
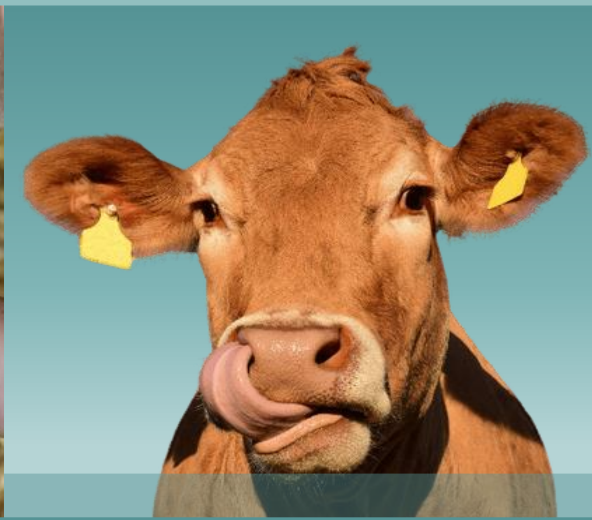


Diana Keller, DVM

Implementation of target-orientated approaches in antibiotic and homeopathic treatment of acute bovine clinical mastitis in dairy practice

Dissertation for the acquisition of the academic degree

Doktorin der Agrarwissenschaften (Dr. agr.)

Submitted to the Faculty of Organic Agricultural Sciences, University of Kassel

Department of Animal Nutrition and Animal Health

Witzenhausen, July 2022

This work has been accepted by the Faculty of Organic Agricultural Sciences at the University of Kassel as a thesis for acquiring the academic degree of Doctor of Agricultural Sciences (Dr. agr.).

Main Supervisor: Prof. Dr. Albert Sundrum, University of Kassel

Co-Supervisor: Prof. Dr. Volker Krömker, University of Applied Sciences and Arts, Hannover

Submission Date: 8 July 2022

Defense Date: 15 December 2022

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Preface

This doctoral thesis is submitted to the Faculty of Organic Agricultural Sciences at the University of Kassel as partial fulfilment of the requirements for the degree of Doctor of Agricultural Sciences (Dr. agr.). The research for this work was mainly carried out within the IMPRO project (Impact matrix analysis and cost-benefit calculations to improve management practices regarding health status in organic dairy farming), which was funded by the European Union's 7th Framework Programme (FP7) for research, technological development and demonstration under grant agreement n° 311824. The Speed Mam Color™ study was gratefully financially supported by the University of Kassel.

The application for the study was reviewed and approved by the Ethical Committee of the European Commission. Ethical concerns were carefully checked in advance by all participating institutions (University of Kassel, IRTA, BIOEPAR/INRA/Oniris). All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The RCT study is registered under NTP-ID 00008011-1-9 at the German Federal Institute for Risk Assessment (BfR).

Acknowledgement

Meinen aufrichtigen Dank möchte ich zunächst meinem Doktorvater Albert Sundrum aussprechen. Durch Sie habe ich gelernt, etablierte Ansätze und meine eigenen Ideen kritisch zu hinterfragen, Probleme und Lösungen präzise zu formulieren, und nie aufzugeben, wenn sich das Projekt in einer scheinbaren Sackgasse befand. Ich danke Ihnen auch für die zielführenden, wenn auch manchmal sehr nervenaufreibenden Diskussionen, welche mir immer wieder den Weg gewiesen haben, wenn ich Gefahr lief vom Thema abzudriften. Herzlich bedanken möchte ich mich des Weiteren für „die zeitliche Freiheit“, die Sie mir in der Endphase gewährt haben, die es mir erlaubte, diese Dissertation zu Ende zu bringen. Durch Sie bin ich zu der Wissenschaftlerin geworden, die ich heute bin.

Des Weiteren möchte ich Caroline Doehring danken, meiner wohl erbarmungslosesten Kritikerin. Aus einer Witzenhäuser Kollegin ist für mich eine wichtige Freundin geworden, die ich nicht mehr missen möchte. Durch dich war der Start in den neuen Lebensabschnitt leichter als befürchtet. Unsere gemeinsame Arbeit hat es uns ermöglicht einige fremde Städte und Länder kennenzulernen, neue Kulturen zu entdecken, zu Kongressen und anderen geistigen Ufern aufzubrechen. Du hast mich immerzu motiviert weiterzumachen und nicht aufzugeben auf dem Weg zur Erlangung der Doktorwürde. Ich danke dir für deine schonungslose Ehrlichkeit hinsichtlich meiner geistigen Ergüsse, die produktiven Schreibworkshops und deine großartigen Denkanstöße. Dir habe ich das Erlernen neuer Fähigkeiten und Entdecken neuer Leidenschaften zu verdanken. Um es kurz zu machen: You are amazing!

Meinen Witzenhäuser Kolleginnen (Susanne Hoischen-Taubner, Margret Krieger und Eva-Marie Schwabenbauer) möchte ich an dieser Stelle für die vielfältige Unterstützung danken. Ihr habt mir den Einstieg ins Forscherleben sehr erleichtert. Ich danke euch für die anregenden Diskussionen, euren wertvollen Rat in allen Forschungsfragen und euer kritisches Feedback zu meinen Studienergebnissen, die mich dazu veranlasst haben, meine Ideen aus verschiedenen Perspektiven zu betrachten. Dies hat meine Dissertation ungeheuer bereichert.

I would also like to express my deep gratitude to Isabel Blanco Penedo and Manon De Joybert who supported me throughout. Thank you for your patience, your motivation and your valuable feedback. I have always appreciated the cohesion and respect of this working group. There is no doubt that this time together with the researchers from the IMPRO project will always be something special to me. I am also sincerely grateful to all participating farmers for welcoming us on their farms and for sharing their thoughts and attitudes towards the use of homeopathy with us. Thank you, too, to the veterinarians from IAVH who participated at the farm visits for their constant support, valuable information in the field of homeopathy and excellent discussions within the IMPRO project.

Vor allem aber möchte ich meiner Familie für ihre immerwährende Unterstützung und den Glauben an mich, diese Doktorarbeit eines Tages zu Ende bringen zu können, danken. Eine größere Motivation, als immer wieder gefragt zu werden, wann ich gedenke meine Promotion zu beenden, gibt es wahrscheinlich nicht^^. Ihr wart immer da, wenn ich die Nähe gebraucht habe und habt mir gleichzeitig die Freiheit gegeben alle meine „Pläne zur Eroberung der Weltherrschaft“ in die Tat umzusetzen. Auch in Zeiten des Misserfolgs und der Schwäche seid ihr immer mit Rat, tröstenden Worten oder einem guten Glas Whisky für mich da gewesen.

Einen besonderen Dank möchte ich auch an Sebastian richten, der wohl am meisten während des Projektes „Doktorarbeit“ zurückstecken musste. Ich danke dir, dass du mich von Anfang an bedingungslos unterstützt und gestärkt hast. Du hast mir den Freiraum gegeben wann immer ich ihn brauchte und mir alltägliche Erledigungen abgenommen damit das Schreiben nicht zu kurz kam. Danke für deine Ausdauer mich ständig zu motivieren und deinen unendlichen Glauben in meine Fähigkeiten. Du hast mir gezeigt, dass die Welt nicht gleich untergeht, wenn man mal eine kurze Pause einlegt. Ich danke dir auch für dein offenes Ohr an Tagen, die nicht von Erfolg gekrönt waren.

Der Weg zur Erreichung der Doktorwürde war länger und steiniger als erhofft, aber dank all der Unterstützung und Anleitung durch die genannten wunderbaren Menschen ist mir die Luft dabei nie ausgegangen.

Vielen Dank.

To Mum

- You will always be loved and never forgotten -

“I was taught that the way of progress was neither swift nor easy”

(Marie Curie, 1924)

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Abstract

Implementation of target-orientated approaches in antibiotic and homeopathic treatment of acute bovine clinical mastitis in dairy practice

The primary objective of this work was to identify initial and boundary conditions (prerequisites) for a high treatment success in antibiotic and homeopathic mastitis treatment on dairy farms and to identify options for increasing cure rates while simultaneously reducing antibiotic consumption.

A double-blind, randomized controlled trial (RCT) was conducted on four dairy farms in Germany involving 180 dairy cows to assess the therapeutic effects of antibiotic and homeopathic mastitis treatment, considering a *lege artis* treatment procedure and the principles of classical homeopathy (Paper I). An investigation into the existing prerequisites for antibiotic and homeopathic treatments of clinical mastitis on dairy farms was carried out on 64 dairy farms in France, Germany and Spain by means of extensive interviews (Paper II). The potential contribution of an on-farm rapid mastitis test (Speed Mam Color™) to a more targeted use of antibiotics in mastitis treatment was examined in a diagnostic accuracy study (Paper III).

The RCT study clearly showed that the antibiotic mastitis treatment was superior to individualised homeopathy. However, even when using antibiotic remedies, only suboptimal cure rates were achieved. The antibiotic treatment method provided bacteriological cure rates of 60–80%, whereas individualised homeopathy only achieved a successful bacteriological cure in 33–43% of cases when a mastitis pathogen was identified. In the case of culture-negative milk samples and *E. coli* infections, the antibiotic (77–100%), homeopathic (50–95%) and placebo treatment (60–100%) showed comparable results. The results suggest that the effectiveness of homeopathy in mastitis treatment does not go beyond a placebo effect (bacteriological cure rate after placebo use: 45–47%). The study results additionally indicate that a successful mastitis treatment is highly dependent on the causative mastitis pathogen, and milk sample analyses for diagnostic purposes are essential for a more targeted treatment strategy.

The evaluation of the status quo survey with regard to the prerequisites for mastitis treatment showed that no *lege artis* treatment procedure (including anamnesis and

clinical examination, formulating a diagnosis, selecting an appropriate remedy, checking treatment outcome and documenting all treatment steps) exist on the dairy farms. Rather, it appears that each farmer developed their own treatment strategy. For selecting a corresponding homeopathic remedy, profound knowledge of the principles of classical homeopathy is essential. The assessment of the knowledge of homeopathic principles was carried out by means of extensive interviews with farmers. The results of the qualitative assessment showed that about three quarters of the farmers interviewed had little or only basic knowledge of the principles of classical homeopathy. Of the farmers interviewed, 22% used complex remedies and 51% made use of "approved indications". It also became evident that about 75% of the homeopathic remedies used were only intended for human medical use, and on 11 dairy farms colchicine and aristolochia were found, which are banned for the treatment of food-producing animals. Furthermore, 50% of the interviewed farmers did not document their homeopathic mastitis treatment.

Antibiotic mastitis treatment is often also not target-orientated in dairy practice since antibiotics are generally used without knowledge of the causative mastitis pathogen. On-farm mastitis tests (e.g., Speed Mam Color™) for the rapid identification of mastitis pathogens in milk samples have been developed for evidence-based mastitis treatment as conventional milk sample analyses in a milk laboratory are time-consuming. However, the use of rapid on-farm mastitis tests is only recommended if these can produce reliable results that are comparable to a standard laboratory test. For this reason, a diagnostic accuracy test – the Speed Mam Color™ test – for identifying mastitis pathogens and determining their susceptibility to antibiotic agents was validated in comparison to conventional milk analysis in a milk laboratory. The study results showed that the Speed Mam Color™ test delivered reliable results in the identification of functional groups of bacteria (sensitivity 88%, specificity 81%). However, the Speed Mam Color™ test was less accurate in determining pathogen susceptibility to antibiotics and should therefore currently not be used when selecting an antibiotic remedy for mastitis treatment.

In summary, it can be stated that the use of homeopathy is not a convincing alternative to antibiotic mastitis treatment due to inadequate treatment prerequisites found on dairy farms and due to no detectable positive therapeutic effect for mastitis treatment. Despite optimised study conditions, the antibiotic treatment method also showed

suboptimal cure rates. There is therefore a need to implement a *lege artis* treatment procedure when using antibiotic remedies. Implementing the use of the Speed Mam Color™ test is recommended, as this can be used for an initial estimation of a treatment's merit and for avoiding contraindicated antibiotic use. In conclusion, it can be stated that there is a high potential for increasing treatment success while simultaneously reducing antibiotic use in mastitis therapy when a *lege artis* treatment procedure is implemented and the Speed Mam Color™ test (including target-orientated mastitis treatment) is used. Whether this treatment strategy leads to an improvement in udder health or a reduced use of antibiotics on dairy farms in the long term can only be assessed by a comprehensive monitoring of treatment methods and cure rates at the herd level. Appropriate control mechanisms need to be implemented in dairy practice to ensure this.

Zusammenfassung

Therapie von klinischen bovinen Mastitiden: Implementierung eines zielorientierten Behandlungsansatzes mit antibiotischen und homöopathischen Arzneimitteln auf Milchviehbetrieben

Übergeordnetes Ziel der Arbeit war die Identifikation der Rahmenbedingungen (Voraussetzungen) für einen hohen Behandlungserfolg bei der antibiotischen und homöopathischen Mastitistherapie auf Milchviehbetrieben und die Identifikation möglicher Optionen zur Erhöhung der Heilungsraten. Darüber hinaus wurde die Steigerung des Behandlungserfolges unter der Maßgabe eines reduzierten Antibiotikaverbrauchs untersucht.

Auf vier Milchviehbetrieben in Deutschland erfolgte eine doppeltverblindete, randomisierte und kontrollierte Studie (RCT) an 180 Milchkühen zur Beurteilung therapeutischer Effekte der antibiotischen und homöopathischen Mastitistherapie unter Berücksichtigung eines *lege artis* Behandlungprozederes und den Prinzipien der klassischen Homöopathie (Paper I). Eine Untersuchung von Behandlungsvoraussetzungen, welche auf Milchviehbetrieben hinsichtlich der Anwendung von antibiotischen und homöopathischen Arzneimitteln bei Behandlung von klinischen Mastitiden vorliegen, erfolgte auf 64 Milchviehbetrieben in Frankreich, Deutschland und Spanien mittels umfangreicher Interviews (Paper II). In einer dritten Studie (Paper III) wurde der potenzielle Beitrag eines on-farm Mastitis Schnelltests (Speed Mam Color™) zu einem zielgerichteteren Einsatz von Antibiotika in der Behandlung von klinischen Mastitiden untersucht.

In der RCT-Studie zeigte sich, dass die antibiotische Mastitisbehandlung dem individualisierten homöopathischen Therapieansatz überlegen war. Allerdings wurden auch mit Anwendung von Antibiotika nur suboptimale Heilungsraten erzielt. In Mastitisfällen, in denen ein euterpathogener Erreger festgestellt wurde, erzielte die antibiotische Behandlungsmethode bakteriologische Heilungsraten von 60-80%, während unter Anwendung der individualisierten Homöopathie nur in 33-43% der Mastitisfälle eine erfolgreiche bakteriologische Heilung erreicht werden konnte. Im Falle von keimfreien Milchproben und bei *E. coli* Infektionen zeigten die antibiotische (77-100%), die homöopathische (50-95%) und die Placebo-Behandlung (60-100%) vergleichbare Heilungsraten. Aus den Ergebnissen wird geschlussfolgert, dass die

Wirksamkeit von homöopathischen Arzneimitteln in der Mastitistherapie nicht über einen Placebo-Effekt hinaus geht (bakteriologische Heilungsrate nach einer Placeboanwendung: 45–47 %). Darüber hinaus deuten die Studienergebnisse darauf hin, dass eine erfolgreiche Mastitisbehandlung in erheblichem Maße vom verursachenden Mastitiserreger abhängt und eine zu diagnostischen Zwecken durchgeführte Milchprobenanalyse unverzichtbar für eine zielführendere Behandlungsstrategie ist.

Die Auswertung der Status Quo-Erhebung hinsichtlich der Behandlungsvoraussetzungen in der Mastitistherapie ergab, dass auf den Milchviehbetrieben kein *lege artis* Behandlungsprozedere (Anamnese, klinische Untersuchung, Diagnosestellung, Arzneimittelauswahl, Behandlungskontrolle und Dokumentation) erkennbar war. Vielmehr hatte es den Anschein, dass jeder Landwirt sich eine eigene Verfahrensweise zu eigen machte. Zur Auswahl eines auf das Krankheitsbild abgestimmten homöopathischen Arzneimittels sind Kenntnisse in den Prinzipien der klassischen Homöopathie essenziell. Eine Beurteilung der Kenntnisse in den homöopathischen Prinzipien erfolgte mittels umfangreicher Interviews mit Landwirten. Das Ergebnis einer qualitativen Beurteilung ergab, dass ca. dreiviertel der befragten Landwirte kaum oder nur grundlegende Kenntnisse in den Prinzipien der klassischen Homöopathie aufwiesen. 22% der befragten Landwirte wendeten Komplexmittel an; 51% griffen bei Mittelauswahl auf „bewährte Indikationen“ zurück. Es zeigte sich auch, dass etwa 75% der zur Anwendung kommenden homöopathischen Arzneimittel nur für den humanmedizinischen Gebrauch bestimmt sind; auf 11 Milchviehbetrieben wurden sogar für die Behandlung von lebensmittelliefernden Tieren verbotene Homöopathika (Colchicin und Aristolochia) gefunden. 50% aller befragten Landwirte verfügten über keinerlei Dokumentation ihrer homöopathischen Mastitisbehandlung.

In der Milchviehpraxis verläuft auch die antibiotische Mastitistherapie oft nicht zielgerichtet. Häufig erfolgt die Anwendung von Antibiotika ohne Kenntnis des verursachenden Mastitiserregers. Da konventionelle Milchprobenanalysen in einem Milchlabor zeitaufwendig sind, wurden für eine evidenzbasierte Mastitistherapie on-farm Mastitis-Schnelltests (e.g., Speed Mam Color™ Test) zur schnellen Identifizierung von Mastitiserregern in Milchproben entwickelt. Der Einsatz dieser Schnelltests ist jedoch nur sinnvoll, wenn sie vergleichbare Ergebnisse im Vergleich zur konventionellen Milchprobenanalyse liefern. Aus diesem Grund erfolgte eine Validierung der diagnostischen Genauigkeit des Speed Mam Color™ Tests bezüglich der Identifizierung von Mastitiserregern in Milchproben und Bestimmung deren Antibiotikaresistenzen im Vergleich zur konventionellen Milchprobenanalyse in einem Milchlabor. Der Speed Mam Color™ Test zeigte zuverlässige Ergebnisse in der Identifizierung von Mastitiserregern: Sensitivität 88% und Spezifität 81%. In der Bestimmung der Antibiotikaresistenzen war der Speed Mam Color™ Test jedoch weniger genau und sollte daher derzeit nicht bei der Auswahl eines Antibiotikums in der Mastitisbehandlung berücksichtigt werden.

Zusammenfassend kann anhand der Studienergebnisse festgestellt werden, dass die Anwendung von Homöopathie auf Grund mangelhafter Behandlungsvoraussetzungen und unzureichender Wirksamkeit keine überzeugende Alternative zur antibiotischen Mastitistherapie darstellt. Auch unter optimierten RCT-Bedingungen zeigte die antibiotische Behandlungsmethode suboptimale Heilungsraten. Daher besteht auch bei deren Anwendung die Notwendigkeit der Implementierung eines *lege artis* Behandlungsprozederes. Der Einsatz des Speed Mam Color™ Test ist zur Vermeidung unnötiger Antibiotikaanwendungen geeignet, da die initiale Entscheidungsfindung, ob eine antibiotische Therapie sinnvoll erscheint, befördert wird. Abschließend kann konstatiert werden, dass ein hohes Potenzial zur Erhöhung des Behandlungserfolgs bei gleichzeitiger Reduzierung des Antibiotikaeinsatzes in der Mastitistherapie besteht, wenn die Implementierung eines *lege artis* Behandlungsprozederes und die Anwendung des Speed Mam Color™ Test inklusive einer zielgerichteten Mastitistherapie erfolgt. Ob diese Behandlungsstrategie langfristig zu einer Verbesserung der Eutergesundheit bzw. reduzierter Anwendung von Antibiotika auf Milchviehbetrieben führt, lässt sich nur mittels eines umfassenden Monitorings der Behandlungsverfahren und der Heilungsraten auf der Herdenebene beurteilen. Dafür müssten geeignete Kontrollmechanismen in der Milchviehpraxis implementiert werden.

List of publications

This doctoral thesis is based on the work of the following publications referred to by Roman numerals in the text:

I. Keller, D.; Sundrum, A. 2018. Comparative effectiveness of individualised homeopathy and antibiotics in the treatment of bovine clinical mastitis: randomised controlled trial. (*Published in Veterinary Record,* DOI: 10.1136/vr.104555)

II. Keller, D.; Blanco-Penedo, I.; De Joybert, M.; Sundrum, A. 2019. How target-orientated is the use of homeopathy in dairy farming? – A survey in France, Germany and Spain. (*Published in Acta Veterinaria Scandinavica*, DOI: 10.1186/s13028-019-0463-3)

III. Keller, D.; Sundrum, A. 2022. Reliability of the Speed Mam Color™ test and its potential for reducing antibiotic use in bovine clinical mastitis: A diagnostic accuracy test? (*Submitted to PLOS ONE in 2022*.)

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List of abbreviations

AP	Apparent prevalence
CV	Coefficient of variation
DVG	German Veterinary Association
e.g.	Latin exempli gratia, meaning “for example”
EMEA	European agency for the evaluation of medicinal products
EU	European Union
FN	False negative
FP	False positive
IAVH	International Association for Veterinary Homeopathy
IDF	International Dairy Federation
i.e.	Latin id est, meaning “that is”
ITT	Intension-to-treat principle
κ	Cohen’s kappa coefficient
μ	Mean value, average
n	Sample size
NPV	Negative predictive value
NSAID	Non-steroidal anti-inflammatory drug
P	P-value
PPV	Positive predictive value
RCT	Randomised controlled trial
s	Standard deviation
SCC	Somatic cell count
Se	Sensitivity
Sp	Specificity
TN	True negative
TP	True positive
TrP	True prevalence

1. General introduction

Bovine clinical mastitis is characterised by an often painful inflammation of the mammary gland mainly caused by pathogenic bacteria (Winter et al., 2009). It is one of the "big three" production diseases in dairy farming with the highest prevalence (Pol and Ruegg, 2007; Ruegg, 2017). According to the International Dairy Federation (2011), clinical mastitis is defined as an udder inflammation characterised by visible abnormalities in the milk or udder. Three severities of clinical cases can be differentiated: mild, moderate and severe. Mild clinical mastitis is characterised by observable abnormalities in milk, generally clots or flakes, with little or no signs of swelling of the mammary gland or systemic illness. Visibly abnormal milk, accompanied by swelling in the affected mammary quarter with an absence of systematic signs of illness is described as moderate clinical mastitis (IDF, 2011). Severe clinical mastitis is described by the IDF (2011) as an udder inflammation characterised by a sudden onset, with grave systemic and local symptoms. Mastitis not only causes pain and suffering in the affected animal (animal welfare aspect), it also has a major impact on herd health, food safety and the cost-benefit analysis of dairy farms. According to the Animal Welfare Act (BMEL, 2021), which stipulates that every animal owner must treat their affected animals, in cases where a cow is suffering from clinical mastitis, an appropriate mastitis treatment should be provided immediately.

The most common treatment approach in dairy production worldwide for treating bacterial infections in the udder of dairy cows is the use of antibiotics (Lago et al., 2011; Mansion-de Vries et al., 2014; Francoz et al., 2017). The mode of action of antibiotics is well known. Their most relevant points of attack against bacteria are cell walls, cell membranes, DNA and RNA synthesis, protein biosynthesis and the metabolism of folic acid (Estler and Schmidt, 2006). Antibiotics can kill the invading bacteria or slow down bacterial growth. Undoubtedly, antibiotics have contributed substantially to animal health and welfare and the relief of suffering, and have significantly decreased morbidity and mortality rates in food-producing animals (Hao et al., 2014). However, the antibiotic treatment method has become a subject of increasing criticism in recent years as the overuse of antibiotics is responsible for a significant increase in the prevalence of antibiotic resistance in animals and humans (Laxminarayan et al., 2013; ECDC/EFSA/EMA, 2015; Kuipers et al., 2016; Lees et al., 2017b). Since 1976, it has been known that livestock animals are a key reservoir for antibiotic-resistant pathogens and that antibiotic use results in the transmission of antibiotic-resistant pathogens to humans (Levy et al., 1976; Marshall and Levy, 2011; Founou et al., 2016; Koch et al., 2017). There is also a risk of toxicity to the treated animal (side effects) and antibiotic residues in food (Lees et al., 2017b; Lago et al., 2011), as well as of spreading resistance factors into the environment (Lees et al., 2017b). Regarding the responsible use of antibiotics in livestock and a reduction in the development of pathogen resistance, there are different legal regulations that apply to farmers and veterinarians. First, there is a legally stipulated withdrawal period according to the Commission Implementing Regulation (2018). A withdrawal period is the time that must be observed after the final administration of a remedy to a food-producing animal until the time of the production of food from the animal in order to ensure that any residues do not exceed certain maximum quantities for pharmacologically active substances. Second, there are also regulations for the maximum level of antibiotic usage in organic livestock (Commission regulation, 2008). Furthermore, there are bans on the prescription of and conditions for the application and rededication of certain antibiotic active substance classes (Commission Implementing Regulation, 2018; EMA, 2022). Rededication is the authorization of medicinal substances that are necessary due to therapeutic emergencies that were not originally authorized for the respective animal species or indication. Because of all these different regulations, farmers see themselves exposed to the problem of being unable to treat their animals suffering from diseases. In the absence of alternatives to antibiotic use, farmers often consider other treatment approaches and turn to homeopathy (Hovi and Roderick, 2000; Hektoen et al., 2004; Leon et al., 2006).

Homeopathy is enjoying increasing popularity as an alternative method of mastitis treatment as it entails some benefits in its use compared to antibiotic treatment, such as low or no withdrawal periods, the rapid availability of remedies (no veterinary consultation is required), low costs and complying with consumers' wishes for residue-free food (Leon et al., 2006). Despite the apparent advantages compared to conventional treatments, the use of homeopathy is controversially discussed. Critics claim that the beneficial effects of homeopathic remedies are based entirely on a placebo effect (Shang et al., 2005; Lees et al., 2017b), whereas proponents are convinced that homeopathy is more effective than placebo (Weiermayer et al., 2020). Until now, in an analysis of peer-reviewed publications, verifying the efficacy of homeopathic remedies has revealed only inconsistent results (Mathie and Clausen, 2014, 2015a; Mathie and Clausen, 2015b; Doehring and Sundrum, 2016).

Notwithstanding the advantages and disadvantages or personal convictions towards conventional and alternative treatment methods, increasing treatment success (total cure of the disease) and reducing the prevalence of mastitis should always be paramount when treating diseased animals. According to the maxim of “preventing avoidable and unnecessary suffering”, the treatment method that can provide best possible treatment success should always be selected. Treatment success can be measured based on cure rates, which depend on the efficacy and effectiveness of the administered active substance and on the prevailing treatment prerequisites. Efficacy measures the effect of a treatment under standardised conditions, whereas effectiveness measures the therapeutic effect under real life conditions (Marchand et al., 2011; Singal et al., 2014). It can be assumed that optimal prerequisites ensure a best possible treatment and can considerably support the treatment success. A *lege artis* treatment procedure includes, inter alia, an anamnesis and clinical investigation, the selection of an appropriate remedy and an evaluation of the therapeutic outcome (Baumgartner and Wittek, 2018). It is the basis for both the antibiotic and the homeopathic treatment approaches. Furthermore, expertise and experience in homeopathy and being familiar with homeopathic principles are essential when treating diseased animals with homeopathic remedies. However, the status of prerequisites for the use of homeopathy on dairy farms has not yet been investigated.

Randomised controlled trials (RCTs) are widely accepted as the gold standard for clinical research on the efficacy or effectiveness of medicinal products as they deliver the most reliable results (Pocock, 1984; Kaptchuk, 2001; Kabisch et al., 2011). Various clinical studies comparing the efficacy or effectiveness of antibiotic and homeopathic remedies are available, but most of them lack a degree of scientific quality, resulting in heterogeneous results (Doehring and Sundrum, 2016). Mathie & Clausen (2014, 2015a; 2015b) strongly indicate the need for new and substantially improved research in veterinary homeopathy. An RCT was therefore conducted in consideration of the best possible treatment conditions and to avoid weak points identified in previous study designs (e.g. risk of bias, small sample size) according to Cochrane’s requirements for RCTs (Ryan et al., 2013), the RCT requirements of the European Agency for the evaluation of medicinal products (EMEA, 2000, 2003) and the basic principles of homeopathy (Hahnemann and Schmidt, 1992).

