Standard deviation	ICC
Herd	1.33	1.15	0.29
c) Effect of teat end score			
Fixed effect variables			
Teat end score	1.75	1.14-2.68	0.011
Cows calm at end of the milking	0.19	0.05-0.72	0.015
Milking time (< 5 min.)	0.53	0.15-1.89	0.317
Hand-milking technique	0.54	0.13-2.15	0.374
Parity	1.10	0.93-1.29	0.247
Milk yield	0.97	0.91-1.03	0.297
Breed	1.18	0.36-3.86	0.766
Random effect variables	Variance	Standard deviation	ICC
Herd	0.96	0.98	0.23

Table 10 (continued)

Variables	Odds Ratio	95%CI	*p*-value
d) Integrated effects			
Fixed effect variables			
Cows calm at end of the milking	0.17	0.05-0.64	0.009
Teat end score	1.75	1.14-2.68	0.011
Hand-milking technique	0.69	0.20-2.39	0.555
Parity	1.10	0.93-1.29	0.248
Milk yield	0.98	0.92-1.03	0.421
Breed	1.25	0.38-4.09	0.697
Random effect variables	Variance	Standard deviation	ICC
Herd	1.00	1.00	0.23

ICC: Intraclass correlation coefficient

3.3.3. Relationships between milking hygiene management and infection with contagious or environmental pathogens in subclinical mastitis (SCM)

Tables 11 and 12 shows the relationships between milking practices and the level of cow hygiene, and the proportions of contagious mastitis quarters as a function of quarters from which microbes were isolated. When CNS were classified as contagious pathogens, use of one towel per cow to wipe teats was a preventive factor for infection with contagious bacteria (OR: 0.27, 95%CI: 0.09-0.85, $p = 0.025$), while good cow hygiene was positively associated with infection by contagious bacteria (OR: 6.21, 95%CI: 1.66-23.29, $p = 0.007$, Table 11). When CNS were classified as environmental pathogens, only cow hygiene was statistically associated with environmental mastitis ($p = 0.002$, Table 12).

Table 11. Relationship between contagious mastitis and milking practices/level of cow hygiene at quarter level, among quarters from which bacteria were isolated, when coagulase-negative staphylococci (CNS) was classified as contagious bacteria

Variables	Percentage of contagious mastitis	Contagious/bacteria isolated quarters	Odds Ratio	95% CI	*p*-value
Disinfecting hands before milking	79.4	27/34	1.00	0.22-4.63	1.000
Not conducted	75.3	192/255	Reference		
Presence of buckets to rinse and disinfect hands	86.3	44/51	1.82	0.45-7.33	0.400
Not present	73.5	175/238	Reference		
Drying hands after washing and before milking	69.2	9/13	0.43	0.03-5.26	0.508
Not conducted	76.3	200/262	Reference		
One towel per cow use for teat wiping	60.5	46/76	0.27	0.09-0.85	0.025
Not used	81.2	173/213	Reference		
Cow hygiene appeared good	80.8	189/234	6.21	1.66-23.29	0.007
Not good	54.5	30/55	Reference		

CI: confidence interval

The numbers of observation were 23/176/289 at herd/cow/quarter levels, other than drying hands before milking (22/167/275).

Table 12. Relationship between contagious mastitis and milking practices/level of cow hygiene at quarter level, among quarters from which bacteria were isolated, when coagulase-negative staphylococci (CNS) was classified as environmental bacteria

Variables	Percentage of contagious mastitis	Contagious/bacteria isolated quarters	Odds Ratio	95% CI	*p*-value
Disinfecting hands before milking	67.6	23/34	2.01	0.69-5.86	0.201
Not conducted	54.1	138/255	Reference		
Presence of buckets to rinse and disinfect hands	68.6	35/51	2.20	0.90-5.35	0.084
Not present	52.9	126/238	Reference		
Drying hands after washing and before milking	46.2	6/13	0.53	0.11-2.66	0.441
Not conducted	57.6	151/262	Reference		
One towel per cow use for teat wiping	44.7	34/76	0.51	0.23-1.12	0.093
Not used	59.6	127/213	Reference		
Cow hygiene appeared good	60.7	142/234	3.71	1.64-8.39	0.002
Not good	34.5	19/55	Reference		

CI: confidence interval

The numbers of observation were 23/176/289 at herd/cow/quarter levels, other than drying hands before milking (22/167/275).

3.4. Discussion

A high prevalence of SCM was seen in the study population at both the cow- (68.6%) and quarter-levels (39.2%). This prevalence was comparable to those obtained in other studies in different regions of Uganda, which yielded cow-level prevalence of 60.7-90% [2, 10, 13, 44, 98]. The prevalence of clinical mastitis was low in this study; however, the burden should be measured as an incidence rate in future as most cases occur at the early lactation stage [61].

The multivariable risk factor analyses for SCM detected a preventive factor, cow's calmness at the end of milking and a risk factor, bad teat score. Teat-end scoring originally was developed in response to the increased occurrence of damaged teats observed due to the mechanical forces of vacuum and collapsing liners following the introduction of machine milking systems [72]. However, our study identified damaged teats as a risk factor for SCM even among the hand-milking dairy farms. In the study areas, cross-breed cows have smaller teats than exotic breeds such as Frisian, and traditional milkers pull teats strongly using two fingers (middle finger and forefinger) or the combination of forefinger and thumb, especially at the end of milking when milk flow decreases (consensus observations by the authors and participating farmers). Such ways of milking might damage teats and upset the cows during milking time, either or both of which could predispose the cows to mastitis; this inference also would explain the preventive factor, cow's calmness at the end of milking. On the other hand, one study reported that a stripping technique of removing milk from the teat by moving the thumb and forefinger distally along the teat is a preventive factor for SCM [42]. In the present study, hand milking technique itself did not have an association with SCM and the milking practice checklist was applied and recorded only for the first milker at each farm. To assess milking techniques, more detailed assessments of milking techniques may be required.

Based on the microbiological cultures, the most prevalent pathogen in the study population was *C. bovis*, followed by *S. aureus* and CNS. Previous studies also found that staphylococci [2, 10, 13, 44, 98] and *Corynebacterium* spp. [44, 98] were the major isolates. In the present study, contagious microbes were isolated at a higher proportion than environmental microbes. Key control options to counteract contagious bacterial transmission are post-milking teat dipping, dry cow therapy, milking hygiene, and culling [11, 37, 54, 71, 80]. In the studied farms, hygiene practices were not performed consistently during milking, which might facilitate the transmission of contagious bacteria. Previous studies reported that level of knowledge of SCM was low in Uganda [13, 44]. Such low awareness of SCM may be a cause of the poor compliance with recommended hygiene practices. Bacterial culture in this study was performed only for milk samples with CMT 2 and higher, which might underestimate the proportion of pathogen which cause mild mastitis.

The CNS bacterial group comprises more than 50 species and subspecies [80]. CNS appear to infect the teat canal and gland from skin sources or the environment [71]. Many CNS infections are transient and the risk of cow-to-cow spread is low [71]. Thus, it is difficult to classify CNS as either contagious or environmental bacteria. In our study, the use of one towel per cow for wiping teats, intended to prevent transmission of pathogens from cow to cow, had a negative association with contagious mastitis when CNS were classified as contagious bacteria. CNS may play a role as contagious bacteria on the studied farms.

Intra-herd correlations with values < 0.2 are common for infectious diseases of animals [21]. The models built in the present study had high ICCs (> 0.2), indicating that the farms exhibited a moderate clustering effect. Herd-level management might affect to SCM, and should be the target of interventions. To deal with the clustering effect, GLMMs were used for the present study, and this statistical approach appeared adequate. However, there may have been a selection bias, given that the studied farms were participants in the Safe Milk Project and so may already have had good relationships with the District Veterinary Office. Such farms may have greater knowledge of diseases than the general population in Mbarara District, and our results might be underestimating the actual mastitis prevalence.

In conclusion, prevalence of SCM, particularly due to contagious pathogens, was high in Mbarara District. Formulation of callosity ring around the teat orifices was a risk factor, and comfortable milking may prevent SCM; the use of one towel per cow to wipe the teats would prevent contagious mastitis; and the maintenance of a clean udder environment would reduce environmental mastitis.

3.5. Abstract of Chapter 3

A high prevalence of subclinical mastitis (SCM) has been reported in Uganda; however its risk factors associated with milking practices were not known. A cross-sectional study was conducted to investigate the risk factors in Mbarara District where hand-milking is dominant. In 30 farms, herd-level milking practices were observed. California Mastitis Tests (CMTs) and teat-end scoring were conducted for 608 cows. Milk samples (n = 576) were collected for microbial culturing. Risk factor analysis for SCM were performed. Among quarters

microbes were isolated, relationships between contagious/environmental microbial infection and milking management were analyzed. The SCM prevalence was 68.6% (95% confidence interval (CI): 64.9-72.2%) and 39.2% (37.3-41.2%) at the cow- and quarter-levels, respectively. Microbes were isolated from 48.8% of the milk samples: *Corynebacterium bovis* (15.3%), *Staphylococcus aureus* (11.3%), coagulase-negative staphylococci (CNS) (9.7%), streptococci other than *Streptococcus agalactiae* (5.0%), and others (7.5%). The preventive factor for SCM was cows calm at the end of milking (odds ratio (OR): 0.20, 95%CI: 0.05-0.79, p = 0.021), and risk factor, rough teat-end (OR: 1.75, 95%CI: 1.14-2.68, p = 0.011). Classifying CNS as contagious bacteria, use of one-towel-per-cow for teat wiping was negatively associated with contagious pathogen (OR: 0.27, 95%CI: 0.09-0.85, p = 0.025), while good cow hygiene positively (OR: 6.21, 95%CI: 1.66-23.29, p = 0.007), suggesting that it is more probable to have environmental pathogens if the cow is dirty. Appropriate hand-milking practices that avoid teat damage and an introduction of one-towel-per-cow practices are expected to reduce SCM in Uganda.

Chapter 4. Current dairy herd management practices and their influence on milk yield and subclinical ketosis in an intensive dairy production region of Uganda

4.1. Introduction

Dairy production in Uganda has recently shown steady annual growth of 8%[7], and increased 2.7-fold from 585,374 tons in 1991 to 1,600,861 tons in 2015[31]. The total population in Uganda has been increasing by 3.0% annually, and showed a 1.4-fold increase from 24.2 million in 2002 to 34.6 million in 2014[104]. This population growth and the almost two-fold increase of annual per capita milk consumption from 28.5 liters in 1997 to 50 liters in 2007[113], as well as a sharp increase of dairy exports from USD 299,032 in 2000 to USD 79,021,937 in 2017[106], are pushing the dairy industry into a more important position for both food security and economy of the country.

Development of the dairy industry is determined by technical interventions: an increase in the cattle population, improved reproductive management such as introduction of exotic breeds and use of artificial insemination (AI), and feeding management. Although indigenous breeds such as Ankole and Zebu still predominate (13 million) over exotic breeds such as Friesian, Guernsey and Jersey (1 million) in Uganda as of 2016[105], the southwestern region of Uganda has intensified dairy production through introduction of exotic and cross-breeds cattle[7]. The demand for AI is high, but the proportion of farms using it is still limited (12.3%)[7], and the insemination rate in the region is still low[113]. A previous report in 2011 characterized the dairy industry in the region further as having a dominance of grazing farms, limited use of commercial supplement feeds, and 2-3 times higher milk yields in exotic and cross-breed cattle which are genetically improved than indigenous breeds[7].

From the viewpoint of milking physiology, dairy intensification involving a change of breeds and management style may increase ketotic risk in dairy herds. During the transition period around calving, from 3 weeks before to 3 weeks after, nutrition requirements for the fetus and milk production exceed energy intake, inducing a physiological state of negative energy balance (NEB)[27]. The adaptation of cows to NEB can be measured as suppressed levels of non-esterified fatty acids and β-hydroxybutyrate (BHB) in the blood, and if adaptation fails, ketosis occurs, characterized by a lack of appetite and decreased milk production[78]. Even without clear clinical signs, cattle are diagnosed with subclinical ketosis (SCK) when cows have >1.2 to 1.4 mM BHB/L blood[68, 78], resulting in reduced milk production[114] and economic loss[68]. Exotic breed cattle with a higher milk yield are more susceptible to NEB than indigenous breeds, and thus require careful feeding management during the transition period. In peri-urban Kampala, Uganda, the prevalence of SCK of dairy cows in early lactation was reported as 18.8% in 2013 and 13.9% in 2014[17]; however, SCK prevalence and feeding management in the southwestern region of Uganda have not been reported.

The objectives of this study were to describe the milk yield and nutritional status (using BHB as an indicator) of dairy cattle, and dairy management practices (in particular feeding management of dairy farms in the Mbarara district, the central area of the region in terms of culture and history), and to investigate the relationships between feeding management practices, and milk production and nutritional status of dairy cows.

4.2. Materials and methods

4.2.1. Study area

This study was conducted in the Mbarara district in southwestern Uganda (Fig. 3). The average annual rainfall is 1,200 mm, with two rainy seasons from February to May and September to December; the temperature ranges between 17°C and 30°C with a humidity of 80-90%[60] at an altitude of mostly 1,000-1,400 m. These conditions provide suitable environment for dairy production, and the area is located in the cattle corridor[49]. Farms participating in the study were located in 10 of the 17 sub-counties in Mbarara district, namely Biharwe, Bubaare, Bukiro, Kagongi, Kakiika, Kakoba, Kashare, Rubaya, Rubindi, and Rwanyamahembe. Mbarara district contains the high cattle population[63] and most of the improved dairy breeds[7] in the country. According to the District Veterinary Office in Mbarara, the total cattle population in Mbarara district is currently estimated to be 185,680 on 10,200 dairy farms (personal communication).

4.2.2. Study design and farm selection

A cross-sectional study was conducted from October 2016 to November 2016, and from the middle of February 2017 to March 2017. Thirty farms belonging to the Uganda Crane Creameries Cooperative Union were selected by purposive sampling in Mbarara district, based on the following criteria: (a) herd size: 5 farms with small herds (<10 adult cows per herd, including both milking and dry cows), 20 with medium herds (between 10 and 40 adults per herd), and 5 with large herds (>40 adults per herd); (b) herd management type: 5 to 10 zero-grazing farms, and 20 to 25 grazing farms; (c) accessibility: farms accessible by the project team during regular visits; (d) geographic distribution of farms: 2 to 5 farms per sub-county and 5 to 6 farms per milk collection center, to facilitate diffusion of techniques; and (e) willingness to continue participation in a dairy development project funded by Japan International Cooperation Agency (JICA) for three years.

