Evaluation of efficacy of homeopathic and antibiotic treatment in scientific research and practice with focus on bovine mastitis

Dissertation for the acquisition of the academic degree

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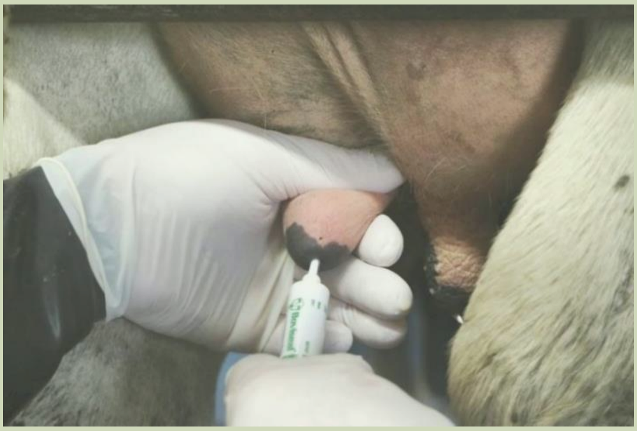

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This work has been accepted by the Faculty of Organic Agricultural Sciences of the University of Kassel as a thesis for acquiring the academic degree of Doctor of Agricultural Sciences (Dr. agr.).

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Preface

This thesis is submitted to the Faculty of Organic Agricultural Sciences (Department of Animal Nutrition and Animal Health) of the University of Kassel as a partial fulfilment of the requirement for the degree of Doctor of Agricultural Sciences. Part of the research (paper I) was carried out within the IMPRO project WP9 (Impact matrix analysis and cost-benefit calculations to improve management practices regarding health status in organic dairy farming) which has received funding from the European Union's Seventh Framework Program for research, technological development and demonstration under grant agreement n° 311824.

Paper II and III received no funding nor any other subsidies and were published while working as a veterinary practitioner extern from the university. Detailed farm records for paper III, collected by the software HERDE by dsp-agrosoft, were provided voluntarily by the farmers participating, which also answered the questionnaires regarding their individual costs (working hour, milk price, price for laboratory diagnostic etc.). The software company dsp-agrosoft friendly supported to find farmers to participate.

Die Entstehung einer Dissertation ist, wie Mastitis, ein multifaktorieller Prozess. Ohne die Mitwirkung einiger wunderbarer Menschen und den geeigneten Umständen wäre diese Arbeit ungeschrieben.

Zuallererst möchte ich mich deshalb bei meinem Doktorvater, Prof. Dr. Albert Sundrum bedanken. Sie haben - von Anfang an - an mich geglaubt und aus der praktischen Tierärztin, eine Wissenschaftlerin gemacht, die sich für evidenzbasierte Veterinärmedizin begeistern konnte und froh ist, diese wieder in die Praxis tragen zu können. Sie wurden nie müde, mich durch Diskussionen herauszufordern, hatten immer Zeit und ein offenes Ohr für ein Gespräch, wenn es nicht richtig weiterging und Ihre Denkanstöße haben jeden Artikel noch besser werden lassen. Glücklich bin ich auch über den zeitlichen Freiraum, den Sie mir bei der Erstellung der Dissertation gegeben haben, wohlwissend, dass die Praxistätigkeit manchmal der Wissenschaft im Wege stand. Man kann sich als Doktorand/in keinen besseren Doktorvater wünschen.

Eine große Unterstützerin war auch meine Mitstreiterin Dr. Diana Keller, aus der durch die gemeinsame Zeit an der Universität Kassel, trotz anschließend getrennter Wege, eine gute Freundin und Vertraute wurde. Dich auf dem Weg zu Deiner Dissertation zu unterstützen, mit gemeinsamen Schreib-Workshops zu quälen, hat auch mir geholfen, regelmäßig fokussiert und in kurzer Zeit voranzukommen. Es ist großartig, dass wir uns gegenseitig motivieren und inspirieren können und gleichzeitig so viel Spaß haben. Von Dir habe ich gelernt, dass Pünktlichkeit nicht alles ist, Sorglosigkeit manchmal weiterhilft und dass man gutes Essen und Trinken immer zelebrieren sollte.

Bedanken möchte ich mich auch bei meinen ehemaligen Witzenhüsser Kolleginnen. Silvia Minke, die gute Seele des Fachgebiets, die immer alles im Blick hat und sofort bereit ist, zu helfen, egal ob mit Tat, einer Umarmung oder einfach mit einem aufmunternden Spruch. Dr. Susanne Hoischen-Taubner, die ungelogen alles beherrscht (Wissenschaft, Kommunikation, Wissenstransfer, Multitasking, Familie, Handwerk, Pferde, Hühner…) und die mir oft mit konstruktiver Kritik und einem anderen Blickwinkel geholfen hat. Danke!

Auch Dr. Margret Krieger möchte ich von Herzen danken, dass Du mich mit Deinem messerscharfen Verstand und deinem routinierten Umgang geholfen hast, sowohl bei der wissenschaftlichen Arbeit als auch bei den Vorlesungen/Übungen und bei Fragen zur Betreuung der Bachelorarbeiten. Susanne Hartmann und Karin Zahnwetzer – dafür, dass Ihr uns alle immer unterstützt habt, egal wie verrückt unsere Ideen, wie stinkend die eingelagerten Organe waren oder ob noch eine „Tierärztin" für den Doktorhut gefehlt hat. Danke!

Also, I would like to express my gratitude for all the great people, I was introduced by working within the European Research project IMPRO from Spain, Sweden, Great Britain, the Netherlands and France. A

great team of brilliant researchers with enthusiasm for animal health and an open mind for new ideas willing to discuss until a good solution has been found. I owe special thanks to Isabel Blanco-Penedo and Lena-Mari-Tamminen: I always enjoyed our meetings and fruitful discussions (on site and online) – please keep in touch from time to time.

Zum Schluss und vor Allem möchte ich Rene Weihrauch, meinem liebenvollen, pragmatischen und geduldigen Freund danken. Du hast, obwohl Dir dieser gläserne Elfenbeinturm von Wissenschaft gehörig zu weit entfernt von der Praxis ist, immer an mich geglaubt, mich unterstützt und mir den Rücken freigehalten. Dafür bin ich Dir unendlich dankbar.

The biggest obstacle to discover is not ignorance,

it is the illusion of knowledge.

Daniel J. Boorstin

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List of Publications

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

I. Efficacy of homeopathy in livestock according to peer-reviewed publications from 1981 to 2014 (Veterinary Record, 2016)

II. Efficacy of antibiotic and homeopathic treatment of bovine mastitis and implications for the effectiveness on farms (submitted at Preventive veterinary medicine)

III. The informative value of an overview on antibiotic consumption, treatment efficacy and cost of clinical mastitis at farm level (Preventive veterinary medicine, 2019)

Abbreviations

CIA	Critically Important Antimicrobials
CONSORT	Consolidated Statement of Reporting Trials
ECCH	European Central Council of Homeopaths
e.g./eg	example given
et al.	et alia / and others
etc.	et cetera
HPCIAs	Highest priority critically important antimicrobials
i.e.	in example
MARAN	Monitoring of Antimicrobial Resistance and antibiotic usage in Animal in the Netherlands
nDD_{ay}	number of daily doses (antibiotics per average herd cow per year)
P	*p*-value
PRISMA	Preferred Reporting Items for Systemic reviews and Meta-Analysis
SCC	Somatic Cell Count (per ml milk)
SEM	Standard Error of Measurement
RCT	Randomized Controlled Trial
REFLECT	Reporting Guidelines for Randomized Control Trials

Abstract

Evaluation of efficacy of homeopathic and antibiotic treatment in scientific research and practice with focus on bovine mastitis

The use of homeopathy and antibiotics in treatment have the same goal: to cure a diseased individual. The first choice should be the most effective treatment and only a repeated treatment success will justify its use. If the chosen treatment is not effective, unnecessary suffering of diseased animals, spreading of disease or chronification of mastitis will be the result. Additionally, in case of antibiotic use, an ineffective use will select and promote the prevalence of multi-resistant bacteria in the environment, humans and animals as well as the loss of effectiveness of critically important antibiotics for treatment.

Therefore, the aim of this thesis was to find out, if homeopathy might help to reduce or replace antibiotics. Consequently, homeopathy must be effective in general and in specific diseases, here in treatment of bovine mastitis, similarly or better compared to the standard antibiotic treatment. But how effective is homeopathic and antibiotic treatment according to scientific trials on bovine mastitis and is this reflected in the use and effectiveness in dairy practice?

A systematic review on general efficacy of homeopathy applied in the treatment of food-producing animals with the aim to reduce antibiotics was performed involving 52 trials within peer-reviewed publications between 1981 and 2014 (paper I). Here, bovine mastitis was the main reason to apply homeopathy instead of antibiotics and the most scientific publications dealt with the efficacy when deployed. Therefore, efficacy and effectiveness of antibiotic and homeopathic treatment in bovine mastitis were evaluated and discussed based on peer-reviewed papers available focussing specifically on their scientific quality and outcome (paper II).

On-farm effectiveness of antibiotic treatment in bovine mastitis and the value of record keeping was the topic of the last paper (III), investigated in a retrospective study with real-world data of 30 dairy farms, considering antibiotic consumption, treatment success and cost of mastitis on individual farm and cow level.

The scientific literature (paper I) did not provide sufficient evidence of efficacy of homeopathy in farm animals on several diseases due to lack of scientific quality, repetition, and performance under various study conditions.

Focussing on bovine mastitis (paper II), antibiotic studies testing efficacy in treatment provided efficacy in 47 % but had a low scientific quality and therefore did not provide valid results. Studies on efficacy of homeopathic treatment of bovine mastitis was positive for 42 % of the trials and had a better scientific quality compared to the former but failed to show efficacy especially in higher quality studies.

In practice, treatment success of antibiotic treatment controlled by somatic cell count ranged between 18 – 59 % on the evaluated farms (paper III). The use of antibiotics for bovine mastitis showed a high variation in antibiotic consumption. About 13 – 53 % of all cows on the investigated farms received antibiotics due to mastitis during lactation with different antibiotic agents adding up from 5 to 17 within one treatment and total cost of mastitis (158 – 483€ per cow and year). The results indicated that using average or incomplete figures risks to lead to considerable misinterpretations. Antibiotics usage was remarkably high on most farms (and over 32 % of them categorized as 'highest priority critically important antimicrobials'), while the poor treatment results signalled a need to improve treatment strategies to increase cure rates and reduce antibiotic consumption.

Finally, when mastitis occurs, appropriate diagnostic including cyto-bacteriological milk samples should be performed to choose an appropriate treatment (applied according to current knowledge), followed by control of treatment outcome on cow and farm level. Any treatment needs to be justified by published results of high-quality studies (efficacy in science) followed by treatment monitoring on farm with outcome control (effectiveness in practice) on individual farms. A comparison between farms externally (benchmarking of treatment frequency, cure rates, disease incidence and costs) is necessary to motivate and support stakeholders (farmers, veterinarians, food retailers and politicians) to improve and change treatments, where necessary and to define treatment guidelines or protocols to reduce unnecessary antibiotic consumption.

Antibiotic treatment of mastitis accompanied with insufficient control can be expected to result in a high antibiotic consumption. Thus, responsible use is not provided without on-going record keeping (treatment details and its outcome) and utilizing real-world data (somatic cell count, milk yield, conductivity etc.) including milk diagnostics (*e.g.,* rapid on-farm milk tests on causing pathogen) to improve treatment strategies, animal health and welfare. The same is true for the use of homeopathy, which cannot be recommended according to the current scientific knowledge as it did not provide evidence for efficacy under optimized conditions and might even be lower under real-world conditions.

Keywords: bovine mastitis, homeopathy, antibiotic, efficacy, effectiveness

Zusammenfassung

Beurteilung der Wirksamkeit therapeutischer Behandlungen mit Homöopathie und Antibiotika in Wissenschaft und Praxis mit besonderem Fokus auf die Mastitis des Rindes

Die Anwendung von Homöopathie und Antibiotika verfolgen dasselbe Ziel: die Heilung eines erkranktes Individuums. Dabei sollte sich die Wahl des Medikaments immer an der voraussichtlich besten Wirksamkeit im jeweiligen Kontext orientieren. Wenn sich die gewählte Behandlungsstrategie jedoch als unwirksam erweist, führt dies möglicherweise zu vermeidbarem Leiden der erkrankten Tiere, zur Chronifizierung und Weiterverbreitung von Krankheiten oder auch frühzeitigen Abgängen aus der Herde. Die Anwendung von Antibiotika fördert zudem das Auftreten und die Selektion multi-resistenter Bakterienstämme in der Umwelt, beim Mensch und beim Tier und kann zum Wirkverlust kritischer Reserveantibiotika führen.

Ziel dieser Arbeit war es herauszufinden, ob homöopathische Behandlungsverfahren helfen können, den Einsatz von Antibiotika zu reduzieren oder diese zu ersetzen. Dafür muss dieses Verfahren eine Wirksamkeit im Allgemeinen, sowie bei speziellen Erkrankungen wie der bovinen Mastitis besitzen, die mindestens gleich oder besser ist als die Standardbehandlung mit Antibiotika. Aber wie wirksam ist die homöopathische und antibiotische Behandlung laut wissenschaftlicher Studien bei der bovinen Mastitis und spiegelt sich dies in der Anwendung und Wirksamkeit auf den Milchviehbetrieben wider?

Zwecks Beantwortung dieser Frage wurde eine Übersichtsarbeit (Review) zur generellen Wirksamkeit der Homöopathie bei Anwendung bei lebensmittelliefernden Tieren bei verschiedensten Krankheiten mit dem Ziel der Antibiotikareduzierung durchgeführt. Es gelangten 52 unabhängig-begutachtete (peer-review) Studien, die zwischen 1981 und 2014 veröffentlicht wurden, zur Auswertung (Artikel I).

Die Mastitis des Rindes war und ist der häufigste Grund für die Anwendung von Homöopathie anstelle von Antibiotika. Auch die meisten wissenschaftlichen Veröffentlichungen beschäftigten sich mit dieser Thematik. Aus diesem Grund wurde sowohl die Wirksamkeit von Homöopathie als auch vergleichend die von Antibiotika bei der bovinen Mastitistherapie in Wissenschaft und Praxis mithilfe der verfügbaren Peer-review-Veröffentlichungen beurteilt, diskutiert und unter besonderer Berücksichtigung ihrer wissenschaftlichen Qualität und ihrer Aussagekraft beurteilt (Artikel 2).

Die Wirksamkeit einer antibiotischen Mastitisbehandlung auf milchviehhaltenden Betrieben und die Relevanz von Datenaufzeichnungen auf dem Betrieb waren Thema des dritten Artikels. In einer retrospektiven Studie gelangten umfangreiche Daten von 30 Milchviehbetrieben unter Berücksichtigung des Antibiotikaverbrauchs, des Behandlungserfolgs und der individuellen Kosten der Mastitis auf Betriebs- und Einzeltierebene zur Auswertung.

Die Auswertung der wissenschaftlichen Literatur (Artikel I) konnte eine Wirksamkeit des homöopathischen Behandlungsverfahren bei Nutztieren bei verschiedenen Krankheiten aufgrund von Mängeln in der wissenschaftlichen Qualität der Studien, fehlender Wiederholungen und divergierender Studienbedingungen nicht ausreichend belegen.

Die Auswertungen der Studien zum Therapieerfolg bei der bovinen Mastitis in Artikel II zeigte, dass in 47 % der untersuchten wissenschaftlichen Studien eine Wirksamkeit der antibiotischen Behandlung nachgewiesen wurde. Allerdings wiesen die Studien nur eine unzureichende wissenschaftliche Qualität auf und waren damit nicht ausreichend valide, um eine Wirksamkeit ableiten zu können. In Studien Behandlung der bovinen Mastitis mit Homöopathie wurde in 42 % der Studien eine Wirksamkeit festgestellt. Obwohl diese Studien zur Homöopathie im Allgemeinen eine höhere wissenschaftliche Qualität als Studien zur Antibiotikatherapie aufwiesen, konnte in den qualitativ besseren Studien kein Nachweis Hinweis auf eine Wirksamkeit der angewandten Homöopathika erbracht werden.

In der Praxis lag der Behandlungserfolg gemessen am Milchzellgehalt nach einer Antibiotika-Therapie in den ausgewerteten Betrieben zwischen 18 und 59 % (Artikel III). Zwischen den Betrieben bestanden große Unterschiede hinsichtlich des Einsatzes von Antibiotika zur Mastitisbehandlung. So erhielten 13 – 53 % aller Kühe Antibiotika zur Mastitisbehandlung während der Laktation. Dabei kamen während einer Einzeltierbehandlung teilweise 5 bis 17 verschiedene antibiotische Wirkstoffe zum Einsatz. Die betrieblichen Gesamtkosten, die durch Mastitis verursacht wurden, variierten zwischen 158 und 483€ pro Kuh und Jahr. Dies weist darauf hin, dass eine Verwendung durchschnittlicher oder unvollständiger Kennzahlen oder Schätzungen abhängig vom betrieblichen Kontext zu erheblichen Fehleinschätzungen führen kann. Der Einsatz von Antibiotika war in den meisten Betrieben bemerkenswert hoch. Über 32 % der eingesetzten Mittel wurden als kritische Reserveantibiotika mit höchster Priorität eingestuft. Gleichzeitig deuten die schlechten Behandlungsergebnisse darauf hin, dass die Behandlungsstrategien verbessert werden müssen.

Die Behandlung von Mastitiden mittels Antibiotika führt ohne eine begleitende Erfolgskontrolle mit hoher Wahrscheinlichkeit zu einem erhöhten Antibiotikaverbrauch. Daher sollte eine verantwortungsvolle Anwendung von Antibiotika mit einer fortlaufenden Dokumentation (Behandlungen, Erfolgskontrolle auf Kuh- und Betriebsebene) unter Verwendung aller verfügbarer Betriebsdaten (Milchzellzahlen, Milchleistung, Leitfähigkeit usw.) einschließlich Milchdiagnostik (z. B. mikrobieller Milchschnelltests auf den Betrieben) und deren Auswertung erfolgen. Erst mit Hilfe einer umfassenden Datenakquise und -auswertung erschließen sich Potentiale, mit denen eine Verbesserung des Behandlungserfolges, der Tiergesundheit und damit des Tierschutzes sowie eine Absenkung des Antibiotikaverbrauches realisiert werden können.

Jede Therapieform sollte durch den Nachweis einer Wirksamkeit innerhalb hochwertiger Studien (Wirksamkeit in der Wissenschaft) gerechtfertigt sein, gefolgt von einem Monitoring der Behandlungen im Betrieb mit Ergebniskontrolle (Wirksamkeit in der Praxis). Erst ein überbetrieblicher Vergleich zwischen landwirtschaftlichen Betrieben (Benchmarking von Krankheitsinzidenz, Behandlungshäufigkeit, Heilungsraten und Kosten) bietet den involvierten Interessengruppen (Landwirte, Tierärzte, Lebensmitteleinzelhandel, Verbraucher, Politiker) die erforderliche Orientierung, um Behandlungen zu verbessern, mögliche Zielvorgaben setzen zu können, Behandlungsrichtlinien oder -protokolle zu definieren und so den Einsatz unnötiger Antibiotikaanwendungen zu reduzieren. Gleiches gilt für den Einsatz von Homöopathie, die nach aktuellem Stand der Wissenschaft nicht empfohlen werden kann, da sie selbst unter optimalen wissenschaftlichen Bedingungen keine Wirksamkeitsnachweise erbringen konnte.

Schlüsselwörter: Rindermastitis, bovine Mastitis, Homöopathie, Antibiotika, Wirksamkeit

1. General Introduction

Cure of disease in farm animals is in general the first and main reason to apply a medical treatment. However, the success of treatment is nothing we can be sure of. Although our knowledge on causes, pathogens, predispositions and their interrelationships increased throughout the years of scientific research, diseases are an ongoing concern and challenge our efforts to keep animals healthy.

One of the main causes for applying a medical treatment on dairy farms is bovine mastitis. Mastitis is defined as an inflammation of the mammary gland. It is a multifactorial disease, as it is influenced by numerous factors (*e.g.,* management, hygiene, herd health, individual cow factors). The disease is mostly caused by bacterial infections, but also fungal or algae pathogens as well as toxins or trauma can be involved.

The detection of mastitis usually occurs by clinical symptoms and visible (flakes, discoloration, blood etc.) or invisible changes in milk (SCC, conductivity etc.) and in the mammary gland (heat, swelling, pain, redness). The detection of the causing pathogen in milk samples plays a key role when trying to increase the treatment success by selection of an effective remedy. In case of chronic/multi-resistant infections it helps to make an early decision on culling to prevent further suffering and a possible spreading of the pathogens within the herd (Mansion-de Vries et al., 2015).