For the improvement of cure rates and a reduction in antibiotic consumption, a target-orientated use is mandatory. This is characterised by the identification of mastitis cases that require antibiotic treatment. In some cases of mastitis, the administration of antibiotic remedies can be classified as contraindicated (Lago and Godden, 2018). Furthermore, the selection of an appropriate remedy according to the functional groups of bacteria that have caused the mastitis is required (Mansion-de Vries et al., 2015; Lago and Godden, 2018). Various studies show that antibiotic treatments are not always successful, or their use is contraindicated for different reasons (Roberson, 2012; Lago and Godden, 2018; Suojala et al., 2013). First, the treatment success for mastitis caused by yeasts (*Aspergillus fumigatus*, *Pseudomonas* spp., *Mycoplasma* spp., *Nocardia* spp., *Klebsiella* spp. and *Prototheca*) is essentially zero (Winter et al., 2009). Second, mastitis cases where the immune system has already cleared the mastitis pathogen (culture-negative) do not benefit from antibiotic therapy, and thus the use of antibiotics is highly questionable (Roberson, 2003, 2012). Third, mild and moderate udder infections caused by *Escherichia coli* that were untreated or antibiotic-treated showed no differences in cure rates (Pyörälä et al., 1994; Suojala et al., 2013; Mansion-de Vries et al., 2015). Finally, there are natural and acquired resistances of pathogens to antibiotics. For all these reasons, an analysis of milk samples is essential before a mastitis treatment is provided.

The gold standard in identifying functional groups of bacteria is a cyto-microbiological analysis in a certified milk laboratory. Nonetheless, the standard milk analysis has several weak points, such as the costs and time involved in taking the milk sample, transporting it, analysing it in the laboratory (Mansion-de Vries et al., 2014; Lago and Godden, 2018), the high technical and financial input required for laboratory equipment and the need for comprehensive diagnostic expertise. To reduce delays and additional inputs, and particularly to improve therapeutic success, rapid on-farm mastitis tests have been developed. The Speed Mam Color™ test is intended to provide dairy farmers or veterinarians with quick and reliable results in identifying the functional groups of bacteria and specifying the pathogens' sensitivity to antibiotics. It is intended for the treatment of cows suffering from clinical mastitis, without the delays arising from conventional laboratory milk analysis, and the selection of an appropriate treatment strategy as rapidly as possible. The improvement of mastitis treatment strategies and mastitis diagnostics is expected to increase udder health and animal welfare and simultaneously reduce antibiotic consumption and antibiotic resistance.

1.1. Basic principles of homeopathy

To better understand certain aspects regarding the use of homeopathy, a brief abstract of the basic principles of classical homeopathy is provided.

The term "homeopathy" is derived from the Greek words for "like" and "suffering". Homeopathy was developed by Samuel Hahnemann (1755-1843), a German physician and philosopher in 1796. The most notable principles of classical homeopathy are:

1. *The law of similars (similia similibus curantur) and proving*.
2. *The law of infinitesimals (potentization): dilution and succussion*.
3. *The minimum dose*.
4. *Totality and the law of individualisation*.

According to the fundamental principal *law of similars*, signs and symptoms can be cured by substances that can cause those signs and symptoms in healthy individuals. The testing of homeopathically-prepared substances in healthy individuals is called *proving*, and reveals the therapeutic effects and possible health disturbances that the substance induces.

The *law of infinitesimals* states that the more diluted an active substance in a medically inert solution (usually water or alcohol) is, the more potent it becomes over a large range of dilutions. A starting solution, called the *mother tincture* of the active substance (herbs/plants, minerals, venom from snakes, blood, pus etc.) is diluted either 1:10 (decimal, D-Potency) or 1:100 (centesimal, C-Potency), with the resulting solution again diluted by the same degree, and so on in a continuous process. The remedy is further processed by a specific type of vigorous shaking at each dilutional stage, which is believed to *potentise* or *dynamise* the homeopathic remedy.

When conducting his proving according to the *law of similars,* Hahnemann recognized that the dosage of the substances he used often caused toxic reactions in the provers and patients. To avoid these toxic reactions, he began to dilute the remedies. The more the medicines were diluted, the gentler and more effective was their curative action.

It is important to treat the individual holistically and not by focussing only on specific disease categories or medical diagnoses. The organism is considered to be a unity of soul, mind and body (Schütte, 2002). Diseases are represented by the totality of the symptoms. Individualised homeopathy is based on the premise that no two individuals are exactly the same and that each medicine has unique characteristics and covers unique symptoms. The most challenging part of a homeopathic treatment is the *individualisation*, as well as finding the corresponding remedy, the *similimum*, that best matches all the symptoms and characteristics in the diseased animal to cure the disease rapidly, gently and sustainably (Hahnemann and Schmidt, 1992; Day, 2001).

The mode of action of homeopathic remedies is not yet known. There are various hypotheses about the mechanism of action of homeopathic remedies. Homeopaths often refer to their remedies as *balancing* unspecified *energies* in the body, correcting a disturbance of the body's *vital force* (Schütte, 2002; Lees et al., 2017a) or stimulating the body's own healing responses (Day, 2001). It is important that the disease passes through all stages only with support of all the self-mobilised defence reactions of the body. Real healing can only be achieved when the real course of the disease has been undergone (Schütte, 2002).

One of the earliest attempts at theoretically explaining the potential effects is the *imprint theory* postulated by Barnard and Stephenson (1967), which states that an imprint of homeopathic information by the molecules dissolved in the remedy is produced by the potentization process, conserved, multiplied by further potentization, and then communicated to the body and taken up as a biologic signal (Barnard and Stephenson, 1967). In 1988, Benveniste claimed that water has an imprint of energies (*memory of water*) to which it has been exposed (Davenas et al., 1988), but other scientists refuted his hypotheses (Maddox et al., 1988; Ovelgönne et al., 1992; Hirst et al., 1993). Chikramane et al. (2010) developed the *nano-particle theory* as the presence of nano-particles of the *active substance* in extreme dilutions was found.

Donauer's first publication in 1815 showed that homeopathy has a tradition in veterinary medicine that is almost as long as in human medicine (Striezel, 2005), although veterinary medicine received only little attention in Hahnemann's extensive literature. However, in a short 12-page essay, Hahnemann postulated that animals can be cured from diseases as safely and confidently as human beings (Schütte, 2002).

2. Aims of the thesis

The overall aim of this doctoral thesis was to provide further knowledge on how to improve treatment success and simultaneously reduce antibiotic consumption in the case of bovine clinical mastitis in consideration of antibiotic and homeopathic treatment methods.

The specific aims were:

I. To conduct a double-blind RCT according to CONSORT guidelines in order to assess the effectiveness of homeopathic treatments in cases of bovine clinical mastitis compared to antibiotic treatments when best possible treatment conditions are present.

II. To determine the status quo on the use of homeopathy in dairy farms.

III. To assess the extent to which farmers consider homeopathic principles and to which degree they implement a *lege artis* treatment concept for antibiotic and homeopathic treatments in cases of bovine clinical mastitis on dairy farms.

IV. To determine the reliability of the Speed Mam Color™ test to detect functional groups of bacteria and pathogen susceptibility to antibiotics in comparison to the standard laboratory analysis of milk samples.

3. I. Comparative effectiveness of individualised homeopathy and antibiotics in the treatment of bovine clinical mastitis: randomised controlled trial

Diana Keller [1, *], Albert Sundrum[1]

* Corresponding author

[1] Department of Animal Health and Animal Nutrition, University of Kassel, 37213 Witzenhausen, Germany

Published in Veterinary Record, DOI: 10.1136/vr.104555

Abstract

Background

Based on the widespread use of homeopathy in dairy farm practice when treating mastitis, a blind randomised controlled trial (RCT) was conducted to assess the effectiveness of homeopathic treatment of clinical mastitis on four dairy farms. The study considered specific guidelines for RCTs as well as the basic principles of individualised homeopathy and involved 180 lactating dairy cows. Evaluation of cure rates was based on clinical investigation of the udder and on laboratory analysis of milk samples. In culture-positive cases, the antibiotic treatment provided suboptimal bacteriological cures (60–81%) but was more effective than individualised homeopathy (33–43%) whose effects appeared little different to those of placebos (45–47%) ($P \leq 0.05$). On the cytological cure level, all three treatment methods were similarly ineffective: antibiotic being 2–21%, individualised homeopathy 0–8% and placebo 3–13% ($P \leq 0.05$; $P=0.13$). Antibiotics, individualised homeopathy and placebo had similar effects on bacteriological and cytological cure in cases of culture-negative milk samples ($P>0.4$) and *Escherichia coli* infections ($P=1.0$). The study results implied that the effectiveness of individualised homeopathy does not go beyond a placebo effect and successful treatment is highly dependent on the specific mastitis pathogen. Thus, antimicrobial or alternative remedies used should be based on the bacterial culture of the milk sample.

Trial registration number NTP-ID: 00008011-1-9

3.1. Introduction

Mastitis is the main reason for antimicrobial use in dairy production worldwide (Erskine et al., 2002; Lago et al., 2011; Mansion-de Vries et al., 2015) and the cause of high economic losses on dairy farms (McCarron et al., 2009; Mansion-de Vries et al., 2015). On the other hand, overuse of antibiotics is responsible for a significant increase in the prevalence of antibiotic resistance (Laxminarayan et al., 2013; ECDC/EFSA/EMA, 2015; Kuipers et al., 2016). Finding alternative treatments is often seen as a way of combating antimicrobial resistance. In this context, homeopathy is enjoying increasing popularity as an alternative mastitis treatment method, particularly on organic farms. On-farm studies (organic farms in Germany and in the south of England and Wales) showed that 34–51% of clinical mastitis cases were treated homeopathically (Hovi and Roderick, 2000; Krömker and Pfannenschmidt, 2005). The European Regulations on organic agriculture also promote the use of homeopathy: homeopathic products shall be used in preference to chemically synthesised veterinary products (Commission regulation, 2008). However, only remedies with positive therapeutic effect for the species of animal, and the condition for which the treatment is intended should be administered. In order to ensure that only effective remedies are administered to diseased animals, medicinal products need proven therapeutic efficacy. Randomised controlled trials (RCTs) are widely accepted as the gold standard for clinical research on the efficacy or effectiveness of medicinal products (Pocock, 1984; Kaptchuk, 2001; Kabisch et al., 2011). A recent review by Doehring and Sundrum (2016) revealed that various clinical studies testing the efficacy or effectiveness of homeopathy returned heterogeneous results. Due to differing scientific approaches and study qualities, some of these studies supported the use of homeopathy while others showed no positive effects. The authors concluded that there was a need to repeat RCTs under various farm conditions before final conclusions on the effectiveness of homeopathic remedies could be drawn. However, when repeating such clinical trials, particular attention should be paid to the study quality. Other authors (Mathie and Clausen, 2014, 2015a; Mathie and Clausen, 2015b) also noted the low number and quality of studies available and strongly indicated new and substantially improved research in both individualised and non-individualised veterinary homeopathy. The present study could contribute to extending current knowledge on the effectiveness of homeopathy by creating one additional high-quality RCT. While considering weak points identified in previous study designs (risk of bias according to Cochrane's evidence-based medicine principles (Ryan et al., 2013), basic principles of classical homeopathy or small sample size)), the aim of the trial (conducted as an RCT) was to examine the comparative effectiveness of treatments for bovine clinical mastitis treated with homeopathic, antimicrobial and placebo remedies on four dairy farms, following homeopathic principles (individualised treatment) and including the best possible treatment conditions (experienced veterinarians in homeopathy, timely and regular follow-up checks and laboratory analyses) in practice.

3.2. Methods

3.2.1. Study sample

The RCT was conducted from June 2016 until the end of December 2016. In total, 180 lactating dairy cows were examined, derived from one organic herd and three conventional herds located in the eastern part of Germany. Herd size varied from 240 to 1500 lactating cows, with a milk yield range from 6.500 to 10.000 kg milk per cow per year. All cows were kept in loose stalls, and the milking routine was conducted in different milking systems (herringbone milking parlour, side-by-side milking parlour, carousel (an internal rotary milking parlour and an external one)). Both pre-milking and post-milking teat disinfection was integrated into the daily milking routine on all farms. Cows were recruited suffering from mild or moderate clinical mastitis according to the definition from the International Dairy Federation (IDF, 2011). Cows exhibiting severe mastitis (presence of fever and/or disturbances of general behaviour) or cows suffering from mastitis in more than one mammary gland were excluded from the study. All animals considered in the study were not suffering from any other clinical disease during the trial period. Furthermore, cows with mastitis caused by *Streptococcus agalactiae* and *Trueperella pyogenes* or with injuries to the teats were excluded because unsuccessful treatment could create long-term damage to the udder. Cows treated with antimicrobial or anti-inflammatory products within the previous 30 days and those with recurrent mastitis were also excluded from the study.

3.2.2. Study design

The study was performed as a double-blind, randomised and placebo-controlled trial which compared the effectiveness of two different treatment strategies (individualised homeopathy and use of antibiotics), taking into account specific guidelines for RCTs (EMEA, 2000, 2003) including CONSORT guidelines for reporting trials (Bennett, 2005) as well as the basic principles of classical homeopathy (Hahnemann and Schmidt, 1992; Day, 2001). The clinical study tried to avoid weak points in study design (blinding or other bias) mentioned by Mathie and Clausen (2015b).

Farmers on four farms, three local veterinarians from one veterinary practice with frequent use of homeopathy, a laboratory assistant and the supervising scientist were involved in the trial. The study enrolled 180 lactating cows using the defined inclusion and exclusion criteria, each with one affected udder quarter. Each case of mild or moderate clinical mastitis was randomly allocated to one of the three treatment strategies: individualised homeopathic (n=60), use of placebo (n=60) or antimicrobial treatment (n=60). Randomisation was ensured by drawing out lots in the form of coloured sticks, stored in opaque boxes. Each stick represented a treatment group and one was drawn before each treatment. In order to keep the number of cows in each treatment group balanced, a total of 60 sticks of each colour were used; 15 sticks of each colour were thus allocated per farm. In comparison with previous studies, the authors implemented standardised homeopathic remedy selection using a predefined procedure, which reduced a possible selection bias towards a favoured remedy to a minimum. The veterinarians' task was the clinical examination of cows suffering from clinical mastitis, repertorisation of symptoms and the assignment of a homeopathic, placebo or antibiotic remedy in each mastitis case, and undertaking the follow-up checks. A pre-test served for the unification of assessment criteria for clinical symptoms and treatment procedures. The farmers were responsible for the administration of the remedies selected, randomisation and observation of animals' health status. The veterinarians and milk laboratory assistants were blinded to the type of treatment over the whole observation period to avoid biased evaluations of treatment success. Farmers were blinded to the homeopathic and the placebo remedies. Knowledge of the antimicrobial products (udder infusions) and the homeopathic/placebo treatments was allowed deliberately for the sake of protection against injuries to the teats or new iatrogenic infections when administering the placebo or homeopathic remedy intracisternally. Differing means of treatment administration were not an issue as the veterinarian was only brought in at the end to evaluate treatment outcome and was blinded beforehand. Correspondingly, the farmers were aware of an antimicrobial treatment when they had to administer the remedies.

3.2.3. Remedies

Twenty-one homeopathic remedies were selected on the basis of the most frequently used pure remedies dedicated for the treatment of animals with mastitis. The selection was made by a software repertory (RadarOpus) and input from a professional veterinary homeopath with long-standing experience in the homeopathic
treatment of food-producing animals: *Aconitum napellus C30, Apis mellifica C30, Belladonna C30, Bryonia alba C30, Calcium fluoratum C30, Calendula officinalis C30, Carbo vegetabilis C30, Cistus Canadensis C30, Conium C30, Hepar sulphuris calcareum C30, Kalium bichromicum C30, Lachesis muta C30, Mercurius solubilis C30, Phellandrium aquaticum C30, Phytolacca decandra C30, Pulsatilla pratensis C30, Pyrogenium C30, Silicea C30, Sulphur C30, Tuberculinum Koch C30 and Urtica urens C30*. Most over-the-counter homeopathic remedies are C30 dilutions, which Hahnemann advocated as the best "usual" dilution of homeopathic remedies (Hahnemann and Schmidt, 1992; Day, 2001; Lees et al., 2017a). All homeopathic remedies (including their clinical remedy picture) were saved in a specially developed software tool which served for standardised repertorisation. Nevertheless, other individual homeopathic remedies which did not appear on the above list were permitted if the veterinarian deemed it necessary. All homeopathic remedies used in the study were produced by Deutsche Homöopathie-Union in Germany. Sugar-based globules without an active ingredient (Globuli Sacchari HAB Gr. 3, Caelo, Germany) were used for the placebo treatment. Both homeopathic and placebo globules—administered in a dosage of 10 globules per day, dissolved in water and administered via syringe (either orally or vaginally), for a period of five days—were identical in their packaging, physical appearance and labelling. Cows allocated to the antibiotic group received the most appropriate antimicrobial product selected by the veterinarian: *Synulox LC Plus, Cloxamycin L, Oxacillin-Na 1000mg-Euter-Injektor, Vetriclox L, Peracef, Ubrolexin, Procain-Penicillin-G Injektor* and *Wedeclox Mastitis*. This was

administered aseptically via udder infusion at the dosage recommended by the manufacturer. The national guidelines for prudent use of antimicrobials in veterinary medicine (Bundestierärztekammer, 2015) were adhered too. Additional remedies such as NSAIDs or udder ointments were not used during the trial.

3.2.4. Treatment procedure

Cows suffering from clinical mastitis as identified by farmers during the daily milking routine (occurrence of clinical symptoms) were subsequently examined by the consultant veterinarian. Those cows which met the inclusion criteria had a milk sample taken from all four udder quarters before the initial treatment. Both clinical and homeopathic symptoms were documented and repertorised individually according to Hahnemann's theory (cross-check of clinical symptom picture with remedy picture) by using a previously developed software tool containing the above-mentioned 21 homeopathic remedies and their corresponding symptoms. The software tool aided standardisation and transparency of the repertorisation procedure. One remedy for each treatment method was allocated to the diseased animal by the veterinarian. Farmers randomised and administered then the allocated remedy — previously determined by the veterinarian — to animal. In order to assess treatment outcomes, the veterinarian, who was completely unaware which treatment method was being used, performed a clinical examination and kept taking milk samples on the 7th, 14th and 28th days post onset of the infection. If the farmer observed a worsening of clinical symptoms or the development of new symptoms during the trial period, the veterinarian examined the affected cow thoroughly and classified the animal as either responsive or non-responsive to the treatment given. The decision whether or not an animal should be excluded from the trial was based on predefined exclusion criteria: body temperature >40°C, considerably reduced thirst or appetite, infection of a second udder quarter, major changes in udder health (occurrence of mammary gland abscesses, gangrenous mastitis) and recumbency. If homeopathic symptoms changed within four days after inclusion or the laboratory results indicated a pathogenic resistance to the antimicrobial agent administered, the veterinarian was allowed to change the remedy while retaining the treatment method. A Consolidated Standards of Reporting Trials flow diagram (see Figure 1) displays the progress of all animals through the trial.

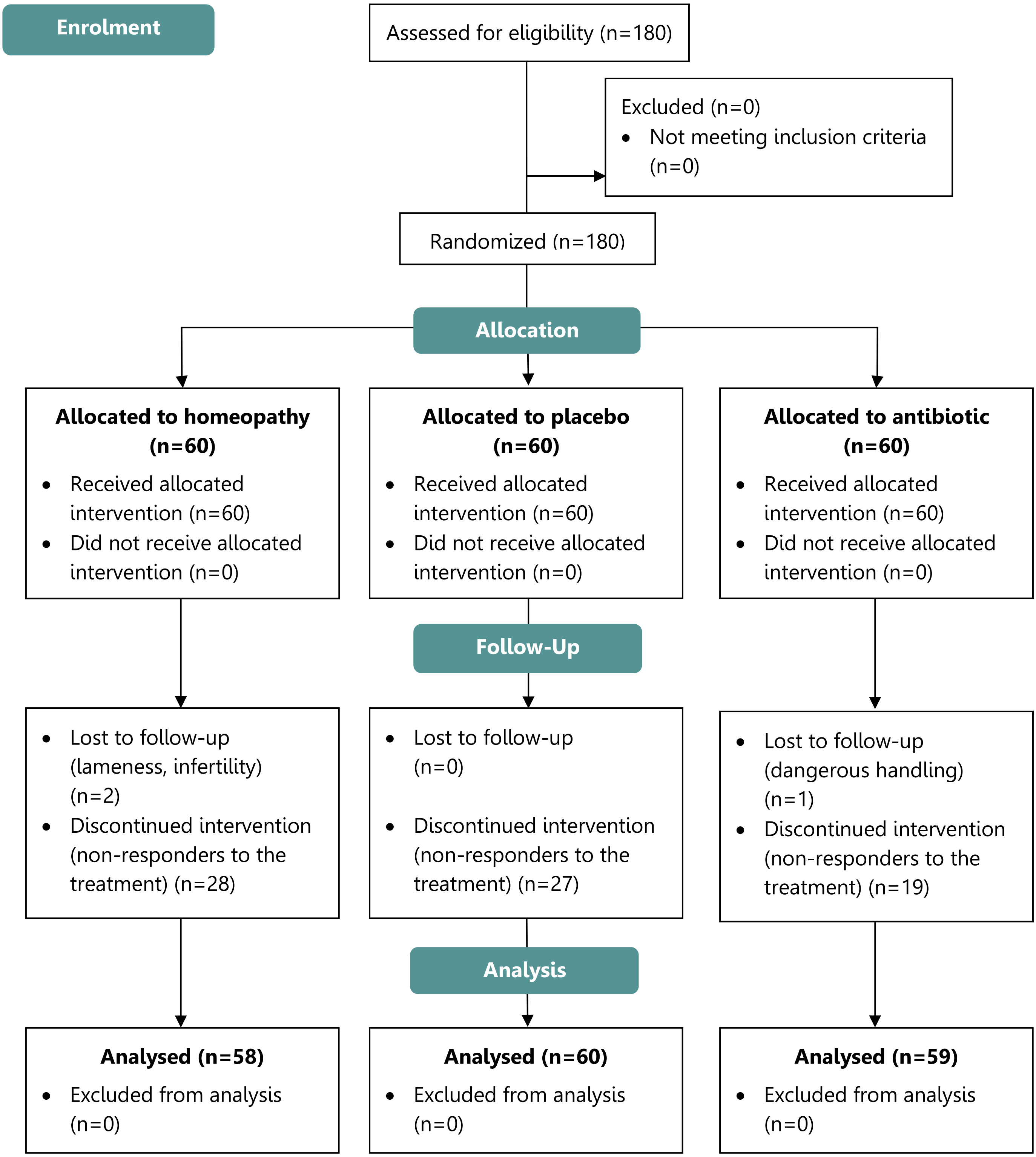


Figure 1. *Consolidated standards according to CONSORT guidelines for reporting trials flow diagram.*

3.2.5. Milk samples and laboratory procedure

According to good clinical practice, milk samples were taken aseptically from all four udder quarters by the veterinarian (Baumgartner and Wittek, 2018) and cyto-bacteriologically analysed by a certified milk laboratory (bovicare, Potsdam, Germany) at days 0, 7, 14 and 28. Pathogens were identified by using a standard agar culture technique, which included bacteriological culture on aesculin blood agar followed by sensory, microscopic and (if necessary) biochemical or serological evaluation of the pathogens. The milk laboratory always ascertained the major pathogen suspected to have caused the clinical mastitis. The somatic cell count (SCC) was also measured by the milk laboratory using a fluorescence method (Integrated Milk Testing MilkoScan FT 6000; Foss, Hamburg). For technical reasons, the SCC could not be determined when the milk deviated significantly from normal (e.g., flocks or clots). In this case, the SCC was assessed with FL+, FL++ or FL+++, depending on the degree of deviation from normal: low, medium or high, respectively.

3.2.6. Classification of outcome

Assessment of the effectiveness of different medical mastitis treatment methods was based on criteria from the European Agency for the Evaluation of Medicinal Products (EMEA, 2003). Despite displaying clinical mastitis symptoms, pathogens were not always identified in routine clinical culture, and in these cases, cows were classified as “culture-negative”. Cure rates were accordingly calculated separately for culture-positive and culture-negative pretreatment milk samples. Cure at a clinical level was ascertained via visual examination of milk and udder palpation and defined as an absence of visible changes in milk and inflammation of the udder. Cows exhibiting no clinical cure were rated as non-responsive to the treatment given. According to the EMEA criteria, bacteriological cure was determined as the elimination of the pathogen present on day 0. Udder quarters were rated as “newly infected” when a new mastitis pathogen (different from the one on day 0) appeared. A newly infected udder quarter was also considered as bacteriological cure. However, from a medical point of view, a newly infected udder quarter cannot be classified as a clinical or bacteriological cure; therefore, criteria for a healthy udder defined by the German Veterinary Association (Fehlings et al., 2012) were also considered. The DVG assessed a bacteriological cure as the elimination of any mastitis pathogens (culture-negative milk sample), but a new udder infection was absence of bacteriological cure. Using DVG's criteria for a healthy udder quarter, a cytological cure was defined when the SCC was below the threshold of 100,000 cells/ml milk (German standard, in other European countries the threshold is below 200,000 cells/ml milk). For the purpose of SCC evaluation, three categories were used: "SCC was higher" (increase of SCC compared with day 0), "SCC was lower" (decrease of SCC until 100,000 cells/ml compared with day 0) and "SCC <100,000 cells/ml" (decrease of SCC below 100,000 cells/ml compared with day 0). The evaluation criteria "total cure" (primary endpoint) was only awarded when a bacteriological, clinical and cytological (SCC <100,000 cells/ml) cure was present at the same time. The primary outcome measure was the elimination of the initial pathogen and a reduction in SCC.

3.2.7. Statistical analysis

SPSS Statistics V.24 (IBM) was used for statistical analysis. All analyses of cure rates were based on udder quarter values and on the intention-to-treat (ITT) principle. Following Gupta (2011), the ITT analysis included all randomised animals in the groups to which they were randomly assigned, regardless of the treatment they actually received and regardless of subsequent withdrawal from treatment. ITT analysis avoids the problems created by omitting dropouts, which can negate randomisation, introduce bias and overestimate clinical effectiveness (Hollis and Campbell, 1999; Gupta, 2011). This means that all 180 animals entered into the study were analysed according to the group they were randomly allocated to at the time of each follow-up check, regardless of whether or not they were excluded from the study before it ended. The evaluation of nominal or categorical parameters was performed by using frequency distribution (contingency table). Significant differences in categorical variables for cure rates within the three treatment groups were tested by chi-squared test and, in case of a frequency distribution of less than five, by using Fisher's exact test. For all comparisons, $P \leq 0.05$ was considered to be significant.

3.3. Results

Cure rates were recorded separately for each follow-up check on days 7, 14 and 28, followed by an analysis of the specific findings. Table 1 shows the initial conditions for mastitis treatment. These did not differ significantly (P>0.30) among treatment groups. In total, 120/180 (66%) pretreatment milk samples showed positive for bacterial growth whereas no bacteria culture could be detected in 60/180 (33%) of cases. Isolated mastitis pathogens were identified as *Streptococcus uberis* (n=45), *Escherichia coli* (n=16) and *Streptococcus dysgalactiae* (n=13), *Klebsiella* spp. (n=9), other aesculin-positive streptococci (n=8), coagulase-negative staphylococci (n=7), *Staphylococcus aureus* (n=7), coliform bacteria (n=5), *Enterococci* spp. (n=5), *Corynebacterium bovis* (n=2), yeasts (n=2) and *Serratia* spp. (n=1).

