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A one health framework to advance food safety and security: An on-farm case study in the Rwandan dairy sector

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ABSTRACT

In Rwanda, cattle and milk hold a cultural and historical significance, providing an opportunity for pro-dairy governmental policies aimed to alleviate food insecurity, malnutrition, and improve livelihoods. The government of Rwanda has identified strategies to grow the dairy sector through strategic investment to achieve these goals. It is estimated two-thirds of lactating cows in Rwanda have clinical or subclinical mastitis, which reduces milk production and increases the risk of milk as a source for zoonotic disease if the milk is consumed undercooked or unpasteurized. This case study outlines the implementation of a One Health framework that integrates education, research, and outreach in Rwanda to improve food safety and food security, for the social, economic, and health benefit of Rwandans and their livestock. Twenty-five Rwandan Extension Specialists participated in the Dairy Dynamic Management education, research, and outreach program. Once trained, the extension specialists supported 30 small holder dairy farmers in performing proper husbandry and animal health practices for mastitis control and reduction of bacterial counts in the udder. Over the 16-week program, 30 small holder dairy farmers and 100 dairy cows were surveyed weekly for animal husbandry, animal health, and mastitis indicators. Outcomes were evaluated by monitoring animal health, foodborne pathogens in milk, and compliance to animal husbandry protocols. Quarter milk samples were collected weekly and evaluated for the presence of bacteria that are common causes of mastitis. We found a statistically significant reduction of mean total bacterial counts and prevalence of bacterial species in quarters over the 16-week training ($P \leq .01$). Smallholders were monitored through observing farmers performing hygienic milking protocols. Farmers conducted the protocol correctly greater than 90% of the time by the end of the 16-week program for 5 of 7 steps for proper hygienic milking procedures, indicating farmers were eager to learn and adopt the procedures. However, follow-up and retraining with Extension Specialists is vital to continued success. We demonstrate that an integrative One Health education, research, and outreach program can be successful in improving animal health, food safety, and food security and this framework can be applied to other agricultural sectors and geographic regions.

1. Introduction

In Rwanda, cows are revered as a symbol of family, friendship, and community and are symbolic of wealth and social status. Cattle are often given as gifts which create a lasting bond of goodwill and camaraderie between the giver and receiver. Since the 15th century, milk has been consumed in social settings similarly to tea and coffee in other cultures and prior to colonization, excess milk produced by cows of wealthy families was shared with poor neighbors [1]. For many Rwandans, milk is a sacred and wholesome food which connects them to their culture and history [2,3]. Furthermore, there is evidence that people in East Africa have been dairying and consuming milk as far back as 3000 years ago [4]. The historical and cultural significance of cattle and milk in Rwanda has established milk as a strong basis among its citizens for pro-

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dairy policies by the government to grow the dairy sector as a means to alleviate food insecurity, malnutrition, and improve livelihoods and pathway for reconciliation following the 1994 genocide [3,5,6].

Economic growth and rising income levels have fueled demand for dairy products worldwide. According to the Food and Agriculture Organization of the United Nations, consumers in developed countries receive about 14% of their calories from dairy products, as compared to 4% in developing countries [7]. In Rwanda, 70 percent of the population drinks milk at a rate of about 40 liters per capita per year [8]. In comparison, consumption of dairy in developed countries is 208 liters per capita per year [7]. The Rwandan government has identified strategies to grow the dairy sector and increase the consumption of dairy products through strategic investments. In 2013, the Ministry of Agriculture and Animal Resources (MINAGRI) of Rwanda established the National Dairy Strategy (NDS) [8]. The NDS provides a framework for development of the dairy industry as a means to improve food security, food quality, and reduce poverty in the country [9]. Investment and expansion of the Rwandan Agriculture Extension System to support smallholders with on-farm dairy production and veterinary services is vital to accomplish increasing production of high quality, safe, and nutritious milk for Rwandans.

The major challenge to achieving the goals set by MINAGRI, are the high rates of mastitis among dairy cattle in the nation. It is estimated that two-thirds of dairy cows in Rwanda have sub-clinical or clinical mastitis [10,11]. Mastitis is inflammation of the udder that reduces milk productivity and alters milk composition and quality and can contribute to foodborne disease [12]. Improperly housing and handling animals leaves them susceptible to contagious mastitis pathogens, those that are highly transmissible from cow to cow, and environmental mastitis pathogens, those that are contracted from environmental surrounding such as bedding, or being housed in dirty or wet environments. Additionally, handlers of animals infected with these pathogens are vulnerable to zoonotic disease [13]. Mastitis pathogens have the potential to cause major and costly disease and affect production in herds worldwide. Both clinical and sub-clinical mastitis have a major negative economic impact on the dairy industry estimated to range from $80 to $125 USD per cow/mastitis event, which is often an underestimate [14]. Direct losses due to mastitis include decreased milk quality, reduced production, costs for veterinary services, drug treatment costs, and losses associated with discarding of milk containing antibiotic residues [15]. Numerous studies document that early detection and treatment, preventive medicine, and culling of chronically infected cows are effective management techniques of mastitis control and when combined with proper husbandry and animal health monitoring can result in an improved economic outcome when compared to no treatment or poor management approaches [14–18]. Milk originating from cows with mastitis, is of lower quality and quantity, and if consumed unpasteurized or undercooked can cause foodborne illness [19]. Rwanda, like many other developing countries, needs investment in infrastructure and extension services for smallholder farmers to improve milk quality and food safety. These are high priority policy goals outlined by MINAGRI in the National Dairy Strategy and they recognize major investment is needed in Rwandan Agriculture Extension System to achieve these goals [8].

According to the Rwandan National Agricultural Extension Strategy, farmers and industry partners cite inefficiency, lack of training materials for extension workers and farmers, and need for demonstration of proper management practices [9]. Dairy Dynamic Management (DDM) is a Hazard Analysis and Critical Control Points (HACCP) based management system, developed at the UC Davis Dairy Food Safety Laboratory, which aims to improve animal health and well-being, milk production and milk quality through a protocol based one-health approach to on-farm management [20–22]. Implementation of DDM has the potential to improve the knowledge and technical skills of Rwandan extension specialists and create a highly trained network of teams specialized in on-farm dairy production management. DDM incorporates One-Health approaches into dairy production medicine to identify problems before they occur and implement changes to sustain production of high-quality milk for a profit. One-Health is a philosophy that can be applied to solve complex problems impacting health and conservation where animals, humans, and the ecosystem intersect to advance public health, food safety, food security, and social justice [20,23,24]. By incorporating this ideology into dairy production management, extension specialists will better understand and be able to approach problems with the awareness that food safety begins on the farm and how diseases can be passed between humans, domestic animals, wildlife, and the ecosystem [20]. This holistic approach is in alignment with the governmental policies established by MINAGRI to increase the availability of milk in an effort to improve health, livelihoods, reduce malnutrition and preserve the natural resources of Rwanda [8].

Here we present a One Health integrated education, research, and outreach program designed to support the Rwandan government's goal to improve food security, nutrition, and poverty alleviation through increased production of high-quality milk by establishing a one health sustainable community network to support food safety and food security in dairy production. This is accomplished by implementing the DDM program within the Rwandan Agriculture Extension Service such that an on-farm dairy production management network is established to support smallholder dairy farmers. The aim of this program was to: 1) train Rwandan Agriculture Extension Service veterinarians and laboratory scientists, University of Rwanda faculty and students, and private industry partners in the DDM program; 2) train smallholder farmers in DDM animal health and hygiene protocols; 3) assess the impact of a DDM curriculum on bacterial counts in the udder and California Mastitis Test score outcomes; and 4) assess compliance to DDM animal health and hygiene protocols by smallholder dairy farmers.

