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Effects of a mastitis treatment strategy with or without on-farm testing

Karien Griffioen,[1]* Annet G. J. Velthuis,[2,3] Gerrit Koop,[1] Theo J. G. M. Lam,[1,2] and 1Health4Food—Dutch Mastitis Diagnostics Consortium†
[1]Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, PO Box 80.151, 3508 TD, Utrecht, the Netherlands
[2]Royal GD, PO Box 9, 7400 AA, Deventer, the Netherlands
[3]Aeres University of Applied Sciences, Postbus 374, 8250 AJ, Dronten, the Netherlands

ABSTRACT

The etiology of mastitis is crucial information to use antimicrobials prudently for control and treatment. This study aimed to evaluate the effects of mastitis diagnosis and treatment strategies with on-farm testing, on cure, new intramammary infections (IMI), somatic cell count (SCC), and antimicrobial use, compared with farmers' current diagnosis and treatment strategies. The on-farm tests used, CHROMagar Mastitis (CHROMagar, Paris, France) and Minnesota Easy Culture System II Tri-plate (University of Minnesota, St. Paul, MN), both had etiological groups of IMI as result, being gram-positive growth, gram-negative growth, or culture negative. Two randomized controlled trials were conducted on 15 herds: trial 1 prospectively enrolled 155 cows with clinical mastitis, and trial 2 cross-sectionally included 78 cows with subclinical mastitis. In both trials, cows were randomly distributed over 3 equal-sized groups: a test group using CHROMagar, a test group using Minnesota, and a control group not using on-farm tests. Farmers decided whether or not to treat, and which antimicrobial treatment would be applied, using information available on the day of enrollment (control group), complemented with the on-farm test result 1 d after enrollment (both test groups). For clinical mastitis, an antimicrobial treatment was given in 58% of cases that used CHROMagar, in 80% that used Minnesota, and in 86% of the controls. For subclinical mastitis, an antimicrobial treatment was given in 50% of cases that used CHROMagar, in 54% that used Minnesota, and in 4% of the controls. Bacteriological cure rate of clinical mastitis was lowest in the CHROMagar group [odds ratio 0.18 (95%CI 0.03–0.99)] compared with the controls. Using the Minnesota on-farm test for subclinical mastitis diagnosis and treatments resulted in fewer new IMI on d 21 [odds ratio 0.06 (95%CI 0.00–0.74)] compared with the controls. Clinical cure rate, percentage of new IMI, and SCC on d 21 of clinical mastitis were comparable among the groups. Using on-farm tests in farmers' decision-making process resulted in more treatments in accordance with the etiology of mastitis than without on-farm testing. A diagnosis and treatment strategy with on-farm testing is advised in cows with clinical mastitis to enhance prudent antimicrobial use. For subclinical mastitis, however, on-farm testing may lead to an unacceptable increase in use of antimicrobials and thus should not be advised as the common approach.

Key words: dairy cattle, on-farm test, treatment strategy, mastitis, prudent antimicrobial use

INTRODUCTION

Prudent use of antimicrobials in animals is key to minimize development of antimicrobial resistance. In dairy farms, most antimicrobials are used to treat mastitis (Pol and Ruegg, 2007; Kuipers et al., 2016). In case of a bacteriological cause of mastitis, antimicrobials may be required. Mastitis cases for which antimicrobial treatment will likely have no effect should be differentiated from those for which antimicrobials likely will be effective. In the latter situation, the most appropriate antimicrobial should be determined. This approach should result in prudent antimicrobial use for mastitis treatments.

Farmers generally use clinical signs and mastitis history to decide upon antimicrobial treatment for mastitic cows (Griffioen et al., 2016). However, clinical signs and mastitis history lack the ability to inform on the etiology of the IMI (Ruegg, 2018). If the etiology of the IMI is known, farmers can target antimicrobial use (Pinzón-Sánchez et al., 2011). In addition to other factors, such as cow and bacterial aspects (Barkema et al.,

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*Corresponding author: k.griffioen@gddiergezondheid.nl
†This consortium also includes D. J. Mevius, F. J. van der Wal, J. B. W. J. Cornelissen, and R. P. Achterberg (Wageningen Bioveterinary Research, Lelystad, the Netherlands); and A. E. Heuvelink, C. G. M. Scherpenzeel, M. M. C. Holstege, and R. Dijkman (Royal GD, Deventer, the Netherlands).

2006), duration of IMI (van den Borne et al., 2010), and formulation of the antimicrobial used, etiology of the IMI may predict the treatment result (Ruegg, 2018). Bacteriological culture in a laboratory (**LBC**) or on-farm is available to diagnose the bacteriological cause of an IMI and to support mastitis treatment decisions. Routine LBC of milk samples, however, is used only sporadically by farmers (Griffioen et al., 2016; Kayitsinga et al., 2017). Nevertheless, farmers are interested in diagnostics for clinical and subclinical mastitis and indicate that they would use diagnostic tools more frequently if quick, inexpensive, reliable, and preferably on-farm tests were available (Griffioen et al., 2016).

Various culture-based on-farm tests are available and seem to contribute to more targeted mastitis treatment when employed under laboratory settings (McCarron et al., 2009; Royster et al., 2014; Ferreira et al., 2018). These on-farm tests most often consist of plates split multiple ways using chromogenic selective media or conventional selective media. In a previous study we evaluated 4 culture-based tests, 2 using chromogenic media (CHROMagar Mastitis, CHROMagar, Paris, France; and VétoRapid, Vetoquinol, 's-Hertogenbosch, the Netherlands), and 2 using conventional selective media (Minnesota Easy Culture System II Tri-plate, University of Minnesota, St. Paul, MN; and Hardy Diagnostic Mastitis Tri-plate, Hardy Diagnostics, Santa Maria, CA) on milk samples routinely submitted to a laboratory for LBC (Griffioen et al., 2018). We concluded that all tests agree more or less equally with LBC to indicate which etiological group of bacteria caused the IMI. Ferreira et al. (2018) also evaluated 4 tests; they found that the chromogenic test Accumast (FERA Animal Health LCC, Ithaca, NY) has higher accuracy, compared with LBC, than the tests using conventional media (the Minnesota test; and the Mastitis Quad plates SSGN and SSGNC, both from DQCI Services, Mounds View, MN).

A US study found that most mastitis treatment decisions made by farmers using the Minnesota Easy Culture Bi-plate, a test with conventional selective media, agree with those that would be taken if the decisions had been based on LBC results (Lago et al., 2011). Additionally, a New Zealand study showed that farmers followed the protocol for 85% of the applied treatments if an on-farm test was incorporated (McDougall et al., 2018). It is unknown whether there is a difference in antimicrobial use and cure when farmers base treatment decisions on chromogenic or on conventional selective culture-based on-farm tests. It has been described that, if a treatment strategy is based on culture results and thus treatment is delayed 24 h, bacteriological cure rate of clinical mastitis is equal to a situation in which all cows are treated immediately with the same antimicrobials (Lago et al., 2011; Vasquez et al., 2017). In both studies, mastitis cases with gram-negative results and with no growth culture results were not treated with antimicrobials, whereas gram-positive results were treated with antimicrobials. Consequently, antimicrobial use has been found to be reduced when a test was used to determine the etiological group of IMI, as opposed to treating all mastitic cows (Lago et al., 2011; Vasquez et al., 2017).

In practice, even without the use of a culture-based test, not all clinical mastitis cases are treated with antimicrobials (Vaarst et al., 2002; Santman-Berends et al., 2016), and subclinical mastitis is hardly treated during lactation (Griffioen et al., 2016). Although the efficacy and economic effects of subclinical mastitis treatments during lactation in general are questionable (Steeneveld et al., 2007; Barlow et al., 2013), such treatments may, for example, be of value in recently acquired subclinical mastitis cases (van den Borne et al., 2010). For those cases, on-farm culture results may be of added value. To our knowledge, the effects of mastitis diagnosis and treatment strategies, as applied by farmers after on-farm tests were used, on antimicrobial use and on cure of clinical and subclinical mastitis, have never been compared with a control group where farmers treated cows using their current decision-making process, without information on the etiological group of the IMI.

In this study, we recorded treatment decisions made by farmers either using an on-farm test, CHROMagar or Minnesota, or using their current treatment decision strategy, often based on clinical signs or SCC, and compared their decisions against the treatments we would advise based on the results of LBC. The aim of this study was to evaluate the effects of 2 diagnosis and treatment strategies of cows with clinical or subclinical mastitis, in which farmers used either of 2 on-farm tests, compared with the current treatment strategy farmers apply, on (1) antimicrobial use, (2) quantitative antimicrobial usage, (3) bacteriological cure, (4) quarter SCC (**QSCC**), (5) intramammary cure (defined as the combination of bacteriological cure and low QSCC), (6) the occurrence of new IMI after treatment (d 21), and, in cases of clinical mastitis, (7) clinical cure of the affected quarter.