In total, 506 adult cows were involved in the study, including primiparous and multiparous cows, and heifers which would deliver within the succeeding month after the survey. All the cows were assumed to be sampled only once.

4.2.3. Data collection

Reproduction data

Cow-level data were collected at the time of farm visits when reproductive and nutritional assessments were being performed. Reproductive information including dates of last calving, first service (AI or natural breeding), last service, and next calving were collected by interviewing owners and/or workers, and the information was updated at following farm visits until December 2017. These data collections were conducted mainly on those animals that delivered within averagely 3 months prior to the visits to avoid recall bias since many of the farms did not keep written reproductive records. From the data, days to first service, days open, and calving interval were calculated. Breeding practice information (use of bulls, AI, or both) was also collected at the herd level.

Nutrition status

Body condition scoring (BCS) [32] was performed for the cows selected based on a factsheet provided by the UK Agriculture & Horticulture Development Board: 5-point scale, 0.25 increments[4]. Training on scoring was conducted before implementation of the study in order to reduce bias among assessors. After body condition scoring of the cows in the early lactation stage, blood samples were collected from coccygeal vessels using 2 mL syringes, and tested for BHB using Precision Xceed (Abbott Japan, Tokyo, Japan) pen-side test kit immediately after sampling. SCK and clinical ketosis (CK) were diagnosed when BHB was more than or equal to 1.2 and 3.0 mM/L blood, respectively[68, 78, 88].

Feeding and herd management

Feeding management data were collected by interviews conducted at the time of farm visits. The recording sheet included: practice of grazing and rotational grazing, size of grazing area, contents and quantity (kg, as sampled basis) of supplementary feed (concentrates or fodder). Average grazing area per cow was calculated from the collected data, by dividing the size of grazing area by the number of adult cows at the farm. The feeding combination pattern was classified into the following categories: a) grazing only (no supplementary feeding), b) grazing and fodder supplement, c) grazing and concentrate supplement, d) grazing and both fodder and concentrate supplement, and e) zero-grazing.

Other herd-level information collected by the interviews included herd size and milking time per day, and at cow-level, daily milk yield (L), parity and breed. Herd size was calculated as the number of adult cows at the farm. Breed was classified as either an exotic dairy breed, mainly Holstein Friesian, or a cross with the local Ankole breed based on the appearance, as there were no pure

Ankole breed cattle on the selected farms.

4.2.4. Data management

All data collected from interviews, scoring, and ketone diagnostic tests were initially recorded on hand-written record sheets in English. Recording criteria for sheet contents, also written in English, were confirmed among the assessors and record keepers before the survey to avoid information bias. Data were then digitized using Excel spreadsheets (Microsoft Office 2013, USA) and imported to Access (Microsoft Office 2013) for assembly.

4.2.5. Statistical analysis

Descriptive statistics

Data collected were summarized at herd and cow levels. For numerical data, the mean, median and range were calculated, and for categorical data, the response counts and proportions. The average number of cows per herd was used for herd-level numerical data. A farm which kept at least one exotic breed cow was categorized as a farm keeping an exotic breed. Prevalence of SCK and CK were calculated for cows within 21 days after calving[88].

Causal diagram for association between feeding management, milk yield and blood β-hydroxybutyrate (BHB) concentration

A causal diagram was built to describe the relationship between feeding management, milk yield and blood BHB concentration with potential confounders (Fig. 6). Biologically, feeding management has an effect on milk yield and nutritional status of cows, and milk yield also affects the nutritional status[27, 35] because feed intake and milk production represent energy intake and output, respectively. Breed, parity, days after calving and milking time per day were selected as potential confounders because these attributes affect milk yield and BHB, and would have an association with the feeding management[26, 35, 59, 77, 114].

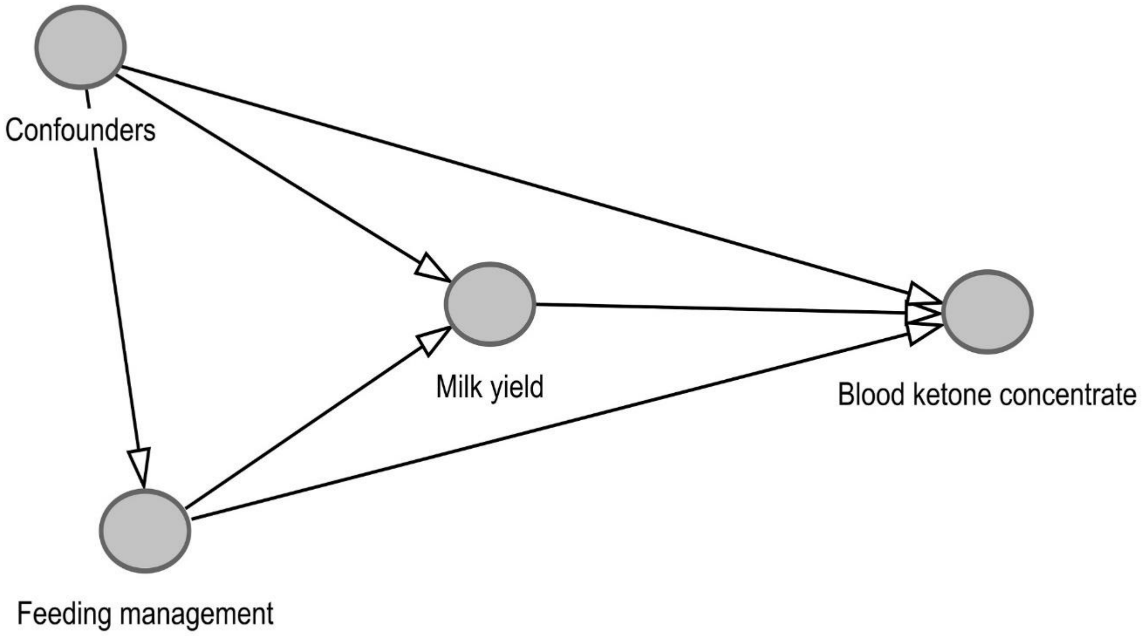


Figure 6. Diagram of the relationships between feeding management, milk yield and blood β-hydroxybutyrate

Effect of feeding management on blood BHB concentration

Univariable linear mixed-effects models (LMMs) were performed selecting the BHB concentration of cows within 21 days after calving as a response variable, and feeding management factors (practice of grazing and rotational grazing, grazing area per cow, concentrate/forage feeding and quantity) as explanatory variables. The herd, as a nominal variable, was set as a random effect. Cows with missing data were excluded from analyses. Next, in order to estimate the total effect of feeding management on BHB, a multivariable model was built using LMMs for each variable whose *p*-value from the univariable analyses above was <0.2, setting the variable of interest (feed management factors) as the exposure variable with potential confounders (breed, parity, days after calving and milking time per day) as the covariates. The logarithm of BHB concentration was used for analyses because the assumption of normality did not meet on the original scale, but on the log scale.

Effect of feeding management on daily milk yield

Total effect of feeding management on daily milk yield per cow was analyzed using linear mixed-effects models (LMMs) setting the herd as a random effect. First, univariable models and second, multivariable models were built. A logarithm of milk yield was chosen as a response variable. The logarithm of milk yield was used for analyses because the assumption of normality did not meet on the original scale, but on the log scale. Practice of grazing and rotational grazing, grazing area per cow, conduct of concentrate/fodder feeding and that of quantity were chosen as the predictors of interest in the univariable analyses. The variables whose *p*-values were <0.2 from the univariable analyses were selected for the multivariable analyses by controlling the potential confounders, which were breed, parity, days after calving and milking time per day to estimate the total effect of each exposure variable. Furthermore, to make milk yield prediction models, the multivariable model including all the variables whose *p*-values were <0.2 with the potential confounders above was built using LMMs. The final model was created by the stepwise backward model simplification, forcing the confounders remained in the model. All these statistical analyses were performed using the statistical software R, version 3.5.1[83].

Power calculation

To ensure the power for multivariable analyses, calculations were performed using the "pwr" package in the statistical software R[14], with a confidence level at 95%, effect size of small (0.2), medium (0.15) and large (0.35)[14], and intraclass correlation coefficient of 0.1 for samples from the same herd. With these assumptions, the milk yield data analysis, including 506 cows, had 99.3% power to detect a medium effect size for a model including 5 explanatory variables, and that of blood ketone body (n = 35) had 56.8% power to detect a large effect size for a model with 5 explanatory variables.

4.2.6. Ethical consideration

This study was performed as the baseline survey of JICA Safe Milk Promotion in Mbarara Project, based on a bilateral agreement between JICA and Mbarara District Local Government. Animals in this study were properly handled using crush during sampling to avoid their stress and injury. Informed consent was obtained from all farms included in this study.

4.3. Results

4.3.1. Dairy production system

The mean herd size of adult cattle was 35.5 cattle (n = 30). The means for herd average days after calving and that of milk yield were 106.8 days (n = 26) and 9.8 L/cow/day (n = 30), respectively (Table 13). The mean milk yields of cross-breed and exotic breed cattle were 7.3 (median: 7, range: 1-20) and 11.2 L/cow/day (median: 10, range: 1-32), respectively (Table 13). The mean herd average blood BHB for cows within 21 days after calving was 0.84 (range: 0.40-1.50) mM/L (Table 13), and that for individual-level was 0.84 (range: 0.20-3.20) mM/L. The relationship between blood BHB and days after calving was shown in Figure 7. The prevalence of SCK and CK within 21 days in milk were 17.1% (6/35, 95% confidence interval (CI): 6.6-33.6%) and 2.9% (1/35, 95% CI: 0.1-14.9%), respectively. The overall proportion of exotic cows among total number of adult cows was 74.5% (377/506), and 83.3% (25/30) of farms kept at least one exotic breed cow. Use of bulls, AI, and both breeding methods were practiced in 60.0% (18/30), 33.3% (10/30) and 6.7% (2/30) of farms, respectively. From reproduction records available on 12 farms, the mean of days to first service after delivery was 120.3 (median: 96, range: 6-549, n = 107). The mean days open was 118.4 (median: 92, range: 17-549, n = 100), and that of calving interval was 415.3, (median: 384, range: 302 - 833, n = 40). Supplementary concentrate and fodder feeding were used in 40% (12/30) and 70% (21/30) of farms, respectively. Brewers waste (30%, 9/30 farms), maize bran (13.3%, 4/30), formula feed (13.3%, 4/30), barley (3.3%, 1/30), and sunflower meal (3.3%, 1/30) were fed in the farms studied and categorized as concentrates. Fresh grass [Napier (*Pennisetum purpureum*), sorghum (*Sorghum bicolor*), Rhodes grass (*Chloris gayana*), lablab (*Dolichos lablab*), 40%, 12/30], corn silage (20%, 6/30), grass silage (10%, 3/30), haylage (16.7%, 5/30), hay (10%, 3/30), and banana peels (10%, 3/30) were fed and classified as fodder. Grazing was conducted at 93.3% of farms (28/30), and 56.7% (17/30) used rotational grazing. The mean grazing area was 2.8 ha/cow/day (n = 26, Table 13). Feeding combination patterns were: grazing only (26.7%, 8/30), fodder (cut and carry) and grazing (33.3%, 10/30), concentrates and grazing (3.3%, 1/30), concentrates, fodder and grazing (30%, 9/30), and zero-grazing (6.7%, 2/30).

Table 13. Herd-level information of cows, farms and feeding management in the study

	n	Mean	Median	Range
Herd size	30	35.5	28.5	4.0-250.0
Average parity	28	2.7	2.8	0.7-4.5
Average days after calving	26	106.8	115.4	13.6-233.7
Average milk yield (L/cow/day)	30	9.8	9.2	1.8-23.3
Average BCS	22	3.0	3.1	2.3-3.4
Average blood BHB concentration (mM/L)	9	0.84	0.80	0.40-1.50
Concentrate feeds[a)] (kg/cow/day)	11	6.6	4.0	1.0-22.0
Fodder[b)] (kg/cow/day)	12	11.2	11	1.0-36.0
Grazing intensity (ha/cow/day)	26	2.8	1.6	0.2-11.9

BCS: body condition score, BHB: β-hydroxybutyrate, *n*: number of observations
[a)]Concentrate feeds: brewers waste, maize bran, formula feed, barley, sunflower meal
[b)]Fodder: fresh grass, corn silage, grass silage, haylage, hay, banana peel

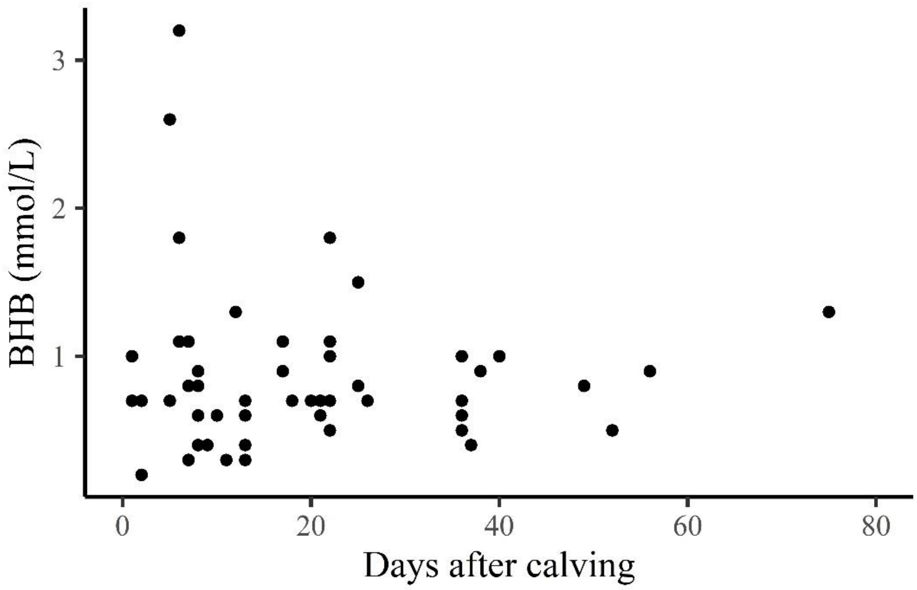


Figure 7. Blood β-hydroxybutyrate (BHB) concentration of dairy cows in early lactation stage in Mbarara, southwestern Uganda

4.3.2. Effect of supplementary concentrates and fodder, their combination, and rotational grazing on the milk yield

From the univariable analysis, variables with *p*-value < 0.2 were: feeding concentrates (p = 0.006) and its quantity (p = 0.050), feeding fodder (p = 0.090) and its quantity (p = 0.124), feeding combination (p = 0.009), and conduct of rotational grazing (p = 0.118). The six multivariable models were built for the variables above by controlling potential confounders, and results are shown in Table 14. Cows fed supplementary concentrates had higher milk yield (9.20 L/cow/day) than those which were not (5.95 L/cow/day, difference in log: 0.44, 95% CI: 0.02-0.85, ratio between groups in original scale (L/cow/day): 1.55, 95% CI: 1.02-2.34, p = 0.041, Model 1 in Table 14). Cows on farms where rotational grazing was conducted had higher milk yield (5.78 L) than those in the farms where rotational grazing was not practiced (3.46 L, difference in log: 0.51, 95% CI: 0.10-0.92, ratio between groups in original scale (L/cow/day): 1.67, 95% CI: 1.11-2.51, p = 0.017, Model 5 in Table 14). The feeding combination did not have a statistically significant effect on milk yield, but it showed a trend that the combination pattern (feeding pasture, fodder and concentrates) had a positive impact on milk yield compared to feeding pasture only (difference in log: 0.60, 95% CI: -0.01-1.21, p = 0.055, Model 6 in Table 14). The rotational grazing remained in the final model, the same model as Model 5 in Table 14.