Table 1 *Initial condition of udder quarters suffering from mild or moderate mastitis by treatment strategy*

			Treatment strategy			
Initial condition		**p-value**	**Individualised homeopathy (n=60)**	**Placebo (n=60)**	**Antibiotic (n=60)**	**All patients (n=180)**
Affected udder quarter*	RF	0.55	11	15	15	41
	RR		20	17	15	52
	LF		10	9	16	35
	LR		19	19	14	52
Lactation number		0.44	2.7 ± 1.63†	2.9 ± 1.7	3.0 ± 1.6	2.9 ± 1.6
Days in milk		0.32	125 ± 943	122 ± 89	106 ± 80	117 ± 88
Bacterial isolates‡	Yes	0.74	40	38	42	120
	No		20	22	18	60

* The udder quarter position is indicated as RF (right front), RR (right rear), LF (left front) and LR (left rear).
† Mean value and corresponding standard deviation (SD)
‡ The presence of mastitis pathogens in milk samples from infected udder quarters at day 0 before treatment.

3.3.1. First follow-up check (day 7)

At the time of the first check-up (see Table 2), the antibiotic treatment method showed an 81% elimination rate of mastitis pathogens which had been found on day 0 (EMEA criteria). This was almost twice as high as those receiving the homeopathic treatment (43%) or the placebo treatment (45%) ($P<0.05$). If using the DVG criteria, the antibiotic treatment was 2.3 or 2.9 times more efficient as either the homeopathic or the placebo treatment. Cows in the homeopathic (15 animals) and placebo groups (17 animals) were more often assessed as non-responsive to the administered remedy than cows in the antibiotic treatment group (3 animals). Where no mastitis pathogen was found at day 0, there was no difference observed in the bacteriological cure rates ($P=0.63$) between the three treatment groups (see Table 2).

The SCC of one milk sample could not be measured because the milk production from the infected udder quarter ceased almost completely. Cytological cure rate results were similar to those for the bacteriological cure (see Table 3). In general, SCC decreased after mastitis treatment in 124/179 cases (69%). The largest decrease in SCC (including SCC <100,000 cells/ml) was recorded in the antimicrobial remedies group (85%), followed by the placebo (55%) and homeopathic treatment groups (45%). In contrast, the SCC was more often higher after a homeopathic treatment on the seventh day post onset of the infection compared with the other treatment methods. A decrease in SCC below the threshold of 100,000 cells/ml milk was detected in 13/180 mastitis cases (7%). For culture-negative milk samples on the day of inclusion (day 0), no significant differences in cytological cure could be found ($P=0.69$). Where a mastitis pathogen was found on day 0, cytological cure rates differed significantly ($P<0.01$) (see Table 3).

3.3.2. Second follow-up check (day 14)

Bacteriological cure rates measured 14 days after the initial treatment (second follow-up check) were similar to those observed at day 7 (see Table 2). The antibiotic treatment method was significantly more effective in eliminating mastitis pathogens found on the day of inclusion than the placebo and homeopathic treatment methods ($P<0.05$). Again, the homeopathic treatment method had the highest number of cases in which the mastitis pathogen identified on day 0 was still present in the milk sample. Although a good proportion of cows in the antibiotic treatment group were evaluated as non-responsive during the second trial week (see Table 2), at the same time, the homeopathic and placebo treatment groups made up the majority of all non-responsive cows. For those cases with a negative result for bacterial growth in the pretreatment milk sample, all three treatment methods came out as equally effective (P=0.72; Table 2).

Two weeks following the initial treatment, the antimicrobial treatment group had achieved the best cytological cure rates with an SCC decrease evident in 45/60 (75%) of quarters (see Table 3), but only 14/60 (23%) of quarters fell below the threshold of 100,000 cells/ml milk. A decrease in SCC was observed in 33/60 (55%) after a homeopathic treatment and in 30/60 (50%) after use of placebo remedies. Furthermore, an increase in SCC had occurred in 12 cases by the time of the second follow-up check: most in the placebo treatment group. When breaking down the cytological cure rates by bacteriological status at day 0, it became evident that significant differences only occurred when an actual mastitis pathogen had been identified on day 0 (P<0.05) (see Table 3). The cytological cure rates for the different treatment methods in a culture-negative pretreatment sample did not differ (P=0.43).

3.3.3. Third follow-up check (day 28)

The antibiotic treatment method had achieved the highest bacteriological cure rates at the time of the third check-up (see Table 2). In contrast, treatment to eliminating mastitis pathogens using a homeopathic or a placebo remedy at day 28 was significantly less successful compared with the antibiotic treatment (P=0.05). The pathogen present on day 0 was still found in four udder quarters, which were treated with antibiotic and placebo remedies (each two animals). Cows categorised as non-responsive at the time of the final check-up on day 28 were mostly those treated with antimicrobial remedies (five out of nine animals). Both the lowest non-responsive and highest bacteriological cure rates for the whole observation period were found after antibiotic treatment. The final check-up revealed no significant differences in treatment success in the three treatment groups where no mastitis pathogen had been found on the day of inclusion (P=0.93; see Table 2).

Table 2 *Bacteriological cure rate by treatment strategy, time of examination and evaluation criteria by EMEA and DVG*

Day of examination and bacterio-logical status at day 0		Homeopathy (n=60)				Placebo (n=60)				Antibiotic (n=60)			
		EMEA†		DVG†		EMEA		DVG		EMEA		DVG	
		n/n	%	n/n	%	n/n	%	n/n	%	n/n	%	n/n	%
Day 7	*Positive (n=120)*												
	BacC*	17/40	42.5	10/40	25.0	17/38	44.7	12/38	31.6	34/42	81.0	30/42	71.4
	NoBacC†	8/40	20.0	8/40	20.0	4/38	10.5	4/38	10.5	5/42	11.9	5/42	11.9
	NewInf‡	-	-	7/40	17.5	-	-	5/38	13.2	-	-	4/42	9.5
	Non-responders§	15/40	37.5	15/40	37.5	17/38	44.7	17/38	44.7	3/42	7.1	3/42	7.1
	Negative (n=60)												
	BacC	19/20	95.0	12/20	60.0	22/22	100.0	17/22	77.3	18/18	100.0	13/18	72.2
	NewInf	-	-	7/20	35.0	-	-	5/22	22.7	-	-	5/18	27.8
	Non-responders	1/20	5.0	1/20	5.0	0/22	0.0	0/22	0.0	0/18	0.0	0/18	0.0
Day 14	*Positive (n=120)*												
	BacC	14/40	35.0	12/40	30.0	18/38	47.4	13/38	34.2	28/42	66.7	23/42	54.8
	NoBacC	5/40	12.5	5/40	12.5	0/38	0.0	0/38	0.0	4/42	9.4	4/42	9.4
	NewInf	-	-	2/40	5.0	-	-	5/38	13.2	-	-	5/42	11.9
	Non-responders	21/40	52.5	21/40	52.5	20/38	52.6	20/38	52.6	10/42	23.8	10/42	23.8
	Negative (n=60)												
	BacC	17/20	85.0	13/20	65.0	18/22	81.8	11/22	50.0	16/18	88.9	13/18	72.2
	NewInf	-	-	4/20	20.0	-	-	7/22	31.8	-	-	3/18	16.7
	Non-responders	3/20	15.0	3/20	15.0	4/22	18.2	4/22	18.2	2/18	11.1	2/18	11.1
Day 28	*Positive (n=120)*												
	BacC	13/39*	33.3	9/39	23.1	17/38	44.7	13/38	34.2	25/42	59.5	23/42	54.8
	NoBacC	2/39	5.1	2/39	5.1	0/38	0.0	0/38	0.0	2/42	4.8	2/42	4.8
	NewInf	-	-	4/39	10.2	-	-	4/38	10.5	-	-	2/42	4.8
	Non-responders	24/39	61.5	24/39	61.5	21/38	55.3	21/38	55.3	15/42	35.7	15/42	35.7
	Negative (n=60)												
	BacC	15/19*	78.9	13/19	68.4	16/22	72.7	14/22	63.6	13/17*	76.5	10/17	58.8
	NewInf	-	-	2/19	10.5	-	-	2/22	9.1	-	-	3/17	17.6
	Non-responders	4/19	21.1	4/19	21.1	6/22	27.3	6/22	27.3	4/17	23.5	4/17	23.5

Results are given in number of total cures out of cases treated (no./no.) and in % of all cases treated.

* Early culling of one cow due to different reasons (dangerous handling, lameness, fertility disorder).

† Evaluation criteria according to EMEA or DVG.

BacC (bacteriological cure: elimination of the pathogen present on day 0); DVG (German Veterinary Association); EMEA (European Agency for the Evaluation of Medicinal Products); NewInf (newly infected udder quarter: pathogen was different from the one on day 0); NoBacC (no bacteriological cure: the pathogen on day 0 was still present in the udder); Non-responders (cows with no clinical cure were rated as nonresponsive to the treatment given).

Table 3 shows the cytological results from 177 milk samples (three animals were previously culled) 28 days after the initial treatment. As already found in the previous check-ups, the antimicrobial treatment method led more often to a decrease in SCC (39%) than the homeopathic (34%) and the placebo treatment strategies (27%) (P=0.13). A total of only 37/177 (21%) quarters saw the SCC drop under the desired threshold value of 100,000 cells/ml milk and most (16 animals) were treated with antimicrobial remedies. An increase in SCC occurred in seven mastitis cases; mainly those treated with placebo remedies (four animals). Significant differences in cytological cure rates between treatments were found neither for culture-negative (P=0.81) nor for culture-positive pretreatment samples (P=0.13).

Table 3 *Cytological cure compared with day 0 by treatment strategy and time of examination*

		Treatment strategy					
	Day of examination and bacteriological status at day 0	**Homeopathy (n=60)**		**Placebo (n=60)**		**Antibiotic (n=60)**	
		n/n	**%**	**n/n**	**%**	**n/n**	**%**
Day 7	*Positive (n=120)*						
	SCC < 100.000 cells/ml	0/40	0.0	1/38	2.6	1/41†	2.4
	SCC was lower	18/40	45.0	20/38	52.6	34/41	82.9
	SCC was higher	7/40	17.5	0/38	0.0	3/41	7.3
	Non-responders	15/40	37.5	17/38	44.7	3/41	7.3
	Negative (n=60)						
	SCC < 100.000 cells/ml	2/20	10.0	6/22	27.3	3/18	16.7
	SCC was lower	13/20	65.0	13/22	59.1	13/18	72.2
	SCC was higher	4/20	20.0	3/22	13.6	2/18	11.1
	Non-responders	1/20	5.0	0/22	0.0	0/18	0.0
Day 14	*Positive (n=120)*						
	SCC < 100.000 cells/ml	3/40	7.5	1/38	2.6	7/42	16.7
	SCC was lower	13/40	32.5	15/38	39.5	23/42	54.8
	SCC was higher	3/40	7.5	2/38	5.3	2/42	4.8
	Non-responders	21/40	52.5	20/38	52.6	10/42	23.8
	Negative (n=60)						
	SCC < 100.000 cells/ml	5/20	25.0	6/22	27.3	7/18	38.9
	SCC was lower	12/20	60.0	8/22	36.4	8/18	44.4
	SCC was higher	0/20	0.0	4/22	18.2	1/18	5.6
	Non-responders	3/20	15.0	4/22	18.2	2/18	11.1

Table 3 *Cytological cure compared with day 0 by treatment strategy and time of examination*

		Treatment strategy					
	Day of examination and bacteriological status at day 0	Homeopathy (n=60)		Placebo (n=60)		Antibiotic (n=60)	
		n/n	%	n/n	%	n/n	%
Day 28	*Positive (n=120)*						
	SCC < 100.000 cells/ml	2/39*	5.1	5/38	13.2	9/42	21.4
	SCC was lower	13/39	33.3	11/38	28.9	17/42	40.5
	SCC was higher	0/39	0.0	1/38	2.6	1/42	2.4
	Non-responders	24/39	61.5	21/38	55.3	15/42	35.7
	Negative (n=60)						
	SCC < 100.000 cells/ml	6/19*	31.6	8/22	36.4	7/17*	41.2
	SCC was lower	7/19	36.8	5/22	22.7	6/17	35.3
	SCC was higher	2/19	10.5	3/22	13.6	0/17	0.0
	Non-responders	4/19	21.1	6/22	27.3	4/17	23.5

Results are given in number of total cures out of cases treated (n/n) and in % of all cases treated.
* Early culling of one cow due to different reasons (dangerous handling, lameness, fertility disorder).
† Low quantity of milk: SCC measurement was not possible.
Non-responders (cows with no clinical cure were rated as non-responsive to the treatment given); SCC (somatic cell count).

3.4. Additional findings

3.4.1. Bacteriological cure rate at pathogen level

An assessment of the results of the bacteriological cure rates at pathogen level showed that the homeopathic and the placebo treatment strategies were less effective in curing mastitis caused by *Streptococcus uberis* (P=0.01) and *Streptococcus dysgalactiae* (P=0.03) than the antibiotic treatment method. In contrast, all treatment methods showed similar bacteriological cure rates when treating an *Escherichia coli* infection (P=1.0) (see Table 4).

Table 4 *Bacteriological cure at pathogen level by treatment strategy at the time of the first follow-up check (day 7*)*

	Treatment strategy					
	Homeopathy (n=60)		**Placebo (n=60)**		**Antibiotic (n=60)**	
Mastitis pathogens	**n/n**	**%**	**n/n**	**%**	**n/n**	**%**
Streptococcus uberis (n=45)						
BacC	1/12	8.3	3/16	18.7	11/17	64.7
Pathogen change	1/12	8.3	1/16	6.3	2/17	11.8
NoBacC	3/12	25.0	3/16	18.7	2/17	11.8
Non-responders	7/12	58.3	9/16	56.3	2/17	11.8
Escherichia coli (n=16)						
BacC	3/6	50.0	3/5	60.0	4/5	80.0
Pathogen change	2/6	33.3	2/5	40.0	1/5	20.0
NoBacC	1/6	16.7	0/5	0.0	0/5	0.0
Non-responders	0/6	0.0	0/5	0.0	0/5	0.0
Streptococcus dysgalactiae (n=13)						
BacC	0/3	0.0	2/3	66.7	6/7	85.7
Pathogen change	0/3	0.0	0/3	0.0	1/7	14.3
NoBacC	1/3	33.3	0/3	0.0	0/7	0.0
Non-responders	2/3	66.7	1/3	33.3	0/7	0.0

Results are given in number of total cures out of cases treated (n/n) and in % of all cases treated.

* An evaluation of bacteriological cure rates at the time of the second/third follow-up check has not been performed due to low sample sizes (occurrence of non-responders and new infections at subsequent follow-up checks).

BacC (bacteriological cure: elimination of the pathogen present on day 0); NoBacC (no bacteriological cure: the pathogen on day 0 was still present in the udder); Non-responders (cows with no clinical cure were rated as non-responsive to the treatment given).

3.4.2. Total cure rate

The total cure rate (see Table 5) generally did not differ between the treatment methods ($P>0.05$), except on 28th day post onset of the infection ($P<0.05$). A total cure in udder health (DVG criteria) was only identified in a few cases: 13 cows (homeopathy: 2, placebo: 7, antibiotic: 4) at the time of the first follow-up check, 26 animals (homeopathy: 8, placebo: 7, antibiotic: 11) at the second and 33 cows (homeopathy: 6, placebo: 13, antibiotic: 14) at the third. Over the whole observation period (28 days), a total cure was only observed in seven cases, mainly after a placebo treatment (five cows). Only one animal in the homeopathic treatment group and one animal in the antibiotic group were declared to be totally cured from mastitis.

Table 5 *Total cure rate by treatment strategy and time of examination*

	Treatment strategy					
Day of examination and bacteriological status at day 0	**Homeopathy (n=60)**		**Placebo (n=60)**		**Antibiotic (n=60)**	
	n/n	**%**	**n/n**	**%**	**n/n**	**%**
Positive (n=120)						
Day 7	0/40*	0.0	1/38	2.6	1/42	2.4
Day 14	3/40	7.5	1/38	2.6	5/42	11.9
Day 28	*1/40*†	*2.5*†	*5/38*†	*13.2*†	*9/42*†	*21.4*†
Negative (n=60)						
Day 7	2/20	10.0	6/22	27.3	3/18	16.7
Day 14	5/20	25.0	6/22	27.3	6/18	33.3
Day 28	5/20	25.0	8/22	36.4	5/18	27.8

Results are given in number of total cures out of cases treated (n/n) and in % of all cases treated.
* Values in roman font did not differ significantly in total cure rates ($P>0.05$).
† Values in italics show significant differences in total cure rates ($P<0.05$).

3.4.3. Changes in SCC

The change in SCC trends by treatment strategy is illustrated in Table 3. The SCC analysis showed that a rapid return to a normal SCC below 100,000 cells/ml milk was more likely when no mastitis pathogen was found in the pretreatment milk sample than when the milk sample on day 0 indicated positive bacterial growth. Even 28 days after the initial treatment (independent of the treatment strategy), only 37 animals had achieved the target value 100,000 cells/ml needed in order to be considered as having achieved a cytological cure.

3.4.4. Differences in cure rates by using DVG or EMEA criteria

Table 2 illustrates that bacteriological cure rates assessed with DVG criteria were always lower than those determined by using the EMEA criteria.

3.5. Discussion

3.5.1. Methodological issues

The present study design fulfilled the RCT criteria required for a comparison of different treatment strategies, including definition of inclusion and exclusion criteria, randomisation and blinding (EMEA, 2003).

All bacteriological and cytological cure rates evaluated in the current study for the homeopathic treatment method were below those of the antimicrobial treatment method, with one exception at day 28 (see Tables 2 and 3). This might be due to various reasons. The lack of an individualised homeopathic treatment (repertorisation) is often claimed to be a major obstacle in clinical studies concerning the effectiveness of homeopathy. The methodological approach of the present study, however, followed the basic principles of classical homeopathy (individualised treatment and repertorisation) as far as possible and reduced possible personal bias using a software repertory. Nevertheless, detection and assessment of individual homeopathic symptoms (such as modalities or peculiar symptoms) can be challenging under practical conditions and can be the cause of uncertainty, even for a veterinary expert in homeopathy. Despite the veterinarian's expertise and the use of the digital repertory, it is possible that an inappropriate homeopathic remedy was selected, resulting in negative influences on cure rates.

Another questionable point might be the standardised dosage of homeopathic remedies. All animals were treated with the same dosage (number of globules and potency) during the milking routine in the milking parlour. This kind of standardisation was used to streamline the treatment procedures which may have had some influence on cure rates. The question of the appropriate potency has often triggered discussions. Some authors (MacLeod and Wolter, 2006; Gnadl, 2008) recommend the use of high potencies, while other authors used both low and high potencies for acute diseases (Schmidt, 2003; Erkens, 2014). It is reported in literature that the correct remedy will act curatively in any dosage or potency (Vithoulkas, 1981; Day, 2001). According to Day (2001) a correct remedy at high potency reaches the centre of the disease and has the potential to result in total cure.

Shang et al. (2005) assumed that any beneficial effects of homeopathic treatment are primarily due to a placebo effect or a non-specific stimulus. For this reason, the authors considered it essential to employ a placebo control group during the clinical trial. While some authors included two control groups (Werner et al., 2010; Hektoen et al., 2004), others had only one control group to compare treatment outcomes (Garbe, 2003; Varshney and Naresh, 2005). However, implementing a control group creates new challenges (blinding and evaluation). Thus, the partial blindness between homeopathic or placebo remedies (globules) and antimicrobial remedies (udder infusions) must be seen critically. In the current study, farmers were aware of the antibiotic treatment, so a possible consequence of farmers' partial impartiality is the risk they may have stopped a homeopathic or placebo treatment at an earlier stage. However, this minor impartiality was deliberately accepted to prevent the risk of iatrogenic harm to animals (injuries of the teats or new infections).

3.5.2. Effectiveness of treatment

In general, a direct comparison of the cure rates achieved in the present study with other clinical mastitis studies is barely meaningful because of large variations in study design, treatment procedure, definition of cure, implementation of homeopathic principles and the use of appropriate control groups (Doehring and Sundrum, 2016). Only two other studies (Hektoen et al., 2004; Werner et al., 2010) conducted a placebo-controlled trial while taking the individual treatment principle into account.

The present study also demonstrates how different evaluation criteria influence cure rates when a mastitis pathogen was identified on the day of inclusion (day 0). The bacteriological cure rates also differed widely (up to 17.5 percentage points) when using EMEA instead of DVG criteria. Both treatment strategies achieved higher cure rates when taking EMEA criteria into account, but the results were inadequate, as the true udder health status was concealed (new udder infections were regularly evaluated as cured). On the contrary, the DVG criteria were not intended to be used for evaluation in scientific studies, but they were seen as more important for udder health and had higher clinical relevance than the EMEA criteria, as they considered the SCC (threshold <100,000 cells/ml) and new udder infections.

In this study, the frequency of non-responsive animals was generally higher in the homeopathy and/or placebo groups than in the antibiotic group. This supports the findings of other studies (2003; Hektoen et al., 2004; Werner et al., 2010) which found higher clinical cure rates after an antibiotic mastitis treatment. However, in the course of the study, the frequency of non-responsive animals after an antibiotic treatment increased steadily. Cows treated with antibiotics were assessed as non-responsive at a later stage of the study; mainly at the time of the second and third check-up. A reason for this could be that many farmers had administered a well-known effective treatment; they believed in the efficacy of antibiotic remedies. It could therefore be assumed that they waited longer before changing the remedy. At the time of the first follow-up check, animals within the homeopathic treatment group were classified as non-responsive five times as often as those after an antibiotic treatment. An explanation could be the "initial worsening": according to homeopathic philosophy, it is a signal that the healing process is under way, generally seen as a favourable response to treatment and is expected to be followed by an improvement of clinical symptoms (Hahnemann and Schmidt, 1992; Swayne, 2000; Owen, 2007). This type of aggravation is described as the optimal reaction to a correct homeopathic remedy (Vithoulkas, 1996). The difficulty is distinguishing whether the worsening of the diseased animals' symptoms are homeopathic aggravations or adverse effects. An incorrect estimate of these symptoms as adverse effects can lead to an early exclusion of animals within the homeopathic treatment group. As the animals' health and welfare was a priority during the clinical study, animals whose symptoms worsened without clearly being linked to homeopathy were therefore excluded. This procedure could have contributed to the high number of non-responsive animals within the homeopathic treatment group.

In terms of the cure rates of mastitis pathogens found on day 0, subsequent follow-up checks found that the antibiotic treatment method was the most effective. This outcome compares well with results found in previous mastitis studies. However, the bacteriological cure rates for all three treatment methods were higher than those reported by Hektoen et al. (2004) and lower than those reported by Werner et al. (2010).

In the present study, a high rate of culture-negative mastitis cases was found, which compares well with studies from other authors (Krömker et al., 2010; Kuehn et al., 2013; Ganda et al., 2016). Culture-negative cases cannot be detected from a change in milk appearance or from clinical symptoms. They cannot thus be excluded as a priori and were therefore included in the analysis. As the trial was a clinical study under practical conditions, culture-negative mastitis cases were also treated as is usual in dairy practice. Independent of the treatment method, almost all culture-negative mastitis cases treated were successfully cured. The study results indicated that the use of antimicrobial remedies in cases of culture-negative pretreatment milk samples is unnecessary and must be seen as contraindicated. The study also confirmed that cure rates are dependent on the pathogen species. As already shown by other authors (Guterbock et al., 1993; Roberson et al., 2004; Suojala et al., 2013) the use of antibiotic remedies in case of mastitis caused by *E. coli* is often contraindicated. In the current study, the cure rates of *E. coli* infections showed no significant differences after a homeopathic, placebo or antibiotic treatment. However, it should be taken into account that the evaluation of *E. coli* infections is based on a small sample size, thus the results may not be representative.

Previous studies on homeopathy provided total cure rates after a homeopathic treatment from 19 up to 36%; from 6 to 16% for a placebo treatment and from 20 to 38% for an antimicrobial treatment (Garbe, 2003; Hektoen et al., 2004; Merck, 2004; Werner et al., 2010). While total cure rates for the placebo and antibiotic treatment method revealed in the present study were on a slightly lower level compared with those from other authors, total cure rates of mastitis cases treated with placebos were similar. In general, the homeopathic treatment method returned a lower effectiveness in treating clinical mastitis than the antibiotic treatment strategy. The mechanism by which homeopathy might work is still unknown. Hahnemann believed that the process of dilution released a spirit-like healing power that is particularly adapted to work on the equally vital force in animal (Ashvin and Mishra, 2007), resulting in stimulating the body's own healing responses and restoring its inner balance (Day, 2001). It was also notable that when using homeopathic remedies, the SCC in 17% of mastitis cases was higher at the time of the first follow-up check than on day 0. Compared with that, an increase in SCC after an antibiotic treatment was found in only 7% of cases and no increase was found after administering a placebo. Somatic cells are a mixture of milk-producing cells shed from the udder tissue (about 2%) and cells from the immune system (leucocytes; 98%). Leucocytes are the cells responsible for identifying bacteria and killing them and are transferred to the udder during infection, resulting in an increase in SCC. As homeopathic remedies are intended to stimulate vital force, it cannot be ignored that the increase of SCC after a homeopathic treatment could be stimulated by this kind of activation. A direct correlation between an increase in SCC and new infection rate was not observed.

3.6. Conclusion

Before homeopathy can be recommended as a serious alternative for the treatment of bovine clinical mastitis, its effectiveness has to be proven. Despite designing the study carefully around the correct use of homeopathy in the current RCT, the homeopathic treatment method was significantly less successful in curing clinical mastitis compared with antibiotic treatment strategy. In culture-positive cases, the antibiotic treatment provided suboptimal bacteriological cures but was more effective than individualised homeopathy whose effects appeared little different to those of placebos. However, on the cytological cure level, all three treatment methods were similarly ineffective. The results of the present study imply that the effectiveness of individualised homeopathy does not go beyond a placebo effect. In more than one-third of all mastitis cases (culture-negative milk samples and *E. coli* infections), antibiotic treatment was contraindicated retrospectively, as antibiotics, individualised homeopathy and placebo had similar effects on bacteriological and cytological cure. In contrast, the antibiotic treatment of mastitis caused by specific mastitis pathogens (except *E. coli*) seems best at promoting successful udder recovery. The study results emphasise the need for bacteriological analysis of milk samples as successful treatment is highly dependent on the specific mastitis pathogen. Homeopathy does not appear to be a universal treatment alternative for cases of mastitis. Instead, good preventive measures (avoiding mastitis) and target-oriented treatment procedures based on bacteriological analysis should be implemented in dairy practice. The use of individualised homeopathy is therefore only recommended under specific conditions inter alia: treatment of mastitis caused by specific mastitis pathogens in combination with antibiotics (complementary therapy), timely and regular follow-up checks, enough time for a homeopathic clinical examination, knowledge of homeopathic principles and use of homeopathic remedies as an initial treatment until culture results suggest other treatment methods. For a target-oriented treatment approach, a reduction in the use of antimicrobial remedies and in order to promote animals' health and welfare (as Roberson et al., 2004 also recommend) culture analyses of milk samples should be made mandatory and be performed regularly before any treatment is undertaken.

3.7. Acknowledgement

The authors are grateful to all farmers who kindly let their animals be included in the study. Special thanks also go to bovicare GmbH who did the fieldwork and the laboratory analysis.

3.8. Funding

The authors were free of vested interest as the project has received funding from the European Union's Seventh Framework Program for research, technological development and demonstration under grant agreement no. 311824 (IMPRO). The clinical trial is registered under NTP-ID: 00008011-1-9 (pre-results) at BfR.

4. II. How target-orientated is the use of homeopathy in dairy farming? - A survey in France, Germany and Spain

Diana Keller [1, *], Isabel Blanco-Penedo [2, 3], Manon De Joybert [4], Albert Sundrum[1]

* Corresponding author

[1] Department of Animal Health and Animal Nutrition, University of Kassel, 37213 Witzenhausen, Germany

[2] Department of Animal Welfare, IRTA, 17121 Monells, Spain

[3] Department of Clinical Sciences, SLU, 75007 Uppsala, Sweden

[4] BIOEPAR, INRA, Oniris, 44307 Nantes, France

Published in Acta Veterinaria Scandinavica, DOI: 10.1186/s13028-019-0463-3

Abstract

Background

Veterinary remedies are intended to support animals in their recovery from diseases. Treatment outcome depends not only on the general effectiveness of the remedies themselves, but also on other treatment prerequisites. This is true for antibiotics, but even more so for treatments with homeopathic products which are characterised by their individualised approach. While the effectiveness of homeopathy has been addressed in various clinical control trials, the practical conditions under which homeopathic products are used on dairy farms have not yet been investigated. This study provides an initial insight into the existing prerequisites on dairy farms for the use of homeopathy (i.e., the consideration of homeopathic principles) and on homeopathic treatment procedures (including anamnesis, clinical examination, diagnosis, selection of a remedy, follow-up checks, and documentation) on 64 dairy farms in France, Germany and Spain. The use of homeopathy was assessed via a standardised questionnaire during face-to-face interviews.