2. Materials and methods

2.1. Study site

Rwanda is a land locked equatorial country in East Central Africa. Rwanda's geography ranges from high altitude forest in the West to semi-arid savanna in the East. This program took place in these two distinct geographical regions of Rwanda. The University of Rwanda, Busogo campus is located in the mountainous Northwest region of Rwanda, adjacent to Volcanoes National Park and borders the country of Congo. The mean annual temperature is 15.9 °C (60.6 °F) and annual rainfall is 1845 mm (72.6 inches per year) [25]. Busogo campus is located within the Gishwati milk shed, which is the top producing milk shed in Rwanda [8]. The University of Rwanda, Nyagatare campus is located in the Northeast region of Rwanda and is comprised of semi-arid plains on low hills. The mean annual temperature is 21 °C (69.7 °F) and annual rainfall is 904 mm (35.6 inches) [26]. The Nyagatare milk shed ranks second in milk production [8]. These campuses are strategically located in the two highest producing milk sheds of the 5 milk sheds identified in the Rwandan National Dairy Strategy and USAID Rwanda Dairy Competitiveness Program II, and local coordination of the project took place at each respective campus to oversee regional activities [8,27]. (Fig. 1).

2.2. DDM curriculum and research study

The DDM curriculum consisted of two parts, learning objectives for both parts can be found in Table 1. First, a two-week face-to-face course that included an overview of the DDM framework (Fig. 2) and clinical milk microbiology (Fig. 3). The course provided knowledge of clinical diagnosis of mastitis and surveillance of foodborne pathogens in milk. The course took place in March 2014, over 2 weeks in Kigali, Rwanda. The second part was a 16-week online course that educated participants in on-farm management, animal husbandry, and animal health and took

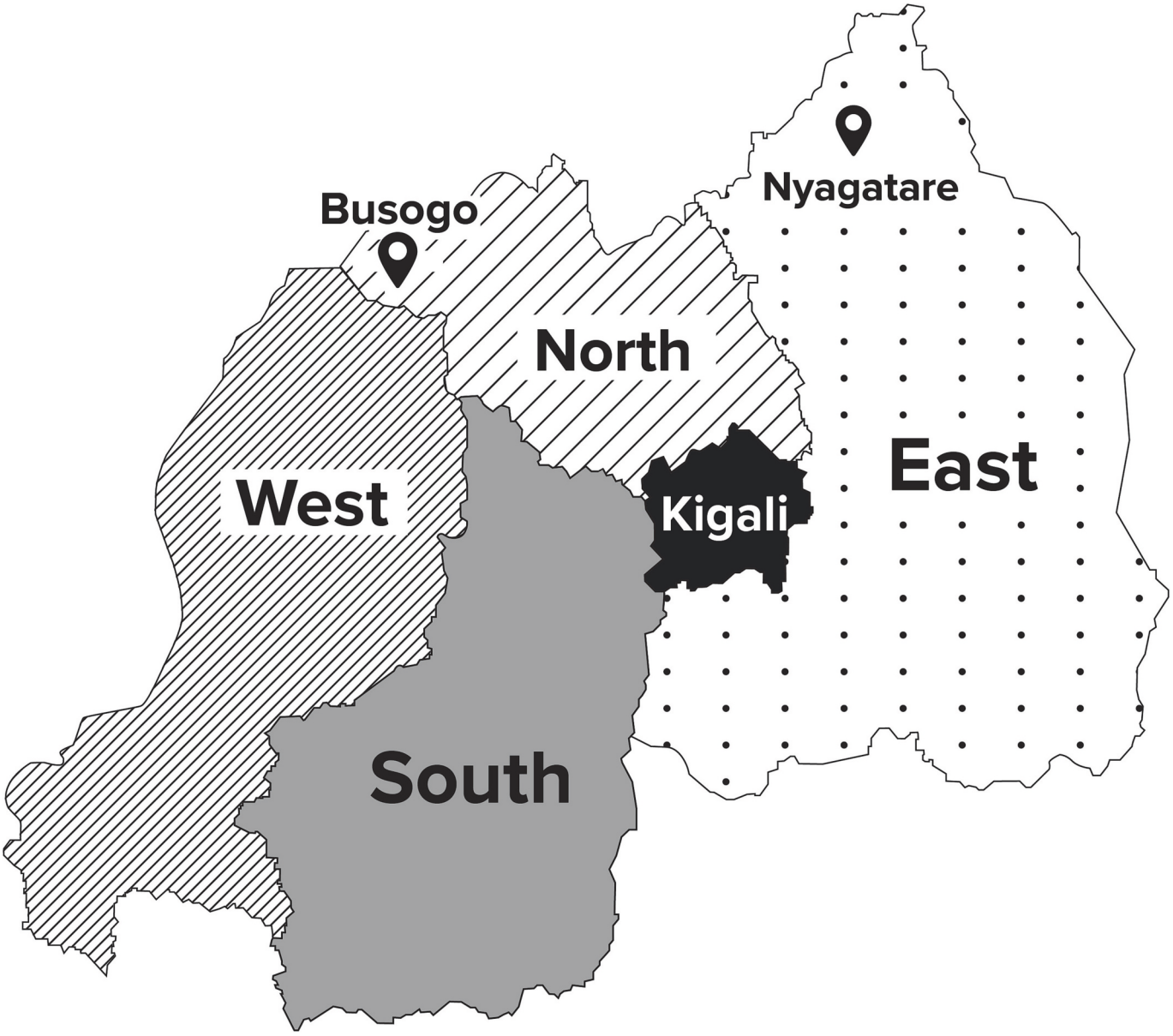

Fig. 1. Milk sheds of Rwanda.
This map illustrates the 5 main milk sheds in Rwanda as outlined by the Rwandan 2013 National Dairy Strategy and the USAID Rwanda Dairy Competitiveness Program II [8,27] and location icons illustrate the location of University of Rwanda, Busogo and University of Rwanda, Nyagatare campuses within the milk sheds.

place from June 2014 to September 2014 concurrently with the 16-week research and outreach component. During the 16-week integrated research and outreach practicum, faculty participants supervised teams of DDM Specialists which included laboratory technicians and students. Teams worked together in educating farmers on conducting hygienic milking procedures, equipment hygiene, animal husbandry, and animal health. The DDM Specialists ensured that farmers followed protocols and animals received proper medical interventions when needed. A total of 100 dairy cattle were enrolled for the 16-week period and each quarter of the udder was sampled every 7 days. The California Mastitis Test (CMT) was performed cow-side as indicator for mastitis, an animal health assessment was conducted, and quarter milk was evaluated for mastitis causing pathogens.

2.3. DDM specialist participants

Participants that were trained in the DDM program included University of Rwanda faculty veterinarians and researchers from the Busogo ($n = 15$) and Nyagatare (n = 15) campuses, and veterinarians and laboratory technicians from Rwanda Agriculture and Animal Resources Development Board (RAB) Health Laboratory ($n = 4$), and the Dairy Quality Assurance Laboratory ($n = 2$), a privately run animal health laboratory in Kigali.

2.4. Smallholder dairy farmer outreach enrollment

Smallholder dairy farmers were selected for participation based on their willingness to adhere to the hygienic milking protocol (Table 3). Over the course of the 16-week outreach program, they were evaluated weekly on their ability to properly perform the hygiene milking protocol. Smallholders were given a score of 0 or 1 for completion of each procedure, where zero was unsatisfactory and 1 was satisfactory. Faculty at University of Rwanda Busogo and Nyagatare campuses selected fifteen smallholder farmers in each region, with each smallholder owning between one and five dairy cows. A total of fifty dairy cows were enrolled at each location. A total of 30 smallholder farmers and 100

Table 1
Dairy dynamic management curriculum for mastitis control in Rwanda. The curricular framework for Dairy Dynamic Management for mastitis control outlines the subject matter, competencies, and learning objectives taught to participants during the education, research, and outreach components of the program.