Unfortunately, mortality/cullings caused by clinical mastitis has increased from 4.7% (1970) to 8.7% (1980), 12.3%(1990) and remained static at 12.9% in 2020 in German dairy cows while incidence of clinical mastitis is on average as high as 31.7% to 47.8% during lactation in constant milk-controlled herds (Martens et al., 2022).

Current studies suggest that evidence-based treatment decisions accompanied by comprehensive treatment documentations, can increase cure rates and reduce antibiotic consumption by detection of the pathogen and their susceptibility towards antimicrobial substances. They enable a selected treatment (*e.g.,* only treating *gram-positive* infections) (Schmenger et al., 2020b; Preine et al., 2022) and shorter treatment durations as enclosed in the Antimicrobial Stewardship approach (Ruegg, 2022).

Efficacy or effectiveness

Before the decision to use a treatment, it is crucial to know how effective it is in a certain disease or pathogen. The terms “efficacy” and “effectiveness” are often used interchangeably, which is misleading. In medicine, efficacy is defined as the ability to produce the intended result or technically how well a particular treatment or drug works under standardised and carefully controlled scientific testing conditions. Effectiveness on the other hand, describes how well a particular treatment or drug works when people (here owner or veterinarians) are actually using it, as opposed to how well it works under scientific testing conditions (Cambridge Dictionary, 2021a). Both is necessary to ensure that an administered remedy or therapy has the desired effects on a diseased patient under real-life circumstances.

Efficacy in science

Support for the efficacy under controlled study conditions, is well defined. Multiple standard protocols *e.g.,* CONSORT (Schulz et al., 2010) or REFLECT statement (Sargeant et al., 2010)) are available to support high quality research, that is transparent, repeatable, with statistical evaluation and aims to reduce different types of bias (*e.g.,* selection bias, performance bias, detection bias, attrition bias, reporting bias). A double-blinded Randomized Controlled Trial (RCT) is currently the gold standard to provide the strongest measure of whether a treatment has an effect (Ryan et al., 2013).

To gain significant results on the efficacy of a specific remedy, scientific trials need to be repeated under the same and under varying conditions. This shows whether result can be confirmed and allows to conduct meta-analyses or systematic reviews on specific scientific questions. Gordon Guyatt introduced the term “Evidence-based-medicine” in 1991 to optimize decision-making on the best treatment of patients in practice, emphasizing the use of evidence from well-designed and well-conducted research (Sur et al., 2011). It classifies evidence into different levels with the highest evidence coming from meta-analyses or systematic reviews that summarize the results of single randomized controlled trials and finally lower levels of evidence (such as case-control studies, expert opinions, etc.). The term was adapted for veterinary medicine under the term “Evidence-Based-Veterinary Science” (EBVS) aiming for the same principles (Cockcroft et al., 2003; Dean, 2014; Arlt, 2016).

Antibiotic treatment

In general, antibiotic treatment in veterinary medicine is accepted as effective. It is the treatment of choice for most bacterial infections and supports the process of healing from an infection by killing or reducing the number of causing bacteria.

On dairy farms bovine mastitis is the main reason to administer antibiotics and thus contributes to high antibiotic consumption in dairy production (Ruegg, 2017). Mastitis treatment is usually administered locally by intramammary infusion in the affected quarter or parenterally by injections, in severe infections often at both administration routes (Barlow, 2011) accompanied by anti-inflammatory drugs. In cases, where *Streptococcus uberis* is involved, intramammary treatment was found to be the most effective treatment for clinical and bacteriological cure and requiring least antibiotics (Hillerton et al., 2002)

As in human medicine, the use of antibiotics in food producing animals is more and more in question due to increasing antibiotic resistance (currently predominantly in human medicine) (Laxminarayan et al., 2013; van Boeckel et al., 2015; Laxminarayan, 2022). Although treatment of diseased animals is unavoidable in order to prevent their suffering and the spreading of diseases between animals as well as to humans, farmers are often blamed as co-responsible for increasing antibiotic resistance by overuse or non-responsible use of antibiotics in food-producing animals (Stoica et al., 2021). This has led to further restrictions for the use in animals - some antibiotics groups are completely banned for the use in animals in most European countries while others require an susceptibility test (antibiogram) before treatment of an individual (European Medicines Agency, 2019).

Homeopathy

Facing the rise of antibiotic resistance and restricting legislation, homeopathy is an often chosen alternatively (Roderick et al., 1999; Krömker et al., 2005), especially in farming practice and bovine mastitis, even if the use is controversial due to a lack of sufficient proof of efficacy (not beyond placebo) and effectiveness (Hektoen, 2005; Lees et al., 2017a, 2017b). Organic farming, Council Regulation (EC) No 834/2007, especially promotes the use of homeopathy and phytotherapy in preference of antibiotics, if it is effective (European Commission, 2007).

Homeopathy was developed by Samuel Hahnemann (1755 - 1843), a German physician and pharmacist (Stanek, 1982). It is based on three principles (Hahnemann, 1869):

1. The law of similars (“like cures like”), means that substances that produce symptoms similar to the symptoms of the disease should be used to cure that disease.

2. The law of potentization and dynamization states that a selected substance is repeatedly diluted (sometimes until no molecule of the original substance is detectable any longer) in order to remove

any harmful effects and shaken vigorously to activate a healing potential. Hahnemann advocates for high dilutions/potencies as they supposed to have a stronger effect without causing harm.

3. The law of individualization states that a *proven* remedy (producing similar symptoms in a healthy human= the *remedy picture* collected in the *Materia Medica*) has to match with the *symptom picture* (collected in the Homeopathic Repertory) of the individual according to its constitution, symptoms, behaviour and feelings in order to apply the most effective treatment.

In case of complex remedies (combination of several substances for one indication) or "approved indications" the third principle is simplified to administering remedies according to common leading symptoms for the disease. The deviation from this principle of classic homeopathy is often criticized and may deteriorate the outcome of treatment compared to individualised homeopathic treatment (Mathie et al., 2017) as individualised homeopathy is considered to be the most effective form by its proponents (Linde et al., 1998).

Cure of mastitis

However, this is true for any type of treatment as it should only be applied when effectiveness can be expected and have at least the same or a better effectiveness than the current standard treatment regimen. A comparison of a new remedy/treatment strategy with an established treatment is only possible when scientific quality and conditions are comparable.

Research on efficacy on antibiotic treatment is available and shows various cure rates in case of bovine mastitis. Cure in bovine mastitis can be defined in several ways (Williamson et al., 2022):

1. clinical cure, when typical symptoms vanish such as changes in milk, signs of inflammation (reddening, swelling) or fever
2. bacterial cure, when the causing pathogen tested before treatment is no longer detectable after treatment and a certain period of time (often 7 or 14 or 21 days after treatment started)
3. cytological cure, when the somatic cell count decreases below 100 or 200.000 cells/ml milk depending on the definition of mastitis within the study
4. full or complete cure, when the udder recovers completely in clinical signs, cytological values and has a negative bacteriological milk sample result

Cure with antibiotic treatment

Schmenger and Krömker (2020a) found an overall bacterial cure rate of 73.3 %, 22.3 % in cytological cure and 21.4 % full cure in bovine mastitis when treated with antibiotics. The average bacterial cure rate can be expected to be around 80% (Owens et al., 2001; Hillerton et al., 2002; Keller et al., 2018; Rossi et al., 2019) for antibiotic mastitis treatment. Wilson et al. (1999) found an average bacterial cure rate by 75% when treated with antibiotics, but it strongly depended on the causing pathogen and the chosen antibiotic.

Cure with homeopathic treatment

For individualized homeopathic treatment 33-43% bacterial cure rate and 0 – 7,5 % full cure depending on the day examined (7, 14, 28 days after treatment started) were found (Keller et al., 2018), similar to Werner et al. (2010) with 43% - 61% bacterial cure and 10 – 31 % full cure. Looking only at clinical cure, rates from 76 - 89% for homeopathy were found comparable with placebo (76 – 87%) within the same trial (Ebert et al., 2017).

“Cure” with placebo treatment

Placebo treatment is usually seen as reliable as it controls for changes by increased attention or other stimuli by the action of treatment. Data show bacteriological cure rates within placebo groups ranged from 44% to 56% but only 0 – 13% for full cure (Poutrel et al., 2008; Werner et al., 2010; Keller et al., 2018).

Self-cure without treatment

Self-healing rates of untreated mastitis were between 0 – 25% (Hillerton et al., 2002; Rossi et al., 2019), 38% (Schukken et al., 2011) and 65% (Wilson et al., 1999) for bacterial cure in trials on mastitis.

According to the published results, antibiotics are expected to have the highest bacterial cure rate for mastitis treatment (73% - 80%) compared to homeopathy, placebo or no treatment. However, these results should be regarded carefully as the term “cure” is a result of many influencing factors (i.e. causing pathogen, resistance pattern, cow characteristics, housing and feeding conditions, treatment type, dose and duration) including the different definitions of cure of the udder (Barkema et al., 2006).

Aiming for evidence-based answers on the question of efficacy of homeopathy in humans or animals several reviews and meta-analyses are available (Linde et al., 1997; Ernst, 2002; Mathie et al., 2015; Lees et al., 2017b, 2017a) with diverging results. Only one review on efficacy of antibiotic treatment in bovine mastitis was available (Winder et al., 2019), in which no conclusions regarding efficacy could be drawn due to lack of power within the included studies.

Effectiveness on farms

When a trial or review suggests a new treatment to be effective under standardized conditions, the same must be tested under real-life-conditions to provide knowledge whether individuals in everyday practice would receive similar outcomes (Krauss, 2018).

How well a treatment works has a big impact on the animal’s and farmer’s life from animal welfare to profit for the farm. Whether a mastitis treatment is effective on a dairy farm depends on many factors, such as feeding, hygiene, housing conditions, prevalent mastitis pathogens, milking techniques, treatment procedures/state-of-the-art treatment, cow-related factors (genetics, age, lactation, immune status, additional diseases) and the interactions between cow and condition related factors. Thus, treatment success should always be captured farm-specific and monitored externally by animal health services or others, that apply similar standards/measures and consider disease incidence at the same time.

However, effectiveness on dairy farms is rarely or only randomly evaluated with prospective or retrospective studies. The studies focus on a specific group and a limited period on a specific farm by defining multiple in-/exclusion criteria. Apart from voluntary reports of adverse reactions towards a remedy (which could also be the absence of effectiveness) by veterinarians or owners, currently no general, permanent monitoring system of treatments including their outcome in practice exists to the authors knowledge. Nationwide records of treatments on a regular base *e.g.,* in dairy cows are kept by veterinarians or/and farmers on farm and animal level in Denmark, Finland, Sweden and Norway (Wolff et al., 2012). However, they do not include treatment outcome. Furthermore, in most European countries reporting is only mandatory for antimicrobial use in food-producing animals, often restricted to fattening animals and used for benchmarking of treatment frequency to lower the antibiotic consumption in general (Sanders et al., 2020), but it do not consider disease incidence at the same time. This means for instance that a farm with a high disease prevalence might simultaneously have a low antibiotic consumption or treatment frequency, when refusing necessary treatment.

Although no mandatory disease or treatment record reporting system in Germany exists, a lot of dairy farms participate in a voluntarily milk record system (analysing milk yield, somatic cell count, milk fat, milk protein, milk urea on cow level once a month) and protocol medical treatments (by their veterinarian or by farmer) on site. Few of them also examine milk samples of cows showing signs of mastitis or before drying off (Ouweltjes et al., 2008; Doehring et al., 2013). Thus, real-world data are available to some extent, but monitoring and control of treatment outcome is rarely done.

Documentation beyond the minimum, requires extra time and work, while benefits are not obvious for many farmers, *e.g.,* only 56% of all farmers frequently or always kept records on mastitis treatments in the USA (Kayitsinga et al., 2017). Motivation to keep and analyse records of treatments to improve treatment strategies could be increased by knowledge on production losses and costs due to mastitis in connection with cure rates and disease incidences to recognize cost-benefits of a better treatment strategy (van Soest et al., 2016).

To reduce antibiotic consumption and possibly replace it by homeopathy, several questions must be answered: Do current studies provide sufficient information about efficacy of homeopathy? Can the current use of antibiotic and/or homeopathic remedies in veterinary practice expected to be effective and responsible? And finally, can the use of homeopathy and antibiotics be (equally) supported?

The evaluation of efficacy and effectiveness of homeopathic and antibiotic treatment in research and practice is inevitable to improve scientific evidence and enable the selection of the most effective remedies in the respective context. Only with this knowledge, a reasonable use in practice and options to replace/reduce antibiotic use while at the same time considering animal welfare aspects are possible.

Aims of the thesis

The present thesis

1. examines the efficacy of homeopathy and the possibility to replace or reduce antibiotics in production diseases in livestock with a comprehensive evaluation of scientific research available,

2. evaluates the efficacy of antibiotic and homeopathic treatment in bovine mastitis with a focus on study design quality and

3. uses the available real-world data of 30 dairy farms to provide knowledge on antibiotic consumption, antibiotic treatment effectiveness and costs of mastitis on individual farm level.

Publications

I. Efficacy of homeopathy in livestock according to peer-reviewed publications from 1981 to 2014 (Veterinary Record, 2016)

II. Efficacy of antibiotic and homeopathic treatment of bovine mastitis and implications for the effectiveness on farms (submitted at Preventive veterinary medicine)

III. The informative value of an overview on antibiotic consumption, treatment efficacy and cost of clinical mastitis at farm level (Preventive veterinary medicine, 2019)

2. Efficacy of homeopathy in livestock according to peer-reviewed publications from 1981 to 2014 (paper I)

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Abstract

Homeopathy is widely used in livestock, especially in order to reduce the use of antibiotics, although it is often seen as controversial. A comprehensive literature review has been conducted to assess the efficacy of homeopathy in cattle, pigs and poultry. Only peer-reviewed publications dealing with homeopathic remedies, which could possibly replace or prevent the use of antibiotics in the case of infective diseases or growth promotion in livestock were included. Search results revealed a total number of 52 trials performed within 48 publications fulfilling the predefined criteria. Twenty-eight trials were in favour of homeopathy, with 26 trials showing a significantly higher efficacy in comparison to a control group, whereas 22 showed no medicinal effect. Cure rates for the treatments with antibiotics, homeopathy or placebo varied to a high degree, while the remedy used did not seem to make a big difference. Looking at all the studies, no study was repeated under comparable conditions. Consequently, the use of homeopathy currently cannot claim to have sufficient prognostic validity where efficacy is concerned. When striving for high therapeutic success in treatment, the potential of homeopathy in replacing or reducing antibiotics can only be validated if evidence of efficacy is confirmed by randomised controlled trials under modified conditions.

1.1 Introduction

Within the scientific community and veterinary practice, the use of homeopathy in food producing animals is highly controversial. On the other hand, there is evidence that homeopathic remedies are widely used in food producing animals (Hovi, 2001; IMPRO, 2015; ECCH, 2017). For organic agriculture, the use of homeopathy is even promoted. According to the Commission Regulation (European Commission, 2008),on organic agriculture, homeopathic and other products should be used in preference to chemically-synthesized allopathic veterinary treatment, provided that the resultant therapy is effective for the species of animal and the condition for which the treatment is intended.

In case of infectious bacterial diseases, antimicrobial drugs have been used in livestock production for decades as the first and often only effective option for individual or group treatment. Nowadays the use of antibiotics in food-producing animals is often unpopular amongst consumers (Midan Marketing, 2014; Mintel Group, 2015; Niamh, 2015). “Antibiotic-free” or “raised without antibiotics” labelled products are enjoying increased popularity in both Europe and the United States. This development is fuelled amongst others by mis- and overuse of antibiotics in human and animal medicine, which has provoked resistant strains of bacteria worldwide (Laxminarayan et al., 2013). Correspondingly, many farmers and also veterinarians see homeopathy as an alternative for treating diseases in farm animals and thus reducing the consumption of antibiotics.

Homeopathy was introduced by Samuel Hahnemann as early as 1796 as a method that treats diseases with dilute remedies that causes the same symptoms undiluted in healthy individuals as the disease does. Those symptoms are seen as very specific and individual, resulting in the requirement for a detailed diagnostic procedure where not only the symptoms and cause of the disease are considered, but also the patient’s behaviour, constitution and conditions that may cause aggravation or amelioration of symptoms, to find the corresponding remedy for each patient (Hahnemann, 1869).

Reviews on the efficacy of homeopathy in humans found that the success of homeopathic treatment in general cannot be completely due to placebo effects, but studies only provided insufficient evidence that homoeopathy is efficacious (Linde et al., 1997). In fact, the quality of the methodology was low (Linde et al., 2001) and the bias high in homeopathic trials on humans. At the same time, there was weak evidence for the particular effectiveness of homeopathy as compared to conventional treatment (Shang et al., 2005). Up till now, only few scientific publications have reviewed the efficacy of homeopathy in animals (Kowalski, 1989; Hektoen, 2005; Rijnberk et al., 2007; Ruegg, 2009), revealing diverse results. Previously, a review and meta-analysis of randomised placebo-controlled trials in veterinary homeopathy with special focus on medical conditions were published by Mathie and Clausen (Mathie et al., 2014a, 2015). Due to the low number and quality of studies available, particularly regarding the risk of bias, they found very limited evidence for distinguishable differences between homeopathy and placebo in clinical interventions, demanding the need for new and higher quality research in veterinary homeopathy. For food-producing animals, there has been no review of peer-reviewed publications conducted on the efficacy of homeopathy. The aim of this review was therefore to systematically evaluate the existing knowledge on the efficacy of homeopathic remedies used for the prevention or treatment of diseases in livestock that are usually treated with antimicrobials by considering more scientific, peer-reviewed publications (incl. doctoral thesis) and a comprehensive timeframe from 1981 to 2014. Within the analysis additional information on trial conditions, used remedies and treated species were taken into account.

1.2 Methods

The search for scientific publications was carried out in the following databases: PubMed, ScienceDirect®, and Web of Science™. Specific databases on veterinary homeopathy were available at Hom(VetCR, 2014) (Carstens Stiftung), Vetion:oekovet.de or AudeSapere and also taken into account. Further publications were found by relying on the reference list of considered studies. The searches were performed in February 2014 and updated up to the end of June 2016. The search terms were defined according to the PICOS approach (Moher et al., 2009) :

- **Population:** “livestock” OR “dairy” OR “cows” OR “cattle” OR “bovine” OR “heifer” OR “calves” OR “pigs” OR “sows” OR “piglets” OR ”poultry” OR “swine” OR “porcine” OR “turkeys” OR “broiler/s” OR “chicken” OR “hen/s”
- **Intervention**: “homeopathy” OR “veterinary homeopathy” OR “homeopathic” OR “dilution” OR “complementary veterinary medicine” OR “isopathy” OR “nosodes”
- **Comparison:** “prevention” OR “prophylaxis” OR “metaphylaxis” OR “treatment/s” OR “therapy” OR “comparison”
- **Outcome:** “efficacy” OR “effectiveness” OR “effect/s” OR “efficiency”
- **Setting:** “clinical trial” OR “review” OR “trial/s” OR “study/ies”.

Only publications in peer-reviewed scientific journals and doctoral theses were taken into account. The studies considered addressed the efficacy of homeopathic drugs in cattle, pigs or poultry in production diseases under European or comparable conditions (in respect to housing, breeds, intensive farming).

Included in the evaluation were only studies dealing with homeopathic remedies, which could possibly replace or prevent the use of antibiotics in the case of infective production diseases or growth promotion. The time frame of publications considered was from year 1981 to 2014. Accessible languages were English and German. Main reasons for excluding publications were: that no peer-review was performed (conference abstracts, journals without peer-review), trials only dealt with companion animals and/or with diseases, that were not infectious *e.g.,* hormonal treated fertility disorders.

The search and filtering process (*see Fig.1*) was performed by the same person following the PRISMA guidelines (Liberati et al., 2009). The following information was extracted from each study according to a predefined protocol (available from the authors): author, publication year and source, research body, species/group, farming system, purpose of application, disease in focus, exclusion criteria, diagnostic method and person diagnosing, remedy used, as well as origin (producer), ingredients and potency of the remedy, way of administration, study design and control groups, methods of measurement, possible risk of bias and outcome of the study. Studies including more than one clinical trial were regarded as separate studies for the purpose of the evaluation and comparison of data.

In medicine, efficacy indicates the capacity for beneficial change (or therapeutic effect) of a given intervention (*e.g.,* a drug). When talking in terms of efficacy vs. effectiveness, effectiveness relates to how well a treatment works in the practice of medicine, as opposed to efficacy, which measures how well treatment works in clinical trials or laboratory studies (Thaul, 2012).