Results

The study revealed that homeopathic treatment procedures were applied very heterogeneously and differed considerably between farms and countries. Farmers also use human products without veterinary prescription as well as other prohibited substances.

Conclusions

The subjective treatment approach using the farmers' own criteria, together with their neglecting to check the outcome of the treatment and the lack of appropriate documentation is presumed to substantially reduce the potential for a successful recovery of the animals from diseases. There is, thus, a need to verify the effectiveness of homeopathic treatments in farm practices based on a *lege artis* treatment procedure and homeopathic principles which can be achieved by the regular monitoring of treatment outcomes and the prevailing rate of the disease at herd level. Furthermore, there is a potential risk to food safety due to the use of non-veterinary drugs without veterinary prescription and the use of other prohibited substances.

Keywords: Dairy cattle, *lege artis* treatment, monitoring treatment outcome, use of homeopathy

4.1. Introduction

The use of homeopathic products has experienced a popular revival in recent years. The reasons for this increased use are manifold and include the high current consumption of antimicrobial products in food-producing animals in Europe, increasing pathogen resistance to antibiotics (ECDC/EFSA/EMA, 2015; Lees et al., 2017b) and expectations consumers have towards foodstuffs without antimicrobial residues. Very low or no withdrawal periods might also contribute to an increased use of homeopathic products in food-producing animals (Leon et al., 2006). European regulations on organic agriculture even promote the use of homeopathy: "homeopathic products shall be used in preference to chemically-synthesized veterinary products provided that their therapeutic effect is effective for the species of animal, and the condition for which the treatment is intended" (Commission regulation, 2008). Homeopathy, as an individualised treatment method, is quite challenging, particularly for lay people. The challenge of homeopathic treatment is to find the remedy that best matches all symptoms and characteristics in the diseased animal. People administering treatment have to select the most appropriate remedy from thousands of different homeopathic medicinal products available on the commercial market (Löscher et al., 2006). The selection of an appropriate remedy therefore requires expertise and experience in homeopathy and being familiar with homeopathic principles. Moreover, a medical treatment comprises several steps as part of a *lege artis* treatment procedure which includes an anamnesis and clinical examination, formulation of a diagnosis, selection of an appropriate remedy and evaluation of the therapeutic outcome (Baumgartner and Wittek, 2018). Documentation also plays a key role in a target-orientated treatment process as it can establish the effectiveness of the treatment and can help to identify systematic errors in treatment process. While the efficacy and effectiveness of homeopathy has been addressed in various studies (Mathie and Clausen, 2014, 2015a; Doehring and Sundrum, 2016), the practical conditions under which homeopathic treatments are being used on dairy farms have not yet been investigated. The objective of this study was to assess the extent to which farmers consider homeopathic principles and implement a *lege artis* treatment concept in cases of mastitis which is, according to Leon et al. (2006) and Roderick and Hovi (1999), often treated homeopathically in dairy farming.

4.2. Methods

4.2.1. Study design

The study was conducted on 49 organic and 15 conventional dairy farms in France (organic n = 20), Germany (conventional n = 5, organic n = 15) and Spain (conventional n = 10, organic n = 14) from January until April 2015. Farmers that were identified as frequent users of homeopathic remedies in a preceding study (Krieger et al., 2017) were invited to participate in the current study. In addition, an internet search (keywords: [organic] dairy farming and use of homeopathy) was performed and veterinary practitioners were contacted followed by a telephone call to the farmers found in order to achieve the required sample size of a minimum of 20 participants per country. The survey was based on a questionnaire with a total number of 25 questions designed specifically to identify the prerequisites when treating an animal using homeopathy. Open- as well as closed-ended questions were used. The questionnaire was developed by scientists and veterinarians experienced in homeopathy from the International Association for Veterinary Homeopathy (IAVH) and was then translated into the respective national languages. The development phase was followed by an on-farm test phase where the questionnaire was employed and revised. The first part of the questionnaire (the researcher's responsibility) focused on general farm management including animal observation practices, diagnostic procedures where disease was suspected, inspection of the stall pharmacy and measures for the early detection of diseases. Questions dealing with the identification of a *lege artis* homeopathic treatment procedure (performance of anamnesis, diagnosis, selection and application of homeopathic remedies, follow-up checks and documentation) implemented by farmers were covered in the second part of the questionnaire and conducted by the veterinarians from IAVH. The questionnaire also addressed the farmers' knowledge of homeopathic principles, their homeopathic education, and their attitude towards seeking veterinary advice. All homeopathic questions were based on the principles of individualised homeopathy. Farmers were interviewed according to a standardised procedure, beginning with the inspection of the stall pharmacy followed by a face-to-face interview with the farmer. Farm visits lasted from approximately 120 to 240 minutes. All respondents' answers were recorded using an online survey tool (LimeSurvey software package©). After completing the data recording, one Excel file was extracted. The farmers' responses to each question addressing certain prerequisites (e.g., anamnesis procedure, selection of remedies, documentation) were subsequently evaluated by one of the researchers.

4.2.2. Content of the questionnaire

A fundamental basic education in homeopathy plays a key role and is expected to have a strong effect on the homeopathic treatment procedure. Farmers were therefore asked what kind of basic training courses they had participated in, how many further training courses (ongoing education) they had attended in the last three years and how long they had been using homeopathy. Although multiple answers to this question were allowed, only the most extensive training course was selected for the evaluation (for example, where "part time, i.e., evening or weekends, totalling 1–2 days" and "full time totalling 1 week to 1 month" were the given answers, only the latter answer was considered in the evaluation).

A thorough anamnesis is essential in formulating a diagnosis which influences the appropriateness of the homeopathic treatment and the corresponding choice of remedy. The process of anamnesis involves, *inter alia*, recalling the most relevant sections of the animal's history and characteristics of the affected animal (age, point of lactation etc.). Respondents were thus asked where they obtained the historical health records of the diseased animals (multiple answers were permitted). Homeopathy requires careful observation of an animal to detect early on the smallest changes in animal health and behaviour, as early treatments may offer the best prospects for success. Thus, farmers were asked how much time per day they spent observing their animals at herd level (results are based on the farmers' self-assessment). Studying the unique signs and symptoms of the diseased animal characterises a homeopathic clinical examination. The more striking, uncommon and peculiar the symptoms found, the higher the chance of selecting the most suitable homeopathic remedy (Hahnemann and Schmidt, 1992). Both general and undefined symptoms (such as loss of appetite and fever) require little attention as they are observed in almost every disease and prompt the use of almost every remedy. Formulating a diagnosis is the process of identifying the nature of an illness and relies on thorough anamnesis and clinical examinations. This process is often challenging for lay people. Hence, the respondents were asked how often they sought the expertise of a homeopathic professional/veterinarian in treatment decisions. Farmers were also asked to illustrate whether, and if so how, they performed a comprehensive clinical examination and what kind of diagnostic measures they used. It is crucial to identify the type of bacteria present in the udder prior to starting any kind of mastitis treatment. Where homeopathic treatment is unsuccessful, the results of the laboratory milk analysis can be used for conventional mastitis treatment. The farmers were therefore asked for their diagnostic procedure before they started a mastitis treatment.

Hahnemann (1992) hypothesised the principle of "*similia similibus curantur*", stating that the characteristics of the diseased animal must be similar to the characteristics of the "remedy picture". A remedy picture is a collection of physiological and psychological symptoms caused by a particular homeopathic remedy in a healthy animal. Homeopathic practitioners use usually repertories containing symptom pictures (a list of signs and symptoms and the corresponding homeopathic remedies that they are thought to be effective for) and Materia Medica containing remedy pictures (a record of different homeopathic remedies and their description of the clinical picture which they cause for selecting an appropriate homeopathic remedy) (Day, 2001; Lees et al., 2017a). In order to achieve the best selection, a repertorisation (a cross-check of the clinical symptom picture with the corresponding remedy picture) is necessary. Selecting the correct remedy requires expertise and experience in homeopathy and various homeopathic principles need to be considered during the selection process. Farmers were thus asked which reference sources they used for choosing homeopathic remedies, due to the challenges in selecting an appropriate homeopathic remedy. The farmers' level of awareness of homeopathic principles was assessed by the veterinary experts in homeopathy and categorised using predefined levels (see Figure 2).

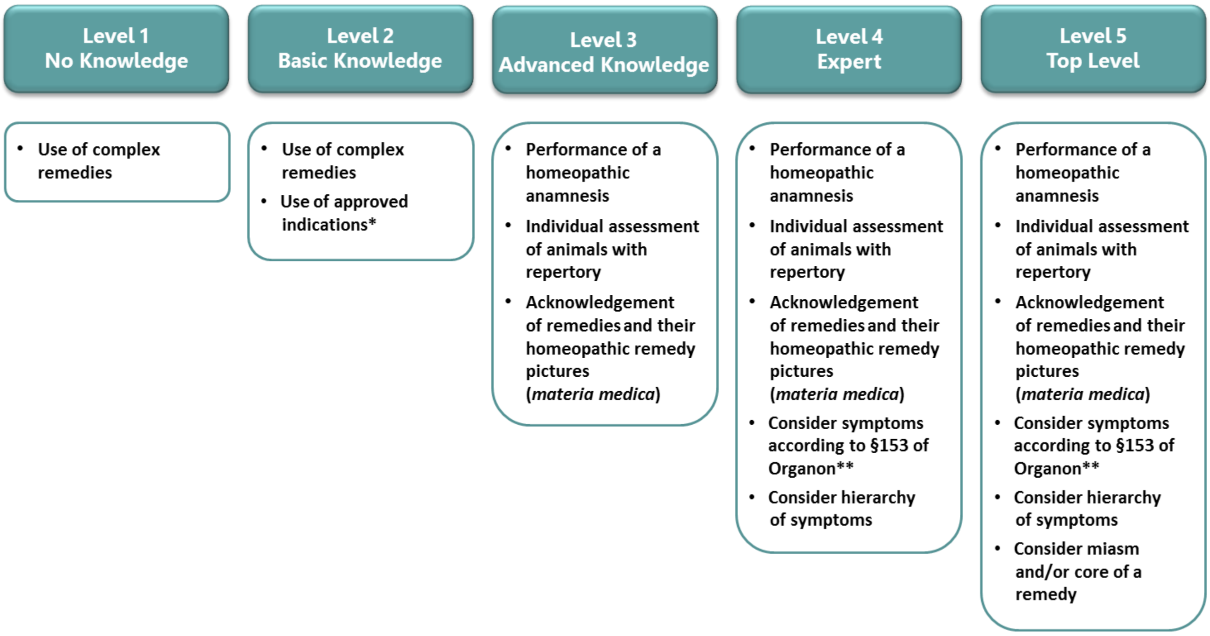


Figure 2. *Levels of awareness of the homeopathic principles.*
** A simplified selection of a homeopathic remedy based on clinical diagnosis and limited leading symptoms.*
*** §153 of Organon of medicine: The more striking, singular, uncommon and peculiar the symptoms found, the higher the chance of selecting the most suitable remedy. General and indefinite symptoms (loss of appetite, debility and fever etc.) require little attention if they cannot be more accurately described.*

A further principle of individualised homeopathy is the prescription of only one remedy at a time as the prescriber cannot distinguish which component of a complex remedy was effective and predicting the interactions which might occur between given remedies is not possible. Therefore, farmers were asked what percentage of homeopathic single remedies and complex remedies they used for treating mastitis. Checking the outcome of the treatment administered is also important when using remedies, since, amongst other things, the switch to other more effective medical treatments could be delayed, independent of the particular treatment method. Delaying treatment which would otherwise have been more effective has a lower prospect of success, since valuable time has elapsed. In this study, the respondents were asked how they check treatment outcomes. Finally, documenting treatments is important for various reasons. Firstly, people who treat food-producing animals are required by EU and national legislation to document every treatment given to diseased animals (Commission Implementing Regulation, 2009b, 2018; BMEL, 2015). This compulsory documentation serves to ensure the protection of public health. Secondly, there is always the risk that treatment is not successful and that the therapy or remedy must be modified. The initial symptoms might have changed due to the previous treatment. Without documenting the initial symptoms, it is difficult – if not impossible – to find an appropriate remedy. Using the documentation, the prescriber is able to review the previous treatment process and alter or optimize the treatment strategy immediately. More importantly, documentation will help the prescriber to ascertain whether the treatment given was successful or not. For these reasons, the questionnaire also dealt with the farmers' documentation procedures. In order to evaluate how comprehensively farmers documented, they were asked to choose from one of three possible options: never, partially or every time (meaning that all treatment steps were documented every time).

4.2.3. Evaluation

For the purpose of the evaluation a frequency distribution, with or without previous categorisation of subject matter in question, was used in the current study. For further information regarding stall construction and other farm conditions (i.e., aspects of farm management and disease prevention) relating to the current study, see the IMPRO project report on www.impro-dairy.eu.

4.3. Results

4.3.1. Demographics of farms and farmers

Most of the farmers who regularly make use of homeopathic remedies were on average 45–54 years old (n=30; 47%) and male (n=44; 69%). Only a few of the users were female (n=20; 31%) or were from other age groups. The results revealed that 63% of the farmers (n=40) were members of an organic association. The estimation of farm size was based on the number of cows. The largest farms visited were in Germany (average [μ]=118, standard deviation [s]=215, coefficient of variation [cv]=1.82), followed by France (μ=61; s=25; cv=0.41) and Spain (μ=34; s=13; cv=0.39). The average farm size of the farms visited in Germany and France was much larger than the nationwide average farm size (Germany n=51; France n=47). The average farm size of the surveyed farms in Spain came close to the nationwide average farm size (n=38) (DG AGRI and European Commission, 2013). Milk records were available on 91% of the farms and an evaluation of individual or detailed milk record data was performed by 85% of the farmers who kept milk records. For a detailed demographic description see Table 6a.

4.3.2. Basic training in homeopathy

The majority (41%) of the farmers have used homeopathy for more than 10 years, followed by using homeopathy from 1 to 5 years (total 33%) and from 5 to 10 years (total 25%) (see Table 6a). Only 1 French farmer answered that he had been using homeopathic remedies for less than 1 year. However, there was a wide variation in the quality and duration of the basic homeopathic training courses. Of the 64 farmers, 25 (39%) stated that they had not attended a specific training course and/or had taught themselves to use homeopathy using books or the Internet. Specific training courses in homeopathy were attended by 61% of farmers. All French and Spanish farmers and 47% of the German farmers who had participated in a professional course were trained by a veterinarian. The remaining 53% of the German farmers were trained in homeopathy by a non-veterinary practitioner.

Table 6a *Status quo of prerequisites for the use of homeopathy present on dairy farms in France, Germany and Spain*

Questions covered by questionnaire	**Number of farms in**		
	France	**Germany**	**Spain**
Demographic description			
Gender			
Female	7	7	6
Male	13	13	18
Age group			
< 26	-	1	-
26-34	3	6	1
35-44	2	3	5
45-54	10	8	12
55-64	5	2	6
Member of Organic farmers association			
Yes	17	16	7
No	3	4	17
Number of cows			
Min	30	26	11
Median	58	75	30
Max	130	1,000	55
Quartile Q1	44.5	49.5	27.0
Quartile Q3	75.5	81.0	46.5
Milk records available			
Yes	15	20	23
No	6	-	9
Basic training in homeopathy			
Duration of using homeopathy			
< 1 year	1	-	-
1 to 5 years	4	2	15
5 to 10 years	6	6	4
More than 10 years	9	12	5
Basic training courses in homeopathy			
No specific training course	1	3	21
Part time: totalling 1-2 days	-	7	1
Part time: totalling > 2 days	-	1	1
Full time: 1 day - 1 week	19	9	1
Full time: 1 week - 1 month	-	-	-
Full time: > 1 month	-	-	-
Supervisor of training course			
Veterinarian	19	8	3
Professor from a university	-	-	-
Professional consultant / Advisors	-	-	-
Members of a homeopathic organisation	-	-	-
Other Homeopaths / “Tierheilpraktiker”	-	9	-

4.3.3. Anamnesis

A highly heterogeneous result emerged on how farmers dealt with the issue of anamnesis. A total of 79% of the Spanish farmers stated that they mostly had no historical information on the diseased animal or that they tried to reconstruct its medical history from their memory. A similar situation was found in Germany where farmers also generally obtained the medical history from memory (70%). Only 8 German farmers used information from health ledgers/cow files. In contrast, 80% of farmers in France used paper files to maintain a medical history. All in all, only 11 out of 64 farmers in the three countries made use of professional herd management software for this procedure. A high variation was also noted in the quantity and quality of animal observations. Only 7 farmers stated that they performed an animal observation while doing nothing else. The time they took to observe their animals differed considerably and ranged from 1 to more than 40 minutes per day. All other farmers stated that they observed the cows in combination with other activities, for example during the milking routine, while feeding or in pasture. While French farmers observed animals for a period of 1 to 30 minutes, Spanish farmers took more time for this process, and claimed to often spend more than 40 minutes for animal observations each day. Further details are provided in Table 6b.

4.3.4. Clinical examination

When using homeopathy, 34 farmers (53%) agreed that a homeopathic clinical examination needs to be performed, whereas 11 farmers (17%) only looked for general clinical signs as commonly performed prior to allopathic treatment (e.g., fever or flaks in milk). In addition, 12 farmers (19%), 6 each from France and Germany, answered that they looked for typical, well-known symptoms and chose a so-called "*approved indication*" (i.e., a simplified selection of a homeopathic remedy based on clinical diagnosis and limited leading symptoms). The remaining 7 farmers either did not perform a homeopathic clinical examination (5%), or they (6%) had assistance from a veterinarian during this process. Quarter milk samples for laboratory cyto-microbiological analysis before farmers treated mastitis were never taken by 53% of farmers. The remaining 30 farmers (47%) only took quarter milk samples depending on the severity of the mastitis, effort and time for labour or course of treatment. In the case of clinical mastitis, 16 out of the 30 farmers collected milk samples (for all animals n=13 farmers; for selected animals n=3 farmers) while in the case of subclinical mastitis, 4 farmers collected quarter milk samples for selected animals. A laboratory milk analysis for both subclinical and clinical mastitis was performed by 10 farmers with different degrees of thoroughness. Table 6b shows a detailed breakdown of the present clinical examination procedure on farms.

4.3.5. Diagnosis

The results of the evaluation show a widespread picture concerning the diagnostic procedure. While French farmers generally never consulted a professional (80%), or only in the case of selected animals (20%), 75% of Spanish farmers consulted a professional in every case of illness. The remaining 25% of Spanish farmers asked for professional advice in specific disease cases. Most German farmers either never consulted a professional (35%) or consulted a professional only in cases where no recovery was foreseeable for the diseased animals (30%) or in specific cases of disease (25%). The remaining 2 German farmers (10%) never consulted a veterinarian or only selected animals were examined by a veterinary practitioner (see Table 6b).

Table 6b *Status quo of prerequisites for the use of homeopathy present on dairy farms in France, Germany and Spain*

Questions covered by questionnaire	**Number of farms in**		
	France	**Germany**	**Spain**
Anamnesis			
Availability of historic health records			
No information exists	-	-	19
From memory	6	14	19
From health ledger papers / cow files	16	8	3
From herd management software	5	5	1
Duration of animal observation			
1 - 10min	12	2	2
11 - 20min	7	6	-
21 - 30min	1	4	2
31 - 40min	-	-	2
> 40min	-	8	18
Combined with other activities (e.g., milking routine, feeding)	19	15	19
Not combined with other activities	1	5	1

Table 6b *Status quo of prerequisites for the use of homeopathy present on dairy farms in France, Germany and Spain*

Questions covered by questionnaire	Number of farms in		
	France	**Germany**	**Spain**
Clinical examination			
Type of clinical examination			
No clinical examination	2	1	-
Homeopathic clinical examination	8	9	17
Use of approved Indication (looking for leading symptoms)	6	6	-
General clinical examination (similar to allopathic treatment)	2	4	5
Help from veterinarian	2	-	2
Taking Quarter milk samples			
No	13	10	11
Yes	7	10	13
In case of clinical mastitis			
For all animals	0	1	2
For selected animals	2	2	9
In case of subclinical mastitis			
For all animals	0	0	0
For selected animals	3	1	0
In case of clinical and subclinical mastitis			
For all animals	0	1	1
For selected animals	2	5	1
Diagnosis			
Consultation of a professional			
Never	16	7	-
In every case of illness	-	1	18
Only at selected animals	4	1	-
Only at specific diseases	-	5	6
Only if no recovery is foreseeable	-	6	-

4.3.6. Availability and selection of a remedy

A high variation in remedies stored on farms was found during the inspection of the stall pharmacy. In total, 324 different homeopathic remedies were identified (among them 240 pure/single remedies, 36 complex remedies and 48 nosodes [homeopathic remedies prepared from pathological material such as blood, pus or pathogens]). While German farmers stored from a minimum of 11 up to a maximum of 218 different remedies, farmers from France and Spain stored from a minimum of 3 up to 20 and 0 up to 24 remedies, respectively (see Table 6c). Homeopathic remedies dedicated for human use were found on 48 farms, mainly in Germany. The majority of farmers (78%)

did not consult a local veterinarian for purchasing human homeopathic remedies, purchasing them instead in pharmacies or via internet.

Furthermore, colchicine and aristolochia, prohibited for use in food-producing animals, were identified on 11 farms. Purchasing homeopathic remedies from a local veterinarian was mainly made by Spanish farmers (63%), whereas this source of acquiring remedies was used by few farmers from France (35%) and Germany (30%). Additionally, 4 German farmers received their homeopathic remedies via the Internet. French and German farmers behaved similarly in the way they used reference materials for selecting an appropriate homeopathic remedy. Both mainly used short manuals (mostly containing "*approved indications*") for the selection of a remedy. As far as the principles of individualised homeopathy were concerned, only 5 farmers from France and 4 from Germany used a repertory in combination with a *materia medica* (repertorisation). In contrast, all Spanish farmers received the advice of a homeopathic veterinarian via telephone or e-mail. Using software for repertorisation of symptoms was not very popular among the farmers. The category "other" included consulting other farmers, other homeopaths or non-veterinary practitioners, as well as notes from homeopathic courses. For detailed information regarding the availability and the selection process of a homeopathic remedy see Table 6c.

Regarding the competence of farmers in selecting the most appropriate remedy, farmers were rated by the IAVH veterinarians most frequently with level 2 (51%), meaning that they had only basic knowledge of homeopathic principles and often used approved indications. Only a small percentage of the farmers (27%) were capable of administering an individualised homeopathic treatment and were rated with level 3. A few farmers, rated with level 1 (22%), only used complex remedies or chose a remedy arbitrarily where disease was identified. The top levels, level 4 and 5, were never assigned. Figure 3 shows the assessment results of the farmers' level of awareness of homeopathic principles. One Spanish farmer was not evaluated as the farmer had never decided which homeopathic remedy to use and consulted the veterinarian in every case.

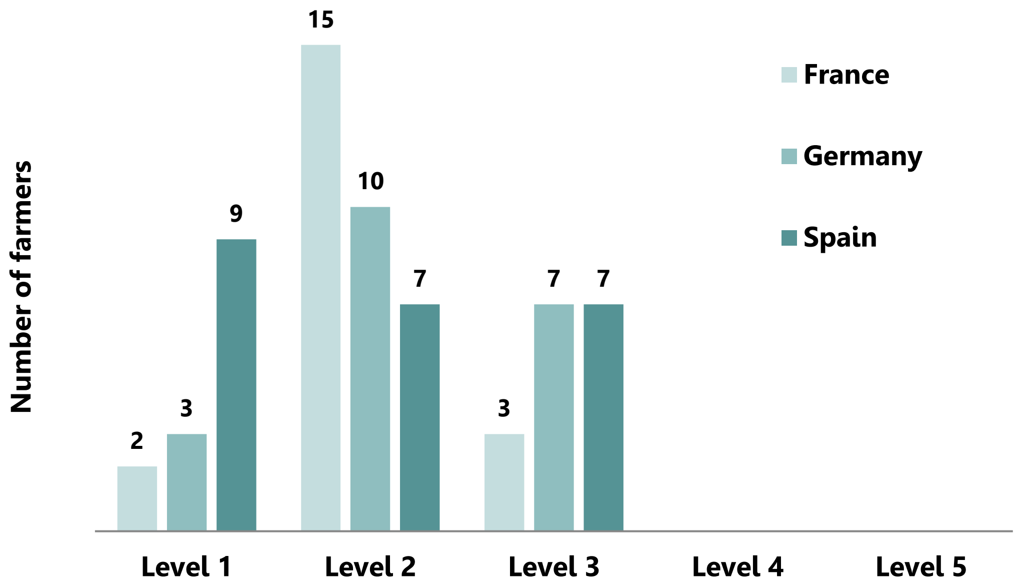


***Figure 3**. Farmers' level of awareness of homeopathic principles assessed by IAVH veterinarian.*

4.3.7. Checking treatment outcome

The majority of farmers (83%) stated that they checked the outcome of the treatment (see Table 6c). However, in most cases, the follow-up checks were only performed visually. Sometimes, the California mastitis test or an udder palpation was carried out. Laboratory investigations were rarely performed. Moreover, farmers were asked if veterinarians regularly checked on the success of their treatments. In total, 70% of all farmers did not consult a veterinarian for the follow-up checks. Assessing the treatment outcome was not (or only in very few cases) performed by local veterinarians in France (0%) and Germany (20%). In Spain, a follow-up check by veterinarians was more common: 63% of farms used this veterinary service and 2 of the Spanish farmers stated that all animals treated were re-checked by a veterinarian. However, the number of animals (all of them or a selection) which were examined by a veterinarian depended on each farmer's criteria and differed considerably between the countries.

4.3.8. Documentation

A heterogeneous result was also found in the field of documentation. The majority of farmers did not adequately document their observations and treatments: half of all farmers (50%) documented nothing at all (see Table 6c). In contrast, 17% of farmers stated that the documentation of treatment (including diagnosis, administration, switching remedies and results of the follow-up check) was always carried out. All other remaining farmers only carried out partial documentation depending on the severity or type of disease, the amount of time available to farmers and the type of treatment. Furthermore, 70% of German, 50% of French and 42% of Spanish farmers did not document homeopathic symptoms. The few remaining farmers took anamnestic records to a varying extent.

Table 6c *Status quo of prerequisites for the use of homeopathy present on dairy farms in France, Germany and Spain*

Questions covered by questionnaire	Number of farms in		
	France	**Germany**	**Spain**
Availability of remedies			
*Source of homeopathic remedies **			
Veterinarian	7	6	15
Pharmacy	19	18	13
Internet	-	4	-
Number of homeopathic remedies stored			
Total number of different remedies	40	314	47
Minimum	3	11	0
Median	14	55	5
Maximum	20	218	24
Selection of homeopathic remedies			
*Use of reference materials **			
Advice of a veterinarian	8	5	24
Internet	-	3	1
Materia medica	7	6	-
Rely on current knowledge alone	2	5	5
Repertory	7	4	-
Short manual for homeopathy	11	19	1
Software	1	1	-
Other people	4	4	1

Table 6c *Status quo of prerequisites for the use of homeopathy present on dairy farms in France, Germany and Spain*

Questions covered by questionnaire	Number of farms in		
	France	**Germany**	**Spain**
Treatment outcome			
Checking treatment outcome by a veterinarian			
Yes	-	4	15
No	20	16	9
Checking treatment outcome by farmer			
Yes	20	20	13
No	-	-	11
*Type of checking treatment outcome by farmer **			
Pure observation (visual)	20	19	20
Clinical investigation (e.g., udder palpation, CMT)	11	15	18
Laboratory investigation	-	3	-
Documentation			
Taking anamnestic records			
Yes	10	6	14
No	10	14	10
Taking treatment records			
No	4	12	16
Yes	16	8	8
Every time	9	-	2
Partial documentation	7	8	6

* More than one answer was permitted

4.4. Discussion

The use of homeopathy is controversially discussed in medical science. Although there are many clinical trials concerning the efficacy and/or effectiveness of homeopathic remedies, a clear result as to whether homeopathy is effective or not could not be provided (Doehring and Sundrum, 2016; Mathie and Clausen, 2014, 2015a; Mathie and Clausen, 2015b). However, randomised controlled trials focus primarily on the efficacy of the homeopathic remedy itself, whereas the conditions of on-farm use are seldom considered and are rarely a subject of scientific investigations. This study provides a first insight into the existing conditions on dairy farms for the use of homeopathy and on current homeopathic treatment procedures in three European countries. As the number of participants was limited, the representativeness of the study results must be treated carefully. The study results are therefore purely descriptive and do not allow the application of statistical analysis, and more extensive studies are needed in this field.