Dairy dynamic management curriculum for mastitis control in Rwanda	
Competencies	Learning objectives
Area 1 - Principles of Dairy Dynamic Management (Competency Level - Foundational)	Understand
Introduction to DDM	Describe the structure of a DDM based approach. Describe each DDM team member of the DDM network. Explain the importance of cooperation, communication, and teamwork in the DDM network. Identify the 10 steps to implement DDM in a variety of dairy production environments. Define One Health as an approach to sustainable dairy production.
The DDM Specialist	Explain the role of the DDM Specialist as a management facilitator. Define the responsibilities of each DDM team member. Discuss the DDM specialist role in facilitating communication and teamwork. Explain the steps and components on how to lead a successful DDM meeting.
Mastitis Control	Describe the economic impact on dairy farmers caused by mastitis. Describe the impact of mastitis on public health, foodborne disease, and animal welfare. Explain how the farm environment is a food production facility. Describe how the environment, animal care, and pathogens contribute to mastitis. Describe the role of the diagnostic laboratory to detect microbial causes of mastitis. Identify which diagnostic tests are needed to identify mastitis. Explain the benefits of herd health surveillance. Identify sources and routes of contamination that can cause mastitis. List the steps in proper milking procedures to prevent mastitis. Select treatments that are appropriate for mastitis. Explain the difference between clinical and sub-clinical mastitis. Identify microbial pathogens that cause clinical mastitis. List the components of a mastitis control program.
Area 2 - Animal Health and Diagnostic Milk Microbiology for Mastitis Control (Competency Level - Technical)	Apply
Laboratory Safety	Demonstrate basic laboratory safety guidelines. Demonstrate proper handling of biohazardous waste. Demonstrate aseptic milk sampling for bulk tank and quarter milk samples. Demonstrate aseptic laboratory techniques.
Clinical Milk Microbiology	Identify supplies and equipment needed for microbiological techniques. Demonstrate ability to make sterile media. Demonstrate proper bench setup to process milk samples and conduct microbiological differential assays. Demonstrate ability to conduct the California Mastitis Test. Demonstrate proper culturing techniques to identify mastitis pathogens. Demonstrate ability to interpret differential microbiological assays for mastitis pathogen identification for both individual samples and bulk tanks.
Animal Health Assessments	Demonstrate ability to conduct animal health assessments according to the written protocol. Demonstrate ability to conduct proper milking procedure according to the written protocol. Demonstrate ability to conduct California Mastitis Test cow-side according to the written protocols. Demonstrate ability to evaluate milk appearance according to the written protocols.
Data Management and Recording	Demonstrate ability to generate accurate records for processed samples. Demonstrate ability to record data and transfer to a database. Demonstrate ability to maintain, interpret, and analyze results to create an electronic report.
Area - 3 Practicum - Outreach and Assessment of Mastitis (Competency Level - Technical)	Apply
Conducting Extension Activities	Demonstrate ability to conduct an outreach workshop to engage stakeholders and deliver relevant information to support their dairy operation. Demonstrate ability to enroll participants for 16-week outreach animal health and mastitis control practicum. Demonstrate ability to deliver structured animal health and mastitis control training program to participants over the course of 16 weeks. Demonstrate ability to accurately collect and maintain records for participants and animals receiving structured outreach. Demonstrate ability to create reports and present findings of 16-week outreach practicum to stakeholders.

dairy cows were initially enrolled and cows were within the first 30 days of lactation. Faculty and students used the DDM framework to oversee training and compliance by creating mission statements, standard operating procedures, training forms, evaluation, and monitoring forms.

2.5. California mastitis test (CMT)

The California Mastitis Test (CMT) was used as an indicator for sub-clinical and clinical mastitis. CMT was conducted cow side at the time of milk collection as a qualitative assessment of somatic cell count. The cows were fore-stripped, then milk from each quarter was expressed into one of four cups on the CMT paddle. An equal amount of CMT reaction

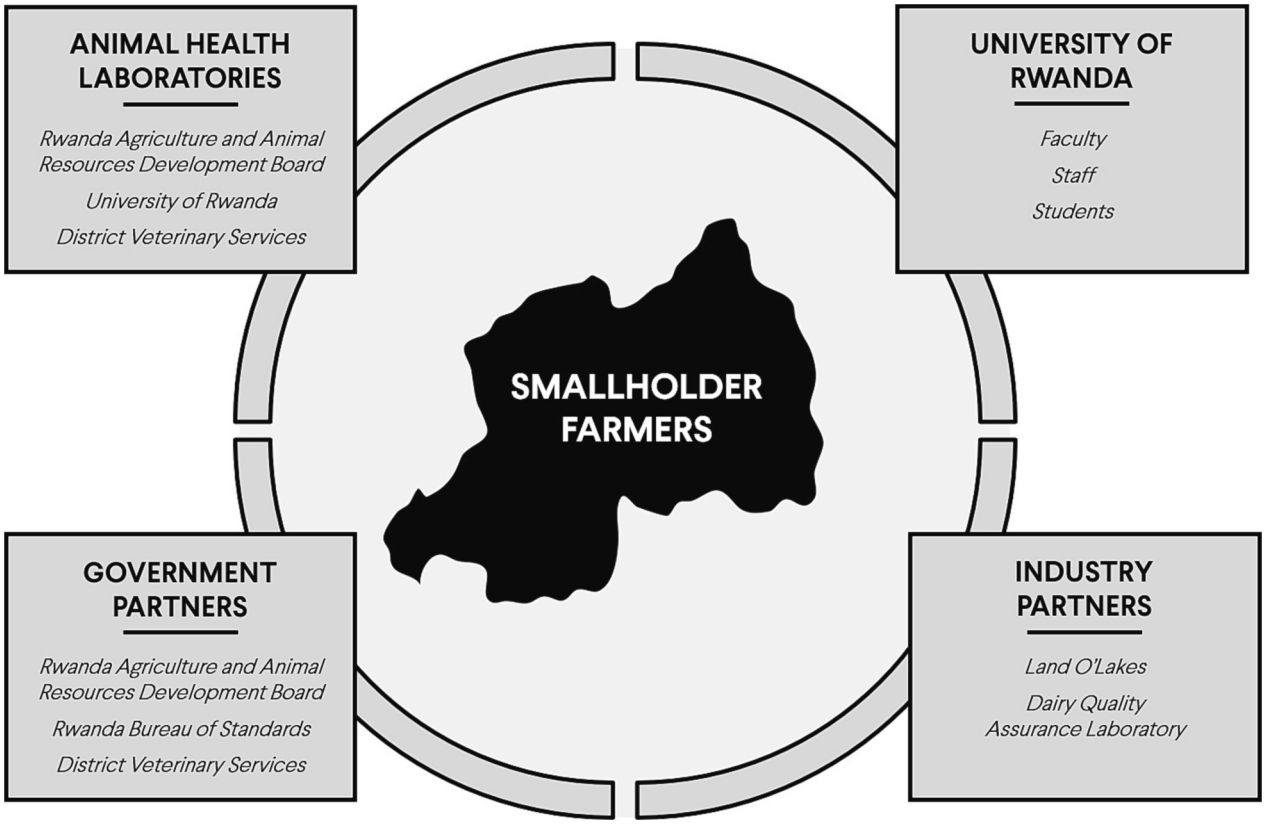

Fig. 2. DDM network for smallholders in Rwanda, Africa.
The DDM network was established in Rwanda to facilitate training and practice in management of animal health to control mastitis in cattle. The goal of the DDM network is to establish a network of teams which facilitate communication, knowledge transfer, and training to support smallholder farmers.

solution to milk (1:1) was added to each cup on the paddle. The paddle is then gently rotated horizontally and mixed for 10–30 seconds. CMT procedure and scoring was performed according to our laboratory video protocol [28].