Table 14. Multivariable analysis results on daily milk yield, adjusting potential confounders as covariates

Variable	Estimate in log	95% CI	*p*-value
Model 1			
Feeding concentrates[a)]	0.44	0.02 - 0.85	0.041
Breed[b)]	0.08	-0.13 - 0.29	0.457
Parity	0.01	-0.01 - 0.04	0.284
Days after calving	-0.00	-0.00 - -0.00	<0.001
Milking time/day	0.12	-0.37 - 0.61	0.618
Model 2			
Concentrates quantity	0.04	-0.01 - 0.09	0.092
Breed[b)]	0.08	-0.14 - 0.30	0.469
Parity	0.01	-0.01 - 0.04	0.296
Days after calving	-0.00	-0.00 - -0.00	<0.001
Milking time/day	0.12	-0.40 - 0.65	0.634
Model 3			
Feeding fodder[c)]	0.33	-0.16 - 0.82	0.181
Breed[b)]	0.06	-0.15 - 0.27	0.554
Parity	0.01	-0.01 - 0.04	0.287
Days after calving	-0.00	-0.00 - -0.00	<0.001
Milking time/day	0.10	-0.44 - 0.65	0.696
Model 4			
Fodder quantity	0.04	-0.01 - 0.09	0.137
Breed[b)]	-0.16	-0.45 - 0.13	0.280
Parity	0.03	0.00 - 0.06	0.096
Days after calving	-0.00	-0.00 - -0.00	<0.001
Milking time/day	-0.10	-0.77 - 0.57	0.754
Model 5			
Rotation[d)]	0.51	0.10 - 0.92	0.017
Breed[b)]	0.08	-0.12 - 0.29	0.428
Parity	0.02	-0.01 - 0.04	0.245
Days after calving	-0.00	-0.00 - -0.00	<0.001
Milking time/day	0.33	-0.14 - 0.80	0.157

Table 14 (continued)

Variable	Estimate in log	95% CI	*p*-value
Model 6			
Feeding combination			
a) Pasture only	Reference		
b) Pasture & fodder	0.19	-0.41 - 0.80	0.513
c) Pasture & concentrates	0.34	-0.80 - 1.47	0.539
d) Pasture, fodder & concentrates	0.60	-0.01 - 1.21	0.055
e) Zero-grazing	0.50	-0.63 - 1.63	0.363
Breed[b)]	0.06	-0.15 - 0.28	0.555
Parity	0.01	-0.01 - 0.04	0.285
Days after calving	-0.00	-0.00 - -0.00	<0.001
Milking time/day	0.06	-0.52 - 0.64	0.826
Final model			
Rotation[d)]	0.51	0.10 - 0.92	0.017
Breed[b)]	0.08	-0.12 - 0.29	0.428
Parity	0.02	-0.01 - 0.04	0.245
Days after calving	-0.00	-0.00 - -0.00	<0.001
Milking time/day	0.33	-0.14 - 0.80	0.157

CI: confidence interval

[a)]Feeding concentrates: feeding or not (not feeding as reference); [b)]Breed: exotic or cross breed (cross breed as reference); [c)]Feeding fodder: feeding fodder supplementary or not (not feeding as reference); [d)]Rotation: practicing rotational grazing or not (not practicing as reference)

4.3.3. Effect of supplementary concentrate feeding on β-hydroxybutyrate (BHB)

From the univariable analysis, variables with *p*-value < 0.2 were: feeding concentrates (p = 0.031) and its quantity (p = 0.010), feeding combination (p = 0.049), and conduct of rotational grazing (p = 0.032). In the multivariable analysis, however, no significant effect of supplementary concentrates on BHB was observed: feeding concentrates (difference in log: 0.56, 95% CI: -0.14-1.26, p = 0.092) and its quantity (difference in log: 0.19, 95% CI: -0.04-0.42, p = 0.082), feeding combination (difference in log: 0.61, 95% CI: -1.99-3.21, p = 0.419), and conduct of rotational grazing (difference in log: 0.59, 95% CI: -0.59-1.77, p = 0.237).

4.4. Discussion

Previous studies reported that the mean herd size of the dairy farms in western Uganda in 2008 was 8.9 cattle[7, 63]. Due to the recent high demand of dairy products, the cattle population has been increasing dramatically, and according to the Mbarara District Veterinary Officer (personal communication), the current mean herd size in Mbarara district is 18.2 cattle, which is about twice larger than that of the western region in 2008. Cattle breeds were also dramatically changed and local Ankole breed is predominantly used for beef production (personal communication with dairy farmers). The exotic-breed cows and their crosses were dominant in the dairy herds. Characteristics of the cattle population including herd size and cattle breed might be a result of the milk demand that has caused an increase in milk production in recent years. However, it should be noted that because of the purposive sampling, the mean herd size of the study population was probably higher than the target population and the proportion of exotic and/or cross-breed cattle might be overestimated, as motivated farmers were invited to the project.

The adaption rate of AI in this study (40% of the farms sampled) was higher than in previous reports (12.3% in southwestern Uganda[7] and 25.1% in Rwanda[59], though the majority of farms in Mbarara still use a bull for breeding. This suggested a potential of rapid improvement of breeds to increase milk yield in Uganda. On the other hand, although the majority of farmers considered that introducing a new breed was crucial to improve milk production, their major concern with new breeds was sensitivity to the local environment and diseases[69]. A study of a decade ago reported the insufficiency of AI service to meet the demand of dairy farmers and limited insemination rate in the southwestern region[113]. Our study showed that the reproductive performance of exotic and/or cross-breed cows (average days to first service, 120.3 and days open, 118.4) was adequate when compared to dairy standards in an industrialized area, although the calculation of reproductive performance was conducted only for farms that kept reproductive records. As an example of a dairy reproduction benchmark, farms in the northeast region of the United States with herd sizes of 25-50 cows described mean days to first service and days open of 91 and 147, respectively[97]. In large commercial dairy herds in Ethiopia which kept only exotic breeds of Holstein or Jersey and used AI only for breeding, the average days open was 192.3[6]. Another study in Ethiopia showed that an experimental farm which kept cross-breed cattle and used a bull for breeding, a nearly similar environment to our study population had average days open of 80 and calving interval of 356[5]. The average days to first service and days open in the study population were very close, indicating a high conception rate. This high reproductive performance might be because the majority of farms (66.7%) used bulls for breeding, which presents higher heat detection rate and quality/quantity of semen compared to AI. However, such good records might suggest a rapid improvement of AI service delivery infrastructure in Mbarara district.

More than 70% of farms in the study fed their cows not only natural pastures, but also fodder and/or concentrates. Our findings show that cows fed concentrates had higher milk yield than those that were not. Also, the trend shown in the results suggests the importance of feeding a combination of concentrates and fodder together with pasture to increase the milk production. Diets should be properly balanced for energy, protein, fiber and minerals, and taking fiber with concentrates is important to digest matter and intake energy properly[65]. Otherwise, lack of rumination, loose manure formation and low milk fat composition occur, and consequently milk yield will not increase adequately. To increase milk production in the current situation in Mbarara, feeding balanced supplementary materials to suffice nutritional requirements of the cows is essential. Besides, supplementary feeding is also important in terms of meeting feeding shortages during the dry seasons. Due to the shortage of natural pasture, milk production decreases and milk price increases during the dry seasons[7]. To cover this shortage, pasture harvesting and storage techniques should be expanded. Applying a rotational grazing system, which increases the feed intake efficiency and saves the pasture resource, had a positive impact on milk yield in this study. The majority (88.2%, 15/17) of farms who used rotational grazing had no rule for the rotation but mainly rotated depending on the grass availability. Applying well-managed rotational grazing techniques should have much better potential for milk production, and detailed technical information about pasture management should be extended to local farmers. Further studies are required to assess the effect of the rotation method, and also to find adequate/reasonable practices that can be applied in the current situation in Uganda.

Cows fed with concentrates had higher milk yield than those which never fed concentrates. The blood BHB concentrate was not significantly different between cows fed with concentrates and those not fed. These facts might suggest that the current feeding strategy in Mbarara had a potential to increase milk production, and at the same time the cows' energy balance were stable with the management. This is reasonable because in a biological sense, an adequate feeding management which enhances nutrient intake can contribute to the milk production and nutritional status of cows[35, 78]. However, this study also revealed that SCK and CK cows were observed with the prevalence of 17.1% and 2.9%, respectively. Moreover, although the effect of feeding supplementary concentrates on BHB was not statistically significant, it showed the trend that feeding concentrates had a positive association with blood BHB concentrate (p = 0.092) indicating that the feeding management tends not to suffice NEB. It is highly possible that the number of SCK/CK cows would be increased in near future since the herd management in Mbarara had been changing into more intensive due to high milk production demands. For those SCK/CK cows, special care such as close observation of their appetite and propylene glycol administration for cows with poor appetite in early lactation is needed in order not to lose production. Proper herd management will help dairy farmers in Mbarara to prevent and control CK/SCK cases and improve milk yield at farm level as the proportion of exotic-breed cows is likely to continue growing. To increase milk production and to overcome the NEB status of cows in the dairy production area in Uganda, knowledge of proper herd management practices should be extended to the farmers.

In conclusion, exotic dairy cattle breeds were dominant and quite a few cross-breeds are present in dairy farms in southwestern Uganda. The present study revealed that exotic and cross-breed cows have the potential to produce more milk, given sufficient supplementary feeding, recommended as concentrates together with fodder. SCK/CK cows were observed in this study area. Cows with high ketone concentrations require special precautions. In order to increase milk yield without nutritional disorders, training of dairy farmers about adequate feeding management practices is important in Uganda where the dairy industry is fast developing.

4.5. Abstract of Chapter 4

Dairy production in Uganda has recently shown steady growth. Development and intensification of dairy production may bring about the issue of negative energy balance (NEB) followed by ketosis in cattle. However, the current dairy herd health and management status in the southwestern region of Uganda has not been reported. The objectives of this study were to describe herd management practices and production status in this region, and to investigate relationships between feeding management practices, nutritional status, and daily milk yield of dairy cows.

Thirty farms participated in this study. Herd attributes, management practices, nutritional and production status of the cows were collected by interviews and inspections from October 2016 to March 2017. In order to estimate

the total effects of feeding management on blood β-hydroxybutyrate (BHB) and milk yield, a causal diagram was created. Multivariable analyses were performed using linear mixed-effects models, setting BHB of cows within 21 days after calving and milk yield as response variables, feeding management factors as exposure variables, potential confounders as covariates, and herd as a random effect variable.

The mean herd size of adult cows on participating farms (n = 30) was 35.5 and average milk yield 9.8 L/cow/day. The proportion of exotic breeds was 74.5% of 506 adult cows. Supplementary concentrates and forage were used in 40% and 70% of farms, respectively; grazing was conducted at 93.3%. The prevalence of subclinical ketosis (SCK) for cows within 21 days after calving was 17.1% (6/35, 95% confidence interval (CI): 6.6-33.6%). From the multivariable models estimating the total effect, cows fed concentrates had higher milk yield (9.20 L/cow/day) than cows not (5.95 L/cow/day, ratio between groups: 1.55, 95% CI: 1.02-2.34, p = 0.041). Cows in the farm where rotational grazing was conducted had higher milk yield (5.78 L) than those in the farms where rotational grazing was not (3.46 L, ratio between groups: 1.67, 95% CI: 1.11-2.51, p = 0.017). No significant effect of feeding management on BHB was estimated (p = 0.092).

Exotic dairy cattle breeds are dominant on dairy farms in southwestern Uganda. This study revealed that exotic and cross-breed cows have the potential to produce higher milk yields, given sufficient nutrition. SCK cows were observed in this study area. Cows with high ketone concentrations require special precautions. In order to increase milk yield without nutritional disorders in Uganda where the dairy industry is fast developing, introduction of adequate feeding management is important.

Chapter 5. General discussion

5.1. Highlights of this thesis

In this PhD thesis research, I conducted problem-oriented field epidemiology studies by targeting dominant problems in dairy production medicine with respect to sustainable dairy farming. I focused on bovine leptospirosis in Japan and East Coast Fever (ECF), mastitis control, and feeding and reproductive management practices in Uganda. For each topic, descriptive epidemiological analyses were performed first to characterize the current dairy farming situation. Second, risk factor analyses were conducted to identify factors associated with problems and determine the degree to which these factors affect problem development. The evidence obtained from the risk factor analyses could facilitate future study opportunities and/or interventions to overcome problems affecting sustainable dairy farming.