This review classified homeopathic treatment as efficacious when the homeopathic remedy presented a statistically significant improvement in comparison to a placebo, a conventionally treated or an untreated group of farm animals. If the impact of a homeopathic treatment did not differ significantly to that from a placebo or an untreated group - or even worse - nil efficacy was reported. Results were classified as ”inconclusive”, when the level of efficacy did not differ significantly between the homeopathic group and the group employing antibiotics (without a further control group as placebo or untreated), when the values of particular parameters improved while others deteriorated or when the authors did not provide a clear result. To assess the possible influence of single dichotomous factors on reporting efficacy of homeopathy in a trial, a sensitivity analysis was performed, expressing the effect size as odds ratio (OR) with 95 percent confidence interval and *P*-values calculated according to Sheskin (2003).

1.3 Results

From the 4448 publications found, 48 publications were identified, which met the given criteria (Fig 1).

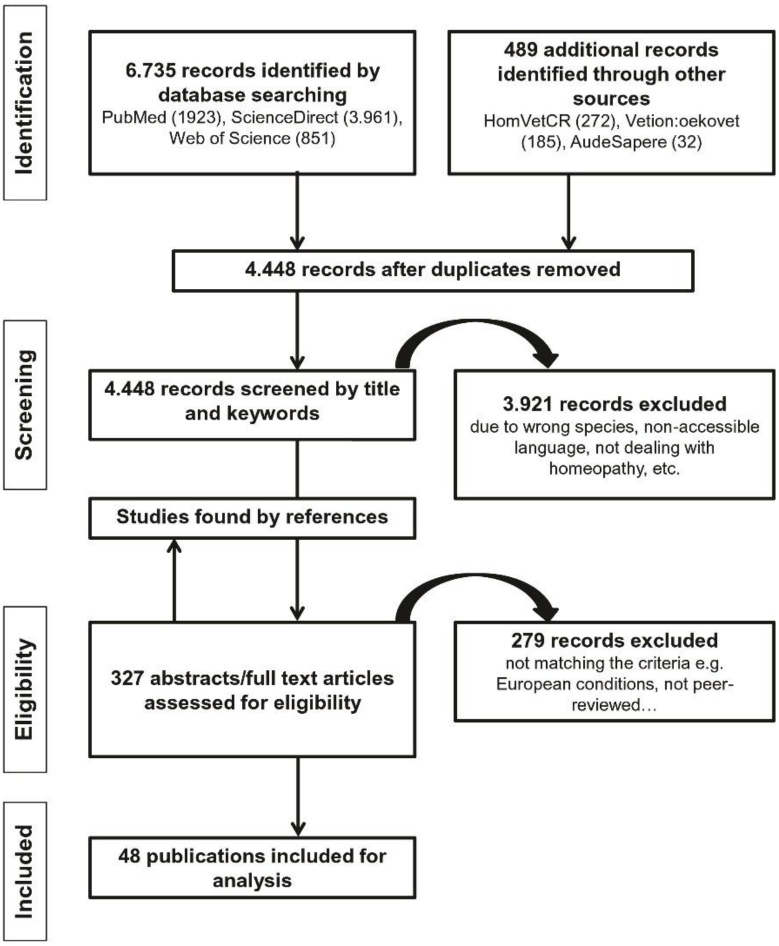

Figure 1: Search process for publication based on PRISMA guidelines

These publications featured 52 individual clinical trials (including 34 trials with cattle, 12 with pigs, six with poultry). One publication included three separate clinical trials (Day 1986), and two publications each included two clinical trials (Schütte, 1991, 1994).

The 36 scientific papers were primarily published in homeopathic journals (e.g. Homeopathy, British Homeopathic Journal, International Journal of High Dilution Research, Alternative Therapies in Health and Medicine, Zeitschrift für Ganzheitliche Tiermedizin), and only a few were found in veterinary journals (e.g. Veterinary Record, Journal of Dairy Science, Journal of Animal Science, Berliner und Münchener Tierärztliche Wochenzeitschrift, Transboundary and Emerging Diseases). Sixteen doctoral theses on homeopathy in livestock were available, having originated from veterinary or agricultural universities. The greater part of the studies were published between the years 2003 and 2014 (n= 21). It seems fewer studies were published before, with 14 publications in the time period 1981 till 1991 and 9 publications between 1992 and 2002.

In most of the studies (54%), the production method was not specified. The remaining studies were defined as either conventional (33% - particularly pig and poultry farms) or as organic (6% - exclusively dairy farms). Seven percent of the studies had looked at both farming systems.

The main purpose of applying homeopathic remedies in clinical trials was prevention of disease (quoted in a total of 30 studies, including 6 studies aiming for good general health or growth promotion). Furthermore, 18 studies refer to the therapeutic treatment of diseases, while four studies focused on metaphylactic treatment (medication administered before the onset of clinical signs in the herd).

Indication

The reason for using homeopathic remedies to treat disease was different for each species (*see Fig.2*). In studies dealing with cattle, treatment was for mastitis or fertility disorders in dairy cows. Calves were only treated for the indication diarrhoea and pneumonia. The studies on pigs ranged between fertility disorders, especially mastitis-metritis-agalactia-syndrome (MMA) in sows, respiratory infections and diarrhoea in piglets, and growth promotion and general health in fattening pigs. In the case of poultry, the main indication for using homeopathy was growth promotion or the treatment of diarrhoea.

Studies which included more than one treatment indication were counted per indication. Nearly all animals treated with remedies had become sick through natural infection. An artificial infection was only induced in two studies on chicken: (Velkers et al., 2005) induced an *E.coli*-infection in the chickens on day 8 and (Berchieri et al., 2006) challenged chickens on the 17th day after birth with a broth culture of *Salmonella enteritidis*.

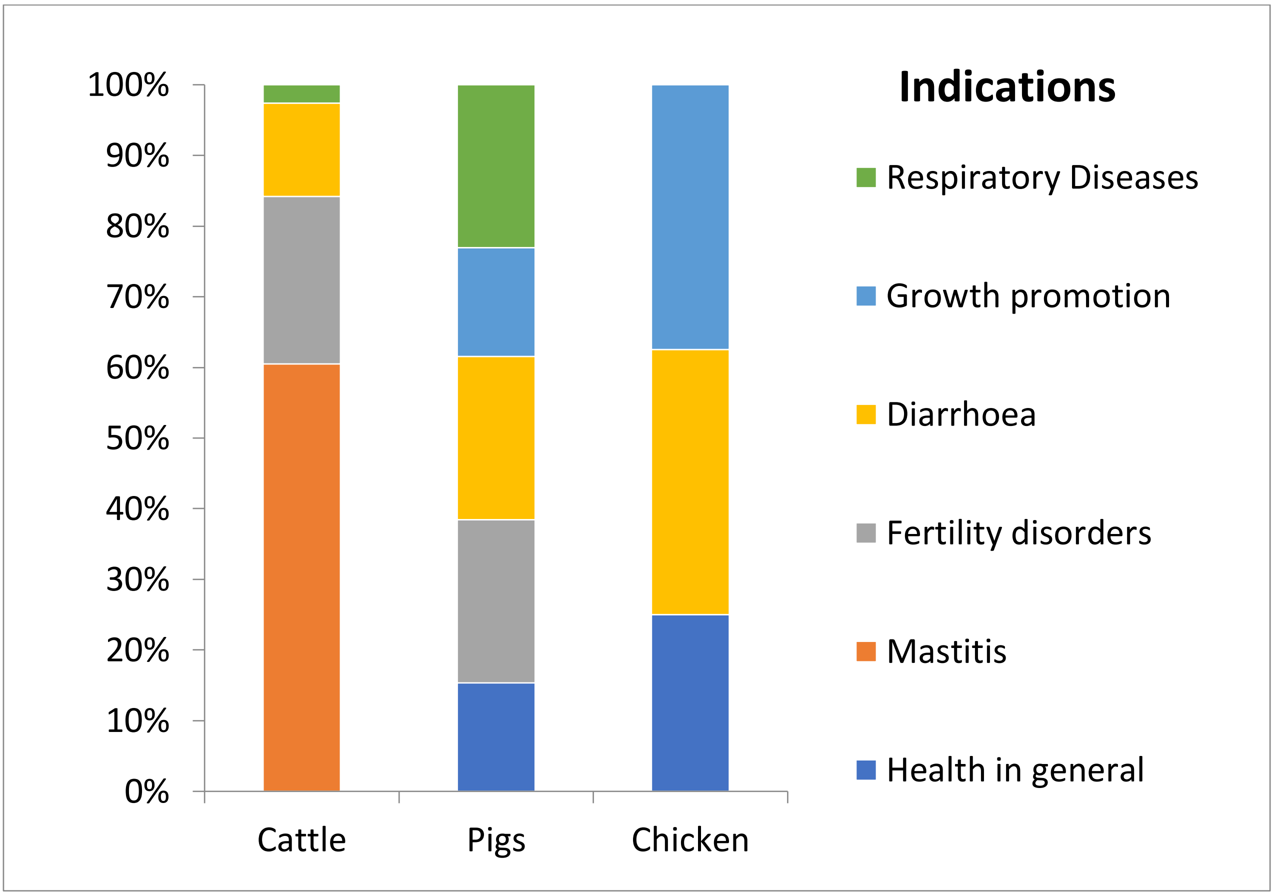

Figure 2: Distribution of the indications in relation to the use of homeopathy

Exclusion criteria

Nineteen of 52 studies defined criteria for the exclusion of animals from trial participation or gave reasons for exclusion of animals during the trial. Animals were primarily excluded for showing systemic symptoms (e.g. fever, loss of appetite) or severe organ damage, which affects the ability of an animal's immune system to react to a stimulus, as well as the presence of additional diseases or any further medication being taken at the time of the study, missing data or because the animals died or were sold prematurely.

Diagnostic procedure

In the various studies diagnostic procedures to evaluate the amelioration or deterioration of health or performance and to measure the effect of the applied remedy were quite heterogeneous. The diagnostic methods were divided into four groups:

1. Direct tests (DT): eg, laboratory tests for detection of the pathogen in milk, faeces, blood, and so on.
2. Indirect tests (IT): eg, California mastitis test or conductivity milk test, rectal palpation of the uterus, general blood parameters, and so on.
3. Clinical signs (CS): eg, optical assessment of milk for clots, observation of clinical signs such as alterations in faeces consistency, coughing, and so on.
4. Measurements (M): eg, body temperature, body weight, milk yield, and so on.

In more than half of the trials, two (n=22) or more parameters (n=9) were considered when diagnosing diseases or the possible effects of using remedies. The most frequently undertaken diagnostic test was the recording of clinical signs, followed by direct tests. Indirect tests, often available as on-site tests, and additional measurements were performed to a lesser degree (Fig 3).

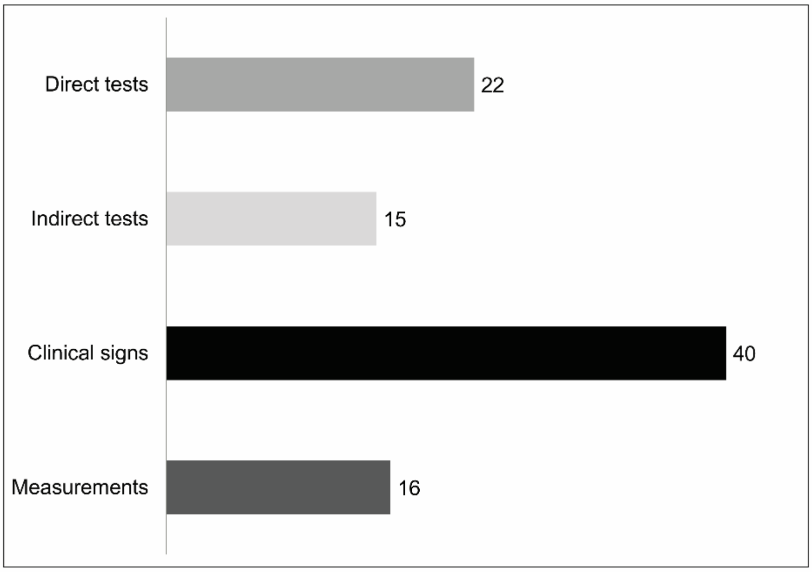

Figure 3: Quality of diagnostic measures

An individualized homeopathic diagnostic procedure enclosing the choice of an appropriate (and often different) homeopathic remedy for the individual were performed within 5 trials (Seifert, 1987; Hektoen, 2004; Werner et al., 2010) with two of them limiting the selection of possible remedies (Sonnenwald, 1986; Williamson et al., 2014). The question of who had diagnosed a disease or observed a symptom remained unanswered in 22 of 52 studies. In 13 studies homeopathic expertise was considered by a veterinarian, trained or experienced in homeopathy or accompanied by a trained homeopath (Hektoen et al., 2004) or supervised by an external veterinarian experienced in homeopathy (Werner, 2006). In 11 trials, a veterinarian diagnosed the clinical signs, in three trials the farmer decided whether the animal improved or not, in one case the diagnosis was made by the veterinarian and farmer together in another, an immunologist and in a further trial an automated milking system gave the diagnosis.

Remedies

In general, two types of homeopathic remedies were available: remedies including only one active ingredient in the initial tincture (used for classic or individualised homeopathy) and remedies combining two or more active ingredients in the initial tincture (used for complex homeopathy). Fifty-six per cent of the studies saw complex remedies (with combined ingredients) being employed and 34 per cent of the studies saw classic single remedies being used. In the remaining studies (10 per cent), the type of homeopathic remedy was not revealed or was impossible to differentiate (eg, parallel use of single and complex remedies). The producers of the applied remedies were not listed in 30 per cent of the studies. In the other cases, the companies Heel (15 per cent) and DHU (13 per cent) were most often listed as producers of remedies, followed by other small producing companies or local pharmacies (42 per cent). The homeopathic dilutions or potencies of the applied drugs were either high (n=21 trials) from D60 (respectively C30, a 1: 10-60 dilution) to D400 (respectively C200, a 1: 10-400 dilution) or low potencies (n=21) from D1 (a 1:10 dilution) to D24 (a 1:10-24 dilution), where the letter 'D' stands for decimal dilution steps and 'C' for centesimal dilution steps In 6 studies, the potency was not mentioned. Four studies considered both high and low potencies (Tiefenthaler, 1990; Vohla, 1991; Knierim, 1992; Egan, 1995). The ingredients used in the studies were of herbal, mineral or animal origin. Nosodes, a specific type of homeopathic preparation often called "homeopathic vaccines" were made up either from the pathogen or from products of the disease (i.e. pus, sputum) and used in 9 trials.

Methods of administration

The means of administration differed depending on whether individuals or groups of animals were treated. For groups, the administration was solely oral (by drinking water or mineral salt). For individuals, the drugs were usually administered orally or infrequently via subcutaneous or intramuscular injection, topical (eg, vulval spray or udder cream) or by intramammary injection.

Study design

The "evidence-based medicine" approach is premised upon the current knowledge level based on clinical studies and scientific publications, which confirm or invalidate the efficacy of a certain measure or remedy (Panesar et al., 2010). In single clinical trials, double-blind Randomised-Controlled-Trials (RCT) are regarded as the "gold standard" for proving the empiric evidence of efficacy of a remedy tested (European Medicines Agency, 2001; Kaptchuk, 2001). With this in mind, the structures of the selected trials were examined with respect to their ability to measure the efficacy of homeopathy on the level of evidence-based-medicine.

Seventy-nine percent of the single clinical trials were studies, including a randomisation and a control group, called Randomised Controlled Trials (RCT) (n=41). The remaining studies were either non-randomized controlled trials i.e. parallel groups (n=6) or observational trials without a control group (n=5). The RCT (n=41) could be divided into 21 non-blind trials, 9 single-blind trials and 11 double-blind trials. "Single-blind" means that the patient is not aware of the true nature of the drug applied. In the case of animals, a single-blind experiment means that the person administering the drug is not aware of the true nature of the applied drug or the ingredients. Double-blind trials include a "blinding" of both the user and the sample assessor (Schulz et al., 2002). The blinding aims to eliminate effects such as observation bias and wishful thinking, which could occur if the samples are evaluated by someone who already knows the group's organisational and treatment methods.

Control groups (eg, an untreated group, placebo group or antibiotic treated group) were also included in this evaluation. More than half of the trials (65 per cent) had a study design with only one control group, 17 per cent included two control groups and four studies compared the experimental group with three control groups. The five observational studies did not use any control or parallel group at all. Out of all 64 control groups, 42 per cent were treated with a placebo, 31 per cent with antibiotics, 10 per cent with a different conventional treatment (eg, teat sealers or a local disinfectant) or a combined treatment (eg, antibiotic combined with a placebo or a homeopathic remedy) and only 17 per cent included an untreated group.

Efficacy

In total, 54 per cent (n=28) of the trials were able to confirm the efficacy of the homeopathic remedy administered, while 42 per cent (n=22) found no benefit compared with the placebo or untreated group. Four per cent (n=2) had inconclusive results. When considering a single species (Table 1) only homeopathic studies dealing with pigs were found to be frequently efficacious, while studies with cattle or poultry were seen to have a similar distribution of efficacious and non-efficacious treatment.

Table 1: Number of trials and outcome regarding the efficacy of the homeopathic remedy applied

Species	Number of Trials	Efficacy	Inconclusive Results	No Efficacy
Cattle	34	15	1	18
Pigs	12	9	1	2
Poultry	6	4	0	2
Summary	52	28	2	22

Trials demonstrating the efficacy of the homeopathic remedy were published mainly in scientific journals (21 out of 28 positive trials), as well as studies with a negative outcome (14 out of 22 negative). In journals focussing on alternative treatments, 15 of 18 trials reported that the homeopathic remedy tested was effective (odds ratio [OR] 3.75, 95 per cent confidence interval [CI] 0.63 to 22.04, P=0.14), while in journals with a broader focus on veterinary medicine, 12 out of 18 trials found the homeopathic treatment was ineffective (OR 0.27, 95 per cent CI 0.05 to 1.57). Results described in a doctoral thesis on homeopathic remedies were nearly evenly distributed between non-efficacious (n=8) and efficacious (n=7), apart from one thesis which revealed inconclusive results.

Treatment success

In order to provide information on cure rates, 18 out of 52 clinical trials which administered homeopathy exclusively for therapeutic reasons were further analysed. Only 10 of these trials gave information on cure rates (Table 2), while eight trials did not define cure rates at all. Instead, the effectiveness of the homeopathic remedy was evaluated on the basis of other criteria, for example, number of recurring treatments, mortality, duration of diarrhoea, fertility parameters, milk yield, and so on. A wide range of different criteria for defining recovery or cure was found, for example, in case of mastitis: ‘full recovery’ with or without cytobacteriological recovery, or ‘clinical recovery’ with or without cytobacteriological recovery, or only ‘bacteriological recovery’, and so on. This made comparison and interpretation of the results more difficult. Correspondingly, the different cure rates could not all be compared directly to one another.

Table 2: Cure rates for therapeutic use of different remedies

Species	Indication	Recovery effect with Homeopathy	Allopathy	Placebo	Author (Year)
Cows	Mastitis	34% [a] 18% [d]	26% [a] 24% [d]	——	Sonnenwald (1986)
Cows	Mastitis	34% [a] 18% [d]	26% [a] 24% [d]	——	Merck et al. (1989)
Cows	Mastitis	33% [a, o] 80% [a, p]	67% [a, o] 50% [a, p]	——	Tiefenthaler (1990)
Cows	Mastitis	19% [a] 47% [e] 29% [f]	20% [a] 45% [e] 35% [f]	6% [a] 56% [e] 13% [f]	Hektoen et al. (2004)
Cows	Mastitis	47% [b] 31% [c]	24% [b] 24% [c]	30% [b] 4% [c]	Werner (2010)
Cows	Mastitis	5% [a,g] 10% [a,h]	17% [a,g] 65% [a,h]	——	Walkenhorst (2006)
Cows	Mastitis	63% [e] 36% [n]	95% [e] 74 % [n]	——	Williamson and Lacy-Hulbert (2014)
Calves	Diarrhoea	83% [l]	——	80% [l]	Kayne and Rafferty (1994)
Calves	Diarrhoea	69-77% [i,m]	——	69% [m]	Hornig (2014)
Sows	MMA	61% [m]	35% [m]	——	Seifert (1987)

a [Full recovery = no pathological findings of milk and udder and negative cyto-bacteriological results of milk samples]
b [Full recovery = no pathological findings of milk and udder with negative bacteriological results of milk samples on 1st day of disease]
c [Full recovery = no pathological findings of milk and udder with positive bacteriological results of milk samples on 1st day of disease]
d [Clinical recovery = no pathological findings of milk and udder and with positive bacteriological or/and positive cytological results of milk samples]
e [Clinical recovery = no pathological findings of milk and udder]
f [Bacteriological recovery = negative bacteriological result of milk samples after treatment and negative California-Mastitis-Test]
g [Cows with clinical mastitis at min. one udder quarter]
h [Cows without clinical signs, but positive bacteriological result of milk samples at min. one udder]
i [Range of results due to the use of different homeopathic remedies]
l [Clinical recovery = change from watery to pasty faeces; animals treated by homeopathy recovered 1 day earlier]
m [Clinical recovery]
n [Bacteriological recovery = negative bacteriological result of milk samples after treatment]
o [test group = cows with acute mastitis with disturbed general condition]
p [test group = cows with acute mastitis without disturbed general condition]

Overall, the cure rates in the seven studies focusing on mastitis ranged from 5 per cent to 80 per cent for a treatment with homeopathic remedies, from 17 per cent to 95 per cent when using a conventional remedy (predominantly antibiotics), and from 4 per cent to 56 per cent for the administration of a placebo. In two studies, the results comparing homeopathic remedies with a placebo for diarrhoea in calves seemed to be the same. The results for MMA in sows favoured homeopathy compared to allopathy.