2.6. Milk sample collection

Teats were treated with a 2% iodine-based teat dip for 30 seconds, dried, and then milk samples were aseptically collected from individual quarters each into sterile 50 ml containers. Samples were placed directly on ice in a cold box then plated on 5% Washed Bovine Blood Agar Plates upon return to the laboratory or held at 4 °C overnight and plated the next morning. Samples were collected every 7 days over 16 consecutive weeks.

2.7. Microbiological analysis

For all samples, milk inoculum was plated on Infusion Agar (Becton Dickinson catalog # 211037) containing 5% washed bovine red blood cells (Hemostat catalog # WBB500). Approximately, 10 µl of milk was aseptically plated and the plates were incubated aerobically at 37 °C for 18–24 hours. Plates were examined for Total Bacterial Count (TBC), and colonies were categorized into *Staphylococcus* spp., *Staphylococcus aureus*, Coagulase Negative *Staphylococcus* (CNS), *Streptococcus* spp., coliforms and other bacterial species following the flow chart in Fig. 3 by using bacterial morphology, gram stain, the KOH string test, catalase test and coagulase test to determine bacterial species present.

2.8. Data analysis

Data analysis was carried out using R version 3.3.2 to generate descriptive statistics, graphics, and tables. Microbiological data was transformed using $\log_{10}$ (CFU/ml +1) for normality and comparisons for before and after hygiene intervention were analyzed with paired *t*-test at week two and week sixteen at a 95% confidence interval [29]. McNemar's test was used to compare frequency counts for microbiological outcomes of paired quarters at week 2 and week 16. Wilcoxon Sum-Rank Test was used to analyze paired CMT data at week two and week sixteen at a 95% confidence interval.

3. Results

3.1. Curriculum results

Over the course of 1 year, 25 students participated in both the face-to-face and online DDM training program. Overall, participants indicated a high degree of satisfaction with the coursework. Results of a questionnaire administered at the end of the face-to-face training to gauge student satisfaction with the course material quality (91% satisfaction), course speaker quality (93% satisfactions), organization of course (82% satisfaction), content and quality of instructors and staff (86% satisfaction). Participant comments indicated they most liked the personal interaction and depth of knowledge provided by the instructors and they felt they learned important new skills by participating. Comments for improving the curriculum included a desire to incorporate more materials on the topics of molecular techniques for diagnostics, antibiotic sensitivity testing, and additional material in the laboratory section on *Mycoplasma* spp. These topics were out of the scope of time allocated for in-person training and at the time the equipment and supplies were not available to conduct this training. However, it can be incorporated into future training programs.

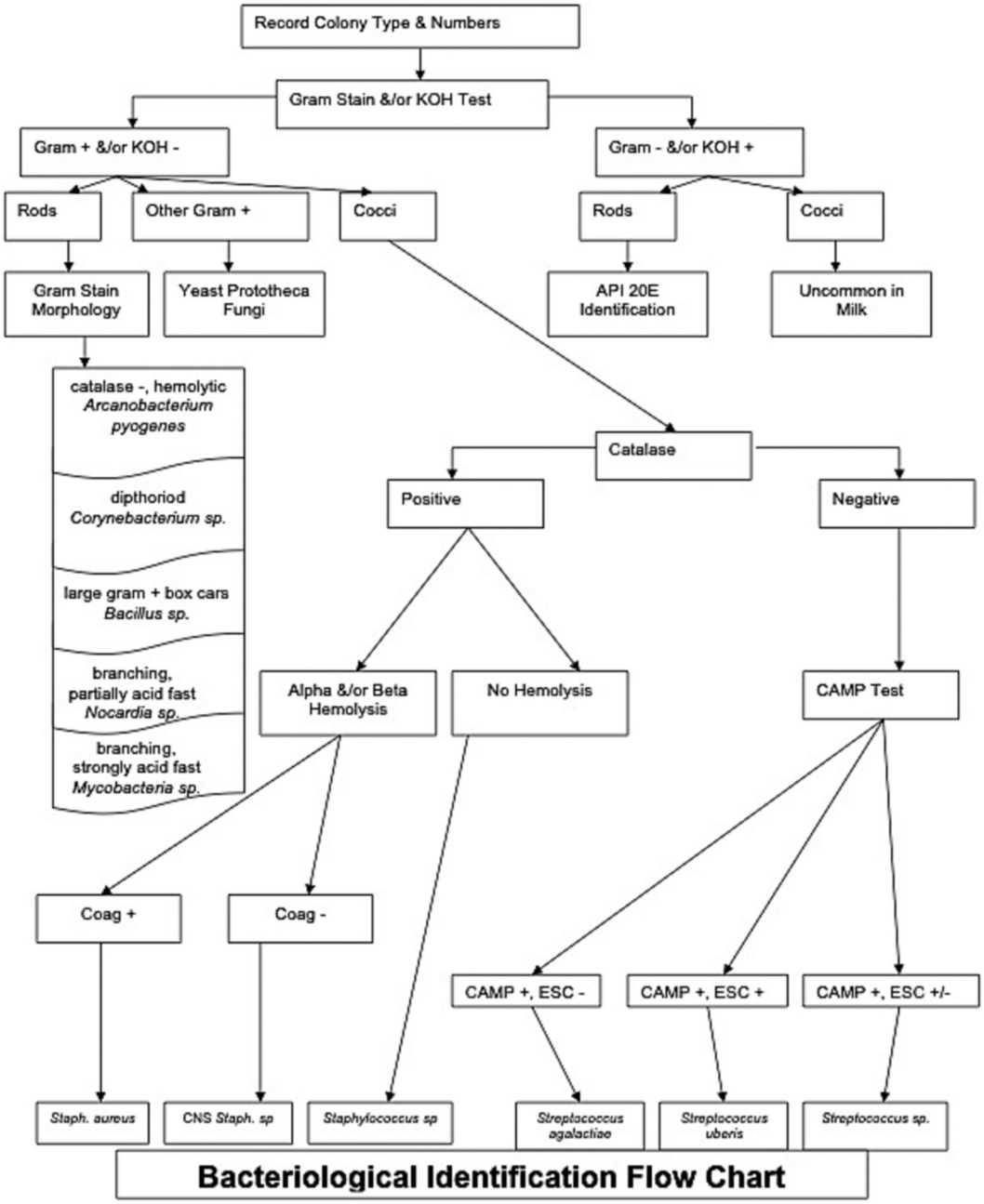

Fig. 3. Bacteriological identification flow chart.
The Bacteriological Identification Flow Chart displays the bacterial morphology and differential assays such as gram stain, the KOH string test, catalase test, coagulase test, CAMP and esculin test used to identify, and categorized bacteria isolated from milk that are associated with mastitis.

3.2. Research results

3.2.1. Microbiological analysis

Of the 100 dairy cows initially enrolled in this study, 93 were sampled for all 4 quarters on weeks 2 and 16. Seven cows were omitted from the study due to missing quarter samples ($n = 5$) or exclusion from the study ($n = 2$). Four cows were dropped from the study in the Busogo dataset, and three cows were dropped from the study in the Nyagatare dataset. Week one data was removed due to procedural errors in sample collection and data recording. Table 2 lists microbiological mean bacterial count, $\log_{10}$ (CFU/ml +1), outcomes for paired quarter samples collected at week 2 and 16 for cows sampled and by region, Busogo and Nyagatare. Table 3 lists prevalence of bacterial species for paired quarter samples collected at week 2 and 16 for cows sampled and by region, Busogo and Nyagatare. Timeseries graphs of microbiological outcomes can be found in Fig. 4. For all cows sampled, Total Bacterial Count (TBC) and prevalence of bacterial species in the quarter, *Staphylococcus* spp., *Staphylococcus aureus*, Coagulase Negative *Staphylococcus* (CNS), and coliform counts were significantly reduced ($p \leq .001$). However, regional differences were observed. Mean TBC for cows in the Busogo region was initially higher than mean TBC for cows in the Nyagatare region, 2.47 $\log_{10}$ (CFU/ml +1) vs 0.48 $\log_{10}$ (CFU/ml +1), respectively. In the Busogo region *Staphylococcus* spp., *Staphylococcus aureus*, Coagulase Negative *Staphylococcus* (CNS), mean CFU per ml was significantly reduced over the course of the 16 weeks as well as prevalence in the quarter, while the Nyagatare region saw significant reductions in *Staphylococcus* spp. ($p \leq .001$)., coliforms ($p < .001$) and other spp. ($p < .05$) for mean CFU per ml and prevalence. After completion of the study