The most obvious result of the on-farm assessments is the large heterogeneity between farms on how homeopathic remedies are used. The reasons for the heterogeneity in the use of homeopathy are manifold and may include, *inter alia*, the different perspectives and interests of the users, the complexity of the homeopathic treatment approach, and the differences in the availability of homeopathic veterinary remedies or local veterinarians experienced in homeopathy. The study revealed results which were not originally expected. During the inspection of the stall pharmacies, many different homeopathic remedies were found. The main problem here is that approximately three-quarters of these remedies are designed for human use and were not prescribed by veterinarians. Furthermore, colchicine and aristolochia, although prohibited for animal treatment (Commission Implementing Regulation, 2009a), were found in the stall pharmacies. According to EU regulations, only veterinarians are permitted to prescribe human medicinal products for treating food-producing animals (European Union, 2018). On the other hand, farmers would like to reduce the use of antimicrobial products (Jones et al., 2015) and may looking for alternatives. The authors are convinced that in the absence of local veterinary advice, farmers find themselves compelled to make decisions on therapy alone or have to resort to pharmacies or non-veterinary practitioners for help. A recently-published study confirmed that the majority of veterinarians had little to no knowledge of the use of alternative therapies, and the majority of veterinarians (72%) were uncomfortable using alternative treatments for livestock (Sorge et al., 2019). Veterinarians also need to be a minimum familiar with alternative treatment methods in order to be more involved in the treatment process and to discuss the given treatment with farmers. Furthermore, most of pharmacies or non-veterinary practitioners have little or no experience or knowledge of farm animal diseases and are even less well-informed on the legislation covering animal welfare, animal health and public health regulations in livestock production (Hovi, 2001). The study also showed that many famers lack basic training in homeopathy and only had limited knowledge of homeopathic principles. Homeopathy treats each animal as a unique individual, and thus requires individual treatment along with expertise in homeopathic principles. Farmers often hesitate to give an individually tailored treatment and often use “*approved indications*” instead, which contradicts one fundamental principle of individualised homeopathy.

Animal observation was mainly considered insufficient as famers were often distracted by other routine work, resulting in a less thorough detection of diseased animals and the relevant symptoms for homeopathic treatment. A further important finding was the absence of documentation of treatment procedures and outcomes for homeopathic treatment. Farmers might be reluctant to do this because they could be liable to prosecution where using human homeopathic remedies without prescription by a veterinarian when the stall pharmacies are inspected by official veterinarians. Some farmers mentioned the additional work and lack of time as a reason for non-documentation, although farmers are legally obliged to document every treatment given to food-producing animals. Without thorough documentation, a successful outcome cannot be evidenced and farmers cannot learn from treatment failures revealed by monitoring.

The current assessment of the treatment outcome was insufficiently performed by farmers and, in addition, is based on the farmers’ subjective perception. But it is a mistake to think that untreated animals never recover and treated ones always do (Fajt, 2016). Various mastitis studies have shown that untreated animals achieved cure rates of up to 69% (Francoz et al., 2017). There is no ultimate guarantee for the recovery of udder health where remedies – independent of the therapy method – were administered. For the purpose of evaluating the actual treatment effect, it is therefore absolutely necessary to undertake a clinical examination of each animal being treated. A treatment effect is the difference between the disease outcome with and without treatment (Fajt, 2016). Thorough follow-up checks and documentation of treatment outcomes are required to assess the effects of a change in treatment procedure and to verify the effectiveness of treatments in farm practices (Sundrum, 2016).

The heterogeneous treatment approaches, together with the often insufficient knowledge of homeopathic principles, do not automatically lead to poor treatment outcomes. A therapeutic success can be achieved in various ways. However, there is an increased risk that factors influencing the outcome of a homeopathic treatment might be overseen or that methodological errors – for example, non-compliance of homeopathic principles during the selection of an appropriate remedy – might occur.

The implementation of a *lege artis* treatment procedure can reduce systematic errors, such as an insufficient clinical examination or not checking the treatment outcome. Finally, precise documentation can be expected to lead to the selection of the most appropriate treatment procedures.

However, the actual cure rates of treatment methods are difficult to ascertain at present due to the lack of appropriate follow-up checks of treatment outcomes and documentation. An appropriate treatment monitoring system which enables the assessment of the effectiveness of treatments in farm practices is needed and should be implemented for medical treatments (Sundrum, 2016). Many veterinarians hesitate to administer sick animal care using alternative therapies, as their efficacy has not yet been proven. They were concerned that the lack of proven effective therapy options would impair livestock welfare (Sorge et al., 2019). An appropriate monitoring system could contribute to the assessment of the effectiveness of the homeopathic treatment approach on farms and could satisfy the veterinarians' need for more data on the efficacy of alternative therapies (Sorge et al., 2019).

After consideration of all the aforementioned facts, the use of homeopathic remedies can currently not be recommended unless a *lege artis* homeopathic treatment procedure and an appropriate initial and boundary conditions on the farm, including the monitoring of treatment outcome, is implemented. These prerequisites are not restricted to homeopathy, but apply also to other alternative treatment methods, especially phytotherapy, and conventional medicine (Tamminen et al., 2018). Without implementing these prerequisites and monitoring systems, it must be assumed that where unsuccessful treatment goes undetected, prolonged suffering of diseased animals will result.

4.5. Conclusion

A target-orientated and successful treatment requires the implementation of a *lege artis* treatment procedure in the use of medicinal products. The study revealed that neither uniform treatment procedures nor a *lege artis* treatment for the use of homeopathy existed on the dairy farms visited. Each farmer seemed to have developed their own homeopathic treatment strategy. This subjective treatment approach using the farmers' own criteria while neglecting documentation and monitoring is suspected to reduce the potential for successful treatment. The current use of homeopathy carries a high risk for the prolonged suffering of diseased animals in cases where unsuccessful treatment goes undetected. There is, thus, a need to verify the effectiveness of homeopathic treatments in farm practice in consideration of a *lege artis* treatment procedure and homeopathic principles. This can be achieved through regular monitoring of treatment outcomes and the prevalence rate of diseases at herd level. Furthermore, there is a potential risk to food safety due to the use of non-veterinary drugs without veterinary prescription and the use of other prohibited substances in food-producing animals.

4.6. Acknowledgement

The authors would like to thank all farmers and their veterinarians who participated in the study. Furthermore, the authors are grateful to all homeopathic veterinarians from IAVH for their support. We extend our gratitude to the Spanish colleagues of IRTA for helping with the translation of the questionnaire.

4.7. Funding

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under Grant Agreement No 311824 (IMPRO).

4.8. Consent for publication

The participation in the study was voluntary, and the farmers were informed about the purpose and methods of the study. They were assured that all information would be treated anonymously and that they could withdraw from the study at any time. All participants involved in the study gave consent for publication.

5. III. Reliability of the Speed Mam Color™ test and its potential for reducing antibiotic use in bovine clinical mastitis: A diagnostic accuracy test

Diana Keller [1,*], Albert Sundrum[1]

* Corresponding author

[1] Department of Animal Health and Animal Nutrition, University of Kassel, 37213 Witzenhausen, Germany

Submitted in PLOS ONE

Abstract

The overuse of antibiotics is responsible for a significant increase in the prevalence of antibiotic resistance. Mastitis is the most frequent reason for antibiotic use on dairy farms worldwide. To achieve a reduction in the use of antibiotics and pathogen resistance, antimicrobial products should not be used indiscriminately and irresponsibly in every mastitis case. A target-oriented use of antibiotics, characterised by the careful selection of an appropriate antibiotic targeting the specific mastitis pathogen, often fails in practice due to lack of information regarding the pathogen. The standard cyto-microbiological diagnosis in a milk laboratory is time-consuming and requires increased technical and financial investments in laboratory equipment, expertise and additional manpower. To reduce delays in treatment and to improve therapeutic success, different rapid mastitis tests, inter alia the Speed Mam Color™ test, are available on the commercial market. The Speed Mam Color™ test is intended for the rapid on-farm identification of mastitis pathogens and the determination of their sensitivity profile. The use of the Speed Mam Color™ test can be justified if it produces accurate results compared to conventional laboratory analyses (standard agar culture technique). The objective of this investigation was to evaluate the accuracy of the Speed Mam Color™ test in comparison with standard laboratory milk analyses. Milk samples of cows suffering from clinical mastitis were analysed microbiologically (identification of pathogens including the microscopic investigation of yeasts) in a certified milk laboratory and simultaneously tested using the Speed Mam Color™ test. The results revealed a high sensitivity (87.7%) and specificity (80.8%) for identifying mastitis pathogens using the Speed Mam Color™ test. However, the Speed Mam

Color™ test was less accurate in determining pathogen sensitivity to antibiotics and should therefore not currently be used for determining the resistances of mastitis-causing pathogens and selecting antibiotic remedies for mastitis treatment.

5.1. Introduction

Mastitis is the main reason for the use of antibiotics in dairy production worldwide (Lago et al., 2011; Mansion-de Vries et al., 2015) and is often accompanied by high economic losses (McCarron et al., 2009; Mansion-de Vries et al., 2015). The early detection and appropriate treatment of mastitis is essential for animal health and welfare as well as reducing economic losses. To simultaneously reduce the use of antibiotics and comply with legal regulations, antimicrobial products should not be used indiscriminately and irresponsibly in every mastitis case as not all mastitis cases benefit from antibiotic treatment. Roberson (2003) concluded that in 50–80% of mastitis cases, antibiotic therapy might not be required. Thus, it is important to correctly identify the need for antibiotic treatment in order to reduce antimicrobial consumption (Roberson, 2003; Mansion-de Vries et al., 2015; Lago and Godden, 2018). Furthermore, numerous studies have emphasised that the success of an antibiotic mastitis treatment depends on the specific pathogens causing the mastitis (Mansion-de Vries et al., 2015). Knowing the cause of mastitis allows selective treatment decisions (treat or not treat) and can also guide differential treatment decisions regarding the type of antibiotic selected or the treatment duration (Lago and Godden, 2018). Determining the pathogen species involved and its sensitivity to antibiotics is usually conducted by trained personnel in milk laboratories. However, the time required for taking the milk sample, transporting it to the laboratory and conducting the analysis is a serious disadvantage of standard cyto-microbiological diagnosis (Mansion-de Vries et al., 2014; Lago and Godden, 2018). In addition, a standard laboratory milk analysis requires increased technical and financial investments in laboratory equipment, manpower and the need for comprehensive microbiological expertise. Therefore, a target-oriented use of antibiotic remedies often fails in practice due to a lack of information on the specific mastitis-causing pathogens. Treatment decisions thus far are generally based on the experience of the person carrying out the treatment, while possibly taking the outcome of previous antimicrobial susceptibility testing into account (Owens et al., 1997). To reduce delays and additional effort, and particularly to improve therapeutic success, various rapid mastitis tests have been developed. The best known include the Minnesota easy culture system II Bi-Plate and Tri-Plate, 3M Petrifilm, VetoRapid, Accumast, mastDecide and Speed Mam Color™. These rapid, on-farm tests use different techniques to identify the functional groups of bacteria in milk samples that might be responsible for the development of mastitis. In comparison to other tests, the main advantage of the Speed Mam Color™ test is its ability to identify *Mycoplasma* spp. and the specific sensitivity of a pathogen to antibiotics: in general, 14 different drug agents and drug combinations are tested in the Speed Mam Color™ test (six of these were investigated in the current study). However, the use of the Speed Mam Color™ test can only be recommended if it produces accurate results compared to conventional laboratory analyses. The objective of the investigation was to evaluate the diagnostic accuracy of the Speed Mam Color™ test for clinical mastitis pathogen identification and antimicrobial susceptibility testing in comparison to standard methodology and to address a more target-orientated use of antibiotics.

5.2. Methods

5.2.1. Milk samples

As part of a clinical study (Keller and Sundrum, 2018), quarter milk samples were taken from animals suffering from mild or moderate clinical mastitis (according to the definition of the International Dairy Federation (IDF, 2011)) throughout the period from June 2016 to the end of December 2016. In total, 180 milk samples were examined from four herds located in eastern Germany. During the daily milking routine, farmers identified cows with mild or moderate clinical mastitis symptoms (e.g., flocks/clots in milk, udder swelling, change of milk) and informed the veterinarian for further diagnosis, taking milk samples and administering mastitis treatment. The quarter milk samples were taken aseptically from the infected udder quarter according to good clinical practice (Baumgartner and Wittek, 2018). The milk samples were transported within a maximum of four hours to a certified milk laboratory (bovicare GmbH, Potsdam, Germany) and were microbiologically analysed using a standard agar culture technique by experienced veterinarians or microbiologists within 24 hours of arrival.

5.2.2. Laboratory milk analysis

Pathogens were identified using a standard agar culture technique involving a microbiological culture on an aesculin blood agar plate (incubated for at least 48 hours at 37°C), followed by a sensory, microscopic and, where necessary, biochemical or serological evaluation of the pathogens. An initial evaluation of the bacterial growth was undertaken after 24 hours. Suspect bacteria colonies were incubated on selective culture media for a further 24 hours. The pathogen suspected of causing the clinical mastitis was subsequently subjected to antibiotic susceptibility testing using the agar disc diffusion test: antibiotic paper discs for each antibiotic tested were applied firmly to the agar plate. In cases of mixed infections, the major pathogens were identified based on microbiological expertise and nationwide guidelines for the isolation and identification of mastitis pathogens (Fehlings et al., 2000). After incubating for a further 24 hours at 37°C, growth on the plates was examined (measuring zone diameter of circular inhibition zones) and categorised as either “sensitive”, “intermediary” or “resistant” to the specific antibiotic agent.

5.2.3. Principle of the Speed Mam ColorTM test

The same 180 milk samples were examined concurrently by the milk laboratory using the Speed Mam Color™ test (Virbac Tierarzneimittel GmbH, Bad Oldesloe, Germany) and the standard agar culture technique. Speed Mam Color™ is a microculture system intended for the rapid, on-farm diagnosis of mastitis, the identification of pathogens and the determination of their sensitivity profile. According to the manufacturer, the colour change observed in the wells for identifying functional groups of bacteria is based on a pH change or the bacterial metabolism of a coloured substance into a different colour substance. Each antibiotic well contains both a lyophilized antibiotic agent and tetrazolium chloride (redox indicator). Tetrazolium chloride is colourless in its initial oxidised state but is metabolised by bacterial enzymes into red formazan (reduced state). This colour change results from a redox reaction.

The test was conducted according to the manufacturer’s instructions. After homogenising the milk sample, three drops of milk were added to a bottle of growth medium followed by a second homogenisation. Thereafter, three drops of the inoculated growth medium were added to each well. Two drops of a “Staph” supplement were added to the “Staph” well. The wells (except for the “E. coli”, “Pseudo” and “Staph” wells) were sealed with two 2 drops of paraffin oil and then incubated. After 24 hours of incubation at 37°C (sensitivity test), the control wells and the antibiotic wells were evaluated. After a further 24 hours (a total of 48h of incubation), the wells for interpreting the bacterial identification were read. The “Mycoplasma” well was interpreted after a total incubation time of 7 days. If a bacterial identification well changed to an intermediate colour after 48 hours of incubation, the well was re-evaluated 24 hours later (a total of 72h of incubation). The interpretation of the results (evaluation of colour changes according to the manufacturer’s instructions) was performed by veterinarians from the milk laboratory.

5.2.4. Evaluation of outcomes

In our study, the evaluation of the outcomes was performed by comparing the bacteriological results, including the pathogen sensitivity to antibiotics, provided by the milk laboratory with the outcomes of the Speed Mam Color™ test. If both the reference method and the Speed Mam Color™ test showed identical results, the sample was considered true positive (bacterial growth) or true negative (no bacterial growth). In cases where the Speed Mam Color™ test results deviated from the reference method results, the case was considered either false positive or false negative. According to the instructions of the Speed Mam Color™ test, an incomplete colour change for bacterial growth should be assessed as negative. Where both mastitis test methods were true positive for bacterial growth, a second evaluation step to identify the functional groups of the bacteria was undertaken. Where the same mastitis pathogen was identified, the category “same pathogen” was denoted, while different functional groups of bacteria were allocated to the category “different pathogen”. An intermediate colour change was considered to be negative. For mixed infections, when, among the other mastitis pathogens, the same bacteria was identified by the reference method (standard agar culture technique) and the Speed Mam Color™ test, it was evaluated as consistent. The final step in the evaluation used only those milk samples which were categorised as “same pathogen” and compared their microbial susceptibility to antibiotics. Verifying the accuracy of identifying pathogen resistance was conducted by comparing six antibiotic agents (see Table 7). Both mastitis diagnostic tests used the same drug concentration for each antibiotic agent tested. For patent protection reasons, the drug concentrations used cannot be specified in detail. In cases where an incomplete colour change or streaking occurred, the bacteria were evaluated as being resistant to the antibiotic agent. An intermediate result for pathogen resistance to antibiotics provided by the milk laboratory was also assessed as resistant. The flow chart in Figure 4 depicts the evaluation process used in the present study.

Table 7 *Antibiotic agents used to verify antimicrobial susceptibility to antibiotics compared to the gold standard (standard agar culture technique)*

	Antibiotic agent tested	
Class of antibiotic agent	**Milk laboratory (Gold standard)**	**Speed Mam Color™**
Beta-lactam-penicillin	Oxacillin	Cloxacillin
Broad spectrum penicillin	Amoxicillin & clavulanic acid	Amoxicillin & clavulanic acid
Cephalosporin 1st Generation	Cefazolin	Cefalexin
Cephalosporin 3rd Generation	Cefoperazone	Cefoperazone
Cephalosporin 4th Generation	Cefquinome	Cefquinome
Fluoroquinolone	Danofloxacin	Marbofloxacin

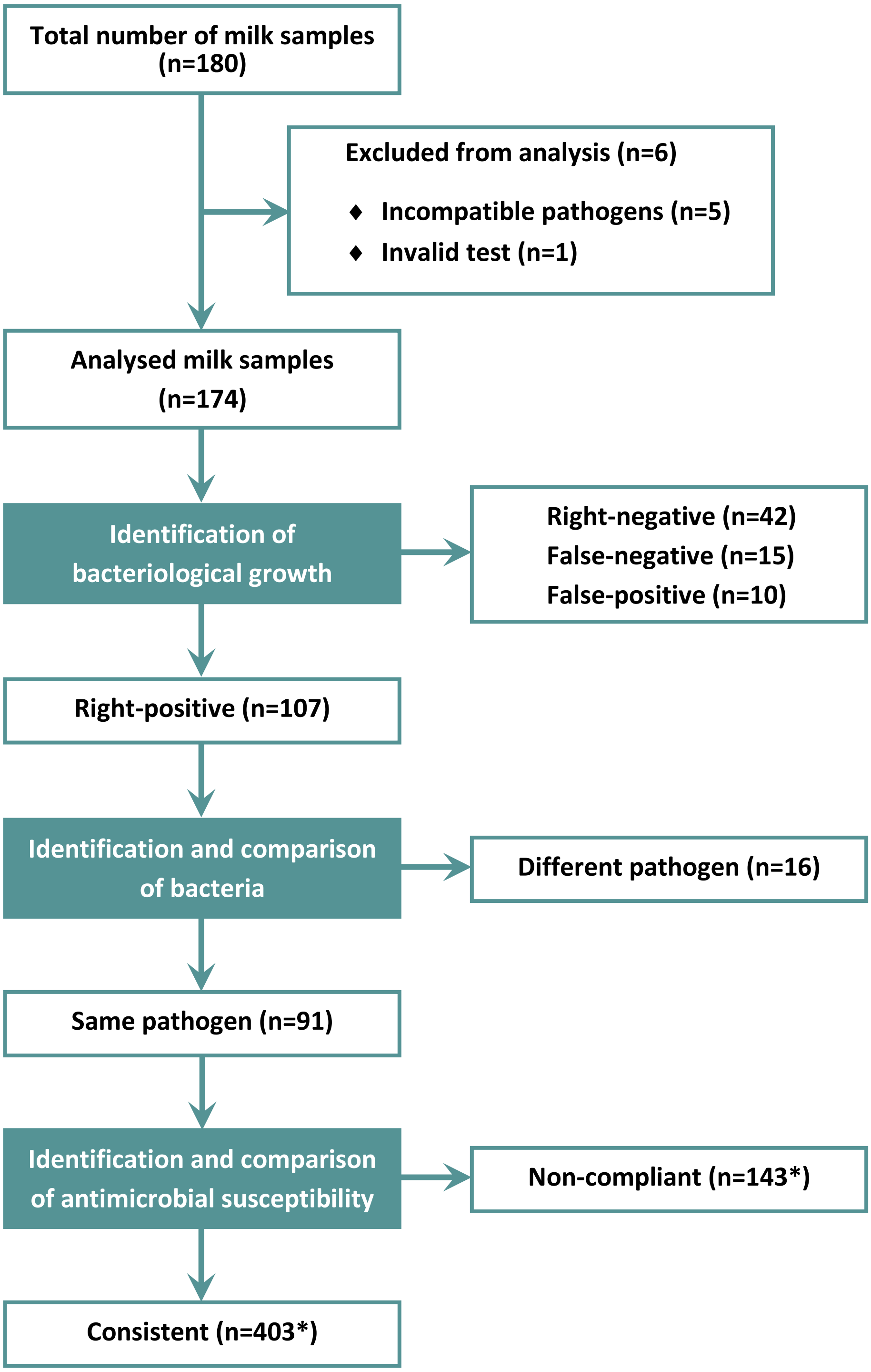


Figure 4. Flow chart of the evaluation process

**Each of the six antibiotic agents were tested per milk sample (in total 546 antibiograms/91 milk samples)*

5.2.5. Statistical analysis

All data was recorded using Microsoft Excel (Microsoft, Redmond, USA). The microbiological results of the Speed Mam Color™ test were compared with the results of the reference method (bacteriological culture on blood agar plates used as the gold standard). Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), true prevalence (TrP) and apparent prevalence (AP) according to Kreienbrock et al. (Kreienbrock et al., 2012) were estimated using calculation formulae (see Figures 5-6) and the epidemiological software WinEpi 2.0 (http://www.winepi.net). Se is defined as the ability of the test to correctly identify the mastitis-causing pathogen; Sp is defined as the ability to correctly identify healthy cows without mastitis (no bacterial growth); PPV is defined as the probability that mastitis is present if the rapid test was positive; NPV is defined as the probability that the animal was healthy if the rapid test was negative; AP is defined as the proportion of animals in the population showing a positive test result regardless of their real status in terms of the disease in question (all positive-test animals); and TrP is defined as the proportion of animals in the population that really did have the disease in question regardless of their test result. From a test result perspective, TrP includes both true positives and false negatives. Each test characteristic and predictive value parameter was considered to have a 95% confidence interval. Accuracy was calculated by dividing the number of true positives and true negatives by the total number of tests, in addition to Cohen's kappa coefficient (k), a statistical coefficient that measures the agreement between alternative methods of categorical assessment when new techniques are being studied, was calculated according to Figure 7. Cohen's kappa coefficient ranges from 0 to 1 and is interpreted as follows: poor agreement (<0.1), slight agreement (0.1–0.2), fair agreement (0.21–0.4), moderate agreement (0.41–0.6), substantial agreement (0.61–0.8) and almost perfect agreement (>0.8) (Cohen, 1960).

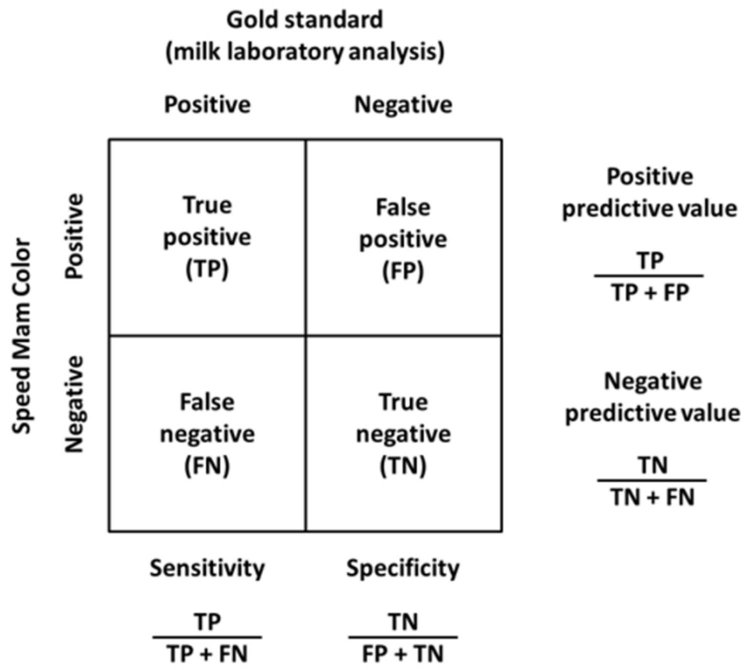


Figure 5. Formulae for calculating sensitivity, specificity, positive and negative predictive value for bacterial growth

TP (true positive); TN (true negative); FP (false positive); FN (false negative)

$$\boldsymbol{Apparent\ Prevalence = \frac{TP + FP}{TP + FP + FN + TN}}$$

$$\boldsymbol{True\ Prevalence = \frac{TP + FN}{TP + FP + FN + TN}}$$

Figure 6. Formula for calculating apparent and true prevalence for bacterial growth

TP (true positive); TN (true negative); FP (false positive); FN (false negative)

$$\boldsymbol{k = \frac{p_0 - p_e}{1 - p_e} = 1 - \frac{1 - p_0}{1 - p_e}}$$

Figure 7. Formula for calculating Cohen's kappa coefficient

p_o is the relative observed agreement among methods
p_e is the hypothetical probability of chance agreement

5.3. Results

5.3.1. Identification of functional groups of bacteria

In total, 174 of the 180 tested milk samples were considered in the evaluation, since six milk samples had to be excluded for two reasons: one invalid test result (colour change in negative control well) and 5 incompatible pathogen species that cannot be identified by Speed Mam Color™ (*Corynebacterium bovis*, *Serratia* spp., yeasts) were found using the standard agar culture technique. The milk laboratory classified 52 of the 174 milk samples (29.9%) as culture-negative and 122 samples (70.1%) as positive for bacterial growth. Table 8 illustrates the frequency distribution of bacteria identified by the milk laboratory, which is similar to the mastitis pathogen distribution from other authors (Krömker et al., 2010; Ganda et al., 2016). The overall Se, Sp, PPV, NPV, accuracy, AP, TrP and Cohen's kappa coefficient for the Speed Mam Color™ test for identifying mastitis-causing pathogens are presented in Table 9. The overall Cohen's kappa coefficient was considered substantial for the standard laboratory culture and Speed Mam Color™ test. The false negative bacteriological results were mainly caused by *Escherichia coli* (4/15, 26.6%) and other aesculin-positive streptococci (3/15, 20.0%). False positive test results were often associated with *Staphylococcus* spp., *Streptococcus* spp. (excluding *Streptococcus uberis*) and *Enterococcus* spp..

Table 8 *Results of the microbiological analysis of 174 milk samples using a standard agar culture technique*

Pathogen	n	%
No bacterial growth	**52**	**29.9**
Gram-positive bacteria	**91**	**52.3**
Streptococcus uberis	43	24.7
Streptococcus dysgalactiae	13	7.5
Other aesculin-positive streptococci	12	6.9
Staphylococcus aureus	9	5.2
Enterococcus spp.	7	4.0
Coagulase-negative staphylococci	7	4.0
Gram-negative bacteria	**31**	**17.8**
Escherichia coli	18	10.3
Klebsiella spp.	8	4.6
Other coliform bacteria	5	2.9

Of the culture-negative mastitis cases, 42 of 52 (80.8%) were correctly detected by the Speed Mam Color™ test. A high accordance between the functional groups of bacteria was observed in the second evaluation step. In 91 of 107 (85.0%) cases, the Speed Mam Color™ test and the standard agar culture technique detected the same species, however, in 16 cases (15.0%) a different pathogen was found by the Speed Mam Color™ test. The calculation of test characteristics and predictive values at the pathogen level was performed for *E. coli*, *Staphylococcus* spp., *Streptococcus* spp. and *S. uberis*, and is presented in Table 9. *Klebsiella* spp. were correctly detected in only 3 of 6 cases. The best agreement regarding the identification of functional groups of bacteria was found for *Streptococcus* spp. and *S. uberis*.