Efficacy and remedy

When the outcomes of the trials were compared with the type of remedy used (single or complex remedies), no differences were found. Remedies with low potencies (D1 to D12) showed the same results as homeopathic treatments with high potencies (more than D24) as far as efficacy was concerned (OR 1,95 per cent CI 0.28 to 3.54, P=1) and did not depend on the purpose of use (prevention, treatment, metaphylaxis) nor the indication (eg, mastitis, diarrhoea), species or the performance of an individualised treatment procedure.

Study design and risk of bias

Figure 4 shows the results of the studies evaluated with a focus on the study designs. The double-blind RCTs reported efficacy (n=5) of the homeopathic treatment almost as often as they reported lack of efficacy of it (n=6). Single-blind RCTs and non-blind RCTs on homeopathy had a tendency to be efficacious. Observational trials or parallel groups (without randomisation) were most likely to present evidence of efficacy.

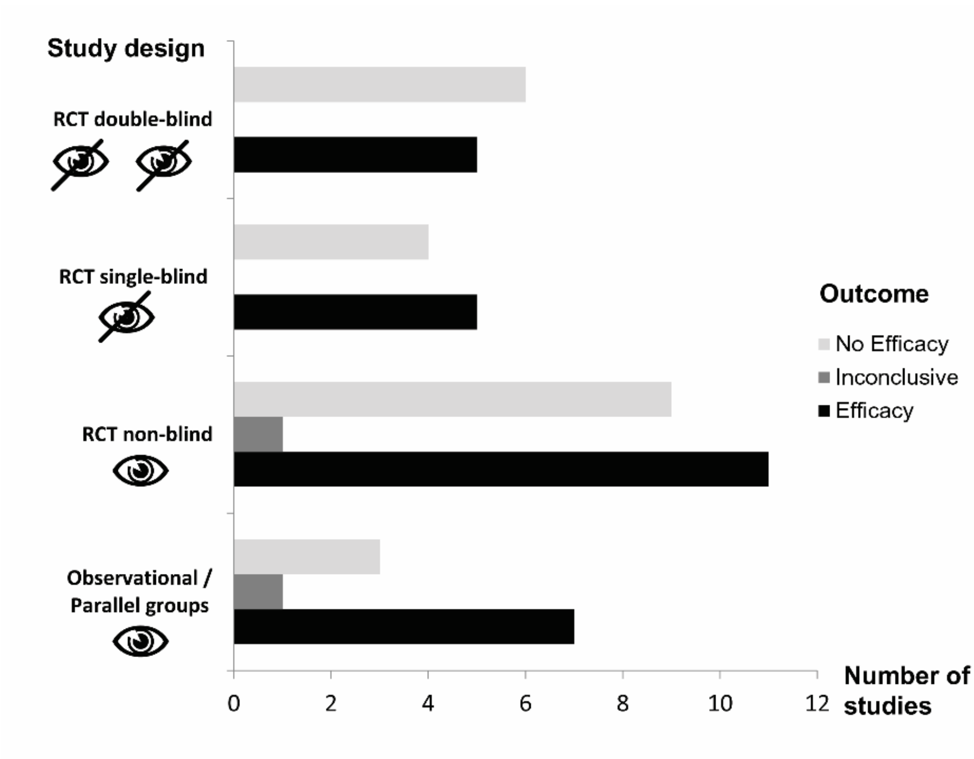

Figure 4: Study design and result for efficacy of homeopathy in the studies

All trials were also analysed for a possible risk of bias, which incorporated both study design and study conduct (Table 3). Studies were regarded as having a 'low' risk of bias when: they included a randomisation, a control group, blinding of the people directly involved in their administration and evaluation, the absence of selective reporting and other shortcomings within the trial, and no direct or indirect funding of the study by the producer of the conventional or homeopathic remedy. Only 13 studies displayed a low or unclear risk of bias. Shortcomings and risk for selective reporting or bias were found in 39 trials to various degrees. Some studies only considered a very small number of animals (eg, 10 to 40 animals) in the trial. Therefore, conclusions drawn could only be very limited. Often, studies were financially supported, eg, by the producer of the homeopathic or conventional remedy (full funding or free provision of remedies), which might have influenced the results (n=12). In one trial, all the researchers worked for the supplier of the homeopathic remedy, which certainly entailed a conflict of interests (Aubry et al., 2013). However, studies with such vested interests reported efficacy of homeopathy less often than studies with no overt vested interest (OR 0.87, 95 per cent CI 0.23 to 3.37, P=0.84 vs OR 1.15, 95 per cent CI 0.30 to 4.42). Further shortcomings were that the origin, contents or potencies of remedies were not presented (n=10), compromising any attempt to repeat a trial. In trials where antibiotics were used as a control, the antimicrobial substance was often chosen without considering guidelines for prudent use or existing knowledge about resistance characteristics of the pathogen in focus, i.e. penicillin treatment for E coli-infection (Coelho et al., 2009). One study also mentioned the exclusion of certain homeopathic remedies and their test results because they were not positive (Berchieri et al., 2006). The intake of the homeopathic remedy was not easy to define, i.e. as the administration was via concentrate or water troughs. Further risks found were: groups were not evenly distributed, measures were not scientifically valid, i.e. subjective or very indirect measures for improvement or cure, homeopathy was only tested in combination with antimicrobials or no control (placebo/untreated) was considered. Altogether, studies with a high possible risk of bias were more prone to report efficacy of homeopathy than studies with a low risk of bias (OR 1.71, 95 per cent CI 0.48 to 6.11, P=0.41 vs OR 0.58, 95 per cent CI 0.16 to 2.09).

Table 3: Risk of bias

Risk of Bias						
Author (Year)	**Randomized**	**Control**	**Blinding**	**Free from vested interest**	**Free of other shortcomings**	**Risk of bias**
Boerms 1981	Y	AB	N	N	potency unknown	High
Frerking 1981	Y	AB, COM	N	U	N	High
Day 1984	N	U	N	U	Intake unsure, some sows didn't receive the full course of medication due to closeness to farrowing,only 20 animals considered	High
Day (1) 1986	N	U	N	U	Uneven distribution of animals	High
Day (2) 1986	Y	P	Y	U	Intake of medication unsure	Low
Day (3) 1986	N	U	N	U	Uneven distribution of cows, missing information on remedy	High
Sonnenwald 1986	Y	AB	N	U	Groups not completely randomized (grazing cows & farmers intervention)	High
Seifert 1987	Y	CON	N	N	U	High
Stopes 1988	N	N	N	U	U	High
Drösemeier 1989	Y	AB, P	Y	N	N	High
Merck 1989	N	AB	N	U	Different times for follow-up checks	High

Risk of Bias (continued)						
Author (Year)	**Randomized**	**Control**	**Blinding**	**Free from vested interest**	**Free of other shortcomings**	**Risk of bias**
Erbe 1990	Y	P	N	U	Uneven distribution, only 42 animals considered	High
Tiefenthaler 1990	Y	AB,COM	N	U	Only 41 animals considered	High
Wirth 1990	Y	P	N	U	Uneven distribution, 42 animals considered	High
Schütte (1) 1991	Y	AB,P	N	U	U	High
Schütte (2) 1991	Y	P	N	U	U	High
Vohla 1991	N	P,U	Y	U	U	High
Knierim 1992	Y	P	Y	U	Only 30 animals considered	Low
Kayne et al. 1994	Y	P	Y	U	20 animals considered	Low
Schütte (1) 1994	Y	P	Y	U	U	Low
Schütte (2) 1994	Y	P	Y	U	U	Low
Egan 1995	N	N	N	U	15 animals considered	High
Searcy 1995	Y	P	N	U	26 animals considered	High
Guajardo-Bernal 1996	Y	P	Y	U	Only 10 animals considered	High
Sandoval 1998	Y	AB	N	U	U	High
Albrecht/ Schütte 1999	Y	P,AB	N	N	U	High
Hümmelchen 1999	Y	P	Y	N	U	High
De Verdier 2003	Y	P	Y	U	U	Low
Garbe 2003	Y	AB, P, COM	Y	N	U	High
Schütte 2003	N	N	N	U	U	High
Hektoen 2004	Y	P,AB	Y	U	Unknown potency	Low
Schlecht 2004	Y	P	Y	N	39 animals considered	High
Holmes 2005	Y	P	Y	U	U	Low
Velkers 2005	Y	U, AB	N	U	U	High
Berchieri 2006	N	U	N	U	Selective reporting (exclusion of ineffective remedies tested)	High
Walkenhorst 2006	Y	AB	N	N	U	High

Risk of Bias (continued)						
Author (Year)	**Randomized**	**Control**	**Blinding**	**Free from vested interest**	**Free of other shortcomings**	**Risk of bias**
Enbergs/ Sensen 2007	Y	CON	N	UN	UN	High
Soto 2008	Y	U,P	N	UN	Uneven distributed initial weight	High
Amalcaburio 2009	Y	U	N	UN	UN	High
Arlt 2009	Y	P	Y	N	UN	High
Coelho 2009	Y	AB	Y	UN	46 animals considered, AB known to be resistant to pathogen in trial	High
Camerlink 2010	Y	P	Y	UN	UN	Low
Klocke 2010	Y	U, CON	N	N	UN	High
Werner 2010	Y	P, AB	Y	UN	UN	Low
Da Silva 2011	N	N	N	UN	remedy data missing	High
Hadipour 2011	Y	AB	N	UN	Potency unknown	High
Kiarazm 2011	Y	P	Y	UN	Potency unknown	Low
Wagenaar 2011	Y	U, CON	N	UN	Remedy data missing	High
Sato 2012	Y	P	Y	UN	UN	Low
Aubry 2013	N	N	N	N	Researcher worked for supplier	High
Hornig 2014	Y	P	Y	UN	UN	Low
Williamson 2014	Y	AB	N	UN	UN	High

Y=Yes; N=No; AB = Antibiotics; HOM=Homeopathy; P=Placebo; U=Untreated; CON = Conventional treatment incl. antibiotics; UN =Unclear; COM = combined treatment of homeopathy and antibiotics

Species specific results

Studies on cattle

Altogether, 34 trials matched the selection criteria described above (tabl.3). The main focus (85%) was on dairy cows (n=29 trials), the other studies dealt with calves (n=5). Homeopathy was proved efficacious in 15 studies, but no evidence was found in 18 studies and inconclusive results were returned for one of them. Five out of eight studies performing double-blind RCTs reported no homeopathic efficacy, while three demonstrated efficacy (Day, 1986; Hümmelchen, 1999; Schlecht, 2004).

Twenty studies dealt exclusively with udder health, including prevention or treatment of acute or subclinical mastitis, drying off (Spranger, 2000) or udder health in general (Schlecht, 2004). Apart from mastitis, fertility disorders (especially around the parturition like retained placenta and endometritis) were addressed in 9 studies. Remarkably, almost all studies on fertility disorders used homeopathy exclusively for prevention and not for therapeutic treatment. Only Enbergs and Sensen (2007) examined the use of two different homeopathic complex remedies to treat chronic endometritis, yielding a positive result. Wirth (1990) and Erbe (1990) applied different metaphylactic homeopathic remedies to their experimental groups to reduce retained placenta and endometritis, both with a positive outcome.

When only taking into account studies on dairy cows, 14 out of 29 publications were able to prove efficacy of the homeopathic remedy, while in the same number of studies no benefit of homeopathy was shown. One of the studies failed to show a clear result: Sonnenwald (1986) struggled with undefined results comparing homeopathic and antibiotic treatment for acute mastitis. The homeopathic treatment showed better results for Gram-negative mastitis-causing bacteria and antibiotics were more effective for Gram-positive *Streptococcus subspecies*.

Four of the five studies on calves dealt exclusively with the treatment of diarrhoea (Frerking et al., 1981; Kayne et al., 1994; Verdier et al., 2003; Hornig, 2014). Of these studies, only Kayne et al. (1994) reported the single homeopathic drug *Arsenicum album* 30C as efficacious, resulting in recovery one day faster in the homeopathically treated calves than in the placebo group. Boerms (1981) tested the effectiveness of the classic homeopathic remedy *Echinacea* in combination with standard allopathic remedies for treating pneumonia and enteritis in calves. This was trialled in comparison with "pure" standard antibiotic treatment without significant differences in cure rates compared to Echinacea.

Table 4: Publications on cattle

Publications on cattle					
Author (Year)	**Species**	**Indication**	**Study design**	**Diagnostic measure**	**Effect of homeopathy?**
Frerking et al., (1981)	Calves	Diarrhoea treatment	RCT non-blind	CS	No: repeated treatments necessary, more deaths, also no effect in combination with antibiotics.
Boerms (1981)	Calves	Pneumonia / Diarrhoea treatment	RCT non-blind	CS	IR: only vague improvement, no sign, differences between antibiotic (AB) & homeopathic group.
Day (1986) – 3 trials	Dairy cows	Postpartum disorders prevention	RCT non-blind	CS	Yes: No dead calf compared to untreated group (7 deaths). 2 of 7 cows (HOM) required assistance during birth & 18/18 in untreated. Fewer cases of mastitis / metritis in HOM (0/4) compared to untreated (9/10).
		Mastitis prevention	RCT non-blind	CS	Yes: Mastitis cases decreased (at high infection risk). Mastitis increased in untreated “low risk” group.
		Mastitis prevention	RCT single-blind	CS	Yes: lower mastitis incidence compared to placebo
Sonnenwald (1986)	Dairy cows	Mastitis (acute) Individualized treatment	RCT non-blind	DT/IT/CS	IR: No significant difference between HOM & AB treatments. *Streptococcus:* better result with AB. Gram-negative bacteria: homeopathy more effective than antibiotics.
Stopes et al. (1988)	Dairy cows	Mastitis prevention	Observational	CS	No: No preventive effect.
Merck et al., (1989)	Dairy cows	Mastitis treatment	Observational	CS	Yes: Good healing rates esp. for *E.coli* infections.

Publications on cattle (continued)					
Author (Year)	**Species**	**Indication**	**Study design**	**Diagnostic measure**	**Effect of homeopathy?**
Wirth (1990)	Dairy cows	Retained placenta / Endometritis metaphylaxis	RCT non-blind	IT/CS	Yes: Occurence of endometritis & retained placenta significantly reduced compared to placebo.
Erbe (1990)	Dairy cows	Retained placenta / Endometritis metaphylaxis	RCT non-blind	IT/CS	Yes: Occurence of endometritis & retained placenta significantly reduced compared to placebo.
Tiefenthaler (1990)	Dairy cows	Mastitis treatment	RCT non-blind	DT/IT	No: No differences between homeopathic group, AB group and combination of both remedy types.
Vohla (1991)	Dairy cows	Puerperal diseases & calf health (prevention)	Observational	CS	No: No differences to untreated and placebo.
Knierim (1992)	Dairy cows	Parturition & puerperal disorders (prevention)	RCT double-blind	IT/CS/M	No: No influence compared to placebo.
Kayne & Rafferty (1994)	Calves	Diarrhoea	RCT double-blind	CS	Yes: Calves treated with homeopathy recovered 1 day earlier than in placebo group.
Schütte (1994) – 2 trials	Dairy cows	Mastitis metaphylaxis	RCT single-blind	M	No: No difference between placebo & homeopathy.
		Retained placenta (prevention)	RCT single-blind	CS	No: No difference between a placebo & homeopathy.
Searcy et al., (1995)	Dairy cows	Subclinical mastitis metaphylaxis	RCT non-blind	IT/M	Yes: 4.5 times less subclinical mastitis than in placebo group.
Egan (1995)	Dairy cows	Subclinical mastitis treatment	Observational	DT/IT	No: No response, mastitis even deteriorated.
Hümmelchen (1999)	Dairy cows	Postpartum disorders (prevention)	RCT non-blind	DT/IT/CS/M	Yes: Better development than placebo for birth, placenta retention, occurrence of mastitis & other infections.
Garbe (2003)	Dairy cows	Mastitis metaphylaxis	RCT double-blind	DT/IT/CS	No: No prophylactic or therapeutic effect in comparison with antibiotic dry off or placebo.
Verdier et al., (2003)	Calves	Diarrhoea treatment	RCT double-blind	CS/M	No: No difference in comparison to placebo.
Hektoen et al., (2004)	Dairy cows	Clinical Mastitis Individualized treatment	RCT double-blind	CS	No: No efficacy beyond placebo or antibiotic group. Antibiotics also comparably poor in effectiveness.
Schlecht (2004)	Dairy cows	Udder health (prevention)	RCT single-blind	DT/IT	Yes: Improved health parameter compared to placebo.
Holmes et al., (2005)	Dairy cows	Mastitis prevention	RCT single-blind	IT	No: No significant differences between homeopathy and placebo on any sample day.

Publications on cattle (continued)					
Author (Year)	**Species**	**Indication**	**Study design**	**Diagnostic measure**	**Effect of homeopathy?**
Walkenhorst (2006)	Dairy cows	Clinical & subclinical mastitis treatment	RCT non-blind	DT/IT/CS	No: No effect, only comparable with self-healing rates, Antimicrobials in all cases more effective.
Enbergs et al., (2007)	Dairy cows	Chronic endometritis treatment	Observational	IT/M	Yes: Culling & conception rate improved, shorter interval until pregnancy, lower insemination index, higher first-service pregnancy rate. Comparable to hormone treatment, less effective than conv. intrauterine treatment.
Arlt et al., (2009)	Dairy cows	Endometritis prevention	RCT double-blind	DT/IT	No: Not effective in prevention or in enhancing reproductive performance.
Klocke et al., (2010)	Dairy cows	Mastitis prevention (at drying off)	RCT non-blind	DT/IT/CS	Yes: Lower SCC and "normal milk secretion" compared to untreated cows, but worse than teat sealer.
Werner et al., (2010)	Dairy cows	Mastitis individualized treatment	RCT single-blind	DT/CS	Yes: Positive over placebo group with mastitis, no difference to antibiotic treatment after 4 and 8 weeks.
Kiarazm et al. (2011)	Dairy cows	Subclinical mastitis treatment	RCT single-blind	DT/IT	YES: SCC and bacterial detection were significantly lower in HOM than placebo group after treatment (day 21 & 28).
Wagenaar et al., (2011)	Dairy cows	Mastitis metaphylaxis	Observational	DT/IT	No: No improvement compared with untreated group.
Aubry et al., (2013)	Dairy cows	Early subclinical mastitis treatment	Observational	IT/M	Yes: Significant reduction of electrical conductivity & increased milk yield 4-7 days after treatment.
Williamson et al., (2014)	Dairy cows	Mastitis individualized treatment	RCT non-blind	CS	No: Curing rate of antibiotics was significantly higher (no placebo or untreated group).
Hornig (2014)	Calves	Diarrhea treatment	RCT double-blind	CS/M	No: No significant difference compared to placebo for all chosen parameters.
DT = Direct Test, IT = Indirect Test, CS = Clinical Signs, M = Measurements / IR = Inconclusive results, AB = Antibiotics, HOM = Homeopathy					

Studies on pigs

Twelve studies on pigs were identified (tab.4). Nine trials found the homeopathic remedy to be effective, two couldn't prove the tested remedy to be efficacious and one had inconclusive results. The various outcomes seemed not to be related to the study design. Observational trials and open RCTs, as well as single- or double-blind RCTs, were performed with either efficacy or no efficacy or inconclusive results for the applied homeopathic remedy reported.

The age of the animals varied considerably between the trials evaluated: Five trials were performed with sows, four trials with mainly fattening pigs and three with piglets. The reasons listed for application of homeopathic remedies were predominantly prevention (n=10). Three studies addressed fertility disorders in sows (Both, 1980; Day, 1984; Seifert, 1987; Drösemeier, 1989), three dealt with respiratory infections and three with diarrhoea, while two focused on growth promotion and one simply aimed for a good general health. The three disease complexes mentioned are amongst the most common reasons for the use of antibiotics on pig farms in practice (Christensen et al., 1995; Petersen et al., 2008). The prevention or treatment of fertility disorders (in particular stillbirth and MMA) with homeopathic remedies was effective in two out of three trials in sows. It should be noted that the trials, which were efficacious were either a parallel group trial (Both, 1980; Day, 1984) or a non-blind RCT (Seifert, 1987), while the one study that failed to yield evidence of efficacy of preventing MMA in sows was a double-blind RCT (Drösemeier, 1989).