Table 2
Mean colony forming units (CFU) for all cows sampled in Rwanda (n = 93). All cows enrolled in the study were at least 30 days in milk. All four quarters were sampled weekly and clinical milk microbiology was conducted on each quarter sample using the flow chart in Fig. 3. A total of 372 quarter milk samples were collected at week 2 and 16 from 93 cows in total, 184 quarters sampled from 46 cows in the Busogo region, and 188 quarters sampled from 47 cows in the Nyagatare region. Microbiological data was transformed using log10 (CFU/ml +1) and week 2 and week 16 were analyzed with a paired *t*-test with a 95% confidence interval.

All quarters sampled ($n = 372$)									
Week	Category	Mean $\log_{10}$ (CFU/ml + 1)	SD	SE	Lower CI	Upper CI	t-stat	df	*P*-value
2	TBC	1.46	1.56	0.04	1.39	1.53	$t = 5.48$	df = 371	$p < .001$***
16	TBC	1.07	1.4	0.03	1.01	1.13			
2	*Staphylococcus* spp.	1.37	1.56	0.04	1.3	1.45	$t = 7.71$	df = 371	p < .001***
16	*Staphylococcus* spp.	0.82	1.28	0.03	0.76	0.88			
2	*Staphylococcus aureus*	0.26	0.84	0.02	0.22	0.3	$t = 4.47$	df = 371	p < .001***
16	*Staphylococcus aureus*	0.05	0.38	0.01	0.03	0.07			
2	CNS	0.17	0.68	0.02	0.13	0.2	$t = 3.26$	df = 371	$p = .001$***
16	CNS	0.04	0.3	0.01	0.02	0.05			
2	*Streptococcus* spp.	0.03	0.29	0.01	0.01	0.04	$t = 0.05$	df = 371	$p = .961$
16	*Streptococcus* spp.	0.02	0.28	0.01	0.01	0.04			
2	Coliforms	0.01	0.15	0	0	0.02	$t = -3.42$	df = 371	p = .001***
16	Coliforms	0.12	0.59	0.01	0.09	0.15			
2	Other	0.12	0.53	0.01	0.1	0.15	$t = 0.28$	df = 371	$p = .778$
16	Other	0.11	0.58	0.01	0.09	0.14			
Busogo region ($n = 184$)									
Week	Category	Mean $\log_{10}$ (CFU/ml + 1)	SD	SE	Lower CI	Upper CI	t-stat	df	P-value
2	TBC	2.47	1.28	0.04	2.38	2.55	$t = 5.66$	df = 183	p < .001***
16	TBC	1.85	1.38	0.05	1.76	1.94			
2	*Staphylococcus* spp.	2.39	1.33	0.04	2.3	2.47	$t = 6.23$	df = 183	p < .001***
16	*Staphylococcus* spp.	1.64	1.39	0.05	1.55	1.73			
2	*Staphylococcus aureus*	0.52	1.14	0.04	0.45	0.6	$t = 5.35$	df = 183	p < .001***
16	*Staphylococcus aureus*	0.06	0.41	0.01	0.03	0.09			
2	CNS	0.3	0.87	0.03	0.24	0.35	$t = 3.21$	df = 183	$p = .002$**
16	CNS	0.06	0.39	0.01	0.04	0.09			
2	*Streptococcus* spp.	0.05	0.41	0.01	0.02	0.08	t = 0.05	df = 183	p = .961
16	*Streptococcus* spp.	0.05	0.39	0.01	0.02	0.07			
2	Coliforms	0.01	0.15	0	0	0.02	$t = 1$	df = 183	$p = .319$
16	Coliforms	0	0	0	0	0			
2	Other	0.15	0.6	0.02	0.11	0.19	$t = -0.94$	df = 183	$p = .350$
16	Other	0.22	0.79	0.03	0.16	0.27			
Nyagatare region ($n = 188$)									
Week	Category	Mean $\log_{10}$ (CFU/ml + 1)	SD	SE	Lower CI	Upper CI	t-stat	df	P-value
2	TBC	0.48	0.55	0.48	0.04	0.41	$t = 1.59$	df = 187	$p = .062$
16	TBC	0.31	0.37	0.31	0.03	0.25			
2	*Staphylococcus* spp.	0.38	0.45	0.38	0.03	0.32	$t = 4.57$	df = 187	p < .001***
16	*Staphylococcus* spp.	0.02	0.03	0.02	0.01	0			
2	*Staphylococcus aureus*	0	0	0	0	0	$t = -1.74$	df = 187	$p = .083$
16	*Staphylococcus aureus*	0.04	0.06	0.04	0.01	0.02			
2	CNS	0.04	0.06	0.04	0.01	0.01	$t = 0.84$	df = 187	$p = .400$
16	CNS	0.01	0.02	0.01	0.01	0			
2	*Streptococcus* spp.	0	0	0	0	0	–	df = 187	–
16	*Streptococcus* spp.	0	0	0	0	0			
2	Coliforms	0.01	0.02	0.01	0	0	$t = -3.70$	df = 187	p < .001***
16	Coliforms	0.24	0.29	0.24	0.03	0.18			
2	Other	0.1	0.13	0.1	0.01	0.07	$t = 2.45$	df = 187	$p = .015$*
16	Other	0.01	0.02	0.01	0.01	0			

and collection of data, the team was informed that smallholders in the Nyagatare region had recently received animal health education and training which included proper milking procedures and mastitis control. This may have contributed to the lower starting bacterial counts observed for Nyagatare.

3.2.2. California mastitis test outcomes

CMT score results for week 2 and 16 can be found in Table 5. From week 2 to week 16 there was a statistically significant reduction in CMT scores for all quarter samples collected, with 279/372 (75%) quarter samples receiving a negative score at week 2 compared to 332/372 (89.2%) of quarter samples receiving a negative score at week 16 ($p <$ .001). In the Busogo region 170/184 (92.4%) of samples received a negative score at week 2 compared to 178/184 (96.7%) at week 16. While there was an increase of quarters receiving a negative CMT score by week 16, overall, it was not a statistically significant change ($p = .07$). In the Nyagatare region there was a statistically significant reduction in CMT scores, with 109/188 (58%) of samples received a negative score at week 2 compared to 154/188 (81.9%) at week 16 (p < .001). However, at week 16, sixteen quarters received a CMT score of 2 and one quarter received a CMT score of 3.

3.3. Outreach results

3.3.1. Farmer compliance to the hygienic milking protocol results

Over the course of the 16 weeks, smallholder farmers improved in their ability to perform the hygienic milking protocol, with greater than 90% of smallholders satisfactorily performing cleaning of the udder, pre-dipping, removal of pre-dip, milking with hygienic equipment, and post-dipping. Roughly half of smallholders satisfactorily completed pre-stripping (53.6%) and monitoring of cattle after milking (56.7%) by the end of the training (Fig. 5).

Table 3
Prevalence of bacteria in paired quarters before and after hygienic milking intervention (n = 372). Contingency table listing the prevalence of bacterial species present in quarter samples collected weekly for 372 quarter milk samples at week 2 and 16 from 93 cows in total, 184 quarters sampled from 46 cows in the Busogo region, and 188 quarters sampled from 47 cows in the Nyagatare region. A quarter was considered positive for a bacterial species when bacterial count was ≥100 CFU/ml. McNemar's test was used to compare microbiological outcomes for paired quarters at week 2 and week 16.