Table 9 *Overall and pathogen-specific test characteristics and predictive values of the Speed Mam Color™ test*

	Se	Sp	PPV	NPV	Accuracy	AP	TrP	K
Overall	**87.7%**	**80.8%**	**91.5%**	**73.7%**	**85.6%**	**67.2%**	**70.1%**	**0.66**
E. coli	64.7%	98.1%	91.7%	89.5%	89.9%	17.4%	24.6%	0.70
Gram-positive bact.	92.3%	82.5%	89.4%	87.0%	88.5%	63.5%	61.5%	0.76
Staphylococcus spp.	81.3%	88.5%	68.4%	93.9%	86.8%	27.9%	23.5%	0.66
Streptococcus uberis	86.0%	96.2%	94.9%	89.3%	91.3%	41.1%	45.3%	0.83
Streptococcus spp.	92.6%	90.4%	92.6%	90.4%	91.7%	56.7%	56.7%	0.83

Staphylococcus spp. (*S. aureus*, coagulase-negative staphylococci); *Streptococcus* spp. (*S. uberis*, *S. dysgalactiae*, other aesculin-positive streptococci)
Se (sensitivity); Sp (specificity); PPV (positive predictive value); NPV (negative predictive value); AP (apparent prevalence); TrP (true prevalence)
K (Cohen's kappa coefficient): 0 denotes poor agreement; 0.01–0.20 denotes slight agreement; 0.21–0.40 denotes fair agreement; 0.41–0.60 denotes moderate agreement; 0.61–0.80 denotes substantial agreement and 0.81–1.00 denotes almost perfect agreement.

5.3.2. Pathogen sensitivity to antibiotics

For comparing the susceptibility of pathogens to antibiotics, a total of 91 antibiograms containing 546 tested antibiotic agents were investigated. Of the mastitis pathogens, 28 showed multidrug resistance (at least 3 resistances per pathogen, see Figure 8). Most multidrug resistances were found for the *Enterococcus* spp., followed by other aesculin-positive streptococci and *S. uberis*.

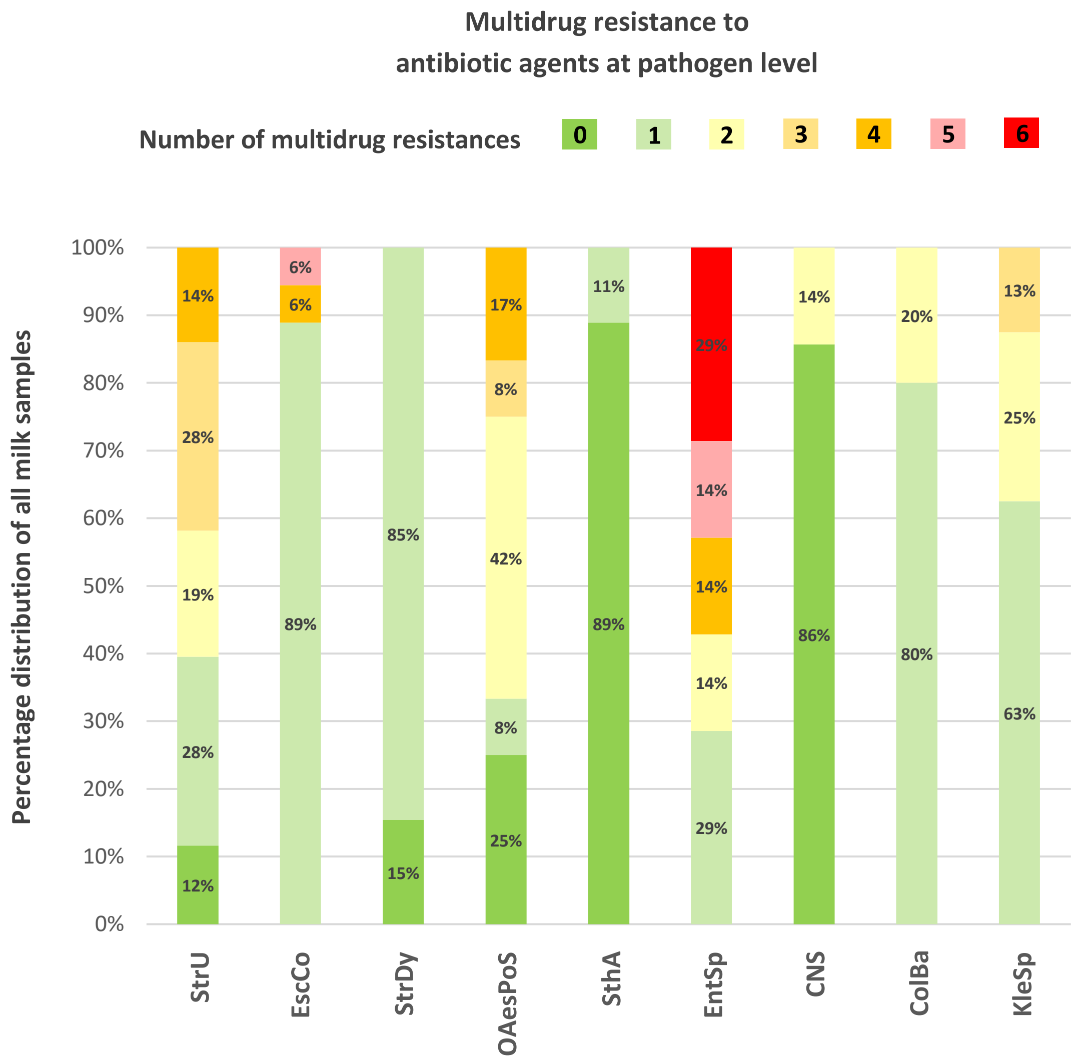


Figure 8. Multidrug resistance to antibiotic agents at pathogen level

StrUb (Streptococcus uberis); EscCo (Escherichia coli); StrDy (Streptococcus dysgalactiae); OaesPoSt (other aesculin-positive streptococci); SthAu (Staphylococcus aureus); EntSp (Enterococcus spp.); CNS (coagulase-negative staphylococci); ColBa (other coliform bacteria); KleSp (Klebsiella spp.)

A standard laboratory culture analysis revealed that, in total, the 91 mastitis pathogens found in the milk samples were resistant 161 times and sensitive to an antibiotic agent 385 times. Most pathogens were resistant to danofloxacin (61/91, 67.0%), followed by oxacillin (41/91, 45.1%) and cefoperazone (34/91, 37.4%). *Enterococcus* spp. resistance was found in 24 of 36 tested antibiotic agents (66.7%), other aesculin-positive streptococci were resistant in 20 of 48 antibiotic agents (41.7%) and *S. uberis* was resistant in 79 of 222 antibiotic agents (35.6%). Most pathogens found in the analysed milk samples were sensitive to a combination of amoxicillin and clavulanic acid (89/91, 97.8%) and cefazolin (86/91, 94.5%). The standard agar culture technique and the Speed Mam Color™ test provided consistent results in 403 out of 546 tested antibiotic agents (73.8%), whereas different results were detected in 26.2% (143/546) of the cases. Figures 9.1 and 9.2 show the overall state of resistance of mastitis pathogens in the current study found by the milk laboratory and the Speed Mam Color™ tests.

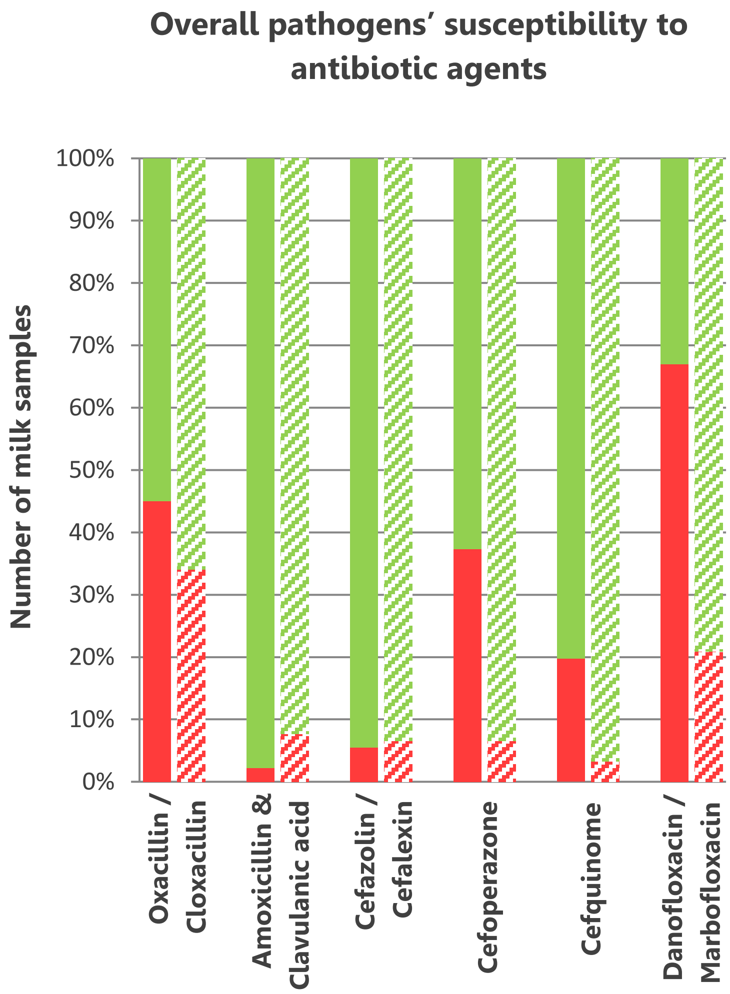


Figure 9.1 Identification of the susceptibility of pathogens to antibiotic agents at antibiotic agent level

■ *Susceptibility of pathogens tested by the milk laboratory*
▨ *Susceptibility of pathogens tested by Speed Mam Color™*
Sensitive to antibiotic agent
Resistant to antibiotic agent

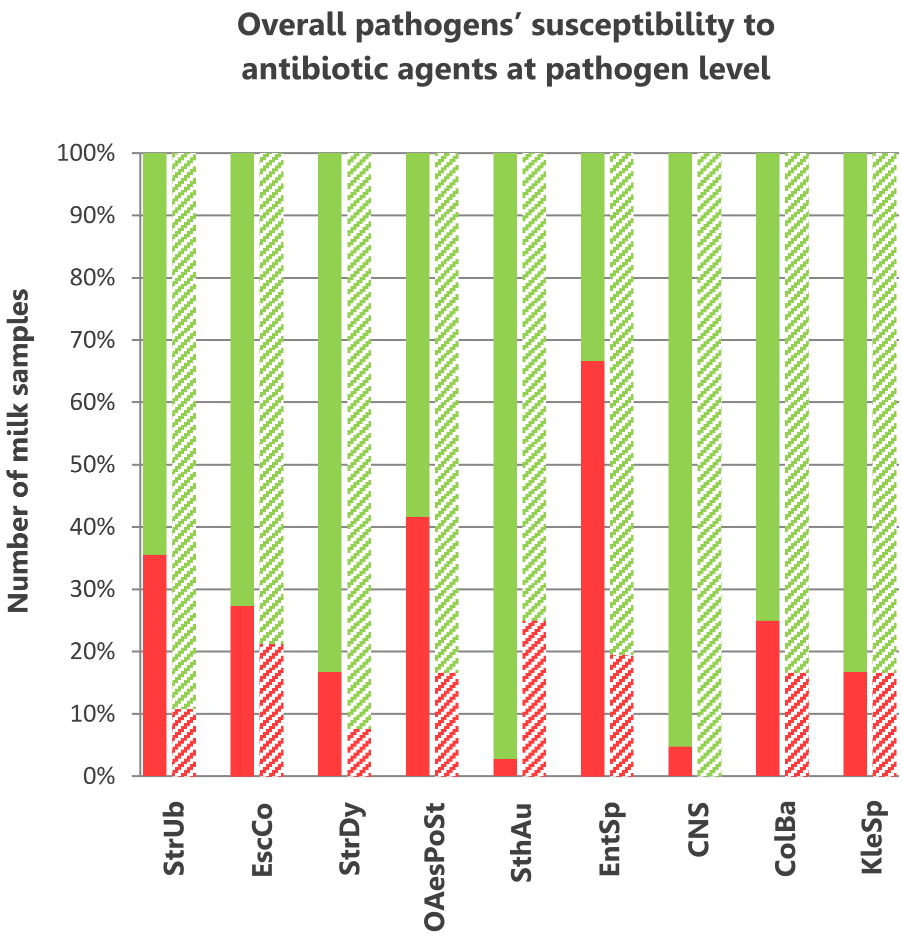


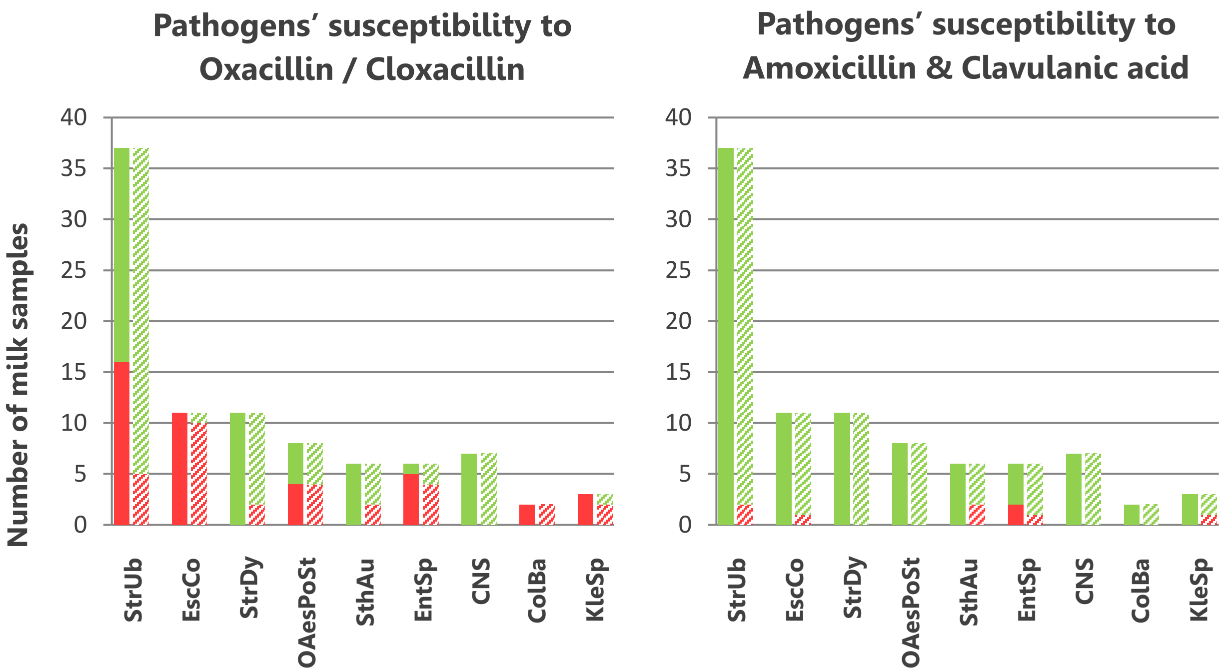
Figure 9.2. Identification of the susceptibility of pathogens to antibiotic agents at pathogen level

Susceptibility of pathogens tested by the milk laboratory
Susceptibility of pathogens tested by Speed Mam Color™
Sensitive to antibiotic agent
Resistant to antibiotic agent

StrUb (Streptococcus uberis); EscCo (Escherichia coli); StrDy (Streptococcus dysgalactiae); OaesPoSt (other aesculin-positive streptococci); SthAu (Staphylococcus aureus); EntSp (Enterococcus spp.); CNS (coagulase-negative staphylococci); ColBa (other coliform bacteria); KleSp (Klebsiella spp.)

Figures 10.1 to 10.6 show the pathogens' susceptibility to each tested antibiotic agent at the pathogen level. The most exact matches were found when cefazolin/cefalexin (86/91, 94.5%), a combination of amoxicillin and clavulanic acid (84/91, 92.3%) and cefquinome (70/91, 76.9%) were tested. Substantial deviations occurred mainly when comparing danofloxacin/marbofloxacin (43/91, 47.3%), cefoperazone (57/91, 62.6%)

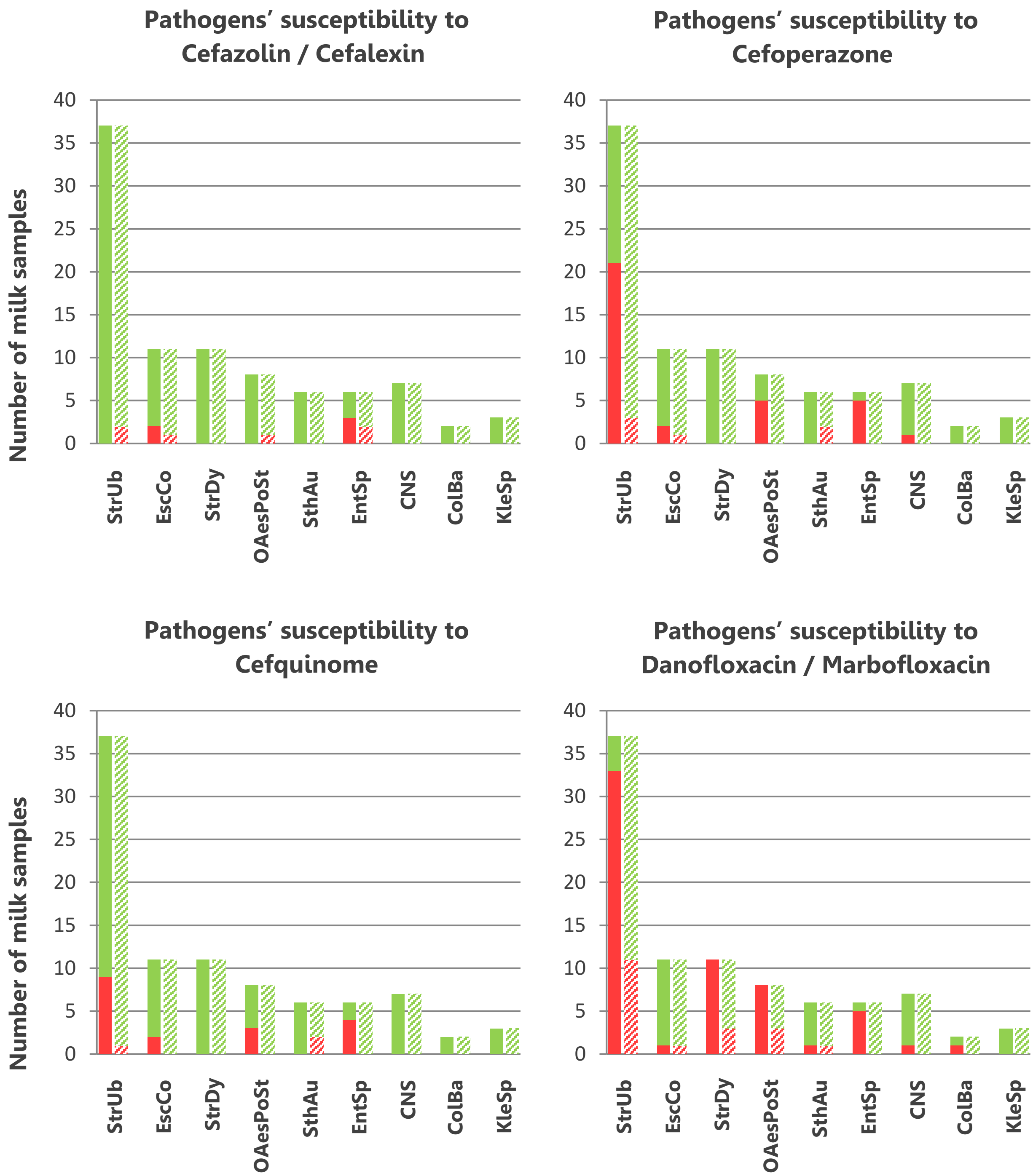
and oxacillin/cloxacillin (63/91, 69.2%). When investigating the pathogen level, it was noted that the majority of deviations in the accuracy of a pathogen's resistance to antibiotic agents was found in *Enterococcus* spp. (19/36, 52.8%), followed by *S. uberis* (75/222, 33.8%) and other aesculin-positive streptococci (16/48, 33.3%) (see Figure 9.2).



Figures 10.1 and 10.2 Identification of the susceptibility of pathogens to different antibiotic agents at pathogen level

Susceptibility of pathogens tested by the milk laboratory
Susceptibility of pathogens tested by Speed Mam Color™
Sensitive to antibiotic agent
Resistant to antibiotic agent

StrUb (Streptococcus uberis); EscCo (Escherichia coli); StrDy (Streptococcus dysgalactiae); OaesPoSt (other aesculin-positive streptococci); SthAu (Staphylococcus aureus); EntSp (Enterococcus spp.); CNS (coagulase-negative staphylococci); ColBa (other coliform bacteria); KleSp (Klebsiella spp.)



Figures 10.3 to 10.6 Identification of the susceptibility of pathogens to different antibiotic agents at pathogen level

Susceptibility of pathogens tested by the milk laboratory
Susceptibility of pathogens tested by Speed Mam Color™
Sensitive to antibiotic agent
Resistant to antibiotic agent

StrUb (Streptococcus uberis); EscCo (Escherichia coli); StrDy (Streptococcus dysgalactiae); OaesPoSt (other aesculin-positive streptococci); SthAu (Staphylococcus aureus); EntSp (Enterococcus spp.); CNS (coagulase-negative staphylococci); ColBa (other coliform bacteria); KleSp (Klebsiella spp.)

5.4. Discussion

The Speed Mam Color™ test showed both advantages and disadvantages for daily use in farm practices. It provided rapid bacteriological results, sometimes even faster than described in the operating instructions (identification of the susceptibility of pathogens within 16–18 hours and the specification of the functional groups of bacteria within 24–36 hours). The Speed Mam Color™ test showed a high sensitivity (87.7%) and specificity (80.8%) in identifying the functional groups of bacteria. Field studies by the manufacturer comparing the Speed Mam Color™ test with standard agar culture techniques to identify functional groups of bacteria, conducted by Benoit and Treilles (2013) and Teich et al. (2012), provided a sensitivity of 92% and 92.5% and specificity of 96% and 94.9%. The overall sensitivity revealed in the study is similar to the results provided by the manufacturer. However, the overall specificity of Speed Mam Color™ (80.8%) was much lower in the current study compared to Benoit and Treilles (2013) and Teich et al. (2012). Compared to other rapid, on-farm mastitis tests, the sensitivity and specificity values in the present study for identifying gram-positive bacterial growth in milk samples were comparable to other rapid, on-farm mastitis test results from other authors: VetoRapid: Se 91%, Sp 78% (Viora et al., 2014); 3M™ Petrifilm™: Se 85–94%, Sp 70–75% (McCarron et al., 2009; Mansion-de Vries et al., 2014); Minnesota easy culture system II Bi-Plate: Se 77–98%, Sp 68–87% (McCarron et al., 2009; Royster et al., 2014); Minnesota easy culture system II Tri-Plate: Se 78–86%, Sp 76–93% (Lago et al., 2006; Royster et al., 2014). Compared to the Accumast system (Se 97%, Sp 84% (Ferreira et al., 2018), the Speed Mam Color™ test achieved lower accuracy rates. The reasons for the deviating test results could be a low number of colony-forming units (microorganism presence was below the detection threshold) or errors in sample selection or culture technique. Caution should be exercised when assessing the test characteristics of the Speed Mam Color™ test as the evaluation was based on a small sample size, although this was higher than reported by the manufacturer (n=39) (Teich et al., 2012). A further study with a greater sample size and a balanced spectrum of functional groups of mastitis-causing bacteria is recommended.

Previous studies have shown that not all mastitis cases, in particular culture-negative cases and mild or moderate *E. coli* infections, both detectable by the Speed Mam Color™ test, require treatment with antimicrobial remedies (Roberson, 2003, 2012;

Suojala et al., 2013). In particular, the on-farm Speed Mam Color™ test correctly detected culture-negative mastitis cases in 80.8%, whereas it was less reliable in detecting milk samples that were contaminated with *E. coli* (Se 64.7%, Sp 98.1%). The authors considered this negligible since bacteriological cure rates for untreated *E. coli* infections are high (80–95%) (23).

Identifying the functional groups of bacteria was limited when compared to conventional laboratory milk analysis, since some pathogens were not covered (e.g., C. bovis, yeasts) (Teich et al., 2012). This resulted in false-negative bacteriological results or in the incorrect identification of functional groups of bacteria. However, this problem can be considered negligible as these minor pathogens occur infrequently in milk samples (Winter et al., 2009; Krömker et al., 2010), spontaneous healing is generally observed or a specific therapy is not possible (Winter et al., 2009). False positive results can lead to an antibiotic treatment that is not required. However, false positive test results were detected in only 5.7% of the cases and thus the risk of increased use of antibiotics is negligible.

Mixed infections, which occur in 2–16% of mastitis cases (Hawari and Al-Dabbas, 2008; Gundelach et al., 2011; Ganda et al., 2016), also posed a particular problem: the interpretation of bacteriological results, including the evaluation of pathogen resistance to antibiotics, was difficult. A milk laboratory can isolate the major pathogen in advance, which can then be tested for its antibiotic susceptibility, whereas this selection step is not available in the Speed Mam Color™ test. Thus, all involved pathogens must be tested and an antibiotic remedy for which all pathogens are sensitive must be selected. A target-oriented antibiotic treatment against the major pathogens is therefore not possible. The success of the Speed Mam Color™ test mainly depends on taking aseptic milk samples in order to avoid mixed infections, which leads to the difficult interpretation of test results and the selection of an appropriate antimicrobial udder infusion.

A low rate of concordance was found when comparing pathogen susceptibility to antibiotics, particularly in cases of *S. uberis* infections and when danofloxacin and marbofloxacin were compared. This deviation in the latter case could be caused by the fact that two different antibiotic agents of the same antibiotic class were used. However, these deviations did not occur when comparing cefoperazone and cefquinome. These deviations could also be caused due to the mixed infections as all mastitis pathogens were tested simultaneously. The literature of other authors does not provide comparative results of the antibiotic susceptibility of pathogens for rapid, on-farm mastitis tests. A qualitative assessment of the ability to reliably detect antimicrobial susceptibility of functional groups of bacteria could therefore not be carried out. A further inconvenience arises from the selection of antibiotic agents to be tested. Nearly all the major antibiotic substances approved in Europe can be tested by Speed Mam Color™, but it lacks a test well for penicillin. Furthermore, many active substances or their combinations are not approved as udder infusions in Germany.

Lago and Godden (Lago and Godden, 2018) reported that the use of on-farm culture systems to guide the treatment strategy of clinical mastitis reduced intramammary antibiotic use by half and tended to decrease milk withholding time by one day, without affecting short or long-term health or the performance of the quarter or cow.

The Speed Mam Color™ test provides rapid bacteriological results and can be used for an initial estimation of a treatment's merit. Mastitis cases that do not need to be treated with antibiotics can be detected reliably and quickly. This on-farm culture system can therefore contribute to a reduction both in the use of antibiotics and in the development of pathogen resistance.

5.5. Conclusion

The present study verified the overall high sensitivity and specificity of the Speed Mam Color™ test and provided a high degree of concordance in determining the functional groups of bacteria (except for gram-negative infections). The accuracy in determining pathogen sensitivity to antibiotics was often low and should be the objective of improvements. The Speed Mam Color™ test cannot fully replace conventional diagnostic tests in milk laboratories and should not be used to determine the susceptibility of pathogens to antibiotics because of its moderate accuracy. Nevertheless, the Speed Mam Color™ test provides rapid bacteriological results and can be used for an initial estimation of a treatment's merit. Mastitis cases that do not need to be treated with antibiotics can be detected reliably and quickly. This on-farm culture system can therefore contribute to a reduction both in the use of antibiotics and in the development of pathogen resistance.