In a study on respiratory disease and general state of health, homeopathy was significantly more effective than low dosage antibiotics or a placebo for fattening pigs, but was no more than an antibiotic metaphylaxis in high dosage (Albrecht and Schütte, 1999). In an earlier non-blind trial of Schütte (1991), a metaphylactic treatment with two different homeopathic complex remedies yielded a significantly higher efficacy compared with a placebo depending on the duration of treatment, but was not as efficacious as an antibiotic treatment.

Two of the studies dealt with growth promotion: Guajardo-Bernal et al. (1996) administered a very high dilution (C201) of *Sulphur* to pregnant sows and examined the weight and performance of the new-born piglets in a single-blind RCT. He found no significant difference in the birth weights and the further development of the piglets in comparison with a placebo group. In an observational trial, Da Silva et al. (2011) treated the pigs directly with a complex remedy in two different treatment schemes aiming for growth promotion. The weight recorded differed between the groups within the study period, but as there was no control group, no clear outcome could be concluded. Studies by Soto et al. (2008), Coelho et al. (2009) and Camerlink et al. (2010) aimed to prevent diarrhoea in piglets. Coelho et al. (2009) and Camerlink et al. (2010) performed a double and single blind RCT, respectively. Both studies yielded a positive result for efficacy of homeopathy in comparison to a control group treated with an antibiotic or placebo. Soto et al. (2008) showed that homeopathically treated piglets lost less weight compared to an untreated control or placebo group, but the difference between the placebo and homeopathy groups was not significant. The only study evaluating the influence of homeopathy on general health in pigs measured by clinical signs, gravity and length of illness, therapeutic results and antibiotic consumption (Schütte, 2003) showed that the consumption of antimicrobials on the participating farms could be reduced by 60% (over three years) when homeopathy was used.

Table 5: Publications on pigs

Publications on pigs					
Author (Year)	**Species**	**Indication**	**Study design**	**Diagnostic measure**	**Effect of homeopathy?**
Day (1984)	Sows	Stillbirth prevention	Observational	M	Yes: Number of stillbirths decreased significantly compared to an untreated group.
Seifert (1987)	Sows	MMA individualized treatment	RCT non-blind	CS/M	Yes: less treatments needed to recover compared to allopathy. Average treatment duration was slightly shorter. Higher litter weight & lower piglet mortality.
Drösemeier (1989)	Sows	MMA prevention	RCT double-blind	DT/CS/M	No: No difference on MMA infection rate compared to placebo or antibiotic group.
Schütte (1991) – 2 trials	Pigs	Respiratory tract diseases prevention	RCT non-blind	CS	Yes: HOM mix over 10 days lessens the sickness rate (18,1%) in comparison to placebo (24,3%), more effective than subtherapeutic AB dose (19,1%) but less than therapeutic AB dose (10,4-8,3%).
			RCT non-blind	CS	Yes: Homeopathic remedy had a significantly lower infection rate (17,7%) than placebo (24,3%) within a 5-day treatment double-dosed.

Publications on pigs (continued)					
Author (Year)	**Species**	**Indication**	**Study design**	**Diagnostic measure**	**Effect of homeopathy?**
Guajardo-Bernal et al., (1996)	Sows	Growth promotion	RCT single-blind	M	No: No difference in birth weight of litters compared to placebo.
Albrecht et al., (1999)	Piglets	General & respiratory disease metaphylaxis	Observation al	CS	Yes: Significantly effective when compared with the placebo & routine low dose antibiotics for reduction of disease & prevention of respiratory diseases - but not better than a therapeutic dose of antibiotics.
Schütte (2003)	Pigs	Health in general	Observation al	CS/M	Yes: Antibiotic use could be reduced by 60% (over 3 years) on participating farms.
Soto et al. (2008)	Piglets	Post weaning diarrhoea & weight loss prevention	RCT non-blind	M	IR: Piglets treated with homeopathy had less weight loss as control group & less but not significant different to placebo. No statistical difference between food consumption or diarrhoea.
Coelho et al., (2009)	Piglets	Coli-diarrhea prevention	RCT double-blind	CS/M	Yes: Highest weight gain & significant reduction of diarrhoea compared to AB control (but medical agent of AB is known to have high resistance to *E. coli*).
Camerlink et al., (2010)	Piglets	Coli-diarrhea prevention	RCT single-blind	DT/CS	Yes: Less transmission & duration of disease shorter and less diarrhoea than in placebo group.
Da Silva et al., (2011)	Pigs	Growth promotion	Observation al	M	IR: Last of 6 measurements was higher, but at slaughter no significant weight differences found ➔ lack of a control group (& only 5 pigs per group).
DT = Direct Test, IT = Indirect Test, CS = Clinical Signs, M = Measurements / IR = Inconclusive results					

Studies on poultry

Only six studies were found dealing with homeopathic treatment in poultry production and they were performed exclusively on chickens (tabl.5). Efficacy of homeopathic treatment was proven in four studies out of the six.

In three trials, homeopathy was administered to treat diarrhoea caused by Salmonella (Sandoval et al., 1998; Berchieri et al., 2006) or *E.coli*-infection (Velkers et al., 2005). The remaining three studies looked at growth promotion (Amalcaburio et al., 2009; Hadipour et al., 2011), and one study combined this with observations on improved immune reactivity (Sato et al., 2012). The four studies where homeopathic treatment was effective were performed as observational (Sandoval et al. 1998), non-blind RCTs (Camphausen, 2002; Berchieri et al., 2006; Hadipour et al., 2011) and double-blind RCT (Sato et al., 2012). Berchieri et al. (2006) induced an infection to test the preventive effect of a *Salmonella* nosode and found a significant lower faecal excretion of *Salmonella enteritis* when compared with an untreated group. Hadipour et al. (2011) compared the growth promoting effects of a combined homeopathic remedy with undefined potency to a group treated with a standard regime of antibiotics and vaccines. They found a 5% lower mortality rate and significantly higher weight gain in the homeopathic experimental group. Sato et al. (2012) performed a double-blind RCT with chicken and noted that the group with *Thymulin* in a D5 ($1:10^{-5}$) dilution benefitted from an immune stimulating and growth promoting effect, compared to an untreated group.

The two studies with a negative outcome were an observational study (Sandoval et al., 1998; Velkers et al., 2005) and a non-blind RCT (Amalcaburio et al., 2009). One of them used high dilutions (C12 or C30) of a combined homeopathic remedy in comparison to an untreated control(Amalcaburio et al., 2009). The other study administered three different combinations of homeopathic drugs combined with an *E.coli* nosode, all in a high dilution (C30, C200), with no effect compared to the untreated or antibiotic-treated control groups (Velkers et al., 2005).

Table 6: Publications on poultry

Publications on poultry					
Author (Year)	**Species**	**Indication**	**Study design**	**Diagnostic measure**	**Effect of homeopathy?**
Sandoval et al. (1998)	Chicken	Salmonellosis treatment	RCT non-blind	DT/CS	Yes: Efficacy not significantly different to antibiotic group.
Velkers et al., (2005)	Chicken	Coli-Diarrhoea (induced) treatment	Observation al	DT/CS/M	No: No difference compared to untreated control.
Berchieri et al. (2006))	Chicken	Salmonellosis (induced) prevention	RCT non-blind	DT	Yes: Faecal excretion of *S.enteritidis* significantly lower than in untreated control.
Amalcaburi et al. (2009)	Chicken	Growth promotion	RCT non-blind	DT/M	No: No difference in growth speed or final weight compared to an untreated control.
Hadipour et al., (2011)	Chicken	Growth promotion	RCT non-blind	DT/CS/M	Yes: Higher growth rate, final weight and food conversion ratio compared to conventional treatment (AB & vaccines).
Sato et al. (2012)	Chicken	Growth promotion & immune system improvement.	RCT double-blind	DT/CS/M	Yes: Less mortality, increased productivity, a higher viability & a possible shunt to B lymphocyte activity & higher weight gain (only for females) than in untreated control.
DT = Direct Test, IT = Indirect Test, CS = Clinical Signs, M = Measurements / AB = Antibiotics					

Heterogeneity among the trials

A classic comparison of the individual clinical trials within a systematic review according to the principles of evidence-based medicine was not possible. These principles involve randomisation, blinding and several clinical trials performed on the same species, disease and remedy to be evaluated in a systematic way. A large heterogeneity among all clinical trials was found. Differences emerged in various areas:

- <u>Patients:</u> The patients included in the trials differed with respect to species. Differences within species were in relation to: age of farm animals, performance level, resilience, and thus different individual abilities to respond to pharmaceutical active ingredients.
- <u>Remedy:</u> The remedies used in the trials differed with respect to: ingredients in classic and combined homeopathic remedies, high and low dilutions (potencies), administration method, pharmaceutically active ingredients (not standardised). Remedies were administered in different ways for various indications in order to achieve preventive, metaphylactic, therapeutic, growth-promoting or immune system stimulating effects.
- <u>Context:</u> The trials were performed in different contexts, distinguished among others by: diseases, pathogens, means of infection (naturally occurring or artificially induced), farm animals' living conditions regarding hygiene standards, feeding regimen and treatment procedures. In 54 per cent of the studies, the living conditions were not described.

- <u>Expertise:</u> Diagnostic procedures in the trials were performed by people who differed considerably in education, expertise and knowledge of treatment strategies. Choice of remedy and means of measuring the therapeutic effect of the homeopathic remedy applied differed between trials. This was also the case regarding assessment of therapeutic effects (eg, visual improvement in milk) compared to an objective analysis (eg, complete clinical examinations accompanied by external laboratory tests) (Fig 3).

Within the studies considered, the use of the same remedy administered to the same species with a comparable medical indication was not repeated. Thus, the results lack any reproducibility.

1.4 Discussion

Taking into account the long existence of homeopathy and use in livestock production, only a comparatively small number of scientific studies focussing on the efficacy of homeopathy have been conducted so far. Four publications summarised a limited number of trials with different search criteria while considering various species, including livestock. Three of them evaluated the efficacy of homeopathy as poor or insufficient; one of the reports did not come to a clear result due to limitations in scientific quality of studies available:

- **Kowalski** (1989) evaluated homeopathic treatment in veterinary literature for a doctoral thesis and found that homeopathy had certain effects, but only one homeopathic trial seemed to have been efficacious. Fourteen controlled, clinical trials (not including experimental trials on single animals and general treatises on remedy pictures) were assessed. Ten of the 14 trials considered cattle or pigs, while the rest dealt with cats, dogs or horses.
- Hektoen (2005) took into account three single clinical trials and the publication of Kowalski (1989) to evaluate the efficacy of homeopathy in animals. The remaining sources covered broader literature and conference proceedings or grey literature on the topic “homeopathy and placebo effect”, predominantly considering human medicine. The resulting “Review of the current involvement of homeopathy in veterinary practice and research” did not emerge with a clear decision on the efficacy of homeopathy, because of the poor quality of the available studies.
- Rijnberk et Ramey (2007) considered ten publications on human medicine, for example homeopathy reviews, while only four clinical trials including animals (three on cattle, one on dogs) were considered. They published a report on the lack of effectiveness of veterinary homeopathy pointing out inter alia the existence of no significant differences of homeopathic remedies and placebos when compared in studies.
- Ruegg (2009) compared different treatment strategies of mastitis and its efficacy. Four publications (Egan, 1998; Hektoen et al., 2004; Holmes et al., 2005; Rijnberk et al., 2007) were considered and concluded that efficacy data for veterinary homeopathy appear to be almost completely lacking.

In comparison to the reports previously published, dealing with the effectiveness of homeopathy in animals, the current review encompasses a much higher number of studies. One reason is the fact that much time has passed between the first review by (Kowalski, 1989) and today. Apart from publications in peer reviewed journals, the current review also included doctoral theses in German. This reflects the situation in German speaking countries, where (for unknown reasons) homeopathy is more frequently used than in other countries (Kayne, 2006; Mathie et al., 2012; Albrecht, 2013; Allensbach Institute for Public Opinion Research, 2014). In their characterization of research literature on veterinary homeopathy in preparation of following publications, Mathie et al. (2012) didn't define doctoral thesis as peer-reviewed as it was done in this review. Due to the extended literature search using multiple sources, it can be assumed that nearly all peer-reviewed articles on homeopathy in livestock matching the search criteria were considered. Thus, the current review can claim to be the most comprehensive review on homeopathy in farm animals so far.

Comparing the previous reports (Kowalski, 1989; Hektoen, 2005; Rijnberk et al., 2007; Ruegg, 2009) with this review's findings, only a very small number of trials on animals were considered and the selection criteria were either unclear or not even stated. These facts may have caused the authors to be biased in their reporting of the results.

Contrary to the summary reports of the previous authors, Clausen et al. (2013) and Mathie and Clausen (2014, 2015) published three articles exclusively on randomized placebo-controlled trials in veterinary medicine built upon each other. The main focus was on medical conditions *e.g.,* randomization, risk of bias according to Cochrane's Evidence-Based-Medicine principles, individualized/non-individualized treatments etc. to assess whether homeopathic interventions are distinguishable from corresponding treatments with placebo. They found two trials of 18 (2014) and 15 (2015) respectively with reliable evidence, free from vested interest precluding general conclusions about efficacy of homeopathy and very limited evidence for differences between homeopathy and placebo within placebo-controlled RCTs.The authors saw a need for a higher quality research on veterinary homeopathy.

Homeopathic trials performed as a single-blind or non-blind RCT, parallel groups or an observational trial (*see Fig.4*) tended to be more frequently efficacious than a double-blind RCT, indicating that positive outcome may partly be due to a bias caused by a conscious or unconscious preference for a certain treatment. The biggest proportion of the trials (79%) were RCTs – a high quality level according to the principles of Evidence-Based-Medicine (EBM) and regarded as the standard for a remedy's clinical proof of efficacy by the scientific community (van Sluijs, 2005). This may lead to the general conclusion that homeopathic remedies are able to support the healing process under certain conditions. However, the EBM-method is also prone to errors (i.e. by a narrow scope of considered studies) producing misleading conclusions or results claiming efficacy where there might be none (Weymayr, 2013).

A critical point in the current methods of performing trials on efficacy is that they are conducted under standardised conditions and do not consider the situation animals actually experience on farms. The focus is mainly on the remedy without particular focus on the interactions between animals and their living conditions, and their ability to react to a certain pathogen. The variability of the living conditions and of the pre-existing situations on farms are not considered either, for instance, regarding the appropriateness of diagnostics to determine the most suitable remedy or treatment procedure. Thus, a certain remedy which proved efficacious in a scientific trial may not be effective under farm conditions.

No differences concerning the outcome were found in relation to the means of publication in scientific journals or as doctoral theses. The number of non-peer-reviewed publications found was more than double the number of peer-reviewed journals. Similar results were found by Clausen et al., 2013), who evaluated all studies, collected by the HomVetCR database on veterinary homeopathy, focusing on the content of placebo-controlled trials. The peer-reviewed studies were more often published in journals dedicated to homeopathy or alternative therapies than in journals on veterinary medicine in general. It can be assumed that journals, which focus on complementary and alternative therapies like homeopathy are rather more likely to receive articles dealing with this therapy. There is an increased risk for publication bias, for example that reports with positive results for a tested method or remedy are usually more likely to be published than those with negative results (Arlt et al., 2010). In this evaluation, articles published in journals focussing on alternative treatments were much more likely to present an efficacy (positive publication bias) while it was vice versa for articles in journals focussing more on conventional therapies (negative publication bias) but failed to provide statistically significant differences (OR 3.75, 95 percent CI 0.63 to 22.04, P=0,14).

Comparing the reasons why homeopathic remedies were used, positive results tended to occur more often for a preventive use (n=16 of 28) than for a therapeutic (n=8 of 28) or a metaphylactic use (n=4 of 28). Remarkably, when strictly following the simile-rules ('like cures like') of homeopathic treatment, a preventive effect is difficult to achieve because a complete homeopathic clinical picture is lacking and so is the matching remedy picture. The results of the trials contradict this principle, but may be an unspecific reaction of the immune system to the stimulus (the remedy). A different case might be the special form of homeopathic remedy called 'nosode', manufactured using a sample of diseased matter as a basis (usually from a certain animal in the herd to be treated), highly diluted and mainly used for prevention. The dilution process reduces the number of infective pathogens to almost zero but could possibly induce unspecific humoral immunity.

The most objective and scientific way to assess the outcome of treating a diseased animal is to perform a direct test (eg, on the presence or absence of the pathogens responsible) and this is best performed by an official laboratory. Starting from this basis, the treatment assessment methods can be listed as follows: direct test > indirect test performed onsite > clinical signs > measurements. The list starts with the most scientific methods and ends with the least scientific ones. The person performing the diagnostic procedures may well have judgement, which varies according to education and experience. The complexity of factors which results in a cure and the performance of different diagnostic measures might distort the resultant efficacy of a remedy applied. Expertise is a prerequisite in finding the appropriate remedy and helps inform any ongoing adaptations of the treatment when necessary. However, when any factors present which cause disease are left undiscovered and are not instantly removed, animals may still remain ill. If farm animals are kept under living conditions where their immune systems are under strain or exhausted, neither conventional nor alternative remedies will be able to provide a sufficient cure rate.

While conventional medication focuses primarily on the indication, homeopathy addresses the individual reactions and symptoms arising in the first place. Correspondingly, to an extent the conventional approach acts in a context-invariant way whereas the homeopathic approach considers the context, particularly observing how animals behave in a specific situation. Thus, an assessment of the effectiveness of homeopathic medication should not exclude the context in which these remedies are employed.

When compared in the same trial, there were differences in the therapeutic effect homeopathy and the placebo had. This ranged between 0% and 27% (Kayne et al., 1994; Hornig, 2014). Sometimes relatively small and insignificant differences were found between the homeopathic remedy and the placebo groups in clinical trials. This raises the question whether the effects of homeopathy are comparable to a placebo effect? Nevertheless, twelve trials were performed above a dilution of $1:10^{24}$ and showed a positive outcome for the efficacy of the homeopathic remedy administered in relation to a control treatment. The efficacy of a placebo generally corresponds to the function of self-healing forces of the body without the influence of an active substance. Therefore, a comparison of homeopathy with a placebo and an untreated group aids the distinction between the effect a placebo has and the self-healing effect. When aiming to find an alternative to an existing medicine, it is necessary to undertake trials with the above remedy, the proposed alternative and a placebo group in order to come up with valid results (European Medicines Agency, 2001). This ensures that animals receive the most effective therapy possible.

In order to be considered as an alternative to antimicrobials in case of bacterial infections, homeopathic remedies ought to be at least as effective. In 18 studies, a homeopathic treatment was compared with an antimicrobial treatment. In several cases, the efficacy of the chosen homeopathic remedy was higher than the conventional treatment with antimicrobials. In the same trial, differences between the cure rate of homeopathic and antibiotic treatment ranged between 2% and 30% in favour of homeopathy in the same trial (Merck et al., 1989; Varshney et al., 2005). However, a comparable amount of trials reported homeopathic remedies as having poorer cure rates compared with the conventional treatment, with differences ranging between 1% to 55% in the same trial (Hektoen et al., 2004; Walkenhorst, 2006). In other studies, no significant differences could be found in the cure between the experimental group treated with homeopathy and an antibiotic control group (Sandoval et al., 1998; Velkers et al., 2005). Furthermore, it has to be considered that in most trials no sensitivity tests on the bacterial resistance patterns of the bacteria responsible were performed before choosing the antimicrobial for treatment. Performing a sensitivity test in the trials might have led to altering the drug selected, thus causing the antimicrobial tested to be more effective overall.

When taking the total number of studies into account, not even one study was repeated under comparable conditions. Consequently, the existing conditions, which enable a systematic review to be carried out completely are not given. The current evidence of studies providing evidence in favour of homeopathy lacks reproducibility and therefore cannot claim to have sufficient prognostic validity. No general conclusions can be drawn as to whether a homeopathic remedy shown to be significantly more effective than a control treatment in a specific context is also effective in a different context or under different conditions (as the previous trial describes). It cannot be concluded whether it is better, worse or ineffective.

The efficacy of a remedy cannot be reduced to the concentration of pharmaceutically active ingredients alone and cannot be isolated from the context in which it is used. It is the result of complex interactions of various factors which include, among others: the individual's immune strength and capacity to react to stimuli, the degree the main and any secondary causes are eliminated, an adequate diagnosis and an appropriate treatment (no matter of the kind), and, last but not least, the farm animals' living conditions, especially in relation to nutrient supply and hygiene conditions.