MATERIALS AND METHODS

Study Design

Two randomized controlled clinical trials were conducted in 15 commercial Dutch dairy herds. Trial 1 evaluated 2 on-farm culture-based mastitis tests, CHROMagar and Minnesota, to be used for treatment decisions for cows with clinical mastitis (Figure 1). Trial

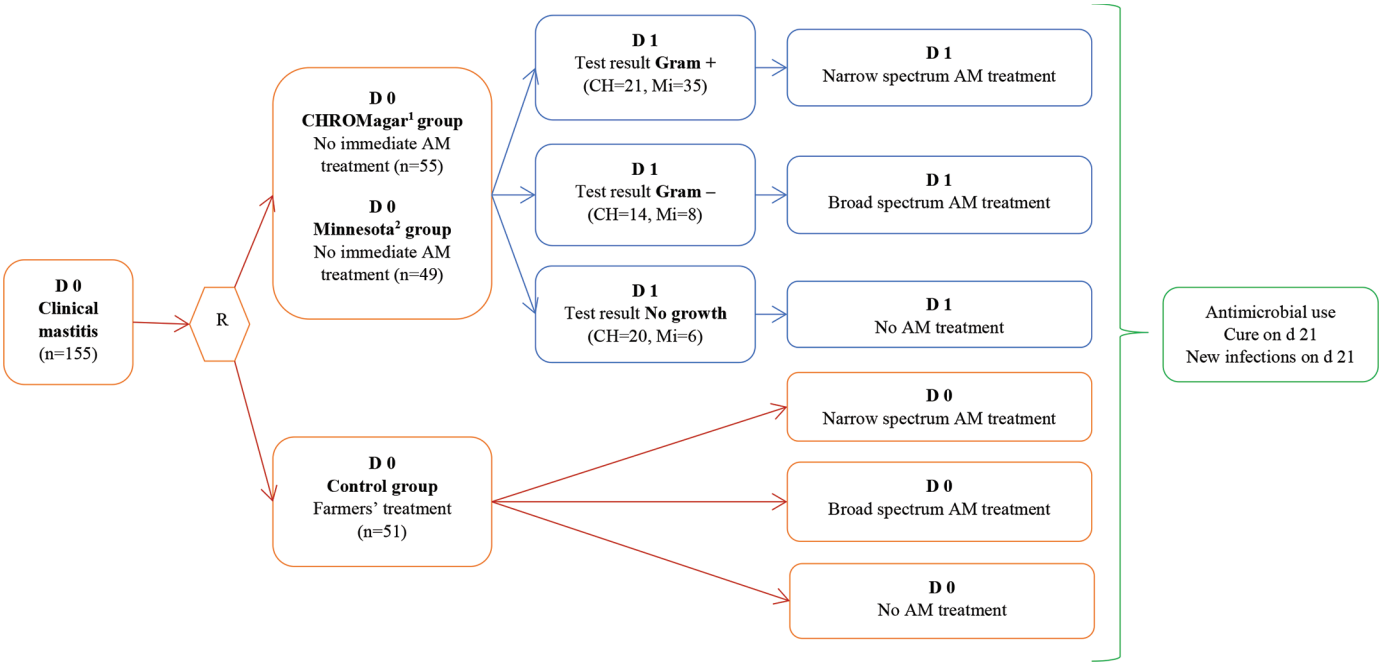

Figure 1. Study design of trial 1. The number of cows with clinical mastitis in 1 quarter that were randomly assigned (R) to 1 of 2 on-farm test groups using Minnesota Tri-plate (Mi) or CHROMagar Mastitis (CH), or to the control group by the farmer randomly selecting an envelope. Two-thirds of the cows were assigned to the on-farm test groups, where a diagnosis and treatment strategy was applied in which the farmer used the on-farm test on d 1, and one-third to the control group, where farmers applied their currently used diagnosis and treatment strategies. The farmer used 1 of the on-farm mastitis tests for half of the anticipated number of clinical mastitis cases during trial 1. After that, the farmer used the other on-farm mastitis test on clinical mastitis cases that were enrolled during the remainder of trial 1. Proportion of cure, new IMI, and antimicrobial use were assessed on d 21 for all cows. The outcomes of the on-farm tests are presented per etiological group on d 1. [1]CHROMagar Mastitis (CHROMagar, Paris, France). [2]Minnesota Easy Culture System II Tri-plate (University of Minnesota, St Paul, MN). AM = antimicrobial.

2 evaluated the same 2 tests, to be used for treatment decisions for cows with subclinical mastitis (Figure 2).

Farm Selection

Farmers were eligible to participate in both trials if they milked at least 100 dairy cows, participated in the routine milk recording program of the Dutch Royal Cattle Syndicate (CRV, Arnhem, the Netherlands) every 4 to 6 weeks, and had conventional management [organic farmers were excluded; farmers with automatic milking systems (n = 5) were allowed to participate]. Farmers were recruited through veterinarians within the authors' network or directly through the network of the authors.

Trial 1: Clinical Mastitis

Fifteen farmers started between May 2017 and March 2018 in trial 1, in which 155 cows were enrolled with clinical mastitis. Enrollment stopped in the period May to July 2018. The study protocol, aseptic sample collection, and on-farm culturing and interpretation of test results were explained to the farmer by study personnel during a farm visit at the start of trial 1. Farmers were asked to include cows with grade 1 clinical mastitis (mild mastitis, only abnormal milk) or grade 2 clinical mastitis (moderate mastitis, abnormal milk and quarter affected). Cows were ineligible to participate if they had participated before, had clinical mastitis in multiple quarters, had been treated with antimicrobials during the last 30 d, or were scheduled to be dried off within 21 d after diagnosing mastitis. Nonsteroidal anti-inflammatory drugs usage was not considered an exclusion criterion. A few possible approaches are available to compare the 3 diagnosis and treatment strategies. We used a design where half of the participating farmers started with an on-farm test group using CHROMagar and the other half started using Minnesota. The number of expected cases on a farm was estimated a few months after the start of the trial, switching to use the other on-farm test when half of the anticipated number of clinical mastitis cases was enrolled on a farm. The protocol for the control group was unchanged. We aimed for 3 equal-sized groups by randomly assigning 2/3 of the mastitis cows to the 2 on-

farm test groups and 1/3 to the control group by farmers taking a numbered envelope. The envelopes were filled in such a way that 1/3 of the envelopes assigned the cow to the control group and 2/3 of the envelopes assigned the cows to the on-farm test group. Because the control group was being filled during the enrollment period for test 1 as well as for test 2, this resulted in approximately equal numbers of animals in the control group as in either of the test groups. For all cows in the trial, farmers aseptically collected 1 milk sample of the affected quarter on d 0. The sample was immediately frozen on farm. A second milk sample was taken at the same time from the quarters in the on-farm test groups and used to be cultured on the on-farm mastitis test by the farmer. In the control group, farmers treated the cows the way they were used to, without testing. On d 21 another milk sample was collected by farmers from all quarters enrolled in the trial, which was also immediately frozen on farm. The frozen samples were collected by study personnel regularly to be cultured in a veterinary laboratory using routine LBC. Farmers were asked to record date and symptoms of all quarters with clinical mastitis that were enrolled in the study, and to classify severity of the mastitis as grade 1, grade 2, or grade 3 (severe mastitis, abnormal milk, quarter affected, and systemic signs of illness) on d 0. Additionally, farmers were asked to record milk yield on d 0 from their milk volume recording system, whether the cow had had clinical mastitis earlier in the current lactation, and the treatment that was applied for cows in the control group. For cows in the on-farm test groups, the farmers also recorded the intended treatment if no on-farm test would have been used on d 0, the on-farm test result, and the actual treatment that was applied on d 1. Furthermore, farmers were asked to record any treatment given to the included cows during the first 21 d after clinical mastitis as well as whether clinical signs were present in the affected quarter on d 21. If after the initial treatment a secondary antimicrobial treatment was given within 21 d, the d-21 sampling date was postponed to 9 d after the last antimicrobial treatment, to avoid interference of antimicrobials in the milk with bacteriological culture of the sample.

Trial 2: Subclinical Mastitis

Trial 2 started on the final enrollment day of trial 1 in each of the 15 herds, in the period May to July 2018.