All cows positive quarters (n = 372)				
Week	Category	Frequency	Proportion (%)	P-value
2	TBC	185	49.7%	$p < .001$***
16	TBC	145	40.0%	
2	*Staphylococcus* spp.	172	46.2%	p < .001***
16	*Staphylococcus* spp.	114	30.6%	
2	*Staphylococcus aureus*	34	9.14%	p < .001***
16	*Staphylococcus aureus*	7	1.88%	
2	CNS	22	5.91%	$p = .005$**
16	CNS	6	1.61%	
2	*Streptococcus* spp.	3	0.81%	–
16	*Streptococcus* spp.	3	0.81%	
2	Coliforms	2	0.54%	$p = .004$**
16	Coliforms	15	4.03%	
2	Other	20	5.38%	$p = .47$
16	Other	15	4.03%	
Busogo (n = 184)				
Week	Category	Frequency	Proportion (%)	P-value
2	TBC	154	83.70%	$p = .001$ ***
16	TBC	125	67.93%	
2	*Staphylococcus* spp.	149	80.98%	p < .001 ***
16	*Staphylococcus* spp.	113	61.41%	
2	*Staphylococcus aureus*	34	18.48%	p < .001***
16	*Staphylococcus aureus*	4	2.17%	
2	CNS	20	10.87%	p = .005**
16	CNS	5	2.72%	
2	*Streptococcus* spp.	3	1.63%	–
16	*Streptococcus* spp.	3	1.63%	
2	Coliforms	1	0.54%	–
16	Coliforms	0	–	
2	Other	11	5.98%	$p = .662$
16	Other	14	7.61%	
Nyagatare (n = 188)				
Week	Category	Frequency	Proportion (%)	P-value
2	TBC	31	16.5%	$p = .081$
16	TBC	20	10.63%	
2	*Staphylococcus* spp.	23	12.23%	p < .001***
16	*Staphylococcus* spp.	1	0.53%	
2	*Staphylococcus aureus*	0	0.00%	$p = .248$
16	*Staphylococcus aureus*	3	1.60%	
2	CNS	2	1.06%	$p = 1$
16	CNS	1	0.53%	
2	*Streptococcus* spp.	0	0.00%	–
16	*Streptococcus* spp.	0	0.00%	
2	Coliforms	1	0.53%	p = .001***
16	Coliforms	15	7.98%	
2	Other	9	4.79%	$p = .027$*
16	Other	1	0.53%	

4. Discussion

This study took place in Rwanda, Africa with the aim of establishing the Dairy Dynamic Management (DDM) as an extension service for smallholder dairy farmers. We trained a multi-sector cohort of veterinary extension agents from University of Rwanda Busogo and Nyagatare campuses, the Rwanda Agriculture and Animal Development Board (RAB) Animal Health Laboratory, and the Dairy Quality Assurance Laboratory (DQAL) in the DDM program. Students participated in a hybrid educational program composed of face-to-face hands-on training and an online course. The hybrid educational program included animal health, animal husbandry, and clinical milk microbiology coursework. Trained participants lead teams of DDM Specialists to conduct outreach and research with smallholder dairy farmers. Thirty smallholder dairy farmers were trained and assessed on following the hygienic milking protocol. We found that when smallholder farmers were trained, monitored, and evaluated and received continued support in DDM procedures and protocols, they were eager to integrate DDM protocols on their farm and significantly lowered the TBC and improved CMT scores in quarter milk aseptically collected from their cows, over the course of the 16-week program.

Effectiveness of the DDM program was evaluated through assessment of bacterial counts of the udder, CMT scoring, and monitoring farmers in adhering to the hygienic milking protocol, overall, results were statistically significant. However, regional differences in outcomes were observed. The mean bacterial counts in milk collected in the Nyagatare region were 2 $\log_{10}$ lower at the start of the study, than milk samples collected in the Busogo region. Upon completion of data collection for this study the authors were informed that the Nyagatare region had just completed training in proper animal health and hygienic milking procedures for dairy cattle. This stark difference in bacterial counts likely can be attributed to a recent training, however other factors may have contributed such as geographic and climate variation. The Nyagatare region is a semi-arid savanna with a higher average annual temperature and lower annual rainfall. This may contribute to lower presence of environmental pathogens that can cause mastitis. Additionally, due to the presence of available grassland in this region, many farmers practice open grazing as opposed to confinement production, which is more prevalent in the Busogo region. Confinement dairy production can contribute to mastitis if production practices do not adhere to stringent animal health and hygiene protocols. In confinement production it is important to maintain clean bedding and stalls to reduce contamination of the udder by feces. While bacterial counts in the Busogo region showed larger reductions across most bacterial categories, bacterial counts for *Staphylococcus* spp., an environmental pathogen, was significantly reduced in the Nyagatare region. Conversely, mean coliform counts and prevalence increased in the Nyagatare region, but not in the Busogo region. Nine cows on 7 farms contributed to the 15 quarters positive for coliforms in the Nyagatare region. Upon inspection of the records for these cattle it was documented that the nine cows belonged to farmers that did not perform each step of the hygienic milking protocol properly by the end of the training program, supporting the need for continued on-farm training and outreach for smallholder dairy farmers.

Conversely, CMT score outcomes were significantly improved in the Nyagatare region over the course of the study with a 24% increase in negative CMT scores. Milk collected from cows in the Busogo region did improve in CMT scoring with a 4.3% increase in negative CMT scores, however this outcome was not statistically significant. The CMT is a qualitative test that is meant to be conducted cow-side. Scores of 1 or higher are indicative of clinical or sub-clinical mastitis and should be followed up with somatic cell count (SCC) testing and microbiological analysis. Microorganisms other than the bacteria identified using the flow chart in Fig. 3, such as *Mycoplasma* spp., or viruses, can cause SCC and CMT scores to be elevated due infections of the udder. In this study we chose to conduct the CMT, and not somatic cell count due to the availability of reagents and supplies in central Africa. Reagents and supplies for the CMT are readily available in Rwanda, however obtaining the necessary reagents, laboratory supplies, and adequate and consistent supply of electricity to conduct weekly SCC on 400 quarter samples was not feasible for this study.

Finally, our study showed that with extension services, training and continued support, small holder dairy farmers were able to adhere to the hygienic milking protocol for this study with greater than 90% of smallholders satisfactorily performing cleaning of the udder, pre-dipping, removal of pre-dip, milking with hygienic equipment, and post-dipping. Pre-stripping the teat before milking and monitoring the cow post milking remained a challenge for half of small holder farmers.

Table 4
Standard operating procedure: hygienic milking protocol. The Standard Operating Procedure for Hygienic Milking provides the user with scope, purpose, materials needed, and step-by-step instructions to properly perform hygienic milking. Additionally, it provides the user with more information on why they are performing each step in the protocol to better facilitate learning and understanding.