In practice, homeopathy may well not be applied according to a certain standard in diagnostic, treatment and education due, amongst other reasons, to the different level of homeopathic education of farmers and veterinarians. However, it is also questionable whether conventional medicine (i.e. antibiotics) is always used according to the guidelines on 'The prudent use of antibiotics in veterinary medicine' in farm practice as the (Federation of Veterinarians of Europe, 2014) recommend. Various publications indicate that farms rarely instigate diagnostic tests to detect the pathogen responsible (Ouweltjes et al., 2008; Doehring et al., 2013).

1.5 Conclusions

In a considerable number of studies, a significant higher efficacy was recorded for homeopathic remedies than for a control group. Therefore, the potential medical efficacy of homeopathy under certain conditions cannot be ruled out. However, this does not necessarily imply that homeopathic remedies are effective under different conditions. This is especially true for the context-sensitive treatment strategy of homeopathy, which considers (beside clinical signs and the pathogen responsible) behaviour, constitution and conditions in which the animal is living in. The review revealed that all studies included were conducted under very specific conditions, but no trial was repeated in a comparable manner. Thus, the previous studies cannot be generalised and have to be regarded as single-case studies. The first priority when medically treating animals should always be to apply the most effective treatment or remedy and thus prevent unnecessary suffering of the animal, if only for the reasons of animal welfare. Due to the unknown level of effectiveness of on-farm homeopathy, this can only be achieved by the appropriate control and monitoring of treatment success in farm practice. Due to a lack of prognostic validity, replacing or reducing antibiotics with homeopathy currently cannot be recommended unless evidence of efficacy is reproduced by RCTs and proven in various farm practice conditions.

2. Efficacy of antibiotic and homeopathic treatment of bovine mastitis and implications for the effectiveness on farms (paper II)

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Submitted at Preventive Veterinary Medicine

Abstract

Whereas there is far-reaching consensus about the efficacy of antibiotics, the efficacy of homeopathy is generally questioned. However, antibiotic use is criticized for promoting antimicrobial resistance. Thus, various regulations limit the use of antibiotics in livestock, possibly counteracting the objective of enhancing healing success for animal welfare reasons. The "One Health" approach to preventing the spread of diseases and resistant bacteria requires the most effective treatment to be chosen. Despite its relevance, no comprehensive knowledge is available on how effective applied treatments on dairy farms really are. This paper examines and discusses current knowledge on efficacy (effect in science/trials) and effectiveness (effect under real farming conditions) in bovine mastitis for both antibiotics and homeopathy.

Twenty-nine trials (12 on homeopathic treatments, 17 on antibiotic treatments) were evaluated for scientific quality and outcome. Study quality was generally poor: only three studies showed sufficient scientific quality. Five of the 12 studies (42%) showed efficacy of the homeopathic treatment, but only one had sufficient scientific quality to provide evidence for efficacy. In the antibiotic treatment studies, eight of the 17 studies (47%) showed increased efficacy of the respective antibiotic compared to a control treatment, but none of the studies showed sufficient scientific quality.

These results question the use of homeopathy in practice as even under ideal scientific study conditions, repeatable evidence for efficacy could not be proven. Also, the extensive use of antibiotics in bovine mastitis can be questioned as the validity of the scientific trials was insufficient from a scientific perspective. Because the effectiveness of both treatments in farming practice has been neither reviewed nor monitored on a larger scale, it is questionable whether current use is reasonable or responsible. To make a clear statement about the efficacy of any treatment, studies must have a good scientific study design, be carried out under comparable conditions within a double-blind RCT and should be openly accessible. However, effectiveness is not only dependent on the efficacy of remedies, but also on the quality of the *lege artis* use in farming practices to identify mastitis, diagnose the responsible pathogen, select the appropriate remedy, follow up on the treatment and improve treatment strategies on an individual and herd basis. Furthermore, the monitoring of treatment outcomes and the analysis of available health and production data are required to formulate general and farm-specific treatment recommendations for veterinarians and farmers.

Keywords: *study design, bovine mastitis, efficacy, homeopathy, antibiotic treatment, evidence*

2.1 Introduction

In medical fields, efficacy is defined as the measurement of a desired effect of a given treatment, drug or intervention compared to no intervention or a placebo (i.e., "how it works in ideal conditions of a scientific trial") within a randomized controlled trial to diminish bias by the surrounding conditions (Cambridge Dictionary, 2021b; European Medicines Agency, 2022). Effectiveness refers to "how the drug works in a real-world situation" (Cambridge Dictionary, 2021b), i.e., on the farm, and is often lower than efficacy because of a number of limiting factors in a multifactorial and complex interrelationship, *e.g.,* additional health issues, different living conditions, insufficient dose rates, uptake or duration of use, or differences in availability, accessibility and affordability (Zinsstag et al., 2011).

Assessing the efficacy of a remedy is a prerequisite for remedy selection when treating a diseased animal. In the case of bovine mastitis, treatment with antibiotics is, in general, the first choice on most dairy farms (Krömker et al., 2017) and is commonly believed to be effective. However, an in-depth investigation of the treatment success is seldom conducted in farming practice. However, antibiotic consumption by farm animals is viewed critically due to an increase in antibiotic-resistant bacteria worldwide and there are demands for a reduction in use (Laxminarayan et al., 2013; Antimicrobial Resistance Collaborators, 2022).

In the search for alternatives to antibiotic treatments, homeopathy is one of the most popular alternatives for the treatment of bovine mastitis (Ruegg, 2009; Hellec et al., 2021). In addition to reducing antibiotic use, the use of homeopathic remedies has many presumed advantages*, e.g.,* low costs, no withdrawal time, availability over the counter, positive reputation amongst consumers, no contribution to antimicrobial resistance, etc. However, the use of homeopathy is highly controversial due to its unclear mode of action and questionable efficacy beyond a placebo effect (Lees et al., 2017a). With respect to animal welfare and the "One Health" approach, the most effective treatment should be chosen as a first option. When there are reasons to replace a standard treatment method, the alternative should be at least equally effective.

Scientific trials are performed to obtain objective and reproducible results on research questions in order to justify the use and improve the success of a treatment. The gold standard for testing the effect of a new drug is the randomized controlled trial (RCT). This study design randomly assigns individuals to an experimental group (receiving the new drug) and a control group (receiving, *e.g.,* a placebo). The only expected difference between the control and experimental group in an RCT is the outcome variable being studied (Hirsh Health Sciences Library, 2019). Specific recommendations on how to design a scientific trial are summarized in statements, i.e. CONSORT (Consolidated Standards of Reporting Trials) (Schulz et al., 2010) or REFLECT (Reporting Guidelines for Randomized Controlled Trials for Livestock and Food Safety) (Sargeant et al., 2010). Both statements require that the remedies being examined must be compared to appropriate control groups (placebo/untreated/standard treatment) with complete information of the applied drugs and their administration, and a randomization of participating individuals, including the allocation of animals.

When a new remedy/treatment has shown efficacy over a control within an individual trial, the effect should be repeatable in similar scientific trials under similar conditions (independently verified by others) to show reliability and rule out random effects. Repeated trials on the same drug/treatment under different conditions will enable a meta-analysis or review to be performed to provide greater evidence of the efficacy. Proof of efficacy under optimized conditions (as in a scientific trial) is an essential prerequisite for justifying its use in farming practice to further test the effectiveness under "real-life" conditions.

To the authors' knowledge, only one review of scientific trials on the overall efficacy of antibiotics in animals have been conducted thus far (Winder et al., 2019), whereas for homeopathy several meta-analyses and systematic reviews on efficacy in humans (Linde et al., 1998; Cucherat et al., 2000; Ernst, 2002; Mathie et al., 2014b) and animals (Hektoen, 2005; Ruegg, 2010; Mathie et al., 2015; Doehring et al., 2016; Lees et al., 2017b, 2017a; Zeise et al., 2019) have been conducted.

The results of these reviews are thus far inconsistent. They have not provided clear evidence of efficacy for antibiotics or homeopathy due to insufficient study quality and a lack of replication of individual studies in the case of veterinary medicine or even due to an absence of efficacy of any kind (Lees et al., 2017b, 2017a).

Although both types of therapies are regularly used, little is known about their actual effectiveness on dairy farms and an evaluation of treatment outcomes seldom takes place in daily practice (Lam et al., 2009). Some countries (*e.g.,* Norway, Sweden, Finland, Denmark) have implemented disease record systems, i.e., reporting on particular antibiotic treatments of diseases by farmers or veterinarians. The data are usually captured by national animal health organizations, but without specifications of the treatment or its outcome. In cases of clinical mastitis, the reporting of cases varied widely between 51% up to 91% of all cases, depending on the country (Wolff et al., 2012).

For homeopathy treatments, even less is known, as remedies are freely available and often used without veterinary consultation. A survey revealed that farmers who made use of homeopathy predominantly had insufficient knowledge of homeopathic principles, the treatment success was seldom assessed and use was not always in compliance with national laws (Keller et al., 2018).

Regular monitoring of treatments and their success depends on farmers' motivation to keep records and spend time and labour on the analysis of those records to improve and adapt existing treatment strategies. Although the implementation of this would promote cost effectiveness and treatment success, less than 41% of 110 Canadian farms (Manglai, 2016) and 56% of 1,700 American farms (Kayitsinga et al., 2017) frequently or always maintained treatment records for their animals.

In the absence of sufficient real-world data on the effectiveness of both types of treatment in mastitis in farming practice, this paper aims to examine the scientific quality of published trials and their outcomes regarding efficacy in bovine mastitis to discuss whether the current use of homeopathy and/or antibiotics is justified by the current scientific knowledge and the implications for the improvement of the effectiveness on farms. An unsuccessfully applied treatment will have inevitable negative consequences concerning animal health and welfare as well as economic losses for the farm, whereas any improvement in the effectiveness of treatments will reduce the consumption of antibiotics and reduce the suffering and spread of bovine mastitis on dairy farms.

2.2 Materials and methods

The requirements of a well-blinded RCT apply to all studies, but difficulties can derive from testing different types of treatments when performed *lege artis.* Antibiotic treatments are chosen predominantly according to the pathogen causing the disease (pathogen-remedy relationship). The gold standard procedure when treating bovine mastitis with antibiotics includes, in addition to an anamnesis and clinical examination, a cyto-bacteriological test of a milk sample of the affected udder quarter. Information on the responsible pathogen and its susceptibility to different antibiotics (by *in vitro* tests using agar plate culturing) helps select the most likely effective antibiotic agent. Although the susceptibility test (also called an antibiogram) helps select specific antibiotics for specific bacteria, it does not necessarily mean that it will definitely be effective in the diseased animal. Thus, following studies *in vivo* is essential to prove the efficacy of an antibiotic treatment in the animal suffering from a disease.

Homeopathy is a treatment method based on a symptom-remedy relationship, where the remedy is usually chosen depending solely on clinical signs, characteristics and behaviour of the individual rather than on the responsible pathogen or cause of disease. A homeopathy treatment requires a thorough anamnesis and an examination of the clinical signs and characteristics of the individual, i.e. body condition, mood and modalities like behaviour and symptom changes under environmental stimuli or external factors forming the "symptom picture". It is matched to a "remedy picture" according to a homeopathic *materia medica*, which leads to the selection of the corresponding and most likely effective homeopathic remedy (including its dilution and administration frequency), called "individualization". The procedure depends strongly on the observation and judgement of the examiner educated in homeopathy.

The different treatment methods require different study designs to obtain optimum results and best possible recovery (Hahnemann, 1869; Stock-Schröer et al., 2009; Sargeant et al., 2010; Schulz et al., 2010) (see Table 7).

Table 7: Requirements for the study design of antibiotic and homeopathic treatments

Optimal study design	**Antibiotics**	**Homeopathy**
Randomization	✓	✓
Control groups	✓	✓
Blinding	✓	✓
Expertise in treatment method	✓	✓
Remedy chosen according to individual symptoms (individualization)	-	✓
Testing of a certain remedy	✓	-
Testing of a certain pathogen	✓	-
Remedy chosen according to antibiogram	✓	-
Definition of clear inclusion/exclusion criteria	✓	✓
Measurement of effect/cure	✓	✓
Statistical analysis	✓	✓
Discussion of possible bias	✓	✓

An internet-based search for scientific publications on the issue was performed using different keywords (antibiotic, antimicrobial, bovine mastitis, cows, dairy, diluted, efficacy, effectiveness, homeopathy, homeopathic, lactation, mastitis, meta-analysis, RCT, review, therapy, trial) in the time frame from January 1985 to January 2020 in English or German. All peer-reviewed papers or doctoral theses matching the topic of efficacy of the therapeutic treatment of bovine mastitis using homeopathy or antibiotics during lactation were considered for the evaluation. Studies without a main focus on antibiotic or homeopathic treatments, *e.g.*, on phytotherapy with an antibiotic control, were excluded from the evaluation. The search and filtering process was performed based on PRISMA guidelines (Liberati et al., 2009) to ensure transparent and complete reporting.

Studies were assessed in detail for their study design, especially regarding the specific requirements for each type of treatment (see Table 1). This included randomization, blinding, control groups, the measure of effect regarding clinical symptoms, indirect tests and cyto-bacteriological milk samples, the consideration of expertise regarding the treatment, the *lege artis* treatment procedure required for mastitis, the treatment method and the critical assessment of the results by statistical analysis and risk of bias. Furthermore, studies were rated using quality points according to the extent to which the requirements of a scientific RCT for the specific treatment type were fulfilled and their outcome regarding efficacy.

2.3 Results

Of the 6,851 publications found, 29 publications (26 publications in English and three in German) meeting the given criteria were identified dealing with the efficacy of homeopathy (n=12) or antibiotics (n=17) when used to treat bovine mastitis.

Efficacy of antibiotic treatments for mastitis

Seventeen studies between 1985 and 2019 on the efficacy of antibiotic treatments for mastitis were found (see Table 8). The sample sizes varied widely between 12 animals (in a crossover study by Pyörälä et al., 1994) and 9,007 cases (Wilson et al., 1999), with group sizes (experimental vs. control) well balanced in 13 of the 17 studies. Apart from three, all the studies randomized the animals or cases to different groups. The experimental group was mainly compared with one or more different antibiotic agents (standard treatment) or a different administration routine or treatment duration (12 of the 17). While seven studies solely compared the antibiotic treatment in question with another antibiotic treatment in the form of a non-inferiority trial, one study had a placebo control, six studies had an untreated control and five studies were compared to a different, non-antibiotic treatment, *e.g.*, oxytocin, anti-inflammatory agents like flunixine or ketoprofen, or frequent milk-out.

Of the 17 studies only three studies concealed the treatment (blinding to personnel). Apart from Pyörälä (1994), all studies defined clear inclusion and exclusion criteria and a measure of effect was fully performed in 14 of the 17 studies, while two performed only laboratory tests without considering the clinical symptoms (*e.g.,* deviations in milk appearance, swelling/soreness of the quarter, body temperature) or performed indirect tests (*e.g.*, California mastitis test, electrical conductivity, NAGase activity).

Expertise in antibiotic treatment, *e.g.*, by a veterinarian or a specific training was only mentioned in six of 17 studies. All the studies examined at least one specific antibiotic agent, while nine studies took into account the responsible pathogen (five naturally occurring infections, three induced the infection and one only differentiated gram-positive/gram-negative bacteria). The susceptibility/resistance patterns of the pathogens were fully determined only by Pyörälä (1998), and a second study restricted the determination of resistance of the bacteria involved to penicillin and methicillin (Ziv et al., 1985).

A statistical analysis was performed in all studies, but the risk of possible bias was only discussed in six of the 17 studies.

A study on antibiotic efficacy could attain up to 11 quality points: randomization (1), blinding (1), placebo or untreated control (1), other control (1), definition of clear inclusion/exclusion criteria (1), performance of clinical (1) and cyto-bacteriological examinations (1), expertise (1), susceptibility testing (1), statistical analysis (1) and a discussion of risk of bias (1).

Schukken et al. (2011) and Poutrel et al. (2008) obtained the highest points for study quality with 8 of 11 points. Descending in quality were six studies with 7 points, five studies with 6 points, three studies with 5 points and the oldest study obtaining only 3 points (Ziv et al., 1985).

The antibiotic tested was found to be effective in eight of the 17 studies, while in six studies efficacy was insufficient compared to the control treatment. Three studies had inconclusive results, *e.g.,* efficacy was only found in some cases depending on the responsible bacteria/bacterial group or the age of the cow. The two highest-quality studies (8 points) found the tested antibiotic to be more effective compared to the control and only half of the following six studies with 7 points showed efficacy.

Table 8: Study design of scientific trials on antibiotics used to treat mastitis during lactation (n=17)

Study	Sample size	Study design						Measure of cure		Expertise in antibiotic treatment	Specific pathogen	Specific antibiotic	Susceptibility of the pathogen tested	Statistical analysis	Risk of bias	Quality (x out of 11)	Efficacy
		Randomization	Blinded	Placebo control	Untreated control	Other control	Definition incl./ exclusion criteria	Clinical signs / indirect tests	Cyto-bacterio-logical Test								
Ziv et al., 1985	**214**						+				+	$+^{3}$	+	+		**3**	**Y**
Guterbock et al., 1993	**254**	+				+	+	+	+			$+^{2}$		+		**6**	**N**
Pyörälä et al., 1994	**12***	+			+			+	+		$+^{i}$	+		+		**5**	**N**
Pyörälä et al., 1998	**543**					+	+	+	+		+	$+^{3}$	+	+		**6**	**I**
Deluyker et al., 1999	**232**	+	+				+	+	+	+		$+^{2}$		+		**7**	**Y**
Wilson et al., 1999	**9.007**				+		+		+			$+^{7}$		+	+	**5**	**Y**
Hillerton et al., 2002	**81**	+			+	+	+	+	+		$+^{i}$	$+^{5}$		+		**7**	**Y**
Roberson et al., 2004	**82**	+			+	+	+	+	+			$+^{2}$		+		**7**	**I**
Wenz et al., 2005	**144**	+					+	+	+			$+^{4}$		+		**5**	**N**
McDougall et al., 2007a	**604**	+					+	+	+		$+^{g}$	$+^{2}$		+	+	**6**	**N**
McDougall et al., 2007b	**1.524**	+	+				+	+	+			$+^{3}$		+		**6**	**N**
Poutrel et al., 2008	**20**	+	+	+			+	+	+	+	$+^{i}$	+		+		**8**	**Y**
Suojala et al., 2010	**132**	+				+	+	+	+	+	+	+		+		**7**	**N**
Schukken et al., 2011	**104**	+			+		+	+	+	$+^{t}$		+		+	+	**8**	**Y**
Schukken et al., 2013	**296**	+					+	+	+	$+^{t}$	+	$+^{2}$		+	+	**7**	**I**
Vasquez et al., 2016	**596**	+					+	+	+	$+^{t}$		$+^{2}$		+	+	**7**	**Y**
Rossi et al., 2019	**226**	+			+		+		+		+	+		+	+	**6**	**Y**

+ = specific procedure was performed; empty field = procedure was not performed; * = cross-over study; $+^{i}$ = induced infection; $+^{g}$ = only gram-positive bacteria considered; $+^{t}$ = trained in antibiotic treatment and examination; $+^{number}$ = number of different treatments groups with, e.g., comparing different antibiotics, different treatment durations, application routes etc.
Y = yes, N = no, I = inconclusive (e.g., efficacy only in certain pathogens or only in younger cows etc.)

Efficacy of homeopathic treatments for mastitis

Twelve studies published between 1989 and 2018 on the efficacy of homeopathy for the treatment of mastitis were found to match all criteria (see Table 9). Sample size ranged between 31 and 300 cases with a well-balanced allocation of sizes between experimental and control groups (apart from two studies with no control group at all). Nine of the 12 studies randomized the cases to different groups. An antibiotic control was chosen in eight studies (three of these included an additional placebo control), two studies compared the experimental group with a placebo control, but no study included an untreated control. Only half of the studies blinded the treatment to farmers, veterinarians or others involved to reduce detection and performance bias.

With the exception of the three older studies (1989, 1990, 1995), all the studies defined clear inclusion and exclusion criteria. The effect of the treatment or remedy was measured by Aubry et al. (2013) using only clinical symptoms and by Egan (1995) and Kiarazm et al. (2011) using only cyto-bacteriological milk samples, while the remaining nine studies included both clinical and bacteriological examinations.

Expertise in homeopathy was mentioned in half of the studies and seven of the 12 studies performed an individualized homeopathic treatment procedure choosing the remedy according to the individual symptom picture of the treated cow as required by homeopathic experts for a successful treatment. The remaining five studies tested specific homeopathic remedies, four a combination of different homeopathic remedies (so-called complex remedies) and Kiarazm et al. (2011) a combination of two nosodes (a solution of *staphylococcus aureus* and *streptococcus dysgalactiae* in unknown dilution).