The risk factor analyses had two primary objectives. The first objective was to predict the outcome of interest and identify factors that contribute to the predicted outcome to provide a general understanding, as shown in the *L.* Hardjo invasion study (Chapter 1). The second objective was addressed via a hypothesis-driven study, which was performed to estimate the causal relationships between various exposures and outcomes by controlling potential confounders. The exposures are commonly (but not necessary) variables conducive to intervention [53]. The latter approach was carried out in the ECF, mastitis control, and feeding and reproductive management studies (Chapters 2-4).

5.1.1. Herd-level risk factors associated with *Leptospira* Hardjo infection in dairy herds in the southern Tohoku, Japan (Chapter 1)

The prevalence of bovine leptospirosis caused by *L.* Hardjo in southern

Tohoku, Japan, is not well known. Bovine leptospirosis is recognized as an economically important disease in agriculture due to reproductive losses [15, 16], and it is important in human health because it is one of the most prevalent zoonoses in the world [3]. This cross-sectional study found that *L*. Hardjo infection at the farm-level was prevalent in southern Tohoku (apparent herd prevalence: 65.1%), and larger herd size and a history of cows staying in Hokkaido Prefecture were identified as risk factors for *L*. Hardjo infection. A disease cluster of *L*. Hardjo-positive farms was detected in the southern part of the study area, where larger herds were aggregated at high density.

The risk factors identified in this study (herd size and sending/introducing cattle to/from Hokkaido) are difficult to control through intervention because changing these states directly affects management style. However, the knowledge obtained from this study would increase farmer awareness of leptospirosis and alternative actions. For example, introducing a vaccination program protects cattle and farmers from the infection. The results of this study also suggested that the presence of cats on farms can help reduce the incidence of *L*. Hardjo infection, although it was not statistically significant. Further study could test the hypothesis that the presence of cats has a preventive effect on *L*. Hardjo infection and determine the extent of any protective effect.

To identify risk factors in this study, a method using a stepwise statistical algorithm was employed. The magnitude of confounding was tested using change-in-estimate criteria. These methods were applied because the purpose of this study was to identify risk factors that are good predictors of *L*. Hardjo infection rather than estimating the magnitude of the causal relationships. For future hypothesis-driven studies such as the one described above (protective effect of cat presence), a causal inference approach could be used [53, 67].

5.1.2. Effect of chemical tick control practices on tick infestation and *Theileria parva* infection in an intensive dairy production region of Uganda (Chapter 2)

ECF is problematic due to economic losses resulting from high morbidity and mortality, declining milk production, and the costs of tick-control and disease-prevention measures [33, 57]. Severe acaricide resistance has been reported in Uganda [109]. Inappropriate farm tick control practices can facilitate and accelerate development of resistance in ticks, but no risk factor analyses have been conducted in Uganda. This study found that the prevalence of *T. parva* infection was 45.2% at the cattle level and 83.3% at the farm level. This study also identified the following preventive factor for tick infestation: use of high-quality measuring cylinders in the farm tick-control infrastructure; the preventive factors for *T. parva* infection were well-managed acaricide agent storage and use of high-quality measuring cylinders for acaricides. Risk factors for *T. parva* infection included longer period of and higher frequency of acaricide use. The effects of these factors were estimated quantitatively after adjusting potential confounders using the causal inference method [19, 53, 67] explained in the general introduction. The odds of a higher tick infestation level for cattle at farms equipped with high-quality cattle crushes was 68% lower than at farms not so equipped (odds ratio (OR): 0.32). The odds of being *T. parva*-positive for cows at farms where acaricides were properly stored were 64% lower than at farms with poor/improper acaricide storage (OR: 0.36). Cows at farms using high-quality measuring cylinders for acaricides had 68% lower odds of being *T. parva* positive than cows at farms using lower-quality cylinders (OR: 0.32). The odds of *T. parva* infection for cows at farms where acaricides were used for a longer period and at a higher frequency were 1.06- and 11.7-times higher than for cows not at such farms, respectively.

This study identified improper and insufficient acaricide use significantly related to tick infestation and *T. parva* infection in the study area of Uganda. Interventions targeting these factors could be expected to lower the incidence of ECF, and this expectation was assessed quantitatively. This differed from the first study discussed in Chapter 1. In the initial study design, the assessment points (tick control countermeasures in this case) were selected based on the hypothesis, and they were all factors considered amenable to intervention. This type of baseline study is useful for designing the next intervention. In future research, the impact of interventions related to acaricide use and the tick-control infrastructure should be evaluated.

Another future study opportunity would be to estimate how strongly *T. parva* infection spreads among cattle in a totally susceptible population (i.e., estimating the basic reproduction number, R_0). The current study found that *T. parva* prevalence was associated with age. From seroprevalence data with age-related information, R_0 can be estimated [45], but the accuracy of estimations using this method depends largely on sample size [45]. Using a sufficient sample size, the R_0 of ECF in Uganda could be estimated.

5.1.3. Prevalence of subclinical mastitis (SCM) and its association with milking practices in dairy herds in Mbarara district, an intensive dairy

production region of Uganda (Chapter 3)

SCM is difficult to detect due to a lack of clinical signs [29]. Mastitis is regarded as the most costly disease affecting dairy farms worldwide [38]. A high prevalence of SCM has been reported in Uganda [2, 10, 13, 44, 98]. However, no risk factor analyses of milking hygiene and practices relating to SCM and no studies of SCM prevalence in Mbarara District, an intensive dairy production region of Uganda, have been reported. This study revealed a high prevalence of SCM (68.8%) at the cow level, and contagious pathogens were the majority of isolated organisms. Use of a one-towel-per-cow approach for teat wiping was negatively associated with contagious pathogen prevalence in the univariable analysis. The major preventive factor for SCM was keeping cows calm at the end of milking, whereas the major risk factor was rough teat end. The effects of these factors were also estimated quantitatively after adjusting potential confounders using the causal inference method. The odds of being SCM positive for cows kept calm at the end of milking were 80% lower than those of cows not kept calm (OR: 0.20); the odds of being SCM positive for cows with rough teat ends were 75% higher than those of cows with normal teat ends (OR: 1.75).

Mastitis control targeting a less-teat-stress strategy, which would contributes to a cow's calmness and good teat-end condition, could be expected to result in a lower SCM prevalence in the target population in Uganda. From the evidence gained in this study, these significant milking practices should be given a higher priority among the milking practice variables assessed. To further evaluate these estimations, an intervention study should be performed. The association between contagious pathogens and the one-towel-per-cow approach was observed only in the univariable analysis. Further study to evaluate the causal effect of contagious mastitis control quantitatively should be performed.

5.1.4. Current dairy herd management practices and their influence on milk yield and subclinical ketosis in an intensive dairy production region of Uganda (Chapter 4)

Subclinical ketosis (SCK) results in reduced milk production [114] and economic losses [68]. In peri-urban Kampala, Uganda, the reported prevalence of SCK in dairy cows during early lactation was 18.8% in 2013 and 13.9% in 2014 [17]; however, SCK prevalence and feeding management practices in the southwestern region of Uganda have not been reported. This study found that the prevalence of SCK in cows during early lactation was 17.1%. Cows fed concentrates had 1.6-times higher milk yield than those not fed concentrates. Cows at farms where rotational grazing was conducted had 1.7-times higher milk yield than cows not at farms practicing rotational grazing. The effect of feeding management on milk yield was quantitatively determined after adjusting potential confounders.

The feeding management interventions of concentrate supplementation and rotational grazing should be instituted. The adequate quantity of concentrate varies based on conditions such as breed, milk yield, days in milk, parity, and management style. Thus, supplementary feeding should be conducted within the plausible quantity. However, this study could not determine the optimal specific quantity of concentrate supplement. By feeding concentrates, milk yield would increase, but at the same time, the number of cows with ketosis might also increase because in the current situation in Mbarara, SCK/clinical ketosis cows were observed more often at high-production farms where supplementary feeding was conducted. The relationship between feeding management and blood β-hydroxybutyrate concentration identified in this study was determined with a limited sample, however. To better assess this effect, further analysis with a sufficient sample size is required.

5.2. Future perspectives

This PhD research epidemiologically described several problems common in dairy production medicine and also identified the risk factors associated with the significant problems. To improve productivity and prevent zoonotic diseases relative to the current baseline in sustainable dairy farming, proper record keeping, monitoring, data description, and the cycle of evaluations and interventions must be performed continuously. Although the intervention studies in Uganda were not included in this thesis, they were conducted apart from the thesis research. Based on the baseline survey results, intervention packages were created (see Appendix), and the extension work was conducted. The results of the interventions are currently being evaluated. Below, I will briefly introduce some of the results reported in the final report of the Japan International Cooperation Agency (JICA) Partnership Program entitled “Safe Milk Promotion in Mbarara Project” [84]. The prevalence of SCM and *T. parva* infection after the interventions declined significantly compared with the baseline; the tick infestation level was significantly lower than at baseline; and milk yield per cow after the interventions was significantly higher than at baseline.

Changing practices after the proper interventions have been carried out or as industry conditions change could introduce new problems. Problem solving is a continuous process in dairy production medicine. The evidence and approaches described in this thesis could be used to solve problems in a wide variety of areas in dairy production medicine.

ACKNOWLEDGEMENT

Firstly, I would like to express my sincere gratitude to my supervisor, Professor Kohei Makita, Head of Veterinary Epidemiology Unit, Graduate School of Veterinary Medicine, Rakuno Gakuen University (RGU) Graduate School for the passionate continuous support of my PhD study. His guidance helped me having high motivation. He gave me broader perspectives to be an autonomous epidemiologist.

Besides my supervisor, I would like to thank the rest of my advisors in the Japan International Cooperation Agency (JICA) Partnership Program “Safe Milk Promotion in Mbarara Project”: Professor Toshihiko Nakao, Professor Hajime Nagahata, Professor Hiroki Nakatsuji, Professor Shin Oikawa, and Professor Yasukazu Muramatsu, for their insightful comments and encouragement. My sincere thanks also goes to Dr. Ryo Murata and Dr. Leo Uchida, Dr. Joseph Byaruhanga, Dr. Ikuo Okamura, Dr. William Mwebembezi, and Dr. Patrick Vudriko. Without their heartful support it would not be possible to conduct this research.

I would like to express my deep appreciation to Dr. Yoshimi Ogata, Dr. Yoshiro Urushiyama, Dr. Eiji Watanabe, and their colleagues, the field veterinarians in Yamagata Prefecture Agricultural Mutual Aid Association who support my research and gave me important knowledge and perception from which I could deeply learn dairy production fields. I thank Yamagata Prefecture Dairy Farming Cooperative and to all of the farmers who participated in the survey.

The studies conducted in Uganda were financially supported by JICA. I am grateful to all the members who involved the project and support my studies. My special thanks goes to Dr. Masahiko Anzai, Dr. Taishi Kayano, Ms. Shizuka Kuroiwa, and Extension Center, RGU for their hard work through the project and their support for my research. I thank the Mbarara District Local Government; the Ministry of Agriculture, Animal Industries, and Fisheries of Uganda; Makerere University; Uganda Crane Creameries Cooperative Union; and the Dairy Development Authority for their support. I am also grateful to the local veterinarians and extension officers for their support with the fieldwork. Special thanks are given to the dairy farmers in Mbarara district who participated in this study.

I thank my fellow, laboratory mates in Veterinary Epidemiology Unit, RGU for the stimulating discussions, hard works, and all the fun we have had during my PhD course.

Finally, I would like to thank my dear wife, Sara, who has devotedly supported me all the time, and my son, Aoto and my baby, Kanna, who encourage me to face any difficulties in my life. I also would like to express my gratitude to my parents who support patiently to complete this PhD course.

REFERENCES

1. Abbas, R. Z., Zaman, M. A., Colwell, D. D., Gilleard, J. and Iqbal, Z. 2014. Acaricide resistance in cattle ticks and approaches to its management: The state of play. http://dx.doi.org/10.1016/j.vetpar.2014.03.006.
2. Abrahmsén, M., Persson, Y., Kanyima, B. M. and Båge, R. 2014. Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. *Trop. Anim. Health Prod.* **46**: 99–105.
3. Adler, B. and de la Peña Moctezuma, A. 2010. *Leptospira* and leptospirosis. *Vet. Microbiol.* **140**: 287–296.
4. Agriculture & Horticulture Development Board 2014. Body condition scoring. https://dairy.ahdb.org.uk/resources-library/technical-information/health-welfare/body-condition-scoring/.
5. Agyemang, K., Astatke, A., Anderson, F. M. and W/Mariam, W. 1991. Effects of work on reproductive and productive performance of crossbred dairy cows in the Ethiopian highlands. *Trop. Anim. Health Prod.* **23**: 241–249.
6. Asseged, B. and Birhanu, M. 2004. Survival analysis of calves and reproductive performance of cows in commercial dairy farms in and around Addis Ababa, Ethiopia. *Trop. Anim. Health Prod.* **36**: 663–672.
7. Balikowa, D. 2011. Dairy development in Uganda: A Review of Uganda ’ s dairy industry.
8. Bernard, F., Vincent, C., Matthieu, L., David, R. and James, D. 2005. Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda). *Prev. Vet. Med.* **67**: 267–281.

9. Bharti, A. R., Nally, J. E., Ricaldi, J. N., Matthias, M. A., Diaz, M. M., Lovett, M. A., Levett, P. N., Gilman, R. H., Willig, M. R., Gotuzzo, E., Vinetz, J. M. and Peru-United States Leptospirosis Consortium 2003. Leptospirosis: a zoonotic disease of global importance. *Lancet. Infect. Dis.* **3**: 757–771.
10. Björk, S. 2013. Clinical and subclinical mastitis in dairy cattle in Kampala, Uganda. https://stud.epsilon.slu.se/5631/.
11. Blowey, R. and Edmondson, P. 2010. Mastitis control in dairy herds, 2nd ed., CAB International, Wallingford.
12. van Buuren, S. 2018. Multilevel multiple imutation. *In*: *Flexible Imputation of Missing Data*, 2nd ed., Chapman & Hall/CRC, Vancouver.
13. Byarugaba, D. K., Nakavuma, J. L., Vaarst, M. and Laker, C. 2008. Mastitis occurrence and constraints to mastitis control in smallholder dairy farming systems in Uganda. *Livest. Res. Rural Dev.* **20**: 5.
14. Cohen, J. 1992. A power primer. *Psychol. Bull.* **112**: 155–159.
15. Dhaliwal, G. S., Murray, R. D., Dobson, H., Montgomery, J. and Ellis, W. A. 1996. Reduced conception rates in dairy cattle associated with serological evidence of *Leptospira interrogans* serovar hardjo infection. *Vet. Rec.* **139**: 110–114.
16. Dhaliwal, G. S., Murray, R. D. and Ellis, W. A. 1996. Reproductive performance of dairy herds infected with *Leptospira interrogans* serovar hardjo relative to the year of diagnosis. *Vet. Rec.* **138**: 272–276.
17. Dickson, T., Nakao, T., Bigirwa, G., Okech, S. G. and Kanyima, B. M. 2015. Prevalence of prepartum negative energy balance and subclinical ketosis in the early lactation of dairy cows in peri-urban Kampala, Uganda. *Jpn. J.*

Vet. Res. **63**: S71.