Standard operating procedure – hygienic milking routines	
Scope: *This Milking Hygiene SOP is for all lactating cows not currently being treated for mastitis or other health diseases. It starts with preparing the udder for milking and ends with monitoring the animal after milking. It does not cover cleaning and sanitizing of equipment.* Purpose: *To milk a clean, dry, pre-sanitized udder in order to provide mastitis control, safe high-quality milk and receive optimum payment for the milk.* Materials Required: 1. Gloves 2. Clean Towels (paper or cloth) 3. Pre-dip teat disinfectant 4. Post-dip teat disinfectant 5. Clean Water	
Step	Instructions
1. Cow Movement	Handle animals in a calm and gentle manner. Do not strike or yell at the cow. *Why? Rough handling stresses cattle and disrupts the milk let down process. Rough handling of cattle should not be tolerated.*
2. Milking Order	Milk cows from most health to least healthy. Milk from unhealthy cows should be diverted from the bulk tank. *Why? This ensures food borne pathogens and pharmaceuticals do not enter the bulk tank or food supply.*
3. Clean Gloves	Wear clean gloves during milking. If your gloves become dirty change them. *Why? Use of clean gloves prevents pathogens from spreading from the handler to the teat ends and* vice versa.
4. Clean Udder	Using clean water, remove any dirt, mud, or debris from teat ends. *Why? This ensures foreign matter does not enter the milking lines or bulk tank. Dirt, mud, and debris contain pathogens that can contaminate the equipment and milk.*
5. Fore-stripping	Fore-strip 2–3 streams of milk from each quarter, checking for irregular milk. *Why? Checking for irregular milk allows for early detection of mastitis, resulting in faster treatment and cure of mastitis.*
6. Isolate Cows with irregular milk	If a cow has irregular milk isolate her for evaluation and treatment after milking. *Why? Isolating a cow with mastitis allows the mastitic milk to be diverted from the bulk tank during treatment and preventing foodborne pathogens and pharmaceuticals from entering the food supply.*
7. Pre-dip	Apply the pre-dip to teats according to manufacturer specifications. *Why? Use of a pre-dip allows the teat to be free of pathogens before milking, preventing the spread of mastitis causing pathogens to other animals and milking equipment.*
8. Pre-dip Removal	Using a clean cloth or paper towel, remove excess teat dip. *Why? Removing excess teat dip prevents chemical residues from entering the bulk tank.*
9. Milking	Begin milking 30–60 seconds after fore-stripping. Continue milking until there is less than 100 ml of milk remaining in each quarter. *Why? Milk let down occurs during this time span if milking begins too late let down is decreased resulting in slower milking and increase chance of mastitis.*
10. Post-dip	Apply the post dip teat disinfectant according to manufacturer specifications. *Why? Post dip ensures any pathogens on the teat end following milking are killed and prevents mastitis.*
11. Post-milking	Following post dipping monitor the cow to make sure she stands for 20 minuets following milking. *Why? Ensuring the cow stands for the following 20* minutes*, allows time for the teat end to close and prevents dirt, mud, and debris from contacting the teat end before closure, and prevents mastitis.*

However, with continued training and education from extension agents these indicators may continue to improve. Additionally, the microbiological and CMT outcomes are in alignment that improved hygienic practices during milking by small holder farmers improved animal health indicators of mastitis. Given the differences in geography, climate, and production practices, we show that an integrated education, research, and outreach approach to dairy production is successful in improving mastitis control with simple and appropriate on-farm interventions and can be successfully implemented in developing countries.

Many factors contribute to improved milk quality and increased dairy production. Focusing on animal health, hygiene, and proper milking procedures are recognized, achievable, and measurable milestones that can be established on smallholder farms with on-farm support by agriculture extension agents [30–32]. The immediate gains to smallholder farmers by this program was access to expanded extension services, technical support to improve production practices and hygiene, an overall reduction of TBC and *Staph* spp. isolated from quarter milk, and improved CMT scores. The foundation of this program's success was based on establishing a One Health network to support education and training for agriculture extension agents and diagnostic laboratory services. Continued long-term program support for education, research, veterinary extension, and animal health diagnostic laboratories is needed to fully assess the impact of this program on milk production [33]. Supporting animal health diagnostic laboratories is essential to protecting public health, food safety, and improving economic outcomes for livestock production [34,35]. Veterinarians rely on the expertise of diagnostic laboratories to determine the etiology of disease so that they can issue a course of treatment and manage herd health. Animal health diagnostic laboratories provide benefits to veterinarians, farmers, and the food supply by ensuring access to diagnostic testing and surveillance of diseases by supporting veterinary extension agents to conduct site visits, train smallholders, collect diagnostic samples and to administer appropriate medical interventions to treat and prevent disease based on laboratory confirmation [34]. Advanced diagnostic testing is needed to continue improving mastitis control, such as somatic cell count, *Mycoplasma* spp. culturing and identification, and molecular methods to conduct surveillance, identification and typing of microbial causes of mastitis and other infectious diseases that impact dairy production. Increasing availability of diagnostic testing and veterinary extension agents would enhance mastitis control and is needed to mitigate other diseases that negatively impact dairy production and improve milk quality and production such that smallholders have access to safe, nutritious milk, that can improve nutrition and economic benefits for their family [8,36,37].

The DDM program established a one health sustainable community network among policy makers in the Rwandan government, the University of Rwanda, and dairy industry partners, Land O'Lakes and Dairy Quality Assurance Laboratory to support education and training in dairy production with an aim to improve food safety and security with on-farm approach to support smallholder farmers [38,39]. Establishing training and education programs among multi-sector partners, facilitated dialog and collaborations which were an immediate benefit to government agencies, industry, and smallholder dairy farmers. These

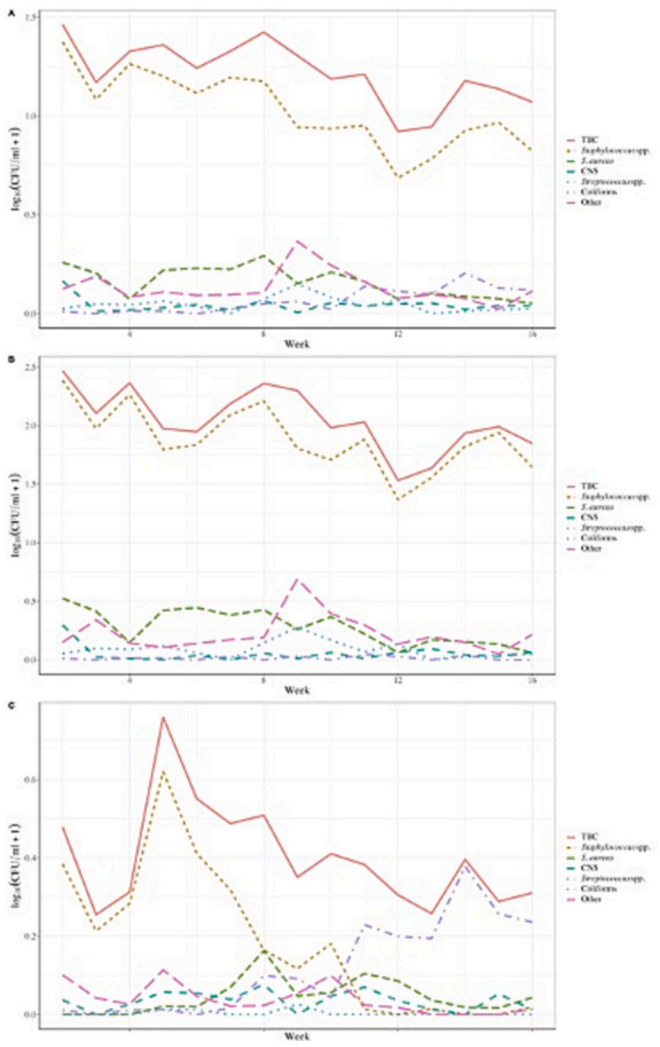

Fig. 4. Herd level mean CFU microbiological outcomes of the udder over 16 weeks for all cows sampled in Rwanda.
A) Microbiological outcomes of quarter samples for all ($n = 93$) cattle sampled. Quarter milk samples were aseptically collected on a weekly basis over the course of 16 weeks. KOH string test, Catalase Test and gram stain tests were conducted to determine bacterial species present. B) Microbiological outcomes for the 46 cattle sampled in the Busogo region. C) Microbiological outcomes for the 47 cattle sampled in the Nyagatare region.

partnerships have set the stage for continued growth of the dairy sector in Rwanda, outlined a framework to establish dairy food safety standards, Rwandan National Mastitis Council, and a pathway for Rwandan smallholder dairy farmers to improve their production practices to see an economic benefit [27,38]. These policies and programs are needed to improve the quality and quantity of milk to allow the formal dairy sector in Rwanda to grow such that value-added milk products like yogurt and cheese can be produced. A market for value added products increases the profitability of dairying and would further improve economic outcomes for small holder dairy farmers, and production of value-added milk products depend on a robust supply of high-quality milk.