Statistical analyses were performed in nine of the 12 studies, all of which were published between 2004 and 2018, but the risk of bias was only discussed in three studies (Williamson et al., 2014; Ebert et al., 2017; Keller et al., 2018).

A study on homeopathic efficacy could attain a maximum of 11 quality points: randomization (1), blinding (1), placebo or untreated control (1), antibiotic control (1), definition of clear inclusion/exclusion criteria (1), performance of clinical (1) and cyto-bacteriological examinations (1), expertise (1), performance of an individualized treatment procedure (1), statistical analysis (1) and a discussion of risk of bias (1).

Egan (1995) had the lowest-quality study design with only 1 point, followed by Aubry et al. (2013) with 3 points. Medium-quality designs included Tiefenthaler (1990) with 5 points and four studies with 6 points. The remaining five studies fulfilled the most criteria for a well-designed RCT, with two studies obtaining 9 points, two studies obtaining 10 points and the highest-quality study with the full 11 points (Keller et al., 2018).

Overall, five studies showed efficacy of the homeopathic treatment, while the remaining seven did not. Nevertheless, only one (Werner et al., 2010) of the five studies with a higher scientific quality (9 to 11 points) found evidence for efficacy of the applied homeopathic treatment.

Table 9: Study design of scientific trials on homeopathy used to treat mastitis during lactation (n=12)

Study	Sample size	Study design						Measure of cure		Expertise in homeopathy	Individualized homeopathic treatment	Test of a specific remedy	Statistical analysis	Risk of bias	Quality (x out of 11)	Efficacy
		Randomization	Blinded	Placebo control	Untreated control	Antibiotic control	Definition of incl./ exclusion criteria	Clinical signs/indirect tests	Cyto-bacteriological tests							
Merck et al., 1989	**100**	+				+		+	+	+	+				**6**	**Y**
Tiefenthaler, 1990	**41**					$+^{2}$		+	+	+	+				**5**	**N**
Egan, 1995	**57**								+			+			**1**	**N**
Hektoen et al., 2004	**57**	+	+	+		+	+	+	+	+	+		+		**10**	**N**
Varshney et al., 2005	**192**	+	+			+	+	+				+	+		**6**	**Y**
Walkenhorst, 2006	**192**	+				+	+	+	+			+	+		**6**	**N**
Werner et al., 2010	**147**	+	+	+		+	+	+	+	+	+		+		**10**	**Y**
Kiarazm et al., 2011	**300**	+	+	+			+		+			+	+		**6**	**Y**
Aubry et al., 2013	**31**						+	+				+	+		**3**	**Y**
Williamson et al., 2014	**227**	+				+	+	+	+	+	+		+	+	**9**	**N**
Ebert et al., 2017	**162**	+	+	+			+	+	+		+		+	+	**9**	**N**
Keller et al., 2018	**180**	+	+	+		+	+	+	+	+	+	+	+	+	**11**	**N**

+ = specific procedure was performed; empty field = procedure was not performed; (number) = number of animals involved in the treatment group; $+^{2}$ = one control of antibiotic treatment and one control with combined treatment of antibiotic and homeopathic treatment; Y = yes, N = no

Comparison of both treatments

In the case of both treatment strategies, no replication or overlap of studies in the condition studied and no comparability of remedies and treatment protocols were found. In general, the study quality of all 29 studies was found to be low and only sufficient (10–11 points) in three trials that performed the required spectrum of an RCT for the treatment method.

Antibiotic treatments showed efficacy in 47% of the studies (eight of 17), but scientific quality in general was very low (maximum 8 of 11 points). No trial achieved the full spectrum required for an optimum study design due to a lack of blinding (82%), insufficient control treatments apart from a comparison with a standard treatment (59%) and no discussion of risk of bias (65%). Even more alarming was the absence of susceptibility testing (88%) of the pathogen and no mention of expertise in antibiotic treatments (65%).

Studies on homeopathy provided efficacy in 42% of the studies and had a substantially better quality, with three studies reaching 10 or 11 points by performing a blinded RCT with individualised homeopathic treatments compared to two control groups (placebo and antibiotic), having the necessary expertise and performing a full assessment of the cure. However, only one of the higher-quality five studies (9 to 11 points) could provide evidence of the applied homeopathic treatment. The remaining seven studies (1 to 6 points) had shortcomings due to a lack of blinding, not considering risk of bias or not considering the requirements of individualized treatment procedures. The main drawback was the lack of expertise in homeopathy in half of the studies, particularly regarding the importance of selecting the correct homeopathic remedy that would determine the success of the treatment according to the principles of homeopathy.

2.4 Discussion

To enable a clear statement on the efficacy of any treatment, studies must include a good scientific study design, meet all the points characterizing the presence or absence of the specific disease (*e.g.*, determination of measures defining the success of mastitis treatment as defined by dairy federations (DVG, 2012; IDF, 2022)) and the treatment method (*e.g.*, using standard protocols for efficacy studies considering different kind of treatments), and be carried out under comparable conditions within a double-blinded RCT.

Studies with a sufficient scientific study design when dealing with the treatment of mastitis were found to be rare within the research and similar results were found for veterinary medicine (Mathie et al., 2014a; Doehring et al., 2016) and human medicine (Mathie et al., 2014b) looking at different diseases treated using homeopathy, and are criticized in the research in general (Mhaskar et al., 2012; Ioannidis, 2014). Major weaknesses found in our study were a lack of blinding, lack of appropriate control groups and an insufficient application of the current gold standard for the selection of remedy (i.e., antibiogram for antibiotic treatments or defining an individual symptom picture for homeopathy), including the requirement for expertise in both treatments. This suggests that the authors, reviewers and editors accord these little importance (Macleod, 2011), although some renowned international journals require statements on study reporting (i.e. CONSORT or REFLECT) in order to be considered for publication.

Studies with the best study design were found solely among the studies on homeopathy but failed to provide evidence for efficacy. The reasons for the higher-quality design compared to trials on antibiotic efficacy may be a result of specific reporting guidelines for basic homeopathic research (REHBAR) (Stock-Schröer et al., 2009; Stock-Schröer et al., 2011), or in defence of the unknown mode of action and a common criticism of homeopathy having no efficacy at all or the same as a placebo (Linde et al., 1997; Shang et al., 2005).

On the contrary, studies on the efficacy of antibiotics had substantially lower quality in study design, but the majority found improved results in comparison to the control treatment. A poor study design implies that the results have only limited evidential significance and a risk of high false-positive rates.

In view of the large number of antibiotic remedies available for bovine mastitis (which means they would have already fulfilled the specific requirements for licensing and approval, including studies on efficacy

(Tollefson et al., 2013)), the number of only 19 peer-reviewed papers on efficacy in bovine mastitis published appears to be small. Additionally, European guidelines requiring the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (European Medicines Agency, 2016) that complement the guideline for the efficacy of intramammary products for use in cattle (EMEA, 2003) ensure the remedy is able to cure the pathogen with respect to animal health and welfare, but ultimately also for consumer protection, environmental impact and the safety of products of animal origin. It is unknown why the majority of these approval studies were not published or made otherwise accessible at the request of the authors. It might be due to, among other reasons, the result of publication biases caused by sponsors'/investigators' tendencies to delay or not submit study results or the motivation to publish only positive results (Lee et al., 2008). Thus, access to information regarding efficacy is biased, making it difficult or even impossible for a veterinary practitioner to form a balanced opinion on a specific remedy.

For homeopathic remedies, a simplified approval procedure without proof of therapeutic efficacy (compared to the official approval required for the registration of conventional remedies) is possible. This takes into account the particular characteristics of homeopathic products, such as the very low level of active substances and the difficulty of applying them to conventional statistical methods (2004/28/EC (20)), but otherwise impedes efforts to obtain valid information on the efficacy of a remedy and might lead to the deterioration of animal health/welfare in cases of insufficient treatment success.

Approval studies for all remedies (including homeopathy) should fulfill all the requirements of a well-designed scientific RCT with sufficient study size and control groups and should be accessible to the public, allowing the replication of trials as well as the performance of reviews and meta-analyses on scientific questions to apply the principles of evidence-based medicine.

Comparing the experimental group with a control group should answer the question of whether the new treatment or medication is similar or more effective compared to the standard treatment and when compared to a placebo or an untreated control group if there is an effect of the tested remedy at all. A non-inferiority trial design is acceptable when there is a licensed treatment that is known to be superior to a placebo and no-treatment from existing data from a double-blind RCT (European Medicines Agency, 2018). The comparison of a new treatment solely with a standard treatment (as in seven studies on antibiotics and five studies on homeopathy) should be seen as unacceptable because it is unknown how effective the standard treatment really is when no publication or monitoring of effectiveness is available. The absence of an untreated control group or a placebo control may be due to the reluctance of farmers/veterinarians to participate in trials with negative controls (Schukken et al., 1995). Second, the non-treatment of an animal with clinical mastitis may not be considered acceptable from an animal welfare perspective and could be rejected by animal welfare committees approving the research protocol.

The 12 studies on homeopathy had sample sizes between 31 and 300 cases, with no study including an untreated control and five studies having a placebo control, but only four of these including enough cases for determining equivalence between the treatments. For statistical significance (95%) and power (80%), at least 120 to 300 cases are necessary when lacking a negative control according to Ruegg (2010). Studies on antibiotic efficacy mostly compared an antibiotic treatment with a different antibiotic treatment or different administration/duration of the same antibiotic (12 of 17) and reached a sufficient number of cases (with one exception by Poutrel et al.,2008), also without an untreated or placebo control. The reasons for choosing an "underpowered" study could be due to cost pressure or approval-related problems reducing the number of animals, but results in studies that are too small to detect a significant effect (Macleod, 2011).

Expertise of the person examining und treating the diseased animals was only available in 50% of the trials for homeopathy and 35% of the trials for antibiotics. It is unknown whether the expertise is simply not mentioned or if it is, in fact, lacking for homeopathy in farming practice, where most farmers uses homeopathy with no or only limited knowledge (Keller et al., 2019) and most veterinarians have no or little experience in the use of alternative therapies like homeopathy (Sorge et al., 2019).

Only three of the 17 studies on antibiotic efficacy performed a susceptibility test of the pathogen found, although the resistance pattern of a pathogen determines the efficacy of the chosen antibiotic substance. Additionally, resistance pattern changes by spontaneous mutation or gene transfer (horizontal or by plasmids) mean that trials with the same bacteria-antibiotic combination might result in different outcomes. Three of the 17 studies induced the udder infection with a specific pathogen, meaning that in these cases the resistance patterns may be known (but were not mentioned).

The risk of bias was discussed only in younger studies from 2014 onwards for homeopathy and from 1999 on for antibiotics, although the criteria were discussed earlier, even for studies on mastitis, *e.g.,* by Schukken and Deluyker (1995) or in general in veterinary clinical trials as early as 1989 (Ribble, 1989).

Due to different requirements for each treatment method and blinding hampered by different administration routines and frequencies of the remedies, the proof of efficacy of homeopathy and antibiotics within one trial considering an optimum study design is difficult. Nevertheless, a common standard for a transparent, reproducible blinded RCT can be applied to all treatments when validating efficacy. In this case, results of good-quality, individual RCTs should be replicated in accordance with evidence-based medicine, even though the current system does not reward replication by investigators and publishers, claiming that studies should be highly novel and significant in their results (Ioannidis, 2014).

Although the studies evaluated on homeopathy had, in general, better scientific quality than the studies on antibiotic efficacy, and three of these had a sufficient study design, only one was able to show evidence for efficacy for the use of an individualized homeopathic treatment. This result questions the use of homeopathy in practice where, even under optimum conditions within a scientific trial, evidence of efficacy could not be convincingly demonstrated.

In studies on the efficacy of antibiotic treatments, 11 of the 17 studies showed efficacy of the antibiotic tested, but none of the studies had sufficient scientific quality, meaning the results cannot be considered valid, although efficacy was claimed. Thus, the extensive use of antibiotics in bovine mastitis is questionable as the internal validity of scientific trials for antibiotic efficacy is not provided from a scientific perspective because efficacy cannot be fully assessed with the studies available.

Ultimately, proof of effectiveness is not only dependent on the quality of scientific studies within a controlled environment, but also on the quality of the *lege artis* use under field conditions on each farm, including follow-ups of each treatment, to improve treatment strategies at the herd and cow level.

Contrary to the conditions of an RCT, “real-world” evidence based on real data outside a trial, *e.g.*, from electronic medical records taken regularly on the farm are less credible compared to RCTs (Kim et al., 2018). Nevertheless, it is important to know that RCTs cannot represent the entire population as they only focus on a selected group (due to inclusion/exclusion criteria) and a specific scenario versus diverse actual scenarios in practice. Also, the duration of trials is limited and can only display short-term effects, whereas long-term effects are usually not described. Thus, it is crucial that accessible medical data and the monitoring of treatments and their success is available to supplement our knowledge on the efficacy of treatments for proper evidence-based veterinary medicine. Providing these monitoring data, *e.g.*, to an animal health service, would allow the formulation of individual and general treatment recommendations or treatment guidelines for more successful treatments.

Currently, effectiveness in farming practice is neither regularly reviewed nor monitored via national programs or private organizations and it is doubtful whether current use is reasonable or responsible. It can be presumed that current treatments could be improved by the monitoring and analysis of treatments and that there is potential for reducing antibiotic use.

Monitoring treatments without including a cure control will not lead to improvements as the prevalence rates of clinical mastitis seem to show no difference between countries with disease recording systems, *e.g.,* Denmark (36–48 cases/100 cow years), Norway (21.3 cases/100 cow years), Sweden (22.6 cases/100 cow years), Finland (17 cases/100 cow years), and without national recording systems, *e.g.,* in Canada (23 cases/100 cow years), France (20.1 cases/100 cow years), England and Wales (47 cases/100 cow years) and the Netherlands (25.2–27.8 cases/100 cow years) (Wolff et al., 2012).

Estimates of the economic impact and costs of mastitis (Lam et al., 2009; van Soest et al., 2016) or comparison with other farms (benchmarking of disease prevalence rates) can be used to motivate farmers and prioritize actions.

2.5 Conclusions

Although 29 studies on the efficacy of antibiotic or homeopathic treatments in bovine mastitis could be found, most of these were of poor quality and only three studies had a good scientific study design. According to the studies evaluated, the use of homeopathy on farms cannot be promoted, when, even under excellent controlled study conditions involving expertise and individualized homeopathic treatment procedures, they fail to show repeatable efficacy and when effectiveness under real-life conditions can expected to be even worse. Studies on efficacy of antibiotics require substantial improvements in study quality to justify the extensive use of antibiotics in farming practice. This should be accompanied by a regular control of the effectiveness of treatments on farms considering that antibiotics are currently the main and first line option in mastitis treatment.

To ensure the effectiveness and ongoing improvement of treatments on farms, all treatments applied should be monitored and evaluated on a regular basis. Financial and legislative support by national governments striving for animal health and welfare, safer products and environmental protection (“One Health”) could help with trade-offs regarding time and costs, as these efforts by the farmer alone do not always have direct or automatic financial rewards.

If the comprehensive and continued evaluation of all treatments and their outcomes is not performed, in the near future antibiotic resistance, or the proportion of bacteria resistant to one or more antibiotics, could intensify through overconsumption and inappropriate use where the chosen antibiotic agent is not or no longer effective. This will not only lead to the unnecessary suffering of animals but also to more untreatable diseases caused by multi-resistant bacteria in humans (“One Health” aspect), especially as we share some pathogens (zoonosis, anthropozoonosis), eat animal products and live with animals, thus increasing the possibility of the transfer of resistant genes between bacteria.

3. The informative value of an overview on antibiotic consumption, treatment efficacy and cost of clinical mastitis at farm level (paper III)

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Abstract

This paper addresses whether it is worthwhile investing time in a more comprehensive documentation and analysis of farm specific data for mastitis treatment. Whilst most farmers keep some records, many refrain from investing more effort in making them more detailed. Therefore, information on farm-specific antibiotic consumption, effectiveness of treatments and the costs of mastitis are lacking; as well as the ability to identify and realise possibilities for improvement. An observational study was conducted on 30 dairy farms, to obtain an overview of farming practice, recording detailed data (using herd management software) on: milk records, diagnostics, preventive and therapeutic treatments and cost of mastitis, on an individual cow level. Out of a total of 30,633 cows, 67% received medication for the treatment or prevention of mastitis over a year. Antibiotics were chosen for 96% of udder treatments, including those for dry cows. Over 32 % of the antibiotics used during lactation belonged to the 'highest priority critically important antimicrobial' category. Success of therapeutic treatment (assessed by individual somatic cell count (SCC)) ranged from 18% to 59% and total costs of mastitis per cow and year from € 158 to € 483. The high variations in antibiotic consumption, treatment outcomes and cost of mastitis between farms, showed that average or incomplete figures risk giving the wrong impression of a farm.

It is thus worthwhile to invest in documentation and analysis of data, so that it is clear where action is required and which investments can be expected to be financially feasible. Benefits emerge from knowledge of antibiotic consumption allowing monitoring and reduction in antibiotic use (as demanded by consumers and legislation), improvements in animal health and welfare thanks to regular checks of how effective a treatment is, and economic benefits due to knowledge of the costs caused by mastitis.

Keywords: *mastitis, treatment, antibiotic, record keeping, cost, systemic analysis.*

3.1 Introduction

Consumers are increasingly concerned about the high consumption of antibiotics in food producing animals and the associated risks of singling out and spreading multi-resistant bacteria. Sales figures of antimicrobials to veterinarians in 25 European countries between 2010 and 2015 revealed an increase in the use of last-resort antibiotics or 'highest priority critically important antimicrobials (HPCIA)' (few antibiotic classes withheld and reserved for severe infections with multi-resistant bacteria) while typical 'first choice' antibiotics decreased consistently (European Medicines Agency, 2017).

In many European countries national initiatives have been established to monitor antimicrobial resistance and consumption using sales figures for antibiotics purchased for veterinary use in kg, i.e. DANMAP (Denmark), GERMAP (Germany), MARAN (Netherlands), NORM-VET (Norway) or SVARM (Sweden). Additionally, the monitoring of antibiotic consumption is harmonized on European level by the EMA and published in the ESVAC report (European Surveillance of veterinary consumption) every year (European Medicines Agency, 2017). In general, these figures cannot be connected to species, indication, number of animals treated or duration of antibiotic treatment. The drug is usually chosen based on general recommendations or experience from previous cases, whereas the pathogen responsible and its resistance pattern are often left to chance (Griffioen et al., 2016). Taking diagnostic samples is seldom regarded as worthwhile as it creates extra-costs and work while the results are not available immediately. Thus, not only is the pathogen responsible often unknown but also the probability of a successful treatment outcome where appropriate choice of an antibiotic substance is concerned and whether the animal will recover in reasonable time often remains unclear.

Mastitis is the most common cause of antibiotic use in dairy production and HPCIAs are used for 22% of all mastitis treatments in Europe (Briyne et al., 2014), increasing the risk of antimicrobial residues and influencing the rise and creation of multi-resistant foodborne pathogens. Multi-resistant bacteria like *methicillin-resistant Staphylococcus aureus* (MRSA) (Spohr et al., 2011; Paterson et al., 2013), *extended-spectrum β-lactamase* (ESBL)-*producing bacteria* (Geser et al., 2012; Randall et al., 2014) and *methicillin-resistant coagulase-negative Staphylococci* (MR-CNS) (Huber et al., 2011) were found in milk samples across Europe resulting in general effort to improve efficacy of treatment and animal health to reduce the need for antimicrobial consumption.

In various scientific studies on clinical mastitis, bacteriological cure rates displayed a wide range, i.e. between 4% and 92% for *Staphylococcus aureus*, greatly depending on the pathogen responsible (Barkema et al., 2006; Mansion-de Vries et al., 2015). In a study by Wilson et al. (1999) on subclinical mastitis comparing seven antibiotic treatments with no treatment about 65% recovered spontaneously (without any treatment) compared to a 75% cure rate with antibiotic treatment, while for 3 out of 7 antibiotic substances tested, the results did not differ from the untreated bacteriological cure rate. Unsuccessful treatment also affects farmer's income. Farmers, their veterinarians and advisors have a considerable tendency to underestimate the actual costs of mastitis (van Asseldonk et al., 2010; Jones et al., 2016). Costs associated with mastitis can be separated into failure costs (*e.g.,* cost of drugs, veterinary service, loss of milk revenue, replacement of culled cows, extra labour) and preventive costs (*e.g.,* hygiene measures). The total cost of mastitis per cow per year may vary from 17 to 261 EUR between farms (van Soest et al., 2016). Although many farmers are well aware of substantial economic losses due to mastitis on their farm, changes in behaviour (*e.g.,* adopting and implementing new measures) may be difficult to achieve. A belief in the efficacy of the new measure is required and the farmer should feel more confident about managing the situation. Some control measures are perceived as too expensive or difficult to implement and that is why a comparison of farm-specific costs of different measures and their expected benefit help the farmer to take action (van Asseldonk et al., 2010).