18. Directorate Agricultural Information Services 2008. Rules for clean hand-milking. http://www.daff.gov.za/daffweb3/Portals/0/InfoPaks/Cattle_handmilking.pdf.

19. Dohoo, I., Martin, W. and Stryhn, H. 2014. Confounding: detection and control. pp. 271–322. *In*: *Veterinary epidemiologic research*, 2nd ed., VER Inc., Charlottetown.

20. Dohoo, I., Martin, W. and Stryhn, H. 2014. Sampling. pp. 33–56. *In*: *Veterinary epidemiologic research*, 2nd ed., VER Inc., Charlottetown.

21. Dohoo, I., Martin, W. and Stryhn, H. 2014. Mixed models for discrete data. pp. 579–606. *In*: *Veterinary epidemiologic research*, 2nd ed., VER Inc., Charlottetown.

22. Dohoo, I. R. Monitoring livestock health and production: service - epidemiology's last frontier?. *Proceedings of the 6th International Symposium on Veterinary Epidemiology and Economics*.

23. Dohoo, I. R., Martin, S. W. and Stryhn, H. 2014. Introduction and causal concepts. pp. 1–32. *In*: *Veterinary epidemiologic research*, 2nd ed., VER Inc., Charlottetown.

24. Dohoo, I. R., Martin, S. W. and Stryhn, H. 2014. Model-building strategies. pp. 365–394. *In*: *Veterinary epidemiologic research*, 2nd ed., VER Inc., Charlottetown.

25. Dreyfus, A., Wilson, P., Collins-Emerson, J., Benschop, J., Moore, S. and Heuer, C. 2015. Risk factors for new infection with *Leptospira* in meat workers in New Zealand. *Occup. Environ. Med.* **72**: 219–225.

26. Dubuc, J. and Buczinski, S. 2018. Short communication: Cow- and herd-level prevalence of hypoglycemia in hyperketonemic postpartum dairy cows. *J. Dairy Sci.* **101**: 3374–3379.

27. Esposito, G., Irons, P. C., Webb, E. C. and Chapwanya, A. 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. *Anim. Reprod. Sci.* **144**: 60–97.

28. Evangelista, K. V and Coburn, J. 2010. *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiol.* **5**: 1413–25.

29. FAO 2014. Impact of mastitis in small scale dairy production systems. http://www.fao.org/3/a-i3377e.pdf.

30. FAO 1983. Ticks and tick-borne diseases : selected articles from the World animal review., FAO.

31. FAO 2018. Livestock Primary, in FAOSTAT. http://www.fao.org/faostat/en/#data/QL.

32. Ferguson, J. D., Galligan, D. T. and Thomsen, N. 1994. Principal Descriptors of Body Condition Score in Holstein Cows. *J. Dairy Sci.* **77**: 2695–2703.

33. Gachohi, J., Skilton, R., Hansen, F., Ngumi, P. and Kitala, P. 2012. Epidemiology of East Coast Fever (*Theileria parva* infection) in Kenya: past, present and the future. *Parasit. Vectors*. **5**: 194.

34. Grooms, D. L. 2006. Reproductive losses caused by bovine viral diarrhea virus and leptospirosis. *Theriogenology*. **66**: 624–628.

35. Grummer, R. R., Mashek, D. G. and Hayirli, A. 2004. Dry matter intake and

energy balance in the transition period. *Vet. Clin. North Am. - Food Anim. Pract.* **20**: 447–470.

36. Hathaway, S. C. 1981. Leptospirosis in New Zealand: an ecological view. *N. Z. Vet. J.* **29**: 109–112.

37. Hogan, J. S., White, D. G. and Pankey, J. W. 1987. Effects of teat dipping on intramammary infections by staphylococci other than *Staphylococcus aureus*. *J. Dairy Sci.* **70**: 873–879.

38. Hogeveen, H., Huijps, K. and Lam, T. J. G. M. 2011. Economic aspects of mastitis: new developments. *N. Z. Vet. J.* **59**: 16–23.

39. Hulsen, J. 2007. Cow signals, UK / Irela ed., Roodbont Publishers, Zutphen.

40. Jonsson, N. N., Mayer, D. G. and Green, P. E. 2000. Possible risk factors on Queensland dairy farms for acaricide resistance in cattle tick (*Boophilus microplus*). *Vet. Parasitol.* **88**: 79–92.

41. Kabi, F., Masembe, C., Muwanika, V., Kirunda, H. and Negrini, R. 2014. Geographic distribution of non-clinical *Theileria parva* infection among indigenous cattle populations in contrasting agro-ecological zones of Uganda: Implications for control strategies. *Parasites and Vectors*. **7**: 1–9.

42. Karimuribo, E. D., Fitzpatrick, J. L., Swai, E. S., Bell, C., Bryant, M. J., Ogden, N. H., Kambarage, D. M. and French, N. P. 2008. Prevalence of subclinical mastitis and associated risk factors in smallholder dairy cows in Tanzania. *Vet. Rec.* **163**: 16–21.

43. Kasozi, K. I., Matovu, E., Tayebwa, D. S., Natuhwera, J., Mugezi, I. and Mahero, M. 2014. Epidemiology of increasing hemo-parasite burden in Ugandan cattle. *Open J. Vet. Med.* **04**: 220–231.

44. Kasozi, K. I., Tingiira, J. B. and Vudriko, P. 2014. High prevalence of

subclinical mastitis and multidrug resistant *Staphylococcus aureus* are a threat to dairy cattle production in Kiboga District (Uganda). *Open J. Vet. Med.* **4**: 35–43.

45. Keeling, M. J. and Rohani, P. 2008. Introduction to simple epidemic models. pp. 15–53. *In*: *Modeling infectious diseases in humans and animals*, Princeton University Press, Princeton.

46. Kelton, D. F. 2006. Epidemiology: a foundation for dairy production medicine. *Vet. Clin. Food. Anim.* **22**: 21–33

47. Kerario, I. I., Simuunza, M. C., Chenyambuga, S. W., Koski, M., Hwang, S. G. and Muleya, W. 2017. Prevalence and risk factors associated with *Theileria parva* infection in cattle in three regions of Tanzania. *Trop. Anim. Health Prod.* **49**: 1613–1621.

48. Kikuchi, N., Toriumi, F., Nakano, F., Moriya, Y. and Takahashi, H. 2013. Prevalence of leptospiral antibodies in dairy cows in Japan. *J. Japan Vet. Med. Assoc.* **66**: 463–467.

49. Kisamba-Mugerwa, W. 1992. Rangeland tenure and resource management : an overview of pastoralism in Uganda.

50. Ko, A. I., Goarant, C. and Picardeau, M. 2009. *Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen. *Nat. Rev. Microbiol.* **7**: 736–747.

51. Konnai, S., Imamura, S., Nakajima, C., Witola, W. H., Yamada, S., Simuunza, M., Nambota, A., Yasuda, J., Ohashi, K. and Onuma, M. 2006. Acquisition and transmission of *Theileria parva* by vector tick, *Rhipicephalus appendiculatus*. *Acta Trop.* **99**: 34–41.

52. Kulldorff, M. 1997. A spatial scan statistic. *Commun. Stat. - Theory*

Methods. **26**: 1481–1496.

53. Kuroki, M. 2017. An introduction to structural causal models, 1st ed., Kyoritsu Shuppan, Tokyo.

54. Lam, T. J., van Vliet, J. H., Schukken, Y. H., Grommers, F. J., van Velden-Russcher, A., Barkema, H. W. and Brand, A. 1997. The effect of discontinuation of postmilking teat disinfection in low somatic cell count herds. II. Dynamics of intramammary infections. *Vet. Q.* **19**: 47–53.

55. Leonard, N., Mee, J. F., Snijders, S. and Mackie, D. 2004. Prevalence of antibodies to *Leptospira interrogans* serovar hardjo in bulk tank milk from unvaccinated irish dairy herds. *Ir. Vet. J.* **57**: 226.

56. Levett, P. N. 2001. Leptospirosis. *Clin. Microbiol.* **14**: 296–326.

57. Marcellino W. L., Salih, D.A., Jull, I. I. and E. H. A. M. 2011. Economic impact of east coast fever in central equatorial state of south Sudan. *Int. Res. J. Agric. Sci. Soil Sci.* **1**: 218–220.

58. Marcellino, W. L., Salih, D. A., Julla, I. I. and El Hussein, A. R. M. 2012. Seroprevalence of East Coast fever in Central Equatoria State, South Sudan. *Vet. Ital.* **48**: 379–385.

59. Mazimpaka, E., Mbuza, F., Michael, T., Gatari, E. N., Bukenya, E. M. and James, O. A. 2017. Current status of cattle production system in Nyagatare District-Rwanda. *Trop. Anim. Health Prod.* **49**: 1645–1656.

60. Mbarara District Local Government 2011. Mbarara District Local Government five-year district development plan. http://npa.ug/wp-content/themes/npatheme/documents/West/Mbarara DDP.pdf.

61. McDougall, S. 1999. Prevalence of clinical mastitis in 38 Waikato dairy herds in early lactation. *N. Z. Vet. J.* **47**: 143–149.

62. Miller, D. A., Wilson, M. A. and Beran, G. W. 1991. Survey to estimate prevalence of *Leptospira interrogans* infection in mature cattle in the United States. *Am. J. Vet. Res.* **52**: 1761–1765.

63. Ministry of Agriculture Animal Industry and Fisheries and Uganda Bureau of Statistics 2009. The national livestock census: a summary report of the national livestock census, 2008.

64. Ministry of Agriculture, F. and F. 2014. The 89th statistical yearbook of Ministry of Agriculture, Forestry and Fisheries. http://www.maff.go.jp/e/data/stat/89nenji/index.html.

65. Moran, J. 2012. Managing high grade dairy cows in the tropics, CSIRO, Melbourne.

66. Morgan, S. L. and Winship, C. 2015. Causal graphs. pp. 77–101. *In*: *Counterfactuals and causal inference (methods and principles for social research)*, 2nd ed., Cambridge University Press, New York.

67. Morgan, S. L. and Winship, C. 2015. Models of causal exposure and identification criteria. pp. 105–139. *In*: *Counterfactuals and causal inference (methods and principles for social research)*, 2nd ed., Cambridge University Press, New York.

68. Mostert, P. F., Bokkers, E. A. M., Van Middelaar, C. E., Hogeveen, H. and De Boer, I. J. M. 2018. Estimating the economic impact of subclinical ketosis in dairy cattle using a dynamic stochastic simulation model. *Animal*. **12**: 145–154.

69. Nalubwama, S., Kabi, F., Vaarst, M., Smolders, G. and Kiggundu, M. 2016. Cattle management practices and milk production on mixed smallholder organic pineapple farms in Central Uganda. *Trop. Anim. Health Prod.* **48**:

1525–1532.

70. National Institute of Infectious Diseases 2016. Leptospirosis in Japan, January 2007-April 2016. https://www.niid.go.jp/niid/en/iasr-vol37-e/865-iasr/6458-436te.html.

71. National Mastitis Council 2017. Laboratory handbook on bovine mastitis, 3rd ed., National Mastitis Council, Inc., New Prague.

72. Neijenhuis, F., Barkema, H. W., Hogeveen, H. and Noordhuizen, J. P. 2000. Classification and longitudinal examination of callused teat ends in dairy cows. *J. Dairy Sci.* **83**: 2795–2804.

73. Norval, R. A. I., Perry, B. D. and Young, A. S. 1992. The epidemiology of theileriosis in Africa, ILRAD, Nairobi.

74. O' Doherty, E., Berry, D. P., O' Grady, L. and Sayers, R. 2014. Management practices as risk factors for the presence of bulk milk antibodies to *Salmonella*, *Neospora caninum* and *Leptospira interrogans* serovar hardjo in Irish dairy herds. *animal*. **8**: 1010–1019.

75. O' Doherty, E., Sayers, R. and O' Grady, L. 2013. Temporal trends in bulk milk antibodies to *Salmonella* , *Neospora caninum* , and *Leptospira interrogans* serovar hardjo in Irish dairy herds. *Prev. Vet. Med.* **109**: 343–348.

76. O' Doherty, E., Sayers, R., O' Grady, L. and Shalloo, L. 2015. Effect of exposure to *Neospora caninum*, *Salmonella*, and *Leptospira interrogans* serovar Hardjo on the economic performance of Irish dairy herds. *J. Dairy Sci.* **98**: 2789–2800.

77. Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. *Vet. Clin. North Am. - Food Anim. Pract.* **20**: 651–674.

78. Oikawa, S. 2015. Recent research trend on subclinical ketosis in dairy cattle. *Japan Var. Med. Assoc.* **68**: 33–42.

79. Pastell, M., Aisla, A. M., Hautala, M., Poikalainen, V., Praks, J., Veermäe, I. and Ahokas, J. 2006. Contactless measurement of cow behavior in a milking robot. *Behav. Res. Methods*. **38**: 479–486.

80. Pyörälä, S. and Taponen, S. 2009. Coagulase-negative staphylococci-emerging mastitis pathogens. *Vet. Microbiol.* **134**: 3–8.