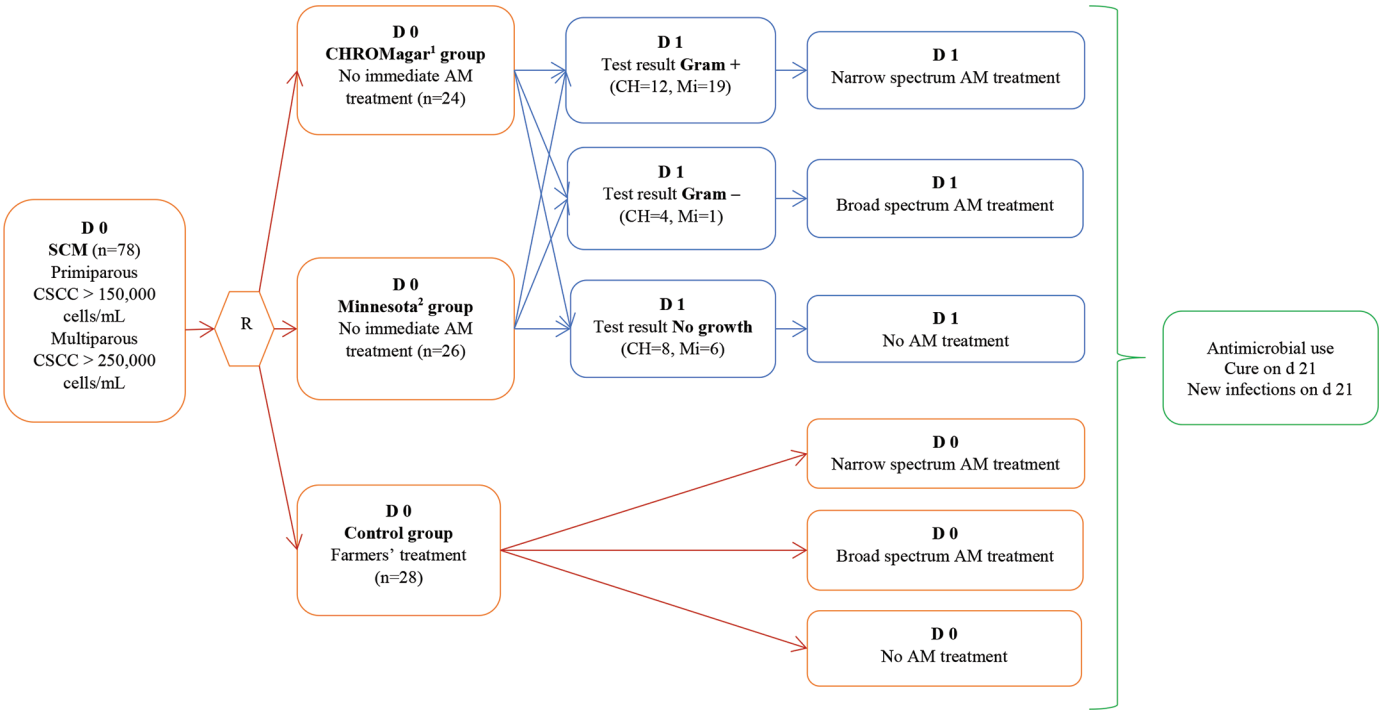

Figure 2. Study design of trial 2. The number of cows with subclinical mastitis in 1 quarter that were randomly assigned (R) to 1 of 3 groups: the CHROMagar group (CH), or the Minnesota group (Mi), in both of which a diagnosis and treatment strategy was applied in which the farmer used the on-farm test on d 1, or to the control group, in which farmers applied their currently used diagnosis and treatment strategies. The outcomes of the on-farm tests are presented per etiological group on d 1. Proportion of cure, new IMI, and antimicrobial use were assessed on d 21 for each of the 3 groups. [1]CHROMagar Mastitis (CHROMagar, Paris, France). [2]Minnesota Easy Culture System II Tri-plate (University of Minnesota, St Paul, MN). AM = antimicrobial.

Seventy-eight cows with subclinical mastitis were enrolled. The study protocol was explained to the farmer at the start of trial 2. On-farm culturing, interpretation of test results, and aseptic sample collection were done the same way as in trial 1. Cases of subclinical mastitis were selected by study personnel, based on the most recent test-day record. Subclinical mastitis was defined as high cow SCC (**CSCC**; primiparous cows CSCC ≥150,000 cells/mL and multiparous cows CSCC ≥250,000 cells/mL; de Haas et al., 2008; Kleiboer and Booij, 2020). The same exclusion criteria applied as in trial 1. In addition, cows that had calved within 5 d before the test-day record were excluded, as well as cows nominated to be sold within 30 d. The sampling day was scheduled within 10 d after milk recording. Samples were collected by the farmer or by study personnel. The California Mastitis Test (**CMT**) was used by the farmer or by study personnel to identify quarters with elevated SCC in selected high-CSCC cows. Trace to +++ were considered positive CMT results. Only cows with 1 CMT-positive quarter were eligible for enrollment, as were cows in which abnormal milk was observed in only 1 quarter. The latter cows were considered to have clinical mastitis and were included in trial 1. A milk sample was taken aseptically from the CMT-positive quarter. Study personnel randomly assigned eligible cows evenly into the CHROMagar group, the Minnesota group, and the control group. For cows in the on-farm test groups, the collected milk sample was cultured on farm on the day of sampling (d 0). On d 1 the farmer read and interpreted the results and treated the cow using the test result. In the control group, cows were treated the way the farmer would otherwise do without testing. All collected samples were transported on ice to the laboratory and frozen at −20°C until LBC. A subsequent milk sample (d 21) was collected aseptically by study personnel or the farmer from all quarters enrolled in trial 2. If collected by the farmer, these samples were frozen on farm and transported later to the laboratory. If samples were collected by study personnel, samples were immediately transported on ice to the laboratory and were frozen at −20°C until LBC. As in trial 1, farmers were asked to record milk yield, mastitis history during the current lactation, and the treatment applied to cows in the control group on d 0. For cows in the on-farm test groups, the farmers recorded the intended treatment if no on-farm test would have been used on d 0, the on-farm test result, and the actual treatment applied on d 1. Any treatment given after the initial treatment was also recorded by the farmers up to d 21. If a secondary treatment was given within 21 d, d-21 sample collection was postponed until 9 d after the end of treatment, as otherwise the concentration of the antimicrobials might interfere with bacteriological culture of the sample.

Follow-Up

Toward the end of the study, the experience of performing the study was discussed with the farmers during farm visits or telephone calls. After the trials were over, farmers filled out a questionnaire regarding whether or not they were willing to continue with a diagnosis and treatment strategy for mastitis including an on-farm test. If they did not express that intention, they were asked why. The farmers who were willing to continue were asked to score their willingness to use an on-farm test on a scale from 0 (never) to 5 (always) for the different grades of mastitis, and which of the on-farm tests they preferred. Additionally, test-day records were collected up to September 2018, from which CSCC and milk production of the enrolled cows were collected.

Routine Bacteriological Culture of Milk

All frozen milk samples were thawed at room temperature in the bacteriological laboratory of Royal GD (Deventer, the Netherlands). Milk samples were bacteriologically cultured, and SCC of the sample was determined using fluorescence flow cytometry (CombiScope 600, Delta Instruments, Drachten, the Netherlands; ISO, 2006). For LBC, 0.01 mL of milk was inoculated onto 6% sheep blood agar (bioTRADING, Mijdrecht, the Netherlands). Plates were incubated aerobically for 48 h at 37°C. Growth was examined after 24 h and 48 h, according to the guidelines of the National Mastitis Council (NMC, 2017). Bacteria present either in pure culture or in equal numbers, with a maximum of 2 morphologically distinct types, were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI Biotyper, Bruker Daltonics GmbH, Bremen, Germany; Barreiro et al., 2010). Samples that had as result after 24 h of incubation either no growth, growth of nonmajor pathogens (NMC, 2017), or *Corynebacterium* species and an SCC of >200,000 cells per mL, or for which the SCC could not be measured because the milk was visually deviating, were re-examined by culturing (0.01 mL) on 6% sheep blood agar after freezing (at least 1 h at −20°C) and subsequent overnight incubation at 37°C (Sol et al., 2002). An IMI was defined as a pure culture or predominance of 1 or 2 types of bacteria. The presence of *Staphylococcus aureus*, *Streptococcus agalactiae*, and hemolytic streptococci was always considered as an IMI. Contamination was defined as growth of more than 2 phenotypically different colony types, without

1 being predominantly present. Bacteriological growth was categorized into 1 of 4 diagnostic categories, being gram-positive growth, gram-negative growth, no growth, or contamination, as described by Griffioen et al. (2018). Readers of LBC were blind to the treatment strategy groups and to the on-farm tests results. Results of LBC were provided to the farmers after trial 2 ended.