Through continued efforts by the Rwandan Government, University of Rwanda, industry partnerships, and UC Davis expertise, the dairy industry in Rwanda has the potential to grow and achieve the goals set by the Rwandan Government National Dairy Strategy [8]. Achieving these goals will allow for increased food security and safety, poverty reduction, and reduced post-harvest loss of dairy products [6]. Milk with higher nutritional content can provide a safe and healthy food for families, improve cognitive development of children, and reduce diarrheal disease, all of which can lead to improved livelihood and health status for the people of Rwanda [40]. Through continued investment in capacity building, education, and training in veterinary extension services and animal health diagnostic laboratories, the Rwandan dairy sector can continue to improve economic outcomes for smallholder farmers.

5. Conclusion

Overall, we demonstrate that an integrated framework of education, research and outreach can be successfully implemented and improve indicators of animal health and dairy production. Integrated programs to improve agricultural productivity which incorporate holistic approaches are needed to address food insecurity, improve nutrition, and create sustainable beneficial economic outcomes for smallholder farmers globally [41,42]. This program aligned with the pro-dairy policies of the Rwandan government and sociocultural aspects of dairy production in Rwanda as well as incorporated regional knowledge, available supplies and facilities, and local expertise in dairy production to ensure successful programmatic outcomes. Tailored educational extension and training programs are needed to meet the specific needs and overcome challenges faced by smallholder farmers to increase food production, animal health, and economic wellbeing. In addition to providing new infrastructure, technology, training and resources, programs aimed at improving food security should support agricultural development of culturally appropriate food that is produced in a sustainable manner and allows local people to provide input, garner buy-in, and advance food sovereignty. Such an approach is necessary to ensure safe, wholesome, and nutritious food is available to meet the nutritional needs of the local population and is produced in a sustainable manner to

Table 5
California mastitis test outcomes. Contingency table listing frequency and proportion of CMT scores for 372 quarter milk samples at week 2 and 16 from 93 cows in total, 184 quarters sampled from 46 cows in the Busogo region, and 188 quarters sampled from 47 cows in the Nyagatare region. Frequency of quarters for each score is given with percent in parenthesis. Wilcoxon Sum-Rank Test was used for statistical analysis of paired samples.

CMT score	Negative	Trace	1	2	3	P-value
All Cows (n = 372)						
Week 2	279 (75%)	69 (18.5%)	24 (6.5%)	0	0	$p < .001$
Week 16	332 (89.2%)	22 (5.9%)	11 (3.0%)	6 (1.6%)	1 (0.3%)	
Busogo (n = 184)						
Week 2	170 (92.4%)	14 (7.6%)	0	0	0	$p = .07$
Week 16	178 (96.7%)	6 (3.3%)	0	0	0	
Nyagatare (n = 188)						
Week 2	109 (58%)	55 (29.2%)	24 (12.8%)	0	0	$p < .001$
Week 16	154 (81.9%)	16 (8.5%)	11 (5.9%)	6 (3.2%)	1 (0.5%)	

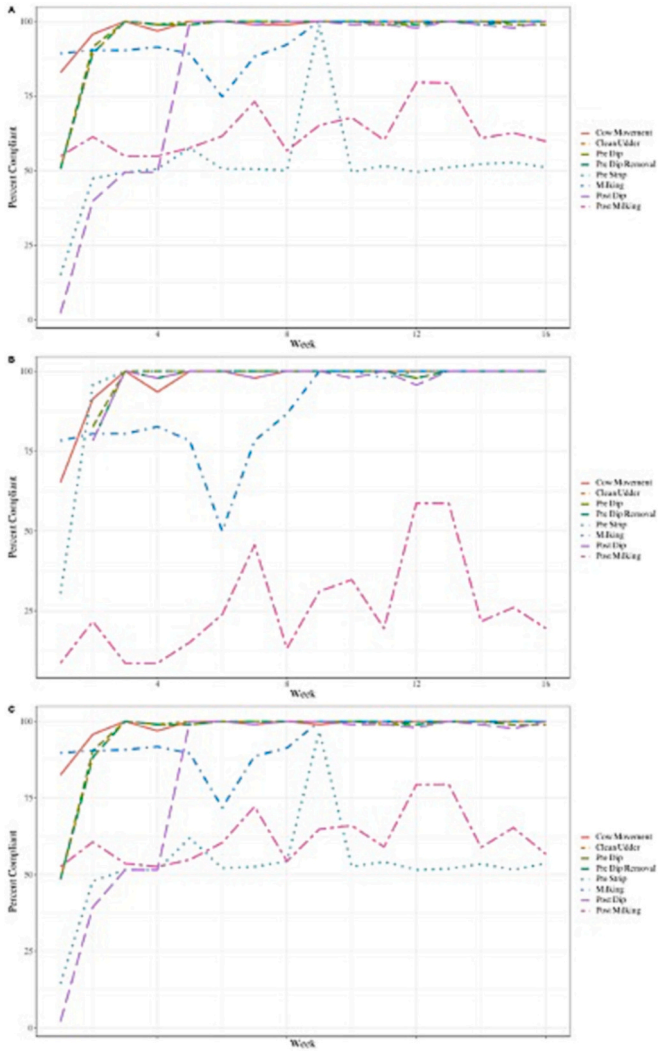

Fig. 5. Compliance to hygienic milking protocol by smallholder farmers. Smallholder farmers were observed by the DDM Specialist performing the hygienic milking protocol (Table 4) for each cow each week over the course of 16 weeks. They were scored for each step in the protocol, score of 0 was given if the step was not performed correctly and a score of 1 was given for performing the step correctly. A) Outcomes of all smallholders in Rwanda for each of the cattle sampled ($n = 93$). B) Outcomes of smallholders in the Busogo region for each of the cattle sampled ($n = 46$). C) Outcomes of smallholders in the Nyagatare region for each of the cattle sampled ($n = 47$).

protect the environment. These outcomes and goals align with multiple UN Sustainable Development Goals (SDGs), which provide a blueprint of seventeen goals which can be applied in developed and developing countries to improve livelihoods and health of people globally, while safeguarding the environment for future generations [43].

One Health approaches for food systems, such as this program, are vital to the success of the overarching goals of the NDS, as it incorporates a holistic, collaborative, and transdisciplinary approach that can be incorporated into educational curriculums to train future practitioners and scientists. Additionally, outcomes of integrative approaches to education, research, and outreach that this program outlines can be applied to inform government policy makers in advancing evidence-based policy decisions. One health approaches for food safety and security are being called for by governments around the world and global public health agencies [44–50]. These frameworks and approaches will be vital to overcome the impending global challenges humanity faces to improve livelihoods, health outcomes, eliminate hunger, and prevent zoonotic disease, and protect the environment.

Ethical considerations

The presented research was conducted in compliance with relevant policies in Rwanda and the United States for ethics, animal handling, animal welfare, and biosafety. Animals enrolled in the practicum component of the study were privately owned and informed consent was obtained for enrollment of smallholder dairy farmers and their animals.

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Author contributions

J.S.C. and S.N. conceived and acquired funding for this work. S.N.G., J.S.C., S.N., designed and created the DDM curricular framework, integrated program, and research and outreach methodologies. J.P.N and P. N implemented and oversaw the outreach practicum with project administration in Rwanda, data curation, and reporting results for the practicum components at the Busogo and Nyagatare, University of Rwanda campuses, respectively. S.N.G. conducted the data analysis and drafted the manuscript. All authors reviewed, edited, discussed, and agreed to the organization and final content of the manuscript.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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