81. R Core Team R 2016. A language and environment for statistical computing. https://www.r-project.org/.

82. R Core Team R 2018. A language and environment for statistical computing. https://www.r-project.org/.

83. R Core Team R 2019. A language and environment for statistical computing. https://www.r-project.org/.

84. Rakuno Gakuen University 2019. JICA partnership program safe milk promotion in Mbarara project final report.

85. Robbertse, L., Baron, S., van der Merwe, N. A., Madder, M., Stoltsz, W. H. and Maritz-Olivier, C. 2016. Genetic diversity, acaricide resistance status and evolutionary potential of a *Rhipicephalus microplus* population from a disease-controlled cattle farming area in South Africa. *Ticks Tick. Borne. Dis.* **7**: 595–603.

86. Ruegg, P. 2005. California mastitis test (CMT) fact sheet 1. http://milkquality.wisc.edu/wp-content/uploads/2011/09/california-mastitis-test-fact-sheet.pdf.

87. Ruegg, P. 2002. Udder hygiene scoring chart. https://milkquality.wisc.edu/wp-content/uploads/sites/212/2011/09/udder-

hygiene-scoring-chart.pdf.

88. Rutherford, A. J., Oikonomou, G. and Smith, R. F. 2016. The effect of subclinical ketosis on activity at estrus and reproductive performance in dairy cattle. *J. Dairy Sci.* **99**: 4808–4815.

89. Ryan, E. G., Leonard, N., Grady, L. O., Doherty, M. L. and More, S. J. 2012. Herd-level risk factors associated with *Leptospira* Hardjo seroprevalence in Beef / Suckler herds in the Republic of Ireland. *Ir. Vet. J.* **65**: 6.

90. Ryan, E. G., Leonard, N., Grady, L. O., More, S. J. and Doherty, M. L. 2012. Seroprevalence of *Leptospira* Hardjo in the Irish suckler cattle population. *Ir. Vet. J.* **65**: 8.

91. Saitoh, H., Koizumi, N., Seto, J., Ajitsu, S., Fujii, A., Takasaki, S., Yamakage, S., Aoki, S., Nakayama, K., Ashino, Y., Chagan-Yasutan, H., Kiyomoto, H. and Hattori, T. 2015. Leptospirosis in the Tohoku Region: re-emerging infectious disease. *Tohoku J. Exp. Med.* **236**: 33–37.

92. Salgado, M., Otto, B., Sandoval, E., Reinhardt, G. and Boqvist, S. 2014. A cross sectional observational study to estimate herd level risk factors for *Leptospira spp.* serovars in small holder dairy cattle farms in southern Chile. *BMC Vet. Res.* **10**: 126.

93. Sanhueza, J. M., Heuer, C., Wilson, P. R. and Benschop, J. 2015. Prevalence and risk factors for *Leptospira* exposure in New Zealand veterinarians. *Epidemiol. Infect.* **143**: 2116–2125.

94. Sargeant, J. M., Kelton, D. F., Martin, S. W. and Mann, E. D. 1997. Evaluation of a bulk-milk ELISA test for the classification of herd-level bovine leukemia virus status. *Prev. Vet. Med.* **31**: 223–230.

95. Schaik, G. Van, Schukken, Y. H., Nielen, M. and Dijkhuizen, A. A. 2002.

Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms : a cohort study. **54**: 279–289.

96. Segura-Correa, V. M., Solis-Calderon, J. J. and Segura-Correa, J. C. 2003. Seroprevalence of and risk factors for leptospiral antibodies among cattle in the state of Yucatan, Mexico. *Trop. Anim. Health Prod.* **35**: 293–299.
97. Smith, J. W., Gilson, W. D., Ely, L. O. and Graves, W. M. 2009. Dairy reproduction benchmarks.
98. Ssajjakambwe, P., Bahizi, G., Setumba, C., Kisaka, S. M. B., Vudriko, P., Atuheire, C., Kabasa, J. D. and Kaneene, J. B. 2017. Milk hygiene in rural southwestern Uganda: prevalence of mastitis and antimicrobial resistance profiles of bacterial contaminants of milk and milk products. *Vet. Med. Int.* **2017**: 8710758.
99. Sustainable Agriculture Initative Platform Working Group 2015. Principles and practices for sustainable dairy farming 2015.
100. Textor, J., Hardt, J. and Knüppel, S. 2011. DAGitty: a graphical tool for analyzing causal diagrams. *Epidemiology*. **22**: 745.
101. Thompson, M. M. and Hrabak, E. M. 2018. Capture and storage of plant genomic DNA on a readily available cellulose matrix. *Biotechniques*. **65**: 285–287.
102. Tsuchimoto, M., Kida, H., Yanagawa, R. and Inui, S. 1983. *Leptospira interrogans* serovar hardjo infection in cows of Hokkaido, Japan--serological evidence. *Nihon Juigaku Zasshi.* **45**: 811–814.
103. Uchida, L., Byaruhanga, J., Okamura, I., Miyama, T., Muramatsu, Y., Vudriko, P. and Makita, K. 2019. FTA-sodium hydroxide-based PCR: an efficient and cheaper option for *Theileria parva* detection in dairy cattle in

Mbarara, Uganda. *J. Vet. Med. Sci.* (in press)

104. Uganda Bureau of Statistics 2016. National Population and Housing Census 2014 - Main Report, Kampala.

105. Uganda Bureau of Statistics Uganda Bureau of Statistics 2017 Statistical Abstract. http://www.ubos.org/onlinefiles/uploads/ubos/statistical_abstracts/2017_Statistical_Abstract.pdf.

106. United Nations 2018. UN comtrade database. https://comtrade.un.org/.

107. Vudriko, P., Okwee-Acai, J., Byaruhanga, J., Tayebwa, D. S., Okech, S. G., Tweyongyere, R., Wampande, E. M., Okurut, A. R. A., Mugabi, K., Muhindo, J. B., Nakavuma, J. L., Umemiya-Shirafuji, R., Xuan, X. and Suzuki, H. 2018. Chemical tick control practices in southwestern and northwestern Uganda. *Ticks Tick. Borne. Dis.* **9**: 945–955.

108. Vudriko, P., Okwee-Acai, J., Byaruhanga, J., Tayebwa, D. S., Omara, R., Muhindo, J. B., Lagu, C., Umemiya-Shirafuji, R., Xuan, X. and Suzuki, H. 2018. Evidence-based tick acaricide resistance intervention strategy in Uganda: Concept and feedback of farmers and stakeholders. *Ticks Tick. Borne. Dis.* **9**: 254–265.

109. Vudriko, P., Okwee-Acai, J., Tayebwa, D. S., Byaruhanga, J., Kakooza, S., Wampande, E., Omara, R., Muhindo, J. B., Tweyongyere, R., Owiny, D. O., Hatta, T., Tsuji, N., Umemiya-Shirafuji, R., Xuan, X., Kanameda, M., Fujisaki, K. and Suzuki, H. 2016. Emergence of multi-acaricide resistant *Rhipicephalus* ticks and its implication on chemical tick control in Uganda. *Parasites and Vectors*. **9**: 254-265.

110. Vudriko, P., Umemiya-Shirafuji, R., Okwee-Acai, J., Tayebwa, D. S.,

Byaruhanga, J., Jirapattharasate, C., Liu, M., Adjou Moumouni, P. F., Fujisaki, K., Xuan, X. and Suzuki, H. 2017. Genetic mutations in sodium channel domain II and carboxylesterase genes associated with phenotypic resistance against synthetic pyrethroids by *Rhipicephalus (Boophilus) decoloratus* ticks in Uganda. *Pestic. Biochem. Physiol.* **143**: 181–190.

111. Walker, A. R., Bouattour, A., Camicas, J.-L., Estrada-Peña, A., Horak, I. G., Latif, A. A., Pegram, R. G. and Preston, P. M. 2014. Ticks of domestic animals in Africa: a guide to identification of species, Bioscience Reports, Edinburgh.

112. WHO 2018. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd ed., World Health Organization, Geneva.

113. Wozemba, D. and Nsanja, R. 2008. Dairy investment opportunities in Uganda- report.

114. Yameogo, N., Ouedraogo, G. A., Kanyandekwe, C. and Sawadogo, G. J. 2008. Relationship between ketosis and dairy cows' blood metabolites in intensive production farms of the periurban area of Dakar. *Trop. Anim. Health Prod.* **40**: 483–490.

115. Yan, K., Ellis, W. A., Mackie, D. P., Taylor, M. J., Mcdowell, S. W. J. and Montgomery, J. M. 1999. Development of an ELISA to detect antibodies to a protective lipopolysaccharide fraction of *Leptospira borgpetersenii* serovar hardjo in cattle. **69**: 173-187.

116. Zhou, H., Hickford, J. G. H. and Fang, Q. 2006. A two-step procedure for extracting genomic DNA from dried blood spots on filter paper for polymerase chain reaction amplification. *Anal. Biochem.* **354**: 159–161.

APPENDIXES

Supplemental tables for Chapter 1. Herd-level risk factors associated with *Leptospira* Hardjo infection in dairy herds in the southern Tohoku, Japan

Table S1. Univariable analysis results for binary and categorical data associated with *Leptospira* Hardjo infection in dairy herds in the southern Tohoku, Japan

Variables	Positive herds	Negative herds	Prevalence (%)	Statistics	*p*-value
Herd management					
Stall style				Fisher's Exact Test	0.09
Free stall	11	1	91.7		
Free barn	1	1	50.0		
Tie stall	55	34	61.8		
Grazing				Chi-squared test	1
Yes	10	5	66.7		
No	54	31	63.5		
Source of feed				Chi-squared test	0.45
Self-supplied	48	29	62.3		
Purchased only	19	7	73.1		
Source of water					
Tap water				Chi-squared test	1
Yes	35	19	64.8		
No	32	17	65.3		
Groundwater				Chi-squared test	0.51
Yes	32	14	69.6		
No	35	22	61.4		
River water				Fisher's Exact Test	1
Yes	4	2	66.7		
No	63	34	64.9		
Bedding material					
Straw				Chi-squared test	0.08
Yes	18	16	52.9		
No	48	18	75.0		
Sawdust				Chi-squared test	0.19
Yes	9	9	50.0		
No	57	25	69.5		
Contact between cows and calves				Fisher's Exact Test	1
Yes	3	1	75.0		
No	64	33	66.0		
Feeding of calf				Fisher's Exact Test	1
Milk replacer	36	67	35.0		
Milk from cows	0	0	0		
Presence of breeding pen				Fisher's Exact Test	0.66
Yes	4	1	80.0		
No	63	35	64.3		

Table S1 (continued)

Variables	Positive herds	Negative herds	Prevalence (%)	Statistics	*p*-value
Placenta cleaning				Chi-squared test	0.51
Just after the calving	28	18	60.9		
The same timing as the pen cleaning	38	17	69.1		
Neonatal calf isolation				Chi-squared test	0.70
Just after the birth	56	31	64.4		
After contact with the dam	11	4	73.3		
Presence of cats				Chi-squared test	0.005
Yes	14	18	43.8		
No	57	20	74.0		
Hygiene management					
Frequency of cleaning pens					
Passage				Fisher's Exact Test	0.74
Daily	58	32	64.4		
Less than above	6	4	66.7		
Stall				Fisher's Exact Test	1
Daily	61	35	63.5		
Less than above	1	1	50.0		
Feed trough				Fisher's Exact Test	1
Daily	60	33	64.5		
Less than above	5	3	62.5		
Water trough				Chi-squared test	0.48
Daily	21	9	70.0		
Less than above	39	26	60.0		
Frequency of disinfecting pens				Fisher's Exact Test	0.56
More than once a year	26	12	68.4		
Less than above	35	23	60.3		
Change of clothing of visitors				Fisher's Exact Test	0.55
Yes	8	6	57.1		
No	59	30	66.2		
Change of boots of visitors				Chi-squared test	0.55
Yes	18	7	72.0		
No	49	29	62.8		
Detection of rodents				Chi-squared test	0.87
Yes	37	22	62.7		
No	24	12	66.7		
Detection of other wild animals				Chi-squared test	0.72
Yes	31	19	62.0		
No	16	13	55.2		

Table S1 (continued)

Variables	Positive herds	Negative herds	Prevalence (%)	Statistics	*p*-value
Vaccination use					
L. Hardjo				Fisher's Exact Test	1
Yes	0	0	0		
No	71	38	65.1		
Other vaccine				Chi-squared test	0.81
Yes	42	25	62.7		
No	19	9	67.9		
Vermicide use				Chi-squared test	1
Yes	22	13	62.9		
No	39	21	65.0		
Disease incidence					
Frequency of vets visits (time/week)				Chi-squared test	0.08
Twice or more	28	8	77.8		
Once or fewer	39	28	58.2		
Abortion within 1 year				Chi-squared test	0.74
Yes	41	24	63.1		
No	26	12	68.4		
Movement of cattle					
Introduction of cows/use of common grazing within 5 years				Fisher's Exact Test	< 0.001
Yes	67	26	72.0		
No	0	10	0		
Introduced/returned from					
Hokkaido				Chi-squared test	
Yes	57	7	89.1		< 0.001
No	8	15	34.8		
Tohoku				Chi-squared test	
Yes	14	16	46.7		< 0.001
No	51	5	91.1		

Table S2. Univariable analysis results for count and continuous data associated with *Leptospira* Hardjo infection in dairy herds in the southern Tohoku, Japan

Variables	Positive herds	Negative herds	Statistics	*p*-value
Herd size			Wilcoxon rank sum test	< 0.001
Mean	60.4	30.1		
Median	39	27		
Range	4 - 500	4 - 160		
Average parity				
Mean	2.7	2.9	Student's *t* test	0.45
Median	2.7	3.1		
Range	1.7 - 4.0	2.0 - 3.5		

The intervention packages of Japan International Cooperation Agency (JICA) Partnership Program entitled “Safe Milk Promotion in Mbarara Project”

Mobile OcOc (One Cloth for One Cow) 1

Before going to a milking parlor,

① Making disinfectant (200 ppm of sodium hypochlorite)

If you have *JIK* which is 3.5% sodium hypochlorite, dilute it into 175 times with water.

- 2L water + 12ml *JIK*
- Recommended temperature of dilution water is below 40°C

② Dip dried towels into the disinfectant, keep them there for 10 min.