On-Farm Culture-Based Mastitis Tests

CHROMagar Mastitis. The CHROMagar test consists of 2 petri dishes, each with a different agar. One agar is specific for gram-positive bacteria (with peptone and yeast extract, salt, and a chromogenic mix), and the other is specific for gram-negative bacteria (with peptone and yeast extract and a chromogenic mix). Because no on-farm manual was available for the use of CHROMagar, a manual was drawn up in Dutch, based on the available online information (CHROMagar Mastitis version 2; CHROMagar, 2014). This manual was provided to the farmers to be used for plating and interpreting CHROMagar. In short, the farmer mixed the milk sample gently, dipped a sterile cotton swab in the milk for 8 to 10 s to become fully saturated, and plated the milk onto the gram-positive plate. To avoid a potential suppressing effect of a selective agar when plating on a subsequent agar, a new sterile cotton swab was then dipped in the milk for 8 to 10 s and streaked onto the gram-negative plate. Both agars were incubated at 37°C for 18 to 24 h. Growth of ≥1 cfu on the gram-positive plate was considered gram-positive growth; growth of ≥1 cfu on the gram-negative plate was considered gram-negative growth. No growth on both plates was defined as no growth.

Minnesota Easy Culture II Tri-Plate. The Minnesota test consists of 1 petri dish split into 3 separate sections (Tri-plate). One section is specific for gram-positive growth (factor agar), one for streptococcal growth (modified TKT agar), and one for gram-negative growth (MacConkey agar). The English manual was translated into Dutch and provided to the farmers, to be used for plating and interpreting Minnesota. In short, the farmer mixed the milk sample gently and dipped a sterile cotton swab in the milk for 8 to 10 s to become fully saturated. The milk was streaked onto the factor agar swab, was dipped in the milk again, streaked onto the MacConkey agar, dipped again, and streaked onto the modified TKT agar. Minnesota was incubated at 37°C, according to the manufacturer's guidelines, for 18 to 24 h (University of Minnesota, 2013). Growth of ≥1 cfu on the factor agar was considered gram-positive growth, with or without growth on TKT agar. Growth of ≥1 cfu on the MacConkey agar was considered gram-negative growth. No growth on the Petri dish was interpreted as no growth.

Treatment

In the on-farm test groups of both trials, a diagnosis and treatment strategy was applied to cows, in which the on-farm test results as obtained by the farmer were included. A gram-positive test result was advised by the authors to be treated with a narrow-spectrum intramammary antimicrobial effective against gram-positive bacteria, in accordance with Dutch guidelines (Formulariumcommissie Melkvee, 2016). A gram-negative test result (regardless of the presence of gram-positive growth) was advised to be treated with a broad-spectrum intramammary antimicrobial effective against gram-positive and gram-negative bacteria (no approved narrow-spectrum antimicrobial effective against gram-negative bacteria is presently available in the Netherlands; Formulariumcommissie Melkvee, 2016). If the test result was no growth, the farmer was advised to not treat the quarter with an antimicrobial. The eventual treatment—for example, the type of antimicrobial chosen—was left to the farmer and the herd veterinarian. For cows in the control group, farmers decided on the type of treatment the way they were used to, based on the herd-specific treatment plan. In the Netherlands, herd-specific treatments plans may vary between herds and are drawn up by the herd veterinarian, where mastitis treatments are categorized based on clinical signs (grade 1 to 3) for clinical mastitis and on CSCC for subclinical mastitis, taking the mastitis history of that specific herd into account. Based on availability of products in all herds, the same narrow-spectrum intramammary antimicrobial is used, whereas there is some variation in the other antimicrobials.

Statistical Analysis

Baseline Characteristics. Baseline characteristics of the 3 diagnosis and treatment strategy groups were compared, using a *t*-test to evaluate equality between the diagnosis and treatment strategy groups for both trials. Differences were considered statistically significant if $P < 0.05$.

Test Characteristics. Results of the on-farm tests as interpreted by the farmers in both trials were compared with the results of LBC as the reference test to calculate the test characteristics of the 2 on-farm mastitis tests. Sensitivity and specificity, with the accompanying binominal exact 95% confidence intervals, were calculated for gram-positive growth, gram-negative growth, and no growth, as described by Dohoo et al. (2009). Furthermore, the proportion of agreement be-

tween LBC and the on-farm tests corrected for chance was calculated, known as Cohen's kappa. The guideline of Landis and Koch (1977) was used to determine the magnitude of agreement. A value of >0.80 was considered to be almost perfect, >0.60–0.80 substantial, >0.40–0.60 moderate, >0.20–0.40 fair, >0.00–0.20 slight, and ≤0.00 poor agreement.

Treatment Decisions. For both trials, farmers' treatment decisions were compared with the advised treatment given the on-farm test result (agreeOFBC) in both on-farm test groups and compared with decisions that would be taken if LBC results were used (agreeLBC) in all 3 diagnosis and treatment strategy groups. AgreeOFBC and agreeLBC were positive if a case with gram-positive growth on the on-farm test as well as on LBC was treated with narrow-spectrum intramammary antimicrobials, if a case with gram-negative growth on the on-farm test as well as on LBC was treated with broad-spectrum intramammary antimicrobials, and if a case with no growth on the on-farm test as well as on LBC was not treated with antimicrobials (Formulariumcommissie Melkvee, 2016).

Proportions of Cure. Outcomes were evaluated for the 3 diagnosis and treatment strategy groups in both trials separately on d 21 after enrollment, irrespective of whether or not an antimicrobial treatment was given. Outcomes determined were bacteriological cure, low QSCC (<100,000 cells/mL in d-21 sample), intramammary cure (combination of the previous 2), and new IMI of the affected quarter. For trial 1, clinical cure was also determined. Bacteriological cure of a quarter was defined as the original bacterial species cultured from the d-0 milk sample not being cultured by LBC from the d-21 sample. For this part of the analysis, quarters with no growth or quarters with contaminated d-0 samples were excluded. A quarter was considered to have a new IMI when a bacterial species was cultured from the d-21 sample that was not cultured from the d-0 sample. Quarters with contaminated d-0 samples were excluded from this part of the analysis. Clinical cure was defined as the absence of clinical signs on d 21 as recorded by the farmers.

Logistic Regression Models. Multivariable logistic regression analysis was performed for all cows in trial 1 and in trial 2 separately, to determine effect of diagnosis and treatment strategy group on the dependent variables intramammary and bacteriological cure, low QSCC, and new infections, using Stata Statistical Software: Release 15 (StataCorp LLC, College Station, TX). For clinical mastitis, clinical cure was determined as well. The explanatory variables tested were the diagnosis and treatment strategy groups (control group, CHROMagar group, or Minnesota group), severity of mastitis (grade 1 or grade 2 clinical mastitis), LBC result (gram-positive growth, gram-negative growth, or culture negative) on d 0, d-0 milk production (continuous), parity (1, 2, 3, or >3), DIM on d 0 (≤100 d or >100 d), whether CSCC on the last test-day record before d 0 was low or high (high: ≥150,000 cells/mL and ≥250,000 cells/mL for primiparous and multiparous cows, respectively; for subclinical mastitis this was the test-day record before the test-day record used for selection of cows). Farm was included as random effect. The diagnosis and treatment strategy group of the cow was always forced into the models. Models were compared based on the goodness of fit (log-likelihood) using Akaike's information criterion (a difference of >2 was considered significant) via stepwise backward selection, where the model with the lowest Akaike's information criterion was considered the best-fitting model. Model selection was done by excluding variables one by one, until a model was obtained including the diagnosis and treatment strategy group and all variables important to the model (including confounders, defined as variables resulting in a change in β-coefficients of the diagnosis and treatment strategy groups of ≥20% when removed from the model).

RESULTS

Participating Farmers and Cows

In total, 17 farmers started participating in trial 1, of which 2 stopped shortly after the start of the trial. One farmer stopped because she was unwilling to delay treatment of clinical mastitis for 18 to 24 h, and 1 farmer because he struggled with record keeping. Data on these 2 herds were not included in the study. Of the 15 farmers, 8 started using Minnesota in the on-farm test group, and 7 started using CHROMagar. The average time farmers participated in the study was 279 d (106–394 d). Herds were spread over the middle of the Netherlands, with 4 herds located in the province of Flevoland, 4 in Overijssel, 3 in Gelderland, and 1 herd in each of the provinces Utrecht, Drenthe, Noord-Brabant, and Zuid-Holland. Five farmers milked with an automatic milking system and 10 with a milking parlor. Participating herds had on average 150 lactating cows (99–270), an average milk production of 9,847 kg/yr (8,200–11,000 kg/yr), and an average bulk tank SCC of 168,429 cells/mL (90,000–325,000 cells/mL).