5.6. Acknowledgement

The authors are grateful to bovicare GmbH who did the fieldwork to a large extent and the laboratory analysis.

6. General discussion

6.1. Evaluating efficacy and effectiveness of treatment methods by conducting randomised controlled trials

The current RCT revealed that the homeopathic treatment method was significantly less successful in curing clinical mastitis compared with antibiotic treatment strategies. The results imply that the effectiveness of individualised homeopathy does not go beyond a placebo effect and that in culture-positive cases (except *E. coli*), the antibiotic treatment method also provided suboptimal bacteriological cures but was more effective than individualised homeopathy and placebo treatments. Furthermore, in culture-negative milk samples and *E. coli* infections, similar effects on bacteriological and cytological cures were detected after using antibiotics, individualised homeopathy and placebo remedies.

RCTs are widely accepted as the gold standard for clinical research on the efficacy or effectiveness of medicinal products due to their repeatability and the assignment of the cause when different results between the experimental and control group occurred (Pocock, 1984; Kaptchuk, 2001; Kabisch et al., 2011). Nevertheless, RCTs have some disadvantages, which makes it difficult to generalise statements regarding the effectiveness of a treatment method under the heterogeneous conditions of farming practise.

The RCT study design has often been criticized for evaluating homeopathic treatment methods (van Haselen, 1998; Ammon and Kösters, 2016). Among other things, the success of the homeopathic treatment method depends on the caregiver/veterinarian: the probability of finding the most appropriate homeopathic remedy depends mainly on the level of knowledge about homeopathic principles. According to Ammon and Kösters (2016), the information available on individual homeopathic remedies differs significantly. There are homeopathic remedies with more than 1,000 known symptoms and remedies with fewer than 10 known symptoms, each with a different degree of validity. In order to minimize the influence of the caregiver/veterinarian in an RCT, Ammon and Kösters (2016) recommend a restriction in the available homeopathic remedies and a standardised treatment protocol. Homeopathic principles, particularly the individualised treatment approach, have also not often been taken into account. According to Doehring et al. (2015) individualised homeopathic treatment procedures were not usually appropriately considered in studies on homeopathy. Almost all RCTs carried out and assessed in the review proved the efficacy of specific homeopathic remedies (Doehring and Sundrum, 2016), meaning that each animal received the same remedy. In contrast, classic individualised homeopathic treatments of bovine clinical mastitis were only examined in two trials. Hektoen et al. (2004) and Werner et al. (2010) treated each cow individually, respecting Hahnemann's theory of classical homeopathy, meaning that a cross-check of the clinical symptom picture with the remedy picture was performed (repertorisation).

Problems can also arise in further courses of mastitis treatment. Changes in clinical symptoms can occur within the natural course of the disease, especially as a reaction to a previously administered homeopathic remedy (Ammon and Kösters, 2016). These changes in clinical symptoms would require a renewed repertorisation of a homeopathic remedy until full recovery has been achieved (iterative approach of classical homeopathy). This iterative approach, however, contradicts the principles of an RCT. According to Ammon and Kösters (2016), difficulties can also arise in assessing cure rates that are primarily based on the improvement of measurable clinical signs (recovery of the milk character/milk yield, reduction in SCC, absence of mastitis pathogens in milk etc.) in RCTS, whereas an improvement in behaviour and general condition is more appropriate in assessing homeopathic treatment methods. This means that if there has been a significant improvement in general condition, the homeopathic treatment is considered to be initially successful even if there is no significant improvement in the clinical symptoms. Experience has shown that an improvement in general condition can be followed by an improvement in the clinical signs (Ammon and Kösters, 2016). However, in contrast to humans, animals cannot speak for themselves, so the assessment of the general condition depends on the observer, who generally makes use of clinical symptoms (local symptoms on the udder, inappetence, fever etc.) of the diseased animal for the assessment of cure rates.

Another main disadvantage of RCT studies is that they represent a different situation than those faced in dairy practice. According to Kim et al. (2018), RCTs are conducted on selective sample sizes (sample population), in tightly controlled study conditions (inclusion and exclusion criteria) and under the premise that the test population is a homogeneous group. Conducting an RCT requires standardised initial and boundary conditions for comparing test results. In practice, animals might not be represented by the animals of the test population because of predetermined inclusion and exclusion criteria. Furthermore, there are other factors which differ between animals and farms, which influences the reliability of RCT test results, including, inter alia, mastitis pathogen spectrum and resistance pattern, individual animal factors (age, lactation number, primary diseases) and management factors (hygiene, manpower). This means that if a remedy or a treatment method shows efficacy in an RCT, it cannot be expected that it is also effective to the same degree in dairy practice. Rather, whereas the results of RCTs can provide information on the potential efficacy of remedies or treatment methods under standardised study conditions, in general, they cannot make a statement on the effectiveness of a treatment method in farming practice due to heterogeneity of the initial and boundary conditions.

Due to these challenges, it is questionable whether RCTs are suitable for assessing the effectiveness of homeopathy in general. For livestock, farmers and consumers, it is not the “efficacy” of a treatment method that is determined in an RCT under standardised study conditions that is relevant, but rather the “effectiveness” that is validated in the specific farm context. A conclusive statement as to the degree to which a mastitis treatment is effective on the individual farm can only be made by validating the RCT results for each individual dairy farm. In this way, the individual requirements of the conventional treatment method (taking milk samples, testing pathogen susceptibility to antibiotic agents etc.) and the homeopathic treatment method (individualisation, repertorisation, iterative treatment approach, consideration of initial worsening of clinical symptoms etc.) can also be met. The evaluation of treatment success is initially carried out at the animal level. Through regular treatment monitoring of cure rates, an analysis at the herd level is possible in a second step. This approach allows a better assessment of the effectiveness of homeopathy and also of the antibiotic treatment method, which is often be claimed to be one of the most successful chemotherapies (Hao et al., 2014). However, the antibiotic treatment method should also be validated for its effectiveness in farming practice because it is a misconception to believe that a high efficacy in RCT studies also ensures a high effectiveness on each dairy farm. A regular monitoring of treatment outcomes can help to improve animal health and welfare by ensuring farmers are aware of the low treatment success. Thus, caregiver/veterinarians can reconsider their treatment strategy and apply more effective treatment methods.

Despite designing the RCT study carefully and providing the best possible study conditions, the homeopathic treatment method was significantly less successful in curing clinical mastitis compared to the antibiotic treatment strategy. The RCT results imply that the effectiveness of individualised homeopathy does not go beyond a placebo effect and that the use of homeopathy is currently not a convincing alternative to antibiotic mastitis treatment. However, since the RCT result was determined under tightly controlled study conditions and the outcome cannot be generalized, it is advisable to validate the effectiveness of both homeopathic and conventional mastitis treatments for each individual dairy farm to ascertain whether the effectiveness of antibiotic mastitis treatment is superior to that of homeopathy.

6.2. Prerequisites for effective mastitis treatment

The study results of the second paper indicate that neither uniform treatment procedures nor a *lege artis* treatment for the use of homeopathy and antibiotic remedies in cases of clinical mastitis were found on the dairy farms visited. Most farmers did not consider or only poorly considered homeopathic principles. Rather, it seemed that each farmer had developed their own homeopathic treatment strategy. Furthermore, checking treatment outcome was inadequately carried out and a thorough documentation of treatment-related data was not or was rarely performed.

The main focus of previous studies regarding homeopathic or antibiotic treatment of bovine clinical mastitis was primarily the assessment of the efficacy of remedies. It can be assumed that treatment success is affected not only by the homeopathic remedy itself, but also by the treatment prerequisites present on dairy farms. RCTs were usually carried out under best possible conditions. In contrast, the prerequisites existing on dairy farms are often unknown. This is true particularly in the case of homeopathic treatment methods. The prerequisites necessary to enable and/or promote treatment success, particularly when animals were treated homeopathically, have thus far not been investigated in clinical studies.

Determining the status quo of the prerequisites present on dairy farms was therefore a first step towards the overall aim of increasing treatment success in cases of clinical mastitis. Special attention was given to the farmers' approach to using homeopathy (the extent to which farmers consider homeopathic principles) and implementing a *lege artis* treatment concept when a cow suffers from clinical mastitis. By using a uniform questionnaire and farm inspection protocol as well as consulting veterinary experts in homeopathy (veterinarians from the International Association for Veterinary Homoeopathy, IAVH) comparable results in respect to the use of homeopathy on dairy farms were obtained.

The representativeness of the study results must be treated carefully as the number of participants was limited. Furthermore, the 64 dairy farms were not selected randomly, but were chosen based on their use of homeopathy for clinical mastitis treatment. These points of bias could have distorted the study results. Nevertheless, the study provides a first insight into the existing prerequisites on dairy farms in three different European countries on the use of homeopathy and on current homeopathic treatment procedures. To the author's knowledge, no further studies in this field of homeopathic treatment procedures on dairy farms are available and a comparison of study results is thus not possible. For this reason, the study results must also be interpreted with caution, but they can, however, serve as the basis for further studies in this field.

Without implementing a *lege artis* treatment procedure, users of homeopathy run a high risk of overlooking important treatment information (anamnesis, clinical symptoms etc.) while considerably diminishing treatment success. In addition, the evaluation of cure rates at the animal and herd level as well as an estimation of the effectiveness of the homeopathic treatment method in the farm-specific context is not possible without adequate treatment checks and documentation. Moreover, a subjective bias of the farmer is very likely. Farmers might assess the mastitis treatment as being successful despite the lack of an objective improvement in the clinical symptoms. Thus, treatment errors cannot be identified and the treatment strategy cannot subsequently be optimized. Regular treatment monitoring, including treatment documentation, and implementing *lege artis* treatment procedures are therefore essential for increasing treatment success.

Cure rates analysed in this study were low despite best possible study conditions (e.g., considering homeopathic principles, regular check-ups, milk sample analyses). The results indicate that inadequate prerequisites in dairy practice (e.g., no *lege artis* treatments and poor knowledge of homeopathic principles) that were often found on the farms visited are a relevant cause for the lower treatment success compared to the results in clinical studies. Since cure rates as well as prerequisites for mastitis treatment in dairy practice are still largely unknown, an analysis of the treatment records at the farm level combined with the regular monitoring of treatment success should be initiated by the responsible authorities. Such an obligation for dairy farms could identify ineffective treatment methods and shorten or prevent the unnecessary suffering of animals due to clinical mastitis.

6.3. The problem of using homeopathy

Nowadays, homeopathy is widely used in dairy practice, particularly on organic farms. Studies on organic farms in Germany and in the south of England and Wales conducted by Krömker and Pfannenschmidt (2005) and Hovi and Roderick (2000) showed that 34% to 51% of clinical mastitis cases were treated with homeopathic remedies. However, various scientific studies assessing the effectiveness of homeopathy (Shang et al., 2005; Doehring and Sundrum, 2016; Lees et al., 2017a, 2017b) show no positive effect of homeopathic treatment methods. Thus, the question arises as to why homeopathy is still so popular despite its lack of effectiveness? Farmers' subjective perception of treatment success and the lack of external control mechanisms might play an important role. During the farm visits conducted in several European countries, it became evident that the farmers were convinced of the effectiveness of homeopathic remedies despite the lack of treatment records. Many farmers reported that they had "successfully" treated family members (especially their own children) with homeopathy. For this reason, farmers also started treating their diseased animals with homeopathic remedies. In addition to the self-referential assessments concerning the effect of homeopathy use, there are other possible reasons for its use. Rationale for the use of homeopathy could also be due to the low or no withdrawal period (Leon et al., 2006) or the advantage of not requiring a veterinary consultation for the medical treatment. Another advantage of using homeopathy is the reduced use of antibiotic remedies in food-producing animals, minimizing the risk of developing pathogen resistance to antibiotics. By making use of homeopathic remedies, the dairy farmer can also meet the expectations many consumers have towards foodstuffs being free of antimicrobial residues.

Homeopaths often argue that homeopathy does not cause direct damage to animals (Lees et al., 2017b). This argument, however, ignores the fact that the animals are harmed by the delayed success of the treatment. The use of individualised homeopathy is characterised by a complex application method (repertorisation) and is challenging, particularly for lay people. Important clinical symptoms might be not recognised and thus a non-corresponding remedy might be selected, or changing to the conventional treatment method in cases of non-recovery might be implemented too late. In principle, treatment success should always be given the highest priority when selecting a remedy. Homeopathy lacks evidence of efficacy in many studies (Shang et al., 2005; Doehring and Sundrum, 2016; Lees et al., 2017a, 2017b). Despite a careful design of the study and despite providing the best possible prerequisites in the RCT, the homeopathic treatment method was significantly less successful in curing clinical mastitis compared to the antibiotic treatment strategy. The study results indicate that the effectiveness of individualised homeopathy does not go beyond a placebo effect. This conclusion was also drawn by Shang et al. (2005). When using homeopathy, avoiding pain, suffering and harm in the diseased cows does not seem to be the focus of decision-making in mastitis treatment. Rather, it appears to be more about minimizing efforts and costs when selecting a remedy. Otherwise, the caregivers/veterinarians would be more likely to choose the treatment method with the best possible treatment success.

The observation that an animal has recovered after a placebo therapy is often a purely subjective perception of the animal owner/veterinarian (“caregiver”). This phenomenon is known as the “caregiver placebo effect” or “placebo by proxy effect”. Caregivers want a medical treatment that will be beneficial and they want the diseased animal to get better. However, according to objectively measurable criteria, the animal has often not recovered at all (Conzemius and Evans, 2012; Gruen et al., 2017). Overlooking a caregiver placebo response can lead to increased animal suffering and divert resources away from treatments that may benefit the diseased animal (Conzemius and Evans, 2012).

Using an ineffective treatment method rather than other effective treatments poses a high risk for prolonging the suffering of diseased animals (Lees et al., 2017b). Animal health and welfare should always be ranked higher than any alleged benefits of homeopathy. As long as the effectiveness of the homeopathic treatment method has not been proven, the results achieved in these studies strongly advise against the treatment of diseased animals with homeopathic remedies. In terms of the animal health and welfare issue of mastitis, there is reason to direct the focus to the implementation of control measures that assess the success of treating diseased farm animals.

6.4. Advantages of a rapid on-farm mastitis test (Speed Mam Color™)

The Speed Mam Color™ test showed an overall high sensitivity and specificity and provided a high degree of concordance in determining the functional groups of bacteria (except for gram-negative infections). But the accuracy in determining pathogen sensitivity to antibiotics was often low. Nevertheless, the Speed Mam Color™ test can be used for an initial estimation of a treatment's merit and can therefore contribute to a reduction both in antibiotic use and the development of pathogen resistances.

According to Ouweltjes et al. (2008) and Doehring and Sundrum (2013), only very few farmers regularly use milk analyses, while the majority of cows suffering from mastitis are treated without any information on the pathogen causing the disease and its susceptibility to antibiotics. Thus, therapy decisions are usually made empirically and are often based on previous susceptibility information for the herd (Owens et al., 1997; Maciel-Guerra et al., 2021). The results obtained by Mansion-de Vries et al. (2015) as well as those of the current study have shown that treatment success depends largely on the specific pathogen causing the mastitis. Knowing the cause of mastitis allows selective treatment decisions (treat or no treat) and additionally provides information for treatment strategies, particularly in the selection of an appropriate antibiotic remedy (Lago and Godden, 2018) in order to increase treatment success. Despite current knowledge on increasing treatment success by means of taking and analysing milk samples, the current study on the use of homeopathy found that quarter milk samples for laboratory cyto-microbiological analysis were never taken by 53% of farmers before treating mastitis. The remaining 47% of farmers took milk samples for all animals (8%) or only for selected animals (39%) in the case of clinical and/or subclinical mastitis. This reluctance to test may be due to the fact that conventional milk sample analyses are expensive (Doehring and Sundrum, 2019) and require considerable effort and time (Lago and Godden, 2018; Mansion-de Vries et al., 2014).

Due to the high time aspect involved when taking the milk sample, transporting it and performing the cyto-microbiological analysis including testing susceptibility, the time-lag before the results are available might extend to up to seven days (LKVBB, 2022). The lack of information about the mastitis pathogen can result in a contraindicated antibiotic treatment. Roberson (2003) concluded that in 50% to 80% of mastitis cases, antibiotic therapy might not be appropriate. Furthermore, it has been reported by other authors that up to 40% of milk samples were culture-negative (Roberson, 2003; Makovec and Ruegg, 2003; Mansion-de Vries et al., 2014) – 33% in the RCT – and thus do not require antibiotic treatment. Approximately 80% of clinical mastitis caused by *E. coli* are mild to moderate cases that are self-limiting and undergo spontaneous cure within a few days (Roberson, 2003, 2012; Suojala et al., 2013). Previous studies have shown that there is usually no difference in cure rates between untreated and antibiotic-treated cows with mastitis caused by *E. coli* (Pyörälä et al., 1994; Lago et al., 2011; Suojala et al., 2013). This result was confirmed in this study as the cure rates of *E. coli* infections showed no significant differences after a homeopathic, placebo or antibiotic treatment.

To find a way out of the dilemma of delayed or contraindicated treatments due to time-consuming conventional milk laboratory analyses, different rapid on-farm tests for mastitis were developed. These include, inter alia, the Minnesota easy culture system II Bi-Plate and Tri-Plate, 3M Petrifilm, VetoRapid and Speed Mam Color™. These on-farm tests offer the benefit that the selection of an effective antibiotic remedy can take place much earlier, the costs for analysis are lower compared to conventional analysis in a milk laboratory and unexperienced diagnosticians can easily use them. Lago and Godden (2018) report that the use of on-farm culture systems to guide the treatment strategy of clinical mastitis reduced intramammary antibiotic use by half and tended to decrease milk withholding time by one day, without affecting short or long-term health or the performance of the quarter or cow.

A study conducted by Neeser et al. (2006) shows that the majority of respondents believed that the use of on-farm bacteriologic culture systems helped them make better treatment decisions and that the use of fewer antibiotics resulted in some cost savings for the farm. But the most important result found by Neeser et al. (2006) was the detection of significant reductions in antibiotic use. The most common disadvantage of on-farm culture systems according to Neeser et al. (Neeser et al., 2006) was that testing antimicrobial susceptibility is not yet covered. In comparison to other rapid on-farm tests available on the commercial market, the Speed Mam Color™ test seems to meet costumers' requirements in every respect. It can identify functional groups of bacteria (including *Mycoplasma* spp.) and determine pathogen sensitivity to antibiotics at the same time. But the use of such rapid on-farm tests is only recommended if they produce similarly reliable results to a standard laboratory test. For this reason, a comparison of the Speed Mam Color™ test and a standard agar culture technique was conducted to check the reliability of the Speed Mam Color™ test and to assess whether it can contribute to a reduction in antibiotic use and the number of pathogens resistance to them.

The study revealed a high sensitivity and specificity of the Speed Mam Color™ test for identifying functional groups of bacteria, particularly when used to classify culture-negative infections. It can thus help to decide whether an antibiotic treatment is appropriate and, in addition, may guide the treatment strategy. Based on the results of the Speed Mam Color™ study, an antibiotic treatment is considered inappropriate in approximately 40% of all mastitis cases (29,9% no bacterial growth, 10,3% mild or moderate *E. coli* infections). As the Speed Mam Color™ test delivers rapid results, a target-orientated mastitis treatment can be provided or a correction of the treatment strategy can take place much earlier compared to conventional milk analyses, which will thus presumably reduce the unnecessary suffering of diseased animals and increase treatment success.

But there are also limitations of the Speed Mam Color™ test for identifying the functional groups of bacteria due to mixed infections and the fact that some pathogens are not covered (e.g., *C. bovis*, yeasts), resulting in a difficult interpretation or false negative results. However, both problems can be considered negligible as minor pathogens (Sargeant et al., 1998; Winter et al., 2009; Mansion-de Vries et al., 2014) as well as mixed infections (Mansion-de Vries et al., 2014; Gundelach et al., 2011) occur infrequently in milk samples. The success of the Speed Mam Color™ test mainly depends on taking aseptic milk samples in order to avoid mixed infections that result in the difficult interpretation of test results and the selection of an appropriate antimicrobial udder infusion.

The unique characteristic of the Speed Mam Color™ test compared to other on-farm mastitis tests lies in testing antimicrobial susceptibility to antibiotics. Despite high sensitivity and specificity with respect to identifying functional groups of bacteria, the Speed Mam Color™ test was less reliable in determining pathogen sensitivity to antibiotics. A low rate of concordance was found when comparing pathogen susceptibility to antibiotics, particularly in cases of *S. uberis* infections. This is particularly problematic because *S. uberis* has been consistently found as the major pathogen in dairy farms around the world (Maciel-Guerra et al., 2021). The literature from other authors does not provide comparative results of the antibiotic susceptibility of pathogens for rapid on-farm mastitis test. A qualitative assessment of the ability to reliably detect the antimicrobial susceptibility of functional groups of bacteria could therefore not be carried out and further scientific studies in this field are required.

6.5. Options for the improvement of udder health

The overall aim of this doctoral thesis was to reflect on options to improve udder health by increasing treatment success and simultaneously reducing the use of antibiotics in cases of bovine clinical mastitis. As already mentioned, milk sample analysis can contribute to increasing treatment success as well as reducing the risk of developing antibiotic resistance. Furthermore, milk sample analysis helps decide whether mastitis treatment is appropriate or not. In cases where an antibiotic mastitis treatment is indicated, the most suitable active ingredient can be selected by testing the pathogen's susceptibility. However, different studies have shown that the success of antibiotic treatments in cases of bovine clinical mastitis is superior to that of homeopathic treatment, but in general is still low (Hektoen et al., 2004; Doehring and Sundrum, 2016). Improving prerequisites (e.g., the use of a *lege artis* treatment procedure including regular check-ups of treatment outcome, knowledge of homeopathic principles, taking regular milk samples, thorough documentation) is therefore mandatory to further increase treatment success. However, despite good study conditions, the study carried out also showed only a moderate treatment success for the antibiotic treatment method. For this reason, improving udder health cannot be achieved with the treatment of clinical mastitis alone. According to Gerber et al. (2020), it is necessary to prevent and control mastitis at the farm level rather than solely treat affected animals due to the high costs caused by mastitis (treatment costs, decreased milk yield, milk withdrawal, premature culling). The incidence of mastitis can be reduced by controlling various factors. One of the most important factors besides high cure rates is hygiene, especially hygiene of the lying surfaces, cleanliness of the animals (udder, claws, legs etc.) and hygiene during the milking routine, followed by the management of drying-off and control strategies of subclinical mastitis (Gerber et al., 2020). The authors (Gerber et al., 2020) showed that a high implementation level of measures to improve udder health can significantly reduce the use of intramammary antimicrobials.

7. General conclusion

The studies carried out clearly show that there is a high potential for increasing treatment success, while simultaneously increasing animal health and reducing antibiotic use in cases of bovine clinical mastitis. The results of regular milk analysis can help to identify mastitis cases where antibiotic treatment is beneficial as well as cases which do not require antibiotic treatment (e.g., culture-negative milk samples or *E. coli* infections). The on-farm Speed Mam Color™ test delivered reliable results for identifying functional groups of bacteria, but it was less reliable in detecting pathogen susceptibility to antibiotics. Nevertheless, the Speed Mam Color™ test is a good option for making rapid treatment decisions (treat or no treat). On the other hand, the implementation of a *lege artis* and target-orientated treatment procedure is also essential for improving udder health and reducing antibiotic use. It became evident that the antibiotic treatment method was more effective compared to the homeopathic treatment method, which did not go beyond a placebo effect. The current arbitrary use of homeopathy in dairy practice, therefore, cannot solve the mastitis problem in dairy production. Furthermore, the use of an ineffective treatment is accompanied by relevant animal welfare problems resulting in prolonged and unnecessary suffering of diseased animals. As the primary objective should always be shortening animal suffering from clinical mastitis, the use of homeopathy is not appropriate and cannot be recommended. However, despite best possible study prerequisites, the cure rates of the conventional treatment method (antibiotics) were also lower than expected. For this reason, appropriate measures to control therapeutic success should be implemented in dairy practice in addition to measures to improve treatment success. Even more important is the fact that all measures must be suited to prevent the development of mastitis. Such measures could include, among other things, improvements in housing and milking hygiene, adequate animal nutrition and drying-off management. The success of the interaction of the optimisation measures outlined for further improvements in udder health and the reduction of antibiotic use in dairy cannot be conclusively assessed within this doctoral thesis as the investigations were carried out under study conditions and at the individual cow level. A conclusion concerning the potential for the improvement of the implemented measures can only be derived by evaluating herd-related health data, which mandatorily requires regular check-ups of treatment success and an almost complete documentation.

8. References

Ammon, K. von, and C. Kösters. 2016. Methodische Probleme von randomisierten Doppelblindstudien in der Homöopathie-Forschung [Methodological problems of randomised double blind studies in homeopathic research]. p. 25–32. In Scientific Society for Homeopathy (ed.). Der aktuelle Stand der Forschung zur Homöopathie [The current state of research on homeopathy], Köthen.

Ashvin, V. D., and S. H. Mishra. 2007. In-vitro antioxidant activity of an adaptogenic homeopathic formulation. Pharmacognosy Magazine 3(10):124–129.

Barnard, G. P., and J. H. Stephenson. 1967. Microdose paradox: a new biophysical concept. Journal of the American Institute of Homeopathy 60:277–286.

Baumgartner, W., and T. Wittek. 2018. Klinische Propädeutik der Haus- und Heimtiere [Clinical propaedeutics of domestic and companion animals]. 9., aktualisierte und erweiterte Auflage. Enke Verlag, Stuttgart.

Bennett, J. A. 2005. The consolidated standards of reporting trials (CONSORT): Guidelines for reporting randomized trials. Nursing Research 54(2):128–132.

Benoit, F., and M. Treilles. 2013. Evaluation of a commercial in-clinic diagnostic test for combined germ identification and antibiotic sensitivity testing in bovine mastitis: Poster presented at European Buiatric Forum, 27.-29. November 2013 in Marseille, France.

BMEL. 2015. Bundesministeriums für Ernährung und Landwirtschaft: Verordnung über Nachweispflichten der Tierhalter für Arzneimittel, die zur Anwendung bei Tieren bestimmt sind [Federal Ministry of Food and Agriculture: Regulation on the animal owner's obligation to provide evidence for the use of medicinal products in animals].

BMEL. 2021. Bundesministeriums für Ernährung und Landwirtschaft: Tierschutzgesetz [Federal Ministry of Food and Agriculture: Animal Welfare Act].

Bundestierärztekammer. 2015. Leitlinien für den sorgfältigen Umgang mit antibakteriell wirksamen Tierarzneimitteln: [Guidelines for the careful use of antibacterial veterinary medicinal products]. Schlütersche Verlagsgesellschaft mbH & Co. KG, Hannover.

Chikramane, P. S., A. K. Suresh, J. R. Bellare, and S. G. Kane. 2010. Extreme homeopathic dilutions retain starting materials: A nanoparticulate perspective. Homeopathy 99(4):231–242. https://doi.org/10.1016/j.homp.2010.05.006.

Cohen, J. 1960. A coefficient of agreement for nominal scales. Educational and Psychological Measurement 20(37):37–46. https://doi.org/10.1177/00131644600 2000104.

Commission Implementing Regulation (EU). 2009a. No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin L 15/1.

Commission Implementing Regulation (EU). 2009b. No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council L 152/11.

Commission Implementing Regulation (EU). 2018. No 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC L 4/43.

Commission regulation (EC). 2008. No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control L 250/1.

Conzemius, M. G., and R. B. Evans. 2012. Caregiver placebo effect for dogs with lameness from osteoarthritis. Journal of the American Veterinary Medical Association 241(10):1314–1319. https://doi.org/10.2460/javma.241.10.1314.

Davenas, E., F. Beauvais, J. Amara, M. Oberbaum, B. Robinzon, A. Miadonna, A. Tedeschi, B. Pomeranz, P. Fortner, and P. Belon. 1988. Human basophil degranulation triggered by very dilute antiserum against IgE. Nature 333(6176):816–818. https://doi.org/10.1038/333816a0.