③ Squeeze them with clean hands, keep them in a bucket with a lid, and prepare another empty bucket to put the towel away

④ Also, prepare a bucket with water for washing hands

Mobilize them to the parlor

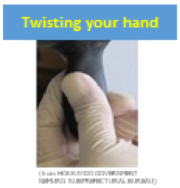
At the parlor,

① Hold a cow which you are about to milk
  * Milk *S. aureus* (+) cows, at the last

② Wash your hand before milking each cow
  a. Wash your hands with a bucket of water
  b. Rinse your hands with clean running water with disinfectant

③ Pick up one towel from the bucket, and use one towel for one cow when you wipe the teats.

④ Wipe each teat by twisting your hand to remove out the dirt
  Use clean part of the towel for each



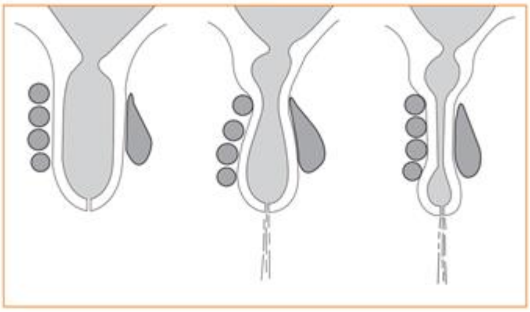
⑤ After finishing all the teats, wipe the teat tips using the other side of the towel

⑥ Put the towel away to the other bucket

⑦ Then, start milking

Mobile OcOc (One Cloth for One Cow) 2

When you milk,



Proper hand milking

- This diagram shows the action of the hand and how milk is drawn out of the teats during milking.
- Milk gently and DO NOT hurt teats.

(Department of Agriculture, Republic of South Africa. 2008.)

Problem of finger milking

- Some dairy farmers squeeze out milk between the folded-over thumb and the fingers, or between the fingers and a straight thumb.
- This milking method causes damage to the teat, hurting and upsetting the cow, thus reducing the milk flow and the production.

After milking,

(from Tokachi-NOSAI)

Post dipping

- After milking, if the cow's calf does not suckle, use a teat dip to prevent mastitis.
- Dip > 75% of a teat length.

Preparation for the next milking

① Wash all the used towels with soap and rinse cleanly

② Dry them under the sunshine

Contacts

- **Dr. Mwebembezi William** (PRINCIPAL VETERINARY OFFICER/DVO)
  0772493143 / 0755744944
- **Mr. Karamuzi Godfrey** (UCCCU)
  0772539175
- **Dr. Byaruhanga Joseph** (PROJECT CONSULTANT)
  0774881544

THE JICA PARTNERSHIP PROGRAM
THE SAFE MILK PROMOTION IN MBARARA PROJECT

Republic of Uganda

Intervention package instruction 1

Feeding strategy — **Start from the step 1. Go ahead (step 2) if you can.**

1. Increase DMI with whatever available

a. Hay:

harvest grasses while they are available (rainy season). Dry, store and feed them when feeding is lacking.

b. Silage:

Ask extension workers and/or specialist on the field how you make silage

c. Agricultural by-product

eg. Banana peal

2. CP / TDN balanced concentrate with quantity of 1/3 of milk yield

The balance and the quantity are ruminologically and nutritionally important for milk production

Brewers waste

Maize bran

Table 1 TDN/CP balanced feeding based on milk yield for brewers waste which looks dry (approximate 85% DM)

Milk yield (kg)	5	10	15	20	25	30
Brewers waste (kg, 85% DM)	0.8	1.7	2.5	3.3	4.2	5.0
Maize bran (kg)	0.8	1.7	2.5	3.3	4.2	5.0

Table 2 TDN/CP balanced feeding based on milk yield for brewers waste of which water drips come out when it is squeezed by hand (approximate 60% DM)

Milk yield (kg)	5	10	15	20	25	30
Brewers waste (kg, 60% DM)	1.1	2.4	3.5	4.7	6.0	7.1
Maize bran (kg)	0.8	1.7	2.5	3.3	4.2	5.0

Intervention package instruction 2

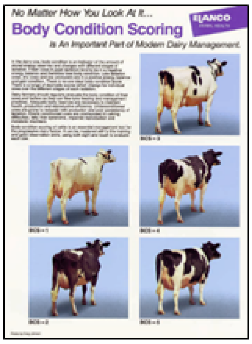
Ketosis management

In terms of ketosis, high producing cow whose body condition score (BCS) changed > 0.5 after calving and bad rumen fill score (RFS) should be aware

BCS check
2 weeks before the expected day → Delivery → BCS and RFS check
2 weeks after the delivery

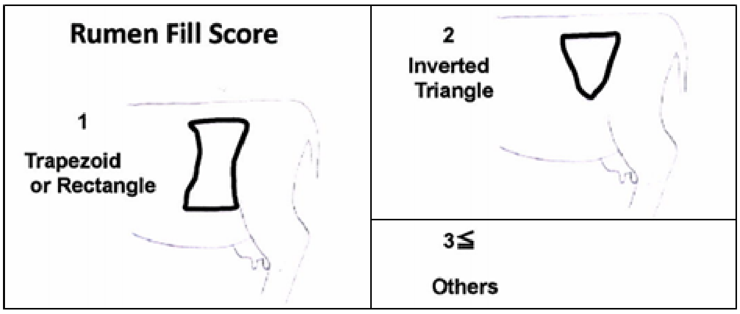
a. Score BCS for cows two weeks before and after the delivery date. Then, see the difference.

b. If the difference is ≥ 0.5, check RFS.



c. If the score is 1 (a sign of non-appetite), give 200 ml of propylene glycol twice a day for 3 days.

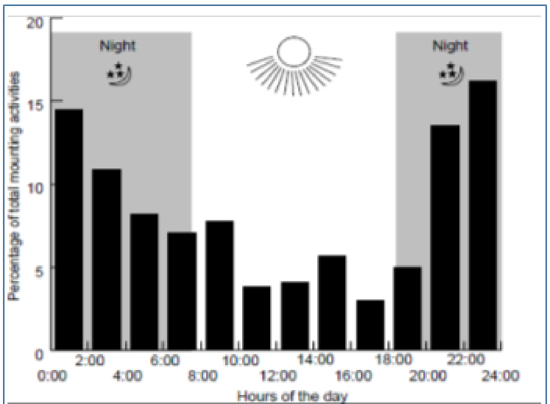
d. If the condition is still bad, call a veterinarian for the further treatment.



Reproduction

a. Set heat detection time every day

1. Assign one specific person to the detection
2. Spend ≥ twice per day for the detection
   eg. Once in the morning, once more in the afternoon after milking
3. Spend ≥ 30 min. for each detection



Distribution of estrus sings in a day Giudice 2007

Heat detection time, frequency, and heat detection rate

Frequency / day	Time (min.)	10	20	30	60
		Heat detection rate			
1		22	31	36	39
2		33	43	55	61
3		45	55	65	71
4		49	61	71	78

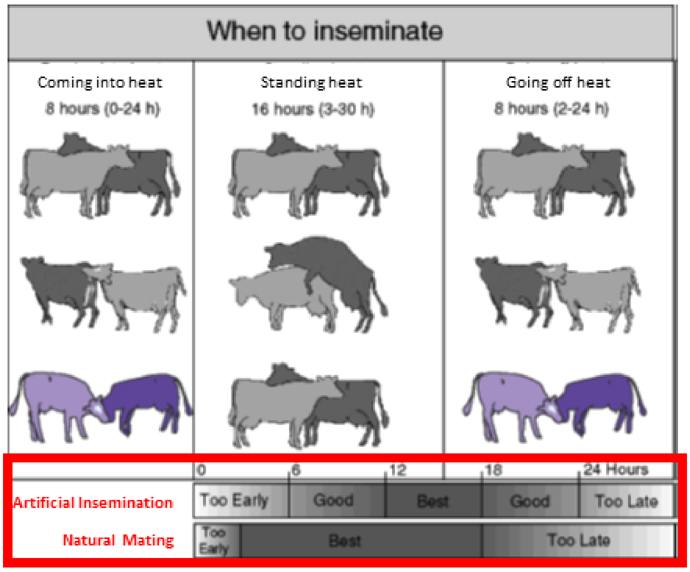
Fertility. 2008. Univ. Arkansas Jodie Pennington

Intervention package instruction 3

Reproduction

b. Keep records of heat symptom

Strength, Type, and Time of detection



Info Dairy Newsletter

Long extended transparent mucus

- Good for both AI and NM

c. Call AI technician or make a cow in heat get together with a bull

Contacts

- **Dr. Mwebembezi William** (PRINCIPAL VETERINARY OFFICER/DVO)
  0772493143 / 0755744944
- **Mr. Karamuzi Godfrey** (UCCCU)
  0772539175
- **Dr. Byaruhanga Joseph** (PROJECT CONSULTANT)
  0774881544

THE JICA PARTNERSHIP PROGRAM

THE SAFE MILK PROMOTION IN MBARARA PROJECT

Republic of Uganda

How to make hay

1. Harvest grass from the first heading time to heading time.
2. Dry it without exposing rain
3. Keep it in a dry place avoiding rain and sunshine until dry season

How to make silage

1. Prepare grass field

2. Harvest grass from the first heading time to heading time


The first heading time


Heading time

3. Cut it into small pieces (length of 1 ~ 3 cm)

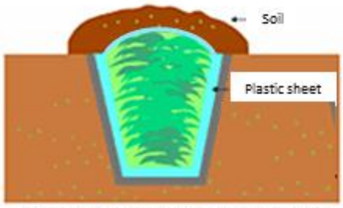

(from Japan Livestock Technology Association)

4. Put it into a silo and press by feet until it will not go down


(from Japan Livestock Technology Association)

Trench silo Container silo

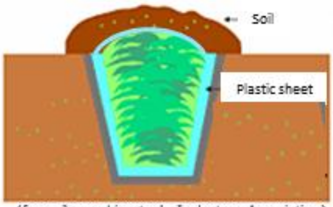
5. Seal the silage with plastic sheets and remove out air as much as possible
6. Cover the silo with soil and put stones or tiers as weights on it



(from Japan Livestock Industry Association)

7. After 20 days, fermentation will stop and you can feed it to cows or keep until dry season

Type of silo



(from Japan Livestock Industry Association)

Trench silo


Stuck silo


(from Japan Livestock Technology Association)

Container silo


Plastic bag silage

Where to make a silo

1. Well drained, 2. Less sunshine, 3. Close to manger, 4. Close to grass field

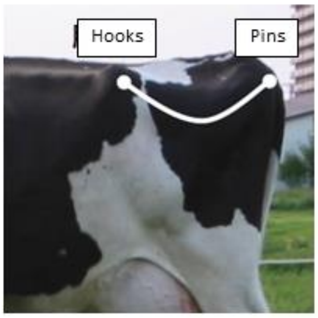
Body Condition Score

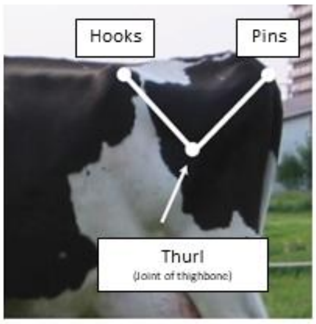
Rump V

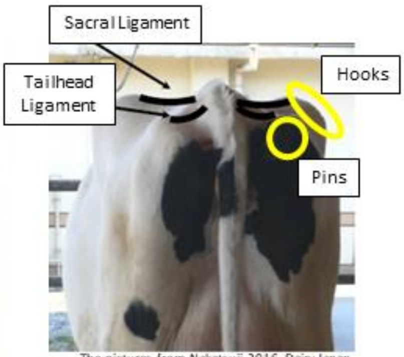
Score	Parts to observe	
	Hooks	Pins
3.00	Fat Pat	Fat Pat
2.75	Angular	Fat Pat
2.50	Angluar	Angular (Barely Fat Pat)
2.50>	Angluar	Angluar

Rump U

Score	Parts to observe	
	Tailhead Ligament	Sacral Ligament
3.25	Visible	Visible
3.50	Barely Visible	Visible
3.75	Not Visible	Barely Visible
4.00	Not Visible	Not Visible
4.25≦	Short Ribs Barely Visible	



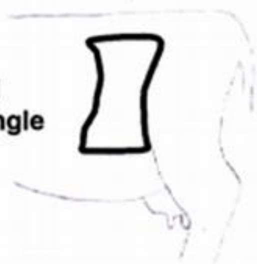




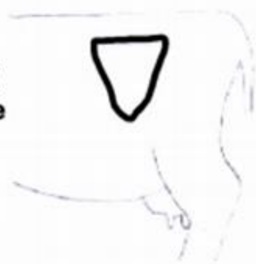
The pictures from Nakatsuji 2016, Dairy Japan

Rumen Fill Score

1
Trapezoid or Rectangle



2
Inverted Triangle



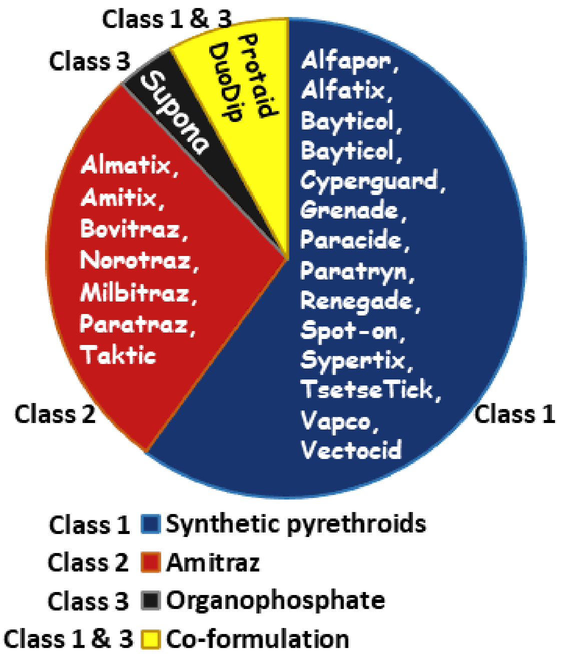
3≦
Others

Goal (1+2) 20%≧

Proper acaricide use

a. Class of acaricide

Know the class of the acaricide you are using at your farm

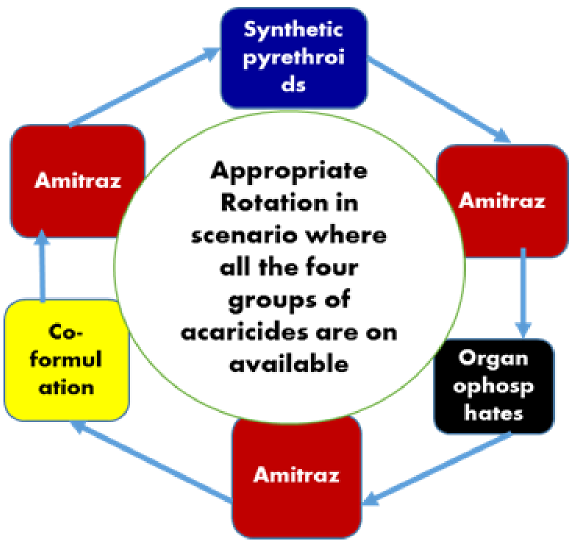


Key points

- There are many acaricides that have different trade names but belong to the same family
- Knowing the family to which an acaricide belongs help in making appropriate decision on prescription, change and/or rotation
- It is recommended that acaricides be procured from licensed veterinary drug outlets