During discussions with the farmers, all indicated they were able to culture and interpret the on-farm test according to the instructions given, and that they appreciated the additional information provided by the test results. Some farmers hesitated to postpone treatment for 24 h for the first enrolled clinical mastitis cases, but they became more secure after experiencing

that severity of mastitis was not affected by a delayed treatment. Moreover, some farmers left cows untreated if they considered them to be clinically cured after 24 h, even if the on-farm test showed bacteriological growth. After the trial, 11 farmers indicated in the short questionnaire that they were willing to continue to use an on-farm test. Four farmers could not decide or did not intend to use the tests in the future, because the time to result was too long or because they had gained too little experience with working with the tests. The interested farmers were willing to use a test for grade 2 clinical mastitis most frequently (scored on average 4.1), followed by subclinical mastitis (on average 3.7), grade 1 mastitis (on average 3.0), and grade 3 mastitis (on average 3.0). Seven farmers preferred CHROMagar and 5 Minnesota, whereas 3 farmers could not indicate their preference.

For trial 1, 163 quarter samples were collected on d 0. Of these quarters, 8 had missing d-21 samples and were therefore excluded from the analysis, resulting in 155 cows included in trial 1 (Table 1). For trial 2, 315 cows were selected as subclinically infected cows based on CSCC on the last test-day record. Of these cows, 90 were ineligible to participate because of recent antimicrobial treatment or because they were nominated to be sold shortly, and 138 had either 0 or 2 or more quarters with a positive CMT result and were, for that reason, not included in the study. Three cows were found to have signs of clinical mastitis and were therefore included in trial 1. After enrollment, 6 cows were excluded due to lack of d-21 samples. In total, 78 cows with 1 CMT-positive quarter fitted the inclusion criteria and were included in trial 2. In total over both trials, 79 cases were enrolled in the CHROMagar group, 75 cases in the Minnesota group, and 79 cases in the control group. The total number of enrolled cases per farm varied from 2 to 39. Two farmers used only CHROMagar during the study; the other farmers used each of both tests at least once. In trial 1, 1 farmer had not enrolled any case of clinical mastitis and 1 farmer only 1 case. In one herd, none of the selected subclinical mastitis cows were eligible for participation in trial 2. In another herd, all d-21 samples of the subclinical quarters were lacking, and this herd was therefore excluded from trial 2. Characteristics of the 3 diagnosis and treatment strategy groups in both trials are shown in Table 1. Farmers categorized 8 cows as grade 3 mastitis. Although we advised not to include grade 3 mastitis cows in the study, we kept these cows in the analysis. We considered these as grade 2 mastitis cows, because based on the recorded symptoms only 2 of them had systemic signs of illness, whereas of the cows categorized as grade 2 mastitis, 13 cows also had systemic signs of illness. Additionally, waiting 1 d before treating these cows apparently was not considered to be an issue by the farmers. Of the grade 3 mastitis cows, 5 were in the CHROMagar group, 1 in the Minnesota group, and 2 in the control group. In trial 1, 15 cows had experienced clinical mastitis earlier in the same lactation, of which 4 were in the CHROMagar group, 8 in the Minnesota group, and 3 in the control group. In trial 2, 5 cows had experienced clinical mastitis earlier in the same lactation, of which 2 were in the CHROMagar group, 3 in the Minnesota group, and none in the control group. In trial 2, the average DIM was significantly higher in the Minnesota group than in both other groups.

Test Results

The results of LBC are shown in Table 2. Almost 20% of all milk samples were culture negative in the laboratory. Significantly more gram-positive bacteria (mainly staphylococci; $P = 0.01$) and significantly fewer gram-negative bacteria ($P = 0.01$) were cultured from clinical mastitis cases in the Minnesota group than in the control group. No other significant differences were found. Table 3 shows the test characteristics of CHROMagar and Minnesota as interpreted by the farmers, compared with the results of LBC. Minnesota had a higher agreement with LBC for all 3 diagnostic categories than did CHROMagar.

Treatment Decisions

The treatment decision of the farmer after using an on-farm test differed from the treatment that would have been applied if no on-farm test were used for 60% of the cows in trial 1. If CHROMagar was used, decisions changed most often from using antimicrobials to not using antimicrobials (45% of the changed decisions), whereas if Minnesota was used, decisions changed most often from using narrow-spectrum antimicrobials to using broad-spectrum antimicrobials (41% of the changed decisions). Using Minnesota, the smallest percentage of changed decisions was for using antimicrobials in the intended treatment to not using them in the applied treatment (24% of the changed decisions). For cows with subclinical mastitis, 30% (Minnesota) to 46% (CHROMagar) of the intended and applied treatments differed. Almost all decisions for subclinical mastitis changed from not using antimicrobials to using antimicrobials.

In trial 1, the percentage of cows with clinical mastitis treated with an antimicrobial was lowest in the CHROMagar group (Table 4). If Minnesota was used to decide on treatment, 77% of the antimicrobial treatments were with a narrow-spectrum antimicrobial. In

Table 1. Average number of cases per farm and characteristics of cows with clinical mastitis or subclinical mastitis at enrollment for 2 on-farm test groups, in which on-farm test results were used by farmers to determine treatment, and for the control group, in which cows were treated as the farmer was used to without testing

	Clinical mastitis			Subclinical mastitis		
	On-farm test group			On-farm test group		
Item	CHROMagar[1]	Minnesota[2]	Control[3]	CHROMagar	Minnesota	Control
Cases (no.)	55	49	51	24	26	28
Average number of cases per farm (minimum to maximum)	3.7 (0 to 9)	3.3 (0 to 8)	3.4 (0 to 9)	1.6 (0 to 6)	1.7 (0 to 5)	1.9 (0 to 6)
Average DIM (minimum to maximum)	120 (0 to 460)	138 (−9 to 354)	136 (4 to 411)	139 (22 to 338)[a]	215 (24 to 737)[b]	122 (11 to 255)[a]
Average parity (minimum to maximum)	3.5 (1 to 8)	3.0 (1 to 9)	3.3 (1 to 7)	3.7 (1 to 8)	2.7 (1 to 5)	3.3 (1 to 7)
Median quarter SCC d 0[4] (×1,000 cells/mL)	—	—	—	1,011	1,076	1,125
Median cow SCC −1 test-day record before event[5] (×1,000 cells/mL)	163	93	76	271	319	233
Median cow SCC −2 test-day record before event[6] (×1,000 cells/mL)	129	79	76	109	305	128
Average milk production d 0 (kg)	32.9	32.7	34.6	35.6	32.7	36.5
Average milk production −1 test-day record before event[5] (kg)	37.5	36.2	36.7	36.0	34.0	36.4
Average milk production −2 test-day record before event[6] (kg)	36.1	37.7	36.2	34.8	33.0	38.8

[a,b]Indicates significant difference between groups for that variable using a *t*-test, with $P < 0.05$ defined as statistically significant.
[1]On-farm test group using CHROMagar Mastitis test results (CHROMagar, Paris, France) on farm to determine IMI status and treatment accordingly.
[2]On-farm test group using Minnesota Easy Culture System II Tri-plate test results (University of Minnesota, St Paul, MN) on farm to determine IMI status and treatment accordingly.
[3]Control group, in which no mastitis test was used. Cases were treated as the farmer was used to, based on the herd-specific treatment plan.
[4]As determined in d-0 milk sample.
[5]As determined on last test-day record before d 0.
[6]As determined on second-to-last test-day record before d 0.

both other groups, approximately 50% of the antimicrobials given were narrow-spectrum intramammary antimicrobials. All narrow-spectrum intramammary treatments were cloxacillin infusions (Orbenin Lactation, Zoetis B.V., Capelle aan den IJssel, the Netherlands). Broad-spectrum intramammary treatments were infusions with amoxicillin/clavulanic acid and prednisone (Avuloxil, Zoetis B.V.), lincomycin and neomycin sulfate (Albiotic Formula, Huvepharma N.V., Antwerp, Belgium), or cefalexin and kanamycin (Ubrolexin, Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany). Narrow-spectrum parenterally applied treatments were treatments with penethamate hydriodide (Mamyzin, Boehringer Ingelheim Vetmedica GmbH) or tylosin (Tylan, Elanco GmbH, Bad Homburg, Germany), and broad-spectrum parenterally applied treatments were various combinations of trimethoprim with sulphadiazine or sulphadoxine. Furthermore, fewer systemic antimicrobials were given to cows in the Minnesota group. In trial 2, the proportion of cows treated with an antimicrobial was higher in the on-farm test groups than in the control group. Almost 100% of the antimicrobial treatments given in trial 2 were narrow-spectrum intramammary antimicrobial treatments. Farmers followed the on-farm test result in their treatment decision in most cases, which is reflected in the percentage of treatments positive for agreeOFBC: 90% of the treatments in trial 1 and 70% of the treatments in trial 2. In situations in which the test result was not followed in both trials, generally no antimicrobials were applied, although bacteriological growth was found in the on-farm test. For clinical mastitis, this was the case 6 times when CHROMagar was used and 4 times when Minnesota was used. For subclinical mastitis, this was the case 3 times when CHROMagar was used and 7 times when Minnesota was used. The 3 groups in both trials were equal with respect to the number of previous clinical mastitis events, the number of quarters dried off up to 21 d after the event, and the number of secondary treatments. Also, with respect to replacement, no differences were found up to 3 test-day records after the event. Milk production was lower if Minnesota was used, compared with CHROMagar, the second (31.7 and 36.1 kg, respectively) and third (31.1 and 34.8 kg, respectively) test-day records after clinical mastitis. No differences were found with respect to CSCC between the 3 groups.