Day, C. 2001. Gesunde Rinderbestände durch Homöopathie: Aufzucht, Haltung und Behandlung [Healthy cattle herds through use of homeopathy: breeding, husbandry and treatment]. Sonntag Verlag., Stuttgart.

DG AGRI, and European Commission. 2013. Facts and figures on organic agriculture: This report has been prepared by Directorate-General Agriculture and Rural Development with the support of Eurostat, Alfio Rodeghiero and the Organic Data Network, Brussels.

Doehring, C., and A. Sundrum. 2013. Mastitisdiagnostik in Deutschland [Mastitis diagnostic in Germany]. p. 91. In Proceeding of BfR-symposium: Antibiotikaresistenz in der Lebensmittelkette [Proceedings of Federal Institute for Risk Assessment symposium: Antimicrobial resistance in the food chain]:, 11.-12. November, Berlin.

Doehring, C., and A. Sundrum. 2016. Efficacy of homeopathy in livestock according to peer-reviewed publications from 1981 to 2014. The Veterinary record 179(24):628. https://doi.org/10.1136/vr.103779.

Doehring, C., and A. Sundrum. 2019. The informative value of an overview on antibiotic consumption, treatment efficacy and cost of clinical mastitis at farm level. Preventive veterinary medicine 165:63–70. https://doi.org/10.1016/j.prevetmed.2019.02.004.

Doehring, C., L.-M. Tamminen, C. F. González, I. Blanco-Penedo, and A. Sundrum. 2015. Report exploring the effectiveness of alternative treatment in livestock systems incorporating a variety of different experts' views and perspectives: Project report of IMPRO Project.

ECDC/EFSA/EMA. 2015. European Centre for Disease Prevention and Control /European Food Safety Authority/ European Medicines Agency first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. 13(1):4006 13, Stockholm/Parma/London.

EMA. 2022. European Medicines Agency: Advice on the designation of antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans - in relation to implementing measures under Article 37(5) of Regulation (EU) 2019/6 on veterinary medicinal products: EMA/CVMP/678496/2021.

EMEA. 2000. European Medicines Agency: Guideline on good clinical practices: VICH Topic GL 9. CVMP/VICH/595/98-Final.

EMEA. 2003. Committee for veterinary medicinal products: Guideline for the conduct of efficacy studies for intramammary products for use in cattle. EMEA/CVMP/344/99-Final-Rev.1.

Erkens, C. 2014. Homöopathie für Rinder [Homeopathy for cattles]. 2nd ed., Eugen Ulmer Verlag, Stuttgart.

Erskine, R. J., R. D. Walker, C. A. Bolin, P. C. Bartlett, and D. G. White. 2002. Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. Journal of dairy science 85(5):1111–1118. https://doi.org/10.3168/jds.S0022-0302(02)74172-6.

Estler, C.-J., and H. Schmidt. 2006. Pharmakologie und Toxikologie [Pharmacology and Toxicology]: Für Studium und Praxis [For study and practice]. 6., vollst. überarb. und erw. Aufl. Schattauer, Stuttgart.

Fajt, V. 2016. Therapeutics & pharmacology. p. 58–59. In World Buiatrics Congress (ed.). The 29th world buiatrics congress, Dublin 2016 - congress proceedings: Cattle health - tomorrow's thinking today, Dublin.

Fehlings, K., B. Baumgärtner, M. Geringer, J. Hamann, K. Knappstein, and M. Zschöck. 2000. Leitlinien zur Entnahme von Milchproben unter antiseptischen Bedingungen und Leitlinien zur Isolierung und Identifizierung von Mastitiserregern [Guidelines for taking milk samples under antiseptic conditions and guidelines for isolating and identifying mastitis pathogens]: Fachgruppe Milchhygiene - AG Eutergesundheit. DVG, Gießen.

Fehlings, K., J. Hamann, W. Klawonn, K. Knappstein, R. Mansfeld, G. Wittkowski, and M. Zschöck. 2012. Leitlinien zur Bekämpfung der Mastitis des Rindes als Bestandsproblem [Guidelines for combating bovine mastitis as a herd problem]: Fachgruppe Milchhygiene - AG Sachverständigenausschuss subklinische Mastitis. 5., überarb. Aufl. DVG, Gießen.

Ferreira, J. C., M. S. Gomes, E. C. R. Bonsaglia, I. F. Canisso, E. F. Garrett, J. L. Stewart, Z. Zhou, and F. S. Lima. 2018. Comparative analysis of four commercial on-farm culture methods to identify bacteria associated with clinical mastitis in dairy cattle. PloS one 13(3): e0194211. https://doi.org/10.1371/journal.pone.0194211.

Founou, L. L., R. C. Founou, and S. Y. Essack. 2016. Antibiotic Resistance in the Food Chain: A Developing Country-Perspective. Frontiers in microbiology 7:1881. https://doi.org/10.3389/fmicb.2016.01881.

Francoz, D., V. Wellemans, J. P. Dupré, J. P. Roy, F. Labelle, P. Lacasse, and S. Dufour. 2017. Invited review: A systematic review and qualitative analysis of treatments other than conventional antimicrobials for clinical mastitis in dairy cows. Journal of dairy science 100(10):7751–7770. https://doi.org/10.3168/jds.2016-12512.

Ganda, E. K., R. S. Bisinotto, D. H. Decter, and R. C. Bicalho. 2016. Evaluation of an on-farm culture system (Accumast) for fast identification of milk pathogens associated with clinical mastitis in dairy cows. PloS one 11(5): e0155314. https://doi.org/10.1371/journal.pone.0155314.

Garbe, S. 2003. Untersuchungen zur Verbesserung der Eutergesundheit bei Milchkühen unter besonderer Berücksichtigung des Einsatzes von Homöopathika [Studies on improving udder health in dairy cows with particular focus on the use of homeopathic remedies]. Doctoral thesis, Berlin.

Gerber, M., S. Dürr, and M. Bodmer. 2020. Reducing antimicrobial use by implementing evidence-based, management-related prevention strategies in dairy cows in Switzerland. Frontiers in veterinary science 7:611682. https://doi.org/10.3389/fvets.2020.611682.

Gnadl, B. 2008. Klassische Homöopathie für Rinder [Classical homeopathy for cattles]. 3., überarb. Aufl. Baumgartner Verlag, Übersee/Feldwies.

Gruen, M. E., D. C. Dorman, and B. D. X. Lascelles. 2017. Caregiver placebo effect in analgesic clinical trials for cats with naturally occurring degenerative joint disease-associated pain. The Veterinary record 180(19):473. https://doi.org/10.1136/vr.104168.

Gundelach, Y., E. Kalscheuer, H. Hamann, and M. Hoedemaker. 2011. Risk factors associated with bacteriological cure, new infection, and incidence of clinical mastitis after dry cow therapy with three different antibiotics. Journal of veterinary science 12(3):227–233. https://doi.org/10.4142/jvs.2011.12.3.227.

Gupta, S. K. 2011. Intention-to-treat concept: A review. Perspectives in clinical research 2(3):109–112. https://doi.org/10.4103/2229-3485.83221.

Guterbock, W. M., A. L. van Eenennaam, R. J. Anderson, I. A. Gardner, J. S. Cullor, and C. A. Holmberg. 1993. Efficacy of intramammary antibiotic therapy for treatment of clinical mastitis caused by environmental pathogens. Journal of dairy science (76):3437–3444.

Hahnemann, S., and J. M. Schmidt. 1992. Organon der Heilkunst [Organon of medicine]: Textkritische Ausgabe der 6. Auflage [Text critical edition of the 6th edition]. Haug, Heidelberg.

Hao, H., G. Cheng, Z. Iqbal, X. Ai, H. I. Hussain, L. Huang, M. Dai, Y. Wang, Z. Liu, and Z. Yuan. 2014. Benefits and risks of antimicrobial use in food-producing animals. Frontiers in microbiology 5:288. https://doi.org/10.3389/fmicb.2014.00288.

Hawari, A. D., and F. Al-Dabbas. 2008. Prevalence and distribution of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Jordan. American Journal of Animal and Veterinary Sciences 3(1):36–39. https://doi.org/10.1111/j.1742-7843.2005.pto960401.x.

Hektoen, L., S. Larsen, S. A. Odegaard, and T. Løken. 2004. Comparison of homeopathy, placebo and antibiotic treatment of clinical mastitis in dairy cows - methodological issues and results from a randomized-clinical trial. Journal of veterinary medicine. Physiology, pathology, clinical medicine 51(9-10):439–446. https://doi.org/10.1111/j.1439-0442.2004.00661.x.

Hirst, S. J., N. A. Hayes, J. Burridge, F. L. Pearce, and J. C. Foreman. 1993. Human basophil degranulation is not triggered by very dilute antiserum against human IgE. Nature 366:525–527.

Hollis, S., and F. Campbell. 1999. What is meant by intention to treat analysis? Survey of published randomised controlled trials. British Medical Journal (Clinical research ed.) 319(7211):670–674. https://doi.org/10.1136/bmj.319.7211.670.

Hovi, M. 2001. Alternative therapy use on UK organic farms - constraints and pitfalls. p. 6–12. In M. Hovi, and M. Vaarst (eds.). Positive health: preventive measures and alternative strategies: Proceedings of the Fifth NAHWOA Workshop, RØdding, Denmark, 11.-13.11.2001.

Hovi, M., and S. Roderick. 2000. Mastitis and mastitis control strategies in organic milk. Cattle Practice 8(3):259–264.

IDF. 2011. International Dairy Federation: Suggested Interpretation of Mastitis Terminology: Revision of Bulletin of IDF No 338/1999 448:1–41.

Jones, P. J., E. A. Marier, R. B. Tranter, G. Wu, E. Watson, and C. J. Teale. 2015. Factors affecting dairy farmers' attitudes towards antimicrobial medicine usage in cattle in England and Wales. Preventive veterinary medicine 121(1-2):30–40. https://doi.org/10.1016/j.prevetmed.2015.05.010.

Kabisch, M., C. Ruckes, M. Seibert-Grafe, and M. Blettner. 2011. Randomized controlled trials: Part 17 of a series on evaluation of scientific publications. Deutsches Ärzteblatt 108(39):663–668. https://doi.org/10.3238/arztebl.2011.0663.

Kaptchuk, T. J. 2001. The double-blind, randomized, placebo-controlled trial: Gold standard or golden calf? Journal of clinical epidemiology 54:541–549. https://doi.org/10.1038/nrd2629.

Keller, D., and A. Sundrum. 2018. Comparative effectiveness of individualised homeopathy and antibiotics in the treatment of bovine clinical mastitis: randomised controlled trial. The Veterinary record 182(14):407. https://doi.org/10.1136/vr.104555.

Kim, H.-S., S. Lee, and J. H. Kim. 2018. Real-world evidence versus randomized controlled trial: clinical research based on electronic medical records. Journal of Korean medical science 33(34):1-7. https://doi.org/10.3346/jkms.2018.33.e213.

Koch, B. J., B. A. Hungate, and L. B. Price. 2017. Food-animal production and the spread of antibiotic resistance: the role of ecology. Frontiers in Ecology and the Environment 15(6):309–318. https://doi.org/10.1002/fee.1505.

Kreienbrock, L., I. Pigeot, and W. Ahrens. 2012. Epidemiologische Methoden [Epidemiological methods]. 5. Aufl. 2012. Spektrum Akademischer Verlag, Heidelberg.

Krieger, M., K. Sjöström, I. Blanco-Penedo, A. Madouasse, J. E. Duval, N. Bareille, C. Fourichon, A. Sundrum, and U. Emanuelson. 2017. Prevalence of production disease related indicators in organic dairy herds in four European countries. Livestock Science 198:104–108. https://doi.org/10.1016/j.livsci.2017.02.015.

Krömker, V., and F. Pfannenschmidt. 2005. Zur Inzidenz klinischer Mastitiden und ihrer Therapie in Milchviehbetrieben des ökologischen Landbaus [Mastitis incidence and therapy in organic dairy farms]. p. 409–410. In J. Heß, and G. Rahmann (eds.). Beiträge zur 8. Wissenschaftstagung Ökologischer Landbau [Proceedings of the 8th scientific conference on organic farming], Kassel, 1.-4.03.2005. Kassel University Press, Kassel.

Krömker, V., C. Zinke, J.-H. Paduch, D. Klocke, A. Reimann, and G. Eller. 2010. Evaluation of increased milking frequency as an additional treatment for cows with clinical mastitis. The Journal of dairy research 77(1):90–94. https://doi.org/10.1017/S0022029909990422.

Kuehn, J. S., P. J. Gorden, D. Munro, R. Rong, Q. Dong, P. J. Plummer, C. Wang, and G. J. Phillips. 2013. Bacterial community profiling of milk samples as a means to understand culture-negative bovine clinical mastitis. PloS one 8(4):1-10. https://doi.org/10.1371/journal.pone.0061959.

Kuipers, A., W. J. Koops, and H. Wemmenhove. 2016. Antibiotic use in dairy herds in the Netherlands from 2005 to 2012. Journal of dairy science 99(2):1632–1648. https://doi.org/10.3168/jds.2014-8428.

Lago, A., and S. M. Godden. 2018. Use of rapid culture systems to guide clinical mastitis treatment decisions. Veterinary Clinics of North America: Food Animal Practice 34(3):389–412. https://doi.org/10.1016/j.cvfa.2018.06.001.

Lago, A., S. M. Godden, R. Bey, P. L. Ruegg, and K. Leslie. 2011. The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. Journal of dairy science 94(9):4441–4456. https://doi.org/10.3168/jds.2010-4046.

Lago, A., M. Jones, S. M. Godden, R. Bey, K. Leslie, R. Dingwel, P. Ruegg, and L. Timms. 2006. Validation of the Minnesota Easy Culture System II: Results from on-farm Bi-plate and in-lab Tri-plate culture vs. standard laboratory culture. p. 111–114. In University of Minnesota (ed.). Minnesota Dairy Health Conference, St. Paul, Minnesota.

Laxminarayan, R., A. Duse, C. Wattal, A. K. M. Zaidi, H. F. L. Wertheim, N. Sumpradit, E. Vlieghe, G. L. Hara, I. M. Gould, H. Goossens, C. Greko, A. D. So, M. Bigdeli, G. Tomson, W. Woodhouse, E. Ombaka, A. Q. Peralta, F. N. Qamar, F. Mir, S. Kariuki, Z. A. Bhutta, A. Coates, R. Bergstrom, G. D. Wright, E. D. Brown, and O. Cars. 2013. Antibiotic resistance - the need for global solutions. The Lancet Infectious Diseases 13(12):1057–1098. https://doi.org/10.1016/S1473-3099(13)70318-9.

Lees, P., L. Pelligand, M. Whiting, D. Chambers, P.-L. Toutain, and M. L. Whitehead. 2017a. Comparison of veterinary drugs and veterinary homeopathy: part 1. The Veterinary record 181(7):170–176. https://doi.org/10.1136/vr.104278.

Lees, P., L. Pelligand, M. Whiting, D. Chambers, P.-L. Toutain, and M. L. Whitehead. 2017b. Comparison of veterinary drugs and veterinary homeopathy: part 2. The Veterinary record 181(8):198–207. https://doi.org/10.1136/vr.104279.

Leon, L., M. Nürnberg, and R. Andersson. 2006. Komplementärmedizin auf ökologischen Betrieben [Complementary medicine on organic farms]. Zeitschrift für ganzheitliche Tiermedizin (20):51–54.

Levy, S. B., G. B. FitzGerald, and A. B. Macone. 1976. Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. The New England Journal of Medicine 11(295):583–588.

LKVBB. 2022. Landeskontrollverband Berlin-Brandenburg eV., FAQ: Was kostet eine Untersuchung und wie lange dauert sie? [State control association Berlin-Brandenburg, FAQ: How much does an examination cost and how long does it take?]. ttps://www.lkvbb.de/fileadmin/Redaktion/Allgemein/Mastitispreise.JPG.

Löscher, W., F. R. Ungemach, and R. Kroker. 2006. Pharmakotherapie bei Haus- und Nutztieren [Pharmacotherapy in pets and livestock]. 7., vollständig überarb. und erw. Aufl. Parey, Stuttgart.

Maciel-Guerra, A., N. Esener, K. Giebel, D. Lea, M. J. Green, A. J. Bradley, and T. Dottorini. 2021. Prediction of Streptococcus uberis clinical mastitis treatment success in dairy herds by means of mass spectrometry and machine-learning. Nature: Scientific reports 11(7736):1–15. https://doi.org/10.1038/s41598-021-87300-0.

MacLeod, G., and H. Wolter. 2006. Homöopathische Behandlung der Rinderkrankeiten [Homeopathic treatment of bovine diseases]. 5., unveränd. Aufl. Sonntag, Stuttgart.

Maddox, J., J. Randi, and W. W. Stewart. 1988. "High-dilution" experiments a delusion. Nature 334:287–290.

Makovec, J. A., and P. L. Ruegg. 2003. Results of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. Journal of dairy science 86(11):3466–3472. https://doi.org/10.3168/jds.S0022-0302(03)73951-4.

Mansion-de Vries, E. M., M. Hoedemaker, and V. Krömker. 2015. Aspekte einer evidenzbasierten Therapie klinischer Mastitiden [Aspects of an evidence-based therapy for clinical mastitis]. Tierarztliche Praxis. Ausgabe G, Grosstiere/Nutztiere 43(5):287–295. https://doi.org/10.15653/TPG-150227.

Mansion-de Vries, E. M., N. Knorr, J.-H. Paduch, C. Zinke, M. Hoedemaker, and V. Krömker. 2014. A field study evaluation of Petrifilm™ plates as a 24-h rapid diagnostic test for clinical mastitis on a dairy farm. Preventive veterinary medicine 113(4):620–624. https://doi.org/10.1016/j.prevetmed.2013.11.019.

Marchand, E., E. Stice, P. Rohde, and C. B. Becker. 2011. Moving from efficacy to effectiveness trials in prevention research. Behaviour research and therapy 49(1):32–41. https://doi.org/10.1016/j.brat.2010.10.008.

Marshall, B. M., and S. B. Levy. 2011. Food animals and antimicrobials: impacts on human health. Clinical microbiology reviews 24(4):718–733. https://doi.org/10.1128/CMR.00002-11.

Mathie, R. T., and J. Clausen. 2014. Veterinary homeopathy: systematic review of medical conditions studied by randomised placebo-controlled trials. The Veterinary record 175(15):373–381. https://doi.org/10.1136/vr.101767.

Mathie, R. T., and J. Clausen. 2015a. Veterinary homeopathy: meta-analysis of randomised placebo-controlled trials. Homeopathy 104(1):3–8. https://doi.org/10.1016/j.homp.2014.11.001.

Mathie, R. T., and J. Clausen. 2015b. Veterinary homeopathy: systematic review of medical conditions studied by randomised trials controlled by other than placebo. BMC veterinary research 11(236):1–16. https://doi.org/10.1186/s12917-015-0542-2.

McCarron, J. L., G. P. Keefe, S. L. McKenna, I. R. Dohoo, and D. E. Poole. 2009. Evaluation of the University of Minnesota Tri-plate and 3M Petrifilm for the isolation of Staphylococcus aureus and Streptococcus species from clinically mastitis milk samples. Journal of dairy science 92(10):5326–5333. https://doi.org/10.3168/jds.2009-2333.

Merck, C.-C. 2004. Etablierung der homöopathischen Mastitistherapie in einem biologisch-dynamisch wirtschaftenden Milcherzeugerbetrieb unter Berücksichtigung ökologischer, epidemiologischer und ökonomischer Gesichtspunkte [Establishment of a homeopathic mastitis therapy on a organic dairy farm taking into account ecological, epidemiological and economic aspects]: Final report on the research project 99UM032, Berlin.

Neeser, N. L., W. D. Hueston, S. M. Godden, and R. F. Bey. 2006. Evaluation of the use of an on-farm system for bacteriologic culture of milk from cows with low-grade mastitis. Journal of the American Veterinary Medical Association 228(2):254–260. https://doi.org/10.2460/javma.228.2.254.

Ouweltjes, W., J. J. Windig, G. de Jong, T. J. G. M. Lam, J. ten Napel, and Y. de Haas. 2008. The use of data from sampling for bacteriology for genetic selection against clinical mastitis. Journal of dairy science 91(12):4860–4870. https://doi.org/10.3168/jds.2008-1355.

Ovelgönne, J. H., A. W. Bol, W. C. Hop, and R. van Wijk. 1992. Mechanical agitation of very dilute antiserum against IgE has no effect on basophil staining properties. Experientia 48(5):504–508. https://doi.org/10.1007/BF01928175.

Owen, D. 2007. Principles and practice of homeopathy: The therapeutic and healing process. Churchill Livingstone, Edinburgh.

Owens, W. E., C. H. Ray, J. L. Watts, and C. H. Yancey. 1997. Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. Journal of dairy science 80(2):313–317.

Pocock, S. J. 1984. Clinical Trials: A Practical Approach. John Wiley & Sons Ltd, Chichester.

Pol, M., and P. L. Ruegg. 2007. Treatment practices and quantification of antimicrobial drug usage in conventional and organic dairy farms in Wisconsin. Journal of dairy science 90(1):249–261. https://doi.org/10.3168/jds.S0022-0302(07)72626-7.

Pyörälä, S., L. Kaartinen, H. Käck, and V. Rainio. 1994. Efficacy of two therapy regimens for treatment of experimentally induced Escherichia coli mastitis in cows. Journal of dairy science 77(2):453–461. https://doi.org/10.3168/jds.S0022-0302(94)76973-3.

Roberson, J. R. 2003. Establishing treatment protocols for clinical mastitis. Veterinary Clinics of North America: Food Animal Practice 19(1):223–234.

Roberson, J. R. 2012. Treatment of clinical mastitis. The Veterinary clinics of North America. Food animal practice 28(2):271–288. https://doi.org/10.1016/j.cvfa.2012.03.011.

Roberson, J. R., L. D. Warnick, and G. Moore. 2004. Mild to moderate clinical mastitis: efficacy of intramammary amoxicillin, frequent milk-out, a combined intramammary amoxicillin, and frequent milk-out treatment versus no treatment. Journal of dairy science 87(3):583–592. https://doi.org/10.3168/jds.S0022-0302(04)73200-2.

Roderick, S., and M. Hovi. 1999. Animal health and welfare in organic livestock systems: identification of constraints and priorities. A report to the Ministry of Agriculture, Fisheries and Food, Reading.

Royster, E., S. Godden, D. Goulart, A. Dahlke, P. Rapnicki, and J. Timmerman. 2014. Evaluation of the Minnesota Easy Culture System II Bi-Plate and Tri-Plate for identification of common mastitis pathogens in milk. Journal of dairy science 97(6):3648–3659. https://doi.org/10.3168/jds.2013-7748.

Ruegg, P. L. 2017. A 100-Year Review: mastitis detection, management, and prevention. Journal of dairy science 100(12):10381–10397. https://doi.org/10.3168/jds.2017-13023.

Ryan, R., S. Hill, D. Broclain, D. Horey, S. Oliver, and M. Prictor. 2013. Supplementary guidance for authors undertaking reviews with the Cochrane consumers and communication review group: Study design guide, https://cccrg.cochrane.org/sites/cccrg.cochrane.org/files/public/uploads/Study_design_guide2013.pdf.

Sargeant, J. M., H. M. Scott, K. E. Leslie, M. J. Ireland, and A. Bashiri. 1998. Clinical mastitis in dairy cattle in Ontario: frequency of occurrence and bacteriological isolates. Canadian Veterinary Journal 39:33–38.

Schmidt, A. 2003. Grundkurs in klassischer Homöopathie für Tierärzte [Basic training course in classical homeopathy for veterinarians]. 3., erw. Aufl. Sonntag, Stuttgart.

Schütte, A. 2002. Homöopathie in der Tiermedizin [Homeopathy in veterinary medicine]. Lohmann Information (2):1–8.

Shang, A., K. Huwiler-Müntener, L. Nartey, P. Jüni, S. Dörig, J. A. C. Sterne, D. Pewsner, and M. Egger. 2005. Are the clinical effects of homoeopathy placebo effects? Comparative study of placebo-controlled trials of homoeopathy and allopathy. The Lancet 366(9487):726–732. https://doi.org/10.1016/S0140-6736(05)67177-2.

Singal, A. G., P. D. R. Higgins, and A. K. Waljee. 2014. A primer on effectiveness and efficacy trials. Clinical and translational gastroenterology 5(e45):1-4. https://doi.org/10.1038/ctg.2013.13.

Sorge, U. S., S. Yamashita, and L. Pieper. 2019. Bovine veterinarians' perspective on organic livestock production in the USA. The Veterinary record 184(12):384. https://doi.org/10.1136/vr.104799.

Striezel, A. 2005. Leitfaden der Nutztiergesundheit. Ganzheitliche Prophylaxe und Therapie [Guide for animal health on farms. Holistic prophylaxis and therapy]. Sonntag Verl., Stuttgart.

Sundrum, A. 2016. Report describing the options and limitations in the use of alternative remedies to reduce the use of antibiotic: Project report of IMPRO Project.

Suojala, L., L. Kaartinen, and S. Pyörälä. 2013. Treatment for bovine Escherichia coli mastitis - an evidence-based approach. Journal of veterinary pharmacology and therapeutics 36(6):521–531. https://doi.org/10.1111/jvp.12057.

Swayne, J. 2000. International dictionary of homoeopathy. Churchill Livingstone, Edinburgh.

Tamminen, L.-M., U. Emanuelson, and I. Blanco-Penedo. 2018. Systematic review of phytotherapeutic treatments for different farm animals under European conditions. Frontiers in veterinary science 5(140):1–11. https://doi.org/10.3389/fvets.2018.00140.

Teich, K., J. Friedrich, J. Morlet, and V. Krömker. 2012. Speed Mam Color - der Mastitis-Schnelltest zur Erreger-Differenzierung und Resistenztestung für die Rinderpraxis - Vorstellung erster Feldversuchergebnisse [Speed Mam Color - the rapid mastitis test for pathogen differentiation and resistance testing in cattle practice - presentation of the first field test results]. p. 112–116. In K. Müller (ed.). Proceedings of 9th Berlin-Brandenburgischer Rindertag, Berlin, 4.-6.10.2012. Cuvillier Verlag, Göttingen.

van Haselen, R. A. 1998. Randomised, placebo-controlled trials of classical homoeopathy: Is there a problem? British Homoeopathic Journal 87(3):128–130. https://doi.org/10.1054/homp.1999.0239.

Varshney, J. P., and R. Naresh. 2005. Comparative efficacy of homeopathic and allopathic systems of medicine in the management of clinical mastitis of Indian dairy cows. Homeopathy 94(2):81–85. https://doi.org/10.1016/j.homp.2004.11.013.

Viora, L., E. M. Graham, D. J. Mellor, K. Reynolds, P. B. A. Simoes, and T. E. Geraghty. 2014. Evaluation of a culture-based pathogen identification kit for bacterial causes of bovine mastitis. The Veterinary record 175(4):89. https://doi.org/10.1136/vr.102499.

Vithoulkas, G. 1981. The science of homeopathy. 1st Evergreen ed. Grove Weidenfeld, New York.

Vithoulkas, G. 1996. True but strange?: Comment letter to Ernst et al. (Nature 381, 361; 1996). Nature 383(6599):383. https://doi.org/10.1038/383383a0.

Weiermayer, P., M. Frass, T. Peinbauer, and L. Ellinger. 2020. Evidenzbasierte Veterinär-/Homöopathie und ihre mögliche Bedeutung für die -Bekämpfung der Antibiotikaresistenzproblematik – ein Überblick [Evidence-based veterinary homeopathy and its possible role in combating the antimicrobial resistance problem - an overview]. Schweizer Archiv für Tierheilkunde 162(10):597–615. https://doi.org/10.17236/sat00273.

Werner, C., A. Sobiraj, and A. Sundrum. 2010. Efficacy of homeopathic and antibiotic treatment strategies in cases of mild and moderate bovine clinical mastitis. The Journal of dairy research 77(4):460–467. https://doi.org/10.1017/S0022029910000543.

Winter, P., C. Burvenich, H. Hogeveen, F. Neijenhuis, and B. de Spiegeleer. 2009. Praktischer Leitfaden Mastitis: Vorgehen beim Einzeltier und im Bestand [Practical guide for mastitis: procedures for individual animals and herds]. 1. Auflage. Enke, Stuttgart.