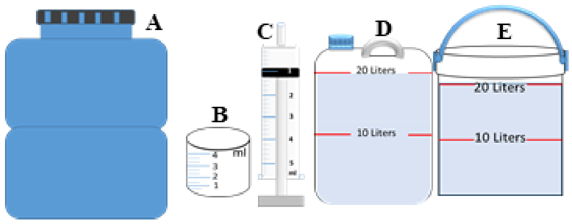
b. Rotational acaricide use

Appropriate acaricide use starts with keeping records of acaricide use at farm level



Appropriate rotation informed by technical guidance is needed

c. Appropriate acaricide dilution



Key points

- You need to have a plan for both materials, labor and volume of acaricides to use for a given herd size.
- Strictly adhere to the manufactures recommended dilution
- Use the right materials for measuring and mixing equipments

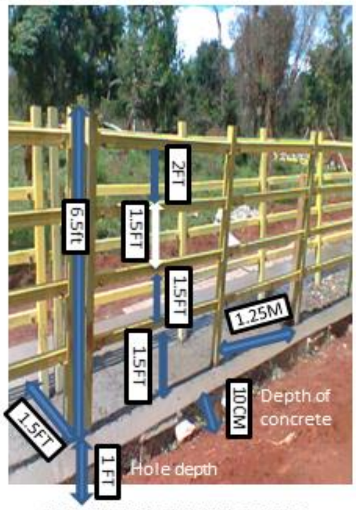
Ticks and TBD intervention package instruction 2

Proper acaricide use (contd.)

d. Appropriate and recommended crush

A good crush with one raw of cattle

FRONT VIEW



SIDE VIEW OF THE CRUSH

e. Appropriate bucket pump

that can spray well all over face of cattle

BUCKET PUMP

Integrated tick control strategies

1. Vaccinate your cattle against ECF
2. Practice Tactical Rotational grazing
3. Practice zero grazing

Strategies of ECF Management

1. Early detection of diseases is encouraged
2. Please submit samples to the laboratory for testing to be sure
3. Ensure early treatment to increase chances of recovery
4. Monitor the progress of treatment
5. Consult a Vet for advice

Contacts

- **Dr. Mwebembezi William** (PRINCIPAL VETERINARY OFFICER/DVO)
  0772493143 / 0755744944
- **Mr. Karamuzi Godfrey** (UCCCU)
  0772539175
- **Dr. Byaruhanga Joseph** (PROJECT CONSULTANT)
  0774881544

THE JICA PARTNERSHIP PROGRAM
THE SAFE MILK PROMOTION IN MBARARA PROJECT

Republic of Uganda

ABSTRACT

Veterinary Epidemiology

Doctoral Course of Veterinary Medicine

Graduate School of Veterinary Medicine

Rakuno Gakuen University Graduate School

Takeshi Miyama

A variety of problems are prevailing in dairy farms, and epidemiological approaches can be used to solve those problems for sustainable dairy farming. In epidemiological study, a researcher often obtains an observed data to estimate the effect of an exposure on the outcome. In observational data, the conditioning in exposure and non-exposure group are different, which biases the observed measure of associations. Our challenge is to identify and control factors that cause the confounding to obtain valid estimates of causal effects. In this PhD thesis, risk factor analyses were conducted for dominant dairy production problems, and the approaches applied in this thesis were discussed. Prediction models were used to predict farm-level bovine leptospirosis invasion in Japan. Causal inference approaches were used for East Coast Fever (ECF), a tick-borne disease, mastitis control, and feeding management in Uganda.

Invasion level of bovine leptospirosis caused by *Leptospira* serovar Hardjo in the south Tohoku, Japan was not well known. This cross-sectional study identified that *L.* Hardjo infection at farm-level was prevalent in the south Tohoku, the apparent herd prevalence: 65.1% (95%CI: 56.2-74.1%), and larger herd size ($p = 0.004$) and cows with a history of staying in Hokkaido Prefecture ($p < 0.001$) were the risk factors of *L.* Hardjo infection. The disease cluster of *L.* Hardjo positive farms were detected (relative risk = 1.87, log likelihood ratio = 9.93, radius = 13.70 km, $p < 0.01$) in the south part of the study area where the larger herds were aggregated with high density. To identify risk factors which well predict *L.* Hardjo infection in this study, a statistical algorithm, the stepwise method was used. Magnitude of confounding was tested using change-in-estimate criteria.

ECF is a problem due to its economic loss resulting from high morbidity and mortality, milk production loss, and control measure cost of ticks and the disease. This study identified that the prevalence of *T. parva* infection at the cattle-level was 45.2% (95%CI: 40.4-50.1) and farm-level was

83.3% (95%CI: 64.5-93.7). This study also detected the preventive factor for the tick infestation, good-quality of cattle crush (OR: 0.32, 95%CI: 0.15-0.63, p = 0.001); the preventive factor for *T. parva* infection, well managed acaricide storage (OR: 0.36, 95%CI: 0.17-0.76, p = 0.008) and good quality of measuring cylinder for acaricide (OR: 0.32, 95%CI: 0.11-0.93, p =0.036).; The risk factors for *T. parva* infection were longer period of (OR: 1.06, 95%CI: 1.01-1.10, p =0.012) and higher frequency of (OR: 11.70, 95%CI: 1.95-70.13, p =0.007) the acaricide use. The effect of these factors was quantitively estimated after adjusting the potential confounders using the causal inference method.

Mastitis is regarded as the most costly disease on dairy farms worldwide. A high prevalence of SCM was reported in Uganda. No risk factor analysis of milking hygiene and practices on SCM, and no SCM prevalence study in Mbarara District, an intensive dairy production region of Uganda had been reported. This study revealed the high SCM prevalence of 68.8% (95%CI: 64.9-72.2%) at the cow-level, and contagious pathogen were majority of the isolated pathogen. Use of one-towel-per-cow for teat wiping before milking was negatively associated with contagious pathogen by the univariable analysis (p = 0.025). The preventive factor for SCM was cows calm at the end of milking (OR: 0.20, 95%CI: 0.05-0.79, p = 0.021), and the risk factor was rough teat-end (OR: 1.75, 95%CI: 1.14-2.68, p = 0.011). The effect of these factors was also detected quantitively after adjusting the potential confounders using the causal inference method.

SCK results in reduced milk production and economic loss. SCK prevalence and feeding management in the southwestern region of Uganda had not been reported. This study identified that the prevalence of SCK for cows in the early lactation was 17.1% (95%CI: 6.6-33.6%). Cows fed concentrates had 1.6 times higher milk yield (95%CI: 1.02-2.34, p = 0.041) than those not. Cows in the farm where rotational grazing was conducted had 1.7 times higher milk yield (95% CI: 1.11-2.51, p = 0.017) than those not. The effect of feeding management on milk yield was quantitively detected after adjusting the potential confounders.

This PhD thesis epidemiologically described the problems prevailing in dairy production medicine, also revealed the risk factors associated with the dominant problems. These risk factor analyses had the two main objectives. One is to predict the outcome of interest, and to identify which factors contribute to the predicted outcome for general understanding as shown in *L.* Hardjo invasion study. The other is hypotheses driven study, which is performed to estimate the causal relationship quantitively between an exposure and outcome by controlling potential confounders. The latter approach was carried out in ECF, mastitis control, and feeding management studies. These approaches could be selected depending on study objectives. The problem solving is a continuous process in dairy production medicine. For sustainable dairy farming, to improve the dairy productivity and to prevent zoonotic diseases from the current baseline, proper record keeping, monitoring, data description, evaluation and intervention cycle should be performed continuously. The evidences and approaches in this thesis must contribute to solve problems in wide variety of area in dairy production medicine.

ABSTRACT IN JAPANESE (和文要旨)

因果推論を用いた酪農生産獣医療における問題指向型実地疫学的研究

酪農学園大学大学院獣医学研究科

獣医学専攻博士課程

獣医疫学　三山豪士

疫学研究は持続可能な酪農を営む上で、生産獣医療現場の問題解決に有用な手法である。疫学研究において、ある暴露因子がアウトカムに及ぼす効果を推定する際、疫学者は観測されたデータを取り扱うことが多くある。観測データでは、暴露群と非暴露群間の条件(背景情報)が異なることが多く、これは交絡を引き起こす。因果効果を推定するためには、この交絡因子を特定し、制御することが重要となる。本博士論文では、酪農生産獣医療における問題点についてリスク因子解析を実施し、その方法について考察した。日本における農場レベルの牛レプトスピラ症浸潤状況は予測モデルを用い、ウガンダにおける東海岸熱(East Coast Fever, ECF)、乳房炎制御、飼料給与管理については因果推論を用いて解析した。

日本の南東北において、*Leptospira* 血清型 Hardjo によって引き起こされる牛レプトスピラ症の浸潤状況の詳細な報告はなかった。本横断研究により有病率が 65.1% (95%CI: 56.2-74.1%)と南東北に広く浸潤していることが明らかにされた。また、牛群規模が大きいこと($p = 0.004$)及び北海道に滞在歴のある牛が農場に在籍すること($p < 0.001$)が *L.* Hardjo 感染のリスク因子であることが明らかとなった。*L.* Hardjo の陽性農場の集積 (relative risk = 1.87, log likelihood ratio = 9.93, radius = 13.70 km, $p < 0.01$) が、大規模農場の密集する研究対象地域の南部に検出された。本研究では、*L.* Hardjo 感染のリスク因子解析に統計的アルゴリズムであるステップワイズ法を用いた。交絡の定量的評価には Change-in-estimate 基準を用いた。

ECF は高い疾病率と致死率、乳生産量の低下、ダニ対策費用等による経済損失のため問題となる。ウガンダ国ムバララ県での本研究による個体レベルの *T. parva* 感染の有病率は 45.2% (95%CI: 40.4-50.1)、農場レベルでは 83.3% (95%CI: 64.5-93.7) であった。リスク因子解析により、高品質の牛保定用枠場は牛体表のダニ浸潤の防御因子であった (OR: 0.32, 95%CI: 0.15-0.63, $p = 0.001$)。質の良い殺ダニ剤保管庫 (OR: 0.36, 95%CI: 0.17-0.76, $p = 0.008$) と殺ダニ剤計測用シリンダー (OR: 0.32, 95%CI: 0.11-0.93, $p =0.036$) は、*T. parva* 感染の防御因子であ

った。また、長期間の同系統の殺ダニ剤の使用 (OR: 1.06, 95%CI: 1.01-1.10, p =0.012) と高い使用頻度 (OR: 11.70, 95%CI: 1.95-70.13, p =0.007) は *T. parva* 感染のリスク因子であった。これらの効果は因果推論を用いて定量的に推定された。

酪農において乳房炎は、世界的に最も経済損失の大きい疾病とされている。ウガンダのいくつかの地域においても潜在性乳房炎 (Subclinical mastitis: SCM)の 高い有病率が報告されているが、酪農集約地域であるムバララ県の SCM の有病率は報告されておらず、また搾乳衛生・手技に関するリスク因子解析の報告はなかった。本研究により高い個体レベルの SCM 有病率 (68.8%, 95%CI: 64.9-72.2%) が認められ、また分離培養検査により伝染性の微生物が優勢であることが明らかにされた。単変数解析において搾乳前の乳頭清拭に一頭一布を利用することと伝染性微生物の検出との間に負の相関が認められた (p = 0.025)。搾乳終盤に牛が落ち着いていることは SCM の防御因子であった (OR: 0.20, 95%CI: 0.05-0.79, p = 0.021)。粗造な乳頭口は SCM のリスク因子であった (OR: 1.75, 95%CI: 1.14-2.68, p = 0.011)。これら効果は因果推論を用いて定量的に推定された。

潜在性ケトーシス(Subclinical ketosis: SCK)は産乳性の低下に伴う経済損失を引き起こす。ウガンダの南西部において SCK の有病率と飼料給与管理方法は報告されてこなかった。本研究において、泌乳初期の牛の SCK 有病率は 17.1% (95%CI: 6.6-33.6%)であった。濃厚飼料を給与した牛は、非給与牛に比べて 1.6 倍高い乳量を示した (95%CI: 1.02-2.34, p = 0.041)。輪換放牧を実施した牛は、非実施牛に比べて 1.7 倍高い乳量を示した (95% CI: 1.11-2.51, p = 0.017)。これら効果は因果推論を用いて定量的に推定された。

本博士論文は、酪農生産獣医療現場に浸潤している問題について記述し、それらの問題のリスク因子を明らかにした。これらリスク因子解析を実施する目的は二つある。一つは、牛レプトスピラ症の研究で認められたように、興味の対象である目的変数を予測し、どの因子が予測に貢献しているかを概念的に理解することである。二つ目は、仮説の検証を目的とし、暴露因子が目的変数に及ぼす因果効果を、交絡因子を調整することで定量的に推定することである。この方法は ECF、乳房炎制御、飼料給与管理それぞれの研究において採用された。これらの手法は研究の目的により適切に選択されるべきである。酪農生産獣医療における問題解決は継続したプロセスである。持続可能な酪農業を目指し、生産性の向上と人獣共通感染症を防ぐには、適切な記録をとること、記録のモニタリング、情報の記述 (視覚化)、評価、介入というサイクルを継続的に実施していく必要がある。本博士論文で用いられた手法と構築された根拠は、生産獣医療の多様な問題解決に役立つものと考える。