Cure of Mastitis

Cows with clinical mastitis that were assigned to the CHROMagar group had a lower bacteriological cure rate [odds ratio (**OR**) 0.18] than cows assigned to the control group (Table 5). In this model, high CSCC on

Table 2. Quarter-level etiology of IMI according to laboratory bacteriological culture from d-0 milk samples from 155 clinical mastitis cases and from 78 subclinical mastitis cases stratified over 2 on-farm test groups in which on-farm test results were used by farmers to determine treatment, and a control group in which cows were treated as the farmer was used to, based on the herd-specific treatment plan without testing

	Cultured bacteria clinical mastitis cases						Cultured bacteria subclinical mastitis cases					
	On-farm test group						On-farm test group					
	CHROMagar (no. = 55)		Minnesota (no. = 49)		Control (no. = 51)		CHROMagar (no. = 24)		Minnesota (no. = 26)		Control (no. = 28)	
Item	No.	Fraction	No.	Fraction	No.	Fraction	No.	Fraction	No.	Fraction	No.	Fraction
1 colony morphology	41	0.75	41	0.84	37	0.73	17	0.71	20	0.77	17	0.61
2 colony morphologies[1]	3	0.05	1	0.02	3	0.06	1	0.04	0	0.00	6	0.21
≥3 colony morphologies	0	0.00	0	0.00	0	0.00	0	0.00	2	0.08	1	0.04
No growth	11	0.20	7	0.14	11	0.22	6	0.25	4	0.15	4	0.14
Gram-positive bacteria	36	0.65	37	0.76	26	0.51	18	0.75	17	0.65	25	0.89
Staphylococcus spp.	9	0.16	16	0.33	12	0.24	13	0.54	9	0.35	13	0.46
Staph. aureus	4	0.07	7	0.14	5	0.10	3	0.13	2	0.08	0	0.00
NAS	5	0.09	9	0.18	7	0.14	10	0.42	7	0.27	13	0.46
Streptococcus spp.	16	0.29	15	0.31	9	0.18	1	0.04	5	0.19	4	0.14
Strep. uberis	8	0.15	8	0.16	4	0.08	0	0.00	1	0.04	0	0.00
Strep. agalactiae	0	0.00	0	0.00	0	0.00	1	0.04	2	0.08	1	0.04
Strep. dysgalactiae	7	0.13	7	0.14	5	0.10	0	0.00	1	0.04	2	0.07
Other streptococci	1	0.02	0	0.00	0	0.00	0	0.00	1	0.04	1	0.04
Other gram-positive bacteria	7	0.13	3	0.06	2	0.04	2	0.08	3	0.12	8	0.29
Enterococcus spp.	4	0.07	3	0.06	3	0.06	3	0.13	0	0.00	0	0.00
Gram-negative bacteria	11	0.20	6	0.12	17	0.33	1	0.04	3	0.12	4	0.14
Lactose fermenting bacteria	11	0.20	5	0.10	16	0.31	0	0.00	0	0.00	0	0.00
Escherichia coli	9	0.16	4	0.08	14	0.27	0	0.00	0	0.00	0	0.00
Klebsiella spp.	1	0.02	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Other coliform bacteria[2]	1	0.02	1	0.02	2	0.04	1	0.04	3	0.12	4	0.14
Other gram-negative bacteria	0	0.00	1	0.02	1	0.02	0	0.00	0	0.00	0	0.00

[1]Up to 2 bacterial species were differentiated if present in a milk sample.

[2]*Serratia* spp., *Enterobacter* spp., and *Citrobacter* spp.

the last test-day record before clinical mastitis also reduced bacteriological cure rate compared with cows with low CSCC at that same point in time. For intramammary cure and low QSCC, no differences were found between the 3 groups.

The numbers were too low to model the effect of diagnosis and treatment strategy group on intramammary cure and low QSCC in cows with subclinical mastitis. Only 4 cows were positive for intramammary cure (2 in the CHROMagar group and 2 in the Minnesota group), and 9 for low QSCC (5 in the CHROMagar group, 3 in the Minnesota group, and 1 in the control group). Cows with subclinical mastitis were less likely to develop new IMI on d 21 in the Minnesota group compared with the controls (Table 6). Lactation length had a confounding effect on the association between diagnosis and treatment strategy group and new IMI on d 21.

DISCUSSION

This study aimed to evaluate the effect of 2 diagnosis and treatment strategies for cows with clinical or subclinical mastitis, each using an on-farm test, on cure and antimicrobial use, compared with farmers' current diagnosis and treatment strategies. Our study showed that farmers' current diagnosis and treatment strategies resulted in similar or higher bacteriological cure rates than when on-farm tests were incorporated in their decision-making process. However, if a diagnosis and treatment strategy including on-farm test results is used, mastitis treatment decisions become more targeted, because 2 to 3 times more treatment decisions were in accordance with the cause of the IMI based on LBC in the on-farm test groups than in the control group.

In human studies it has been found that diagnostic interventions often fail to improve patient outcomes. Only 18% of the studies compared in this review reported improved patient outcomes after using a diagnostic test, even though diagnostic tests were evaluated as performing well. (Siontis et al., 2014). Even if the accuracy of tests is good, their clinical utility may be unknown. Whether testing will affect the outcome is determined by what physicians—or, in our case, farmers—do with the results and thus how test results affect treatment decisions. These authors therefore encourage performing randomized trials that determine the effect of additional testing. Thus, the effect of the whole test-treatment-outcome pathway can be evaluated.

A study that determined the effect of a culture-based on-farm treatment program on bacteriological cure of clinical mastitis in cows also reported a reduction, although nonsignificant, in the OR for bacteriological cure in the culture-based treatment group (OR 0.6) compared with the control group, in which all cows were treated with antimicrobials (Lago et al., 2011). In that study, over 80% of the decisions made on farm after an on-farm test was applied were in accordance with bacteriological culture results from the laboratory. We also found a lower bacteriological cure rate if an on-farm test was used compared with use of no test, with a high percentage of treatments in accordance with the cause of the IMI. Because we found very high bacteriological cure rates in our controls (96% if CSCC on the last test-day record was low, based on the high constant in the model assessing bacteriological cure for clinical mastitis), a lower OR could be expected for the 2 on-farm test groups. Even with the low OR, cows with clinical mastitis had over 80% chance to cure bacteriologically if a diagnosis and treatment strategy using an on-farm test was applied, which is higher than the 60% bacteriological cure found in a US study using treatment strategies with on-farm testing (Lago et al., 2011). The high bacteriological cure rates found for the controls may be caused by the difference in treatments applied, the lower CSCC on the last test-day record, or the high proportion of gram-negative IMI found in the controls compared with the other 2 groups. Gram-negative cases have relatively high bacteriological cure probabilities (Ruegg, 2018) and are less often associ-

Table 3. Sensitivity (Se), specificity (Sp), and agreement (κ) of 2 on-farm tests, CHROMagar mastitis test (CHROMagar, Paris, France) and Minnesota Easy Culture System II Tri-plate (University of Minnesota, St Paul, MN), used by farmers on milk samples collected from cows with clinical and subclinical mastitis, as compared with laboratory bacteriological culture

	CHROMagar			Minnesota		
Result	Se (95% CI)	Sp (95% CI)	κ (95% CI)	Se (95% CI)	Sp (95% CI)	κ (95% CI)
Gram-positive growth	0.57 (0.46–0.68)	0.68 (0.58–0.78)	0.20 (0.11–0.29)	0.91 (0.84–0.97)	0.59 (0.48–0.70)	0.52 (0.41–0.64)
Gram-negative growth	0.67 (0.56–0.77)	0.85 (0.77–0.93)	0.42 (0.31–0.53)	0.56 (0.44–0.67)	0.94 (0.89–0.99)	0.49 (0.38–0.61)
No growth	0.47 (0.36–0.58)	0.69 (0.59–0.80)	0.12 (0.05–0.19)	0.45 (0.34–0.57)	0.89 (0.82–0.96)	0.33 (0.23–0.44)

Table 4. Treatments applied to cows with clinical mastitis and subclinical mastitis in 2 on-farm test groups in which the on-farm tests CHROMagar Mastitis or Minnesota Tri-plate were used by farmers to determine treatment, and in the control group in which cows were treated as the farmer was used to without testing

	Clinical mastitis						Subclinical mastitis					
	On-farm test group						On-farm test group					
	CHROMagar[1]		Minnesota[2]		Control[3]		CHROMagar		Minnesota		Control	
Item	No./ total	Fraction	No./ total	Fraction	No./ total	Fraction	No./ total	Fraction	No./ total	Fraction	No./ total	Fraction
Antimicrobial use	32/55	0.58	39/49	0.80	44/51	0.86	13/24	0.54	13/26	0.50	1/28	0.04
Udder: narrow, Parenteral: none[4]	15/32	0.47	24/39	0.62	16/44	0.36	10/13	0.77	8/13	0.62	1/1	1.00
Udder: narrow, Parenteral: narrow	1/32	0.03	3/39	0.08	5/44	0.11	3/13	0.23	3/13	0.23	0/1	0.00
Udder: narrow, Parenteral: broad	1/32	0.03	3/39	0.08	2/44	0.05	0/13	0.00	0/13	0.00	0/1	0.00
Udder: broad, Parenteral: none[5]	2/32	0.06	3/39	0.08	10/44	0.23	0/13	0.00	1/13	0.08	0/1	0.00
Udder: broad, Parenteral: narrow	3/32	0.09	1/39	0.03	3/44	0.07	0/13	0.00	0/13	0.00	0/1	0.00
Udder: broad, Parenteral: broad	10/32	0.31	5/39	0.13	8/44	0.80	0/13	0.00	1/13	0.08	0/1	0.00
No antimicrobial use	23/55	0.42	10/49	0.20	7/51	0.14	11/24	0.46	13/26	0.50	27/28	0.96
Alternative treatment[6]	7/55	0.13	3/49	0.06	4/51	0.08	1/24	0.04	3/26	0.12	2/28	0.07
Treatment positive for AgreeOFBC[7]	45/55	0.82	44/49	0.90	—	—	17/24	0.71	18/26	0.69	—	—
Treatment positive for AgreeLBC[8]	30/55	0.55	37/49	0.76	19/51	0.37	15/24	0.63	13/26	0.50	6/28	0.21
Total	55/155	0.35	49/155	0.32	51/155	0.33	24/78	0.31	26/78	0.33	28/78	0.36

[1]On-farm test group using CHROMagar Mastitis test (CHROMagar, Paris, France) results on farm to determine IMI status and treatment accordingly.

[2]On-farm test group using Minnesota Easy Culture System II Tri-plate test (University of Minnesota, St Paul, MN) results on farm to determine IMI status and treatment accordingly.

[3]Control group, in which cases were treated as the farmer was used to, based on the herd-specific treatment plan.

[4]Narrow-spectrum intramammary antimicrobial specific against gram-positive bacteria. Udder: cloxacillin. Parenterally: penethamate hydroiodide or tylosin.

[5]Broad-spectrum intramammary antimicrobial against both gram-positive and gram-negative bacteria. Udder: amoxicillin clavulanic acid with prednisolone, lincomycin with neomycin sulfate, or cephalexin with kanamycin. Parenterally: trimethoprim combined with sulphadoxine or sulphadiazine.

[6]An alternative treatment was applied, such as anti-inflammatory drugs or udder mint, but no antimicrobials were applied.

[7]Treatment agreed if gram-positive on-farm test results were treated with a narrow-spectrum antimicrobial, if gram-negative on-farm test results were treated with a broad-spectrum antimicrobial, and if no growth on-farm test results were not treated with an antimicrobial.

[8]Treatment agreed if gram-positive growth on laboratory bacteriological culture was treated with a narrow-spectrum antimicrobial, if gram-negative growth was treated with a broad-spectrum antimicrobial, and if no growth was not treated with an antimicrobial.

Table 5. Logistic regression models for the odds ratio (OR) of intramammary cure, bacteriological cure, low quarter SCC (QSCC), new IMI, and clinical cure of cows with clinical mastitis determined on d 21 after mastitis detection, with the number of cows positive for the outcome and the total number of cows per variable[1]

	Intramammary cure[2] (n = 83)		Bacteriological cure[3] (n = 83)		Low QSCC[4] (n = 118)		New IMI (n = 97)		Clinical cure (n = 110)	
Item	Cured	OR (95% CI)	Cured	OR (95% CI)	Low QSCC	OR (95% CI)	New IMI	OR (95% CI)	Cured	OR (95% CI)
Treatment strategy group[5]										
Control	9/26	Referent	24/26	Referent	10/36	Referent	10/32	Referent	25/32	Referent
CHROMagar	7/30	0.83 (0.24–2.88)	18/30	0.18 (0.03–0.99)	10/42	1.04 (0.34–3.19)	10/35	0.88 (0.31–2.51)	32/41	1.43 (0.42–4.90)
Minnesota	6/27	0.65 (0.18–2.30)	20/27	0.23 (0.04–1.36)	12/40	1.36 (0.45–4.10)	10/30	1.10 (0.34–3.19)	33/37	2.53 (0.61–10.56)
Grade of mastitis										
1	5/39	Referent	—	—	10/59	Referent	—	—	—	—
2	17/44	4.13 (1.31–12.98)	—	—	22/59	3.12 (1.20–8.10)	—	—	—	—
Cow SCC test-day record −1[6]										
Low	—	—	47/53	Referent	—	—	—	—	67/73	Referent
High	—	—	15/30	0.15 (0.05–0.48)	—	—	—	—	23/37	0.14 (0.05–0.43)
Constant	—	0.18 (0.05–0.64)	—	25.58 (4.95–132.02)	—	0.16 (0.05–0.52)	—	0.45 (0.22–0.96)	—	7.53 (2.62–21.64)

[1]Treatment strategy group was forced into the models. Farm was included as random effect.

[2]Quarter was positive for bacteriological cure and had a low QSCC.

[3]The colony-forming unit cultured from the d-0 sample was not cultured from the d-21 sample (no growth and contaminated d-0 samples were excluded).

[4]QSCC in d-21 sample <100,000 cells/mL.

[5](1)Control group in which cases were treated as the farmer was used to, without testing; (2) test group using CHROMagar Mastitis (CHROMagar, Paris, France) results on farm to determine treatment; (3) test group using Minnesota Easy Culture System II Tri-plate (University of Minnesota, St Paul, MN) results on farm to determine treatment.

[6]Cow SCC (CSCC) as recorded on the last test-day record before d 0 (low CSCC was defined for primiparous cows <150,000 cells/mL and for multiparous cows <250,000 cells/mL).

Table 6. Logistic regression models for the odds ratio (OR) of bacteriological cure, and new IMI of cows with subclinical mastitis determined on d 21 after enrollment, with the number of cows positive for the outcome and the total number of cows per variable[1]

	Bacteriological cure[2] (n = 38)		New infections (n = 45)	
Item	Cured	OR (95% CI)	New IMI	OR (95% CI)
Treatment strategy group[3]				
Control	8/11	Referent	5/12	Referent
CHROMagar	7/13	0.41 (0.06–2.92)	4/16	0.25 (0.04–1.72)
Minnesota	4/14	0.20 (0.03–1.34)	1/17	0.06 (0.00–0.74)
Days in lactation				
≤100 d	—	—	5/13	Referent
>100 d	—	—	5/32	0.19 (0.03–1.17)
Milk production (kg)				
Continuous	19/38	1.14 (1.02–1.27)	—	—
Constant	—	0.02 (0.00–1.23)	—	15.26 (0.42–551.83)

[1]The treatment strategy group was forced into the models. Farm was included as random effect.

[2]The colony-forming unit cultured from the d-0 sample was not cultured from the d-21 sample (no growth and contaminated d-0 samples were excluded).

[3](1) Control group in which cases were treated as the farmer was used to, without testing; (2) test group using CHROMagar Mastitis (CHROMagar, Paris, France) results on farm to determine treatment; (3) test group using Minnesota Easy Culture System II Tri-plate (University of Minnesota, St Paul, MN) results on farm to determine treatment.

ated with chronic IMI than gram-positive IMI (Lam et al., 1996). The CHROMagar and Minnesota groups, however, had higher CSCC on the last test-day records and higher proportions of IMI caused by *Staph. aureus* or *Strep. agalactiae*, which are more often associated with chronic IMI (Lam et al., 1996). For practical reasons, milk samples were frozen before being subjected to LBC, which may have affected the recovery of pathogens. For *Staph. aureus* and *Escherichia coli*, for example, evidence exists that more and less, respectively, bacteria may be recovered after freezing of the samples (Schukken et al., 1989). Thus, the true infection status of the udder was not perfectly determined. Also, farmers may have influenced the bacteriological cure rate. Even though cows were randomized to the various treatment strategies, farmers assigning, for example, recurrent cases of mastitis to the treatment strategies with on-farm testing cannot be ruled out. Furthermore, all cows with high SCC in the control group cured bacteriologically, whereas only part of the high-SCC cows assigned to the treatment strategies with on-farm testing cured. We found that the odds for bacteriological cure of mastitis was affected by cow factors such as CSCC on the last test-day record, with high CSCC resulting in lower bacteriological cure rates. Farmers likely include that type of information in their treatment strategy, which is not reflected in the result of an on-farm mastitis test, but which is of high value to be incorporated in the decision-making process to enhance cure probabilities (Krömker and Leimbach, 2017; Ruegg, 2018). Thus, cow factors that affect bacteriological cure should be considered before deciding whether an antimicrobial treatment should be applied.

Literature advises leaving subclinical infected cows untreated during lactation (Steeneveld et al., 2007; Barlow et al., 2013). However, we studied the effects on cure in these cases, as farmers indicated interest in using on-farm tests for subclinical mastitis. If farmers used on-farm tests in their decision-making process for subclinical mastitis, more treatments were in accordance with the cause of the IMI, compared with the control group, but antimicrobial usage increased from almost 0% to over 50%. Although most of the cows with subclinical mastitis were left untreated in the control group, bacteriological cure was as good as in the groups in which on-farm tests were used. For specific cows with recently acquired subclinical IMI, for example, treatment may be attractive (van den Borne et al., 2010). In those cases, the effect of antimicrobial treatment on bacteriological cure should outweigh the negative effect of increased antimicrobial usage. Because no beneficial effect was found on bacteriological cure of subclinical mastitis if an on-farm test was used in the diagnosis and treatment strategy, whereas antimicrobial usage increased, this is not advised as a general approach for subclinical mastitis cases. However, if farmers do want

to treat cows with subclinical mastitis, thorough examination of the (sub)clinical mastitis history of the cow should be performed (Krömker and Leimbach, 2017) to select cows that may benefit from treatment. For that, the etiology of the IMI should be incorporated in the decision-making process, because cure probabilities differ with etiology (Ruegg, 2018). In those situations, knowledge of the cause of the IMI likely is of value to enhance prudent antimicrobial use. Furthermore, the probability of having a new IMI on d 21 after inclusion was high in the controls. If the diagnosis and treatment strategy incorporated on-farm testing, the OR for new IMI decreased considerably (OR 0.06), which may be a positive outcome of this diagnosis and treatment strategy. Further research should be performed to determine how to incorporate the results of on-farm tests in the selection of cows with high cure probabilities to enhance optimal and prudent treatment of cows with subclinical mastitis.

Next to the information farmers already use when making mastitis treatment decisions, such as history of clinical mastitis or history of CSCC from the current lactation (Vaarst et al., 2002; Neeser et al., 2006), they will add the information from test results. Consequently, cure is affected not only by test characteristics but also by the way in which test results are used in the diagnosis and treatment strategy, as was described in a literature review on human diagnostic randomized trials (Siontis et al., 2014). The type of information used may differ for farmers using automatic milking systems, compared with farmers using conventional milking systems, as they might detect cases differently. We enrolled both types of farmers in our study, which might have masked effects, but the effects found were not considered to be caused by bias. In our study, farmers followed the recommendations based on the on-farm test results in most clinical mastitis treatment decisions (82–90%). Cases where the on-farm test result was not followed often were culture-positive results that were left untreated. These may have been cows that were already clinically cured during the 24 h pending test results. By the time test results became available, farmers felt no urge to treat these cows any more. This may be due to clinical self-cure of mastitis, as earlier described (Ruegg, 2018), which does not necessarily mean that the quarter was also cured bacteriologically. Some farmers seemed to be unaware of the phenomenon of self-cure, as we noticed that farmers initially hesitated to postpone treatment. However, after observing the apparent self-cure, some farmers left these cows untreated, even though bacterial growth was detected in the on-farm test. A minority of the farmers were already familiar with the possible self-cure of cows and anticipated that by a delayed decision on treatment of cows in the control group. This type of delayed treatment, while watching the cow closely in the meantime, has earlier been described as "watchful waiting" (Ruegg, 2018) and may in part explain why not all cows in the control group in trial 1 were treated. Because cure was not found to be improved after using an on-farm test, although more targeted treatments were applied, we checked whether strictly following the on-farm test results in the applied treatments would have resulted in an improved cure, but found no positive effect (data not shown). Farmers may need to gain experience using on-farm tests, specifically on how to incorporate the test results into their current treatment decision-making process, to enhance prudent use of antimicrobials for mastitis.

Farmers were able to work with both on-farm tests evaluated and had no specific preference for either of the tests. Culturing milk samples of cows with mastitis is advised, to determine whether and which treatment to apply for mastitis (Formulariumcommissie Melkvee, 2016; Lago and Godden, 2018; Ruegg, 2018). In our study the improved treatments did not lead to improved cure. For low QSCC, new IMI, and clinical cure, however, implementing on-farm tests tended to have a positive effect. Even though farmers' treatments were found to result in high cure probabilities, treatment protocols may improve treatments in terms of targeted antimicrobial use. Such protocols, however, should be easy to follow, to support compliance. We consider incorporating on-farm tests in the diagnosis and treatment strategy for cows with clinical mastitis of help to support farmers to improve antimicrobial use in 3 ways. First, using on-farm tests may lead to lower usage of antimicrobials in clinical mastitis because cases that likely do not benefit from an antimicrobial treatment, such as culture-negative cases, can be selected. In our study in both on-farm test groups in trial 1, antimicrobials were less often applied to cows with culture-negative LBC results (11% of the culture-negative cows in the CHROMagar group; 8% in the Minnesota group), compared with the control group (22% of the culture-negative cows). If cows with gram-negative on-farm test results were also left untreated, as is done in many parts of the world, 69.1% of the clinical mastitis cases would not have received antimicrobials, which is comparable to the 68.5% reported in other studies in which these cases were left untreated (Lago et al., 2011; Vasquez et al., 2017). Therefore, information on the etiology of IMI from an on-farm test can help in applying antimicrobials targeted to cases that will likely benefit from them and, thus, to reduce antimicrobial use in cases that will not. Second, on-farm tests help farmers to use fewer systemic antimicrobials and, therefore, to apply antimicrobials more prudently. When Minnesota was used, the percentage of cows treated with antimicrobials was re-

duced slightly, but the percentage of cows that received systemic antimicrobials was reduced considerably [12 of 49 cows (24%) in the Minnesota group, compared with 18 of 51 cows (35%) in the control group]. This may improve prudent antimicrobial use, as parenteral mastitis treatments are less targeted than intramammary treatments (Hillerton and Berry, 2005) and may have other collateral effects on antimicrobial resistance. Third, applying on-farm tests might help farmers to postpone treatment and, thus, decrease antimicrobial usage. Although some farmers feel the urge to treat clinical mastitis immediately, treatment of clinical mastitis can be postponed 24 h without affecting clinical cure (Krömker and Leimbach, 2017; Vasquez et al., 2017). In this study, through experimenting with postponed treatments, farmers experienced in practice that delayed treatment is possible without a detrimental effect. Therefore, using on-farm tests may help farmers to gain confidence in the watchful waiting approach, postponing treatment, which allows cows the possibility to self-cure IMI. However, because the observed self-cure is not equal to bacteriological cure, on-farm test results could be used to decide on the wisdom of leaving a "self-cured" case untreated. Thus, both tests can be used by farmers in an on-farm environment, to quickly differentiate IMI in 3 broad diagnostic etiological groups and therapeutic categories and to better target treatment decisions.

In conclusion, farmers are able to work with the 2 evaluated mastitis tests on-farm. Incorporating the results in their treatment decision-making process resulted in more targeted treatments than when no on-farm test results were used. Based on this study, cure of cows with clinical mastitis was not improved by adding on-farm testing to the diagnosis and treatment strategy of farmers, but because treatments were more focused, such an approach may be advised. For subclinical mastitis, a common diagnosis and treatment strategy based on on-farm testing is not advised because it would lead to an unnecessary increase in antimicrobial usage. On-farm testing may, however, have added value to optimize antimicrobial treatment of cases the farmer decided to treat based on other information. Given the importance of host factors on cure, further research is needed to determine how additional information, such as on-farm test results and farmers' experience, can be combined in the diagnosis and treatment strategy of farmers to enhance prudent antimicrobial use and cure outcomes of cows with clinical and subclinical mastitis.

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ORCIDS

Karien Griffioen https://orcid.org/0000-0003-1499-6948
Annet G. J. Velthuis https://orcid.org/0000-0002-5869-8144
Theo J. G. M. Lam https://orcid.org/0000-0003-4601-2229