In face of increasing consumer concerns regarding (on the one hand) the issues of animal health and welfare and the use of antibiotics and the economic pressure on production costs (on the other); there is an urgent need to improve the efficiency of preventive and therapeutic measures. Such improvements require a general overview of the current situation (especially in relation to production diseases) and knowledge of the general conditions (potentials and limitations, costs, milk revenues and penalties, etc.) under which the specific farm operates (Sundrum et al., 2016). Comprehensive records are required to determine the current state. Most dairy farmers keep some level of records connected to their cows, *e.g.*, individual ID and birth dates of cows and culling records (required by EC regulation No. 1760/2000 for identification and registration of bovine animals), records on medical treatments (often required by national law) or milking records (voluntarily), but few collect ‘systemic farm data’, meaning the documentation of sufficient information on all farm issues which influence the farm as a system with interrelated or interacting elements. In a study of 110 Canadian farms, less than 41% employed detailed record keeping for data on health (Manglai, 2016). Despite new ‘precision dairy farming’ technologies (with automated systems collecting sensor-derived data from the individual cow), the information garnered from this data is not often utilised fully by the farmers. Further analyses are often restricted to specific areas and not amalgamated to create a comprehensive picture of the farm situation.

Collection and analysis of systemic data is time consuming. On the other hand, gaining an overview based only on subjective memory on number of cases and average treatment costs is unlikely to result in accurate estimations. The aim of this study was to link farm-specific information, gained from herd management software and the farmer in order to put together a systemic analysis. The overall aim was to assess the value of record keeping and a systemic analysis (enabling the major shortcomings to be identified) for mastitis and to ascertain the money available by reducing mastitis incidence to improve the situation on the specific farm.

3.2 Material and methods

Dairy farms using the herd management software HERDE® dsp-agrosoft were invited to participate voluntarily with a flyer presenting the aims of the study: 30 dairy farms located in mid- and northern Germany agreed to fully share their data. Four farms had to be excluded in hindsight due to incomplete documentation. A software-data backup of each farm including:

1. individual IDs,
2. milk records (milk yield, SCC, milk fat / protein / urea on cow level),
3. detailed medication protocols (daily records of type, amount and frequency of each drug administered) for each cow,
4. culling and mortalities (including their causes and had to be reported to authorities as a legal requirement in Germany) and
5. results of diagnostic milk samples (pathogen found)

was the basis for the evaluation. Additional data was provided by the farmers, who replied to a questionnaire asking for labour costs per hour, average costs per case of veterinarian visits (excluding drugs), milk prices, costs of a diagnostic milk-sample including antibiogram.

The time frame covering all farms was March 1, 2013 to February 28, 2014. Data were extracted from the herd software data backups and analysed by the same person with the help of Excel 2010/ Access 2010 (Microsoft®), Python 3.5.2 (Python Software Foundation®) and SPSS Statistics 24.0 (IBM®) in the form of a retrospective study to prevent any external influence. The following details were assessed for each farm separately: number of dairy cows kept, average milk yield per 305 days, average SCC of the herd and individual SCC of each cow/month, frequency and average duration of mastitis treatment per cow treated, frequency and type of drug administered as connected to the cow treated, price and withdrawal time for each drug, number of cases treated, number of cows culled and deaths caused by mastitis, number of diagnostic milk samples taken within the given time period and individual costs for labour/hour, veterinarian per case, costs per milk sample and the milk price received.

Treatments

All medicinal preparations used to treat mastitis and documented by the farmer under the diagnosis "mastitis" (without further definition) within the herd management software were assigned to the following groups: antibiotics (used during lactation or dry period), teat sealants, anti-inflammatory drugs, homeopathy, plant-based remedies or complementary/supportive therapies. Drugs not applied for the treatment of mastitis in first line were excluded, i.e. diuretics, remedies stimulating milk flow, appetite promotors, feed additives etc.

Antibiotic consumption

Both the number of cows receiving antibiotic treatment during lactation or dry period and the number of different antibiotic substances used were assessed. The different antibiotic substances were grouped according to their antibiotic classes. Cephalosporins (3^{th} and 4^{th} generation), fluoroquinolones and macrolides were classified as 'highest priority critically important antimicrobials' as defined by the WHO (2016) for human medicine meeting 3 priorities: 1. The antimicrobial class is the sole, or one of limited available alternatives to treat serious infections with a high absolute number of people affected, 2. …has a high frequency of use for any indication, 3. …is used to treat infections in people for which there is evidence of transmission of resistant bacteria or resistance genes from non-human sources. Furthermore, the number of daily doses (nDD_{ay}) for antibiotic treatment during lactation were quantified, indicating the number of days per year that an average cow in a herd is given an antibiotic treatment (according to Hemme et al., 2016). It was calculated using the total sum of daily doses of each antibiotic substance administered during lactation, divided by the average number of cows present on the farm within the timeframe of one year. Drugs containing multiple antibiotics were divided into separate substances for the calculation.

Effect of therapeutic treatment on SCC

For each mastitis case, the effect of therapeutic treatment was assessed by the SCC in the monthly milk records from 5 to 30 days after the last day of medication and cows were grouped according to their SCC (Hamann, 2003) in:

- 0 to 99,000 somatic cells/ml (likely to be undisturbed, cured).
- 100,000 to 200,000 somatic cells/ml (possible for infection, cure uncertain).
- 201,000 cells/ml and higher (high probability of infection, not cured).

A treatment episode started on the first day a drug was administered to the individual cow (diagnosed with mastitis) and continued until the last day it received a drug. When a break in treatment lasted longer than 5 days, any additional administration of a drug was seen as start of a new treatment episode. The pre-treatment SCCs of the monthly milk records were not included in the evaluation as the record occurred at different time points relative to the beginning of the mastitis episode being studied and SCC rise varies in level and speed depending on pathogen and individual cow factors.

Costs of mastitis

The monetary losses associated with mastitis were calculated separately for each farm. Contributors to these failure costs were: expenditure such as drugs, veterinary service, laboratory costs, extra labour for the farmer; and losses such as discarded milk, a decrease in milk yield due to mastitis, culling/deaths and the resultant replacement costs; and preventive costs involved in treatment for dry cows.

Farm specific data were available for: the average 305day-milk yield on the farm by milk records, wage per hour (modelled on an average 15 minutes per treatment day for each case requiring extra labour) given by the farmer, the average treatment duration calculated from all mastitis treatments during lactation, the average withdrawal time for the drugs used for mastitis treatment during lactation, the cost per mastitis case of veterinary and laboratory service (as indicated by the farmer or laboratory) combined with the number of milk samples taken. The price of each specific drug used on a farm (selling price of veterinarians as recommended by the pharmaceutical companies) was calculated according to the product information, in terms of the daily dose advised for a cow with 600kg body weight. The expected reduction in 305d-milk yield was calculated as 5% per case acc. to Seegers et al. (2003). The milk price was set as 38 cent/kg according to the average price received by conventional farms at that time. Assessing the replacement costs caused by mastitis involved taking the average sum paid for a slaughtered cow as € 500 per cow (excluding cows predeceased or euthanized) and deducted from the cost of a replacement heifer with an average market price of € 1500 for the time in question.

Calculation formula

$$Total\ cost\ of\ mastitis = c_W + c_r + c_{vet} + l_{prod} + l_{dis} + c_{dia} + c_{dry} + c_{new}$$

$$c_w = cost\ of\ extra\ work = wage/hour \times\ 0{,}25h/case \times average\ treatments\ days \times number\ of\ mastitis\ cases$$

$$c_r = cost\ of\ drugs = price\ of\ all\ drugs\ used\ during\ lactation\ in\ a\ dose\ for\ a\ 60kg\ cow$$

$$c_{vet} = cost\ of\ veterinarian = cost\ of\ vet/case \times number\ of\ cases$$

$$l_{prod} = losses\ of\ reduced\ production = (average\ 305d\ milk\ yield \times 5\%) \times numberof\ cases \times milk\ price/kg$$

$$l_{dis} = losses\ from\ discarded\ milk = (avarage\ days\ of\ treatment + average\ days\ of\ withdrawal) \times number\ of\ cases \times average\ daily\ milk\ yield (based\ on\ average\ 305d\ milk\ yield) \times milk\ price/kg$$

$$c_{dia} = cost\ of\ diagnostics = price/milk\ sample \times number\ of\ samples\ taken$$

$$c_{dry} = cost\ of\ dry\ cow\ treatment = price\ of\ all\ drugs\ used$$

$$c_{new} = cost\ of\ replacement = number\ of\ slaughtered\ cows \times €1000 + number\ of\ predeceased\ cows \times €1500$$

In order to factor in different farm sizes, the total costs of mastitis were calculated for the average cow within a herd (divided by the number of cows kept), per case treated (divided by the number of cases) and per animal treated (divided by the number of cows treated) as cows often developed several cases of mastitis in the same lactation period. Finally, the calculation of the cost due to mastitis per case treated (excluding cost of replacements and dry cow treatment) was used to calculate how much money for investment (in total and per cow) in udder health could be made available by decreasing clinical mastitis cases by only 5% on each farm. Management measures have shown to decrease the incidence of clinical mastitis from 3% (*e.g.,* milkers wearing gloves) to 37% (*e.g.,* post-milking teat disinfection) in a contagious situation (Huijps et al., 2010).

Statistical analysis

Data are presented as arithmetic mean values (+/- SEM). Pearson correlation was conducted for the herd size, the average milk yield and the treatment incidence (percentage of cows treated for mastitis within one year) as well as for the average SCC of the herd (based on monthly individual SCC of all cows) and the treatment outcome and also for herd size and the cost per cow/per treated cow in order to illustrate possible correlations.

The intention of data evaluation was to gain an overview of: 1. the actual consumption of antimicrobials and whether there were opportunities to reduce usage without risking a decrease in animal health status, 2. how effective treatment strategies applied were in reality and where changes might be necessary or beneficial, 3. economic benefit by reducing the number of mastitis cases and thus which financial investments for improving animal health could be made.

3.3 Results

Farms

Study farms showed sizes ranging from 103 cows to 3560 cows (median 1118 cows, mean 1178 cows +/-156 SEM) with an average 305d-milk yield ranging between 7381kg and 11072kg (median 9059 kg, mean 9195kg +/-184 SEM). All farms were conventional (non-organic) and mainly kept the breed 'Holstein Friesian'. The average SCC of the herds for the year in question varied between 133,000 somatic cells/ml and 459,000 somatic cells/ml (median 261,000 cells/ml, mean 271.000 cells/ml +/-18,000 SEM). The percentage of cow deaths and cullings that were exclusively caused by mastitis ranged from 3% to 37% (median 22%, mean 20% +/-1.85 SEM).

Treatment of mastitis

A total of 30,633 cows were under review; of which 67% received medication for therapeutic treatment or prevention of mastitis over the course of the year. Antibiotics were the main medication choice for 65% (n = 19,794) of all cows, making up 96% of all udder treatment, including dry cow therapy. A total of 20,577 cows received 127,172 daily doses of a medical preparation over the year. 69% of the doses applied were antibiotics used during lactation and 12% were antibiotics for the dry period. Homeopathy accounted for 7% of the doses applied while teat-sealants for the dry period (6 %) and anti-inflammatory drugs (4%) were used to a small extent. Two percent used supportive preparations like enzyme-based products. Phytotherapy such as camphor ointments or garlic intramammary injectors were seldom administered (< 1%). During lactation, 37% of all cows received a therapeutic mastitis treatment, where 85% of the drugs administered were antibiotics, 8% were homeopathic remedies, 4% anti-inflammatory drugs and 3% were supportive medications. Out of the 52% cows treated during the dry period: 55% received dry-cow treatment with antibiotics only, 40% got a combination of antibiotics and teat-sealer, whilst 5% were treated solely with a teat-sealer.

Antibiotics in use

Taking all farms into account, 49% of cows received antibiotic treatment during the dry period. During lactation, an average 35% of all cows received antibiotic mastitis therapy, ranging from 13% to 53% between farms. On average, each cow was treated for 3.7 days (+/-0.36 SEM, lowest nDD_{ay} 0.7 to highest nDD_{ay} 8.7 days) with an antibiotic substance during lactation in the study period. Over 32% of the chosen antibiotic substances belonged to the group of 'highest priority critically important antimicrobials' (see Table 10).

*Table 10: Distribution of classes of antibiotic used for treatment of mastitis during lactation in German dairy herds (*defined as 'Highest Priority Critically Important Antimicrobials' by the WHO (2016))*

Antibiotic Classes	Antibiotic Agents	Distribution of Antibiotic Use
Aminoglycosides	Gentamycin Kanamycin Neomycin Streptomycin	**11%** (12,613 doses)
Cephalosporins 1. Generation	Cefacetrile Cefalexin Cefazolin	**11%** (12,245 doses)
Cephalosporins 3. Generation*	Cefoperazone Ceftiofur	**6%** (6,372 doses)
Cephalosporins 4. Generation*	Cefquinome	**20%** (23,628 doses)
Fluoroquinolones*	Danofloxacin Enrofloxacin Marbofloxacin	**6%** (6,523 doses)
Macrolides*	Erythromycin Tylosin	**< 1%** (107 doses)
Lincosamides	Lincomycin Pirlimycin	**1%** (1,319 doses)
Penicillins	Amoxicillin Ampicillin Benzylpenicillin Cloxacillin Oxacillin Penethamate Hydroiodide	**44%** (50,698 doses)
Pyrimidines	Trimethoprim	**1%** (1.681 doses)
Sulfonamides	Sulfadimidin Sulfadoxin	**< 1%** (795 doses)
Tetracyclines	Oxytetracyline	**< 1%** (42 doses)

Individual farms administered different pharmaceutical preparations (enclosing injectable solutions and intramammary suspensions) adding up from 5 to 17 different antibiotic agents within the same mastitis treatment episode during lactation. Out of 116,023 single antibiotic doses, penicillins (at 44%) were the first choice for mastitis treatment. The last resort antibiotic, 4th generation cephalosporins, were the second favourite choice at 20%, followed by aminoglycosides (11%) and 1st generation cephalosporins (11%). Third generation cephalosporins and fluoroquinolones were used in 6% of treatments while the remaining antibiotics were only rarely used (around 1%). An average of 33% of cows treated with antibiotics had milk samples taken in order to detect pathogens and their resistance patterns before conducting dry cow or mastitis treatment. On nine farms, farmers did not sample and assess milk as they felt they were aware of the common pathogens in their herd. Other farmers assessed up to 91% of their herd via cyto-bacteriological milk samples within a year.

In total, 19,843 treatment episodes were conducted during lactation and administered to 11,311 cows. The incidence of mastitis measured by treatment episodes was 0.63 (+/- 0.05 SEM, min. 0.26; max. 1.03) per cow and year on average. Correspondingly, each cow treated for mastitis received 1.67 treatments (+/- 0.07 SEM, ranging from 1.24 to 2.09) per year on average, indicating a higher risk of mastitis recurrence. The average duration of treatment per farm was between 1.19 days to 7.48 days (mean 4.14 +/- 0.27 SEM) per case.

Effect of treatment on SCC

For 12,125 of the 19,843 treatments (61.1 %), a further evaluation of the treatment effect during lactation based on the SCC values of the milk recordings (between day 5 and 30 after the end of treatment) was possible. Again, a large disparity between the farms was obvious (see Figure 5).

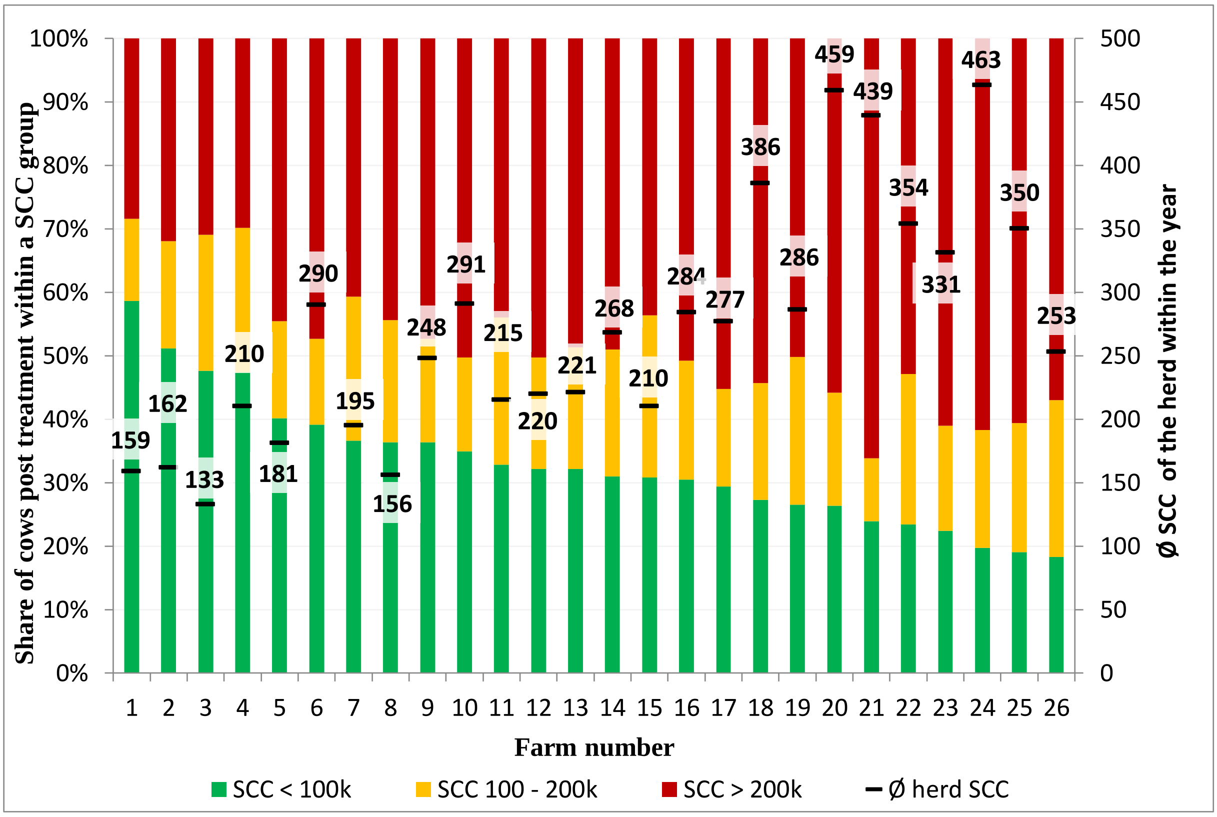

*Figure 5: Distribution of classes of antibiotic used for treatment of mastitis during lactation in German dairy herds (*defined as 'Highest Priority Critically Important Antimicrobials' by the WHO (2016))*

After treatment, cows with a SCC below 100,000 cells/ml milk (indicating a healthy udder) ranged from 18% to 59% between farms, while the group of cows with an SCC greater than 200,000 cells (indicating persistent udder infection) the range was 28% to 66%. When the average SCC value of the whole herd was low (< 200,000 cells/ml milk), cows predominantly showed a positive treatment effect with a bigger proportion of cows below 100,000 cells/ml after treatment, while this was vice versa for herds with an average high SCC (>300,000 cells/ml) within the herd. The higher the average yearly SCC in a herd was, the lower was significantly the number of cows below 100,000 cells/ml after treatment (Pearson correlation p=0.01, r=-

0.73) whereas the number of cows above 200,000 cells/ml increased (r=0.82). Farms with an average herd SCC above 300,000 cells/ml had considerably fewer cows with a SCC below 100,000 cells/ml after treatment and more than 50% of the cows were above 200,000 cells/ml once treatment was completed.

No significant correlation was present between herd size and treatment incidence (p=0.5; r=0.14) nor between the average 305-days milk yield and the treatment incidence (p=0.42; r=0.17).

Costs of mastitis

Total costs included the cost of every case of mastitis treated, the costs for dry cow medication and the replacement costs due to mastitis. Financial losses were displayed in three different forms:

1. The total cost per mastitis case ranged from € 323 to € 662 (mean € 474 +/- 16 SEM).

2. The total cost per cow treated ranged from € 546 to € 1012 (mean € 784 +/- 22 SEM). The average number of treatments per diseased cow were considered for each farm, as a cow treated once ran a higher risk of needing a second treatment.

3. The total cost per average cow within one herd ranged from € 158 to € 483 (mean € 289 +/- 14 SEM) for the considered year.

The highest cost factors connected with mastitis were losses in milk production (median 37%), ranging from 27% to 52% and discarded milk (median 20%), ranging from 13% to 30%. These losses were followed by replacement costs (median 22%) due to culling and deaths caused by mastitis (see Figure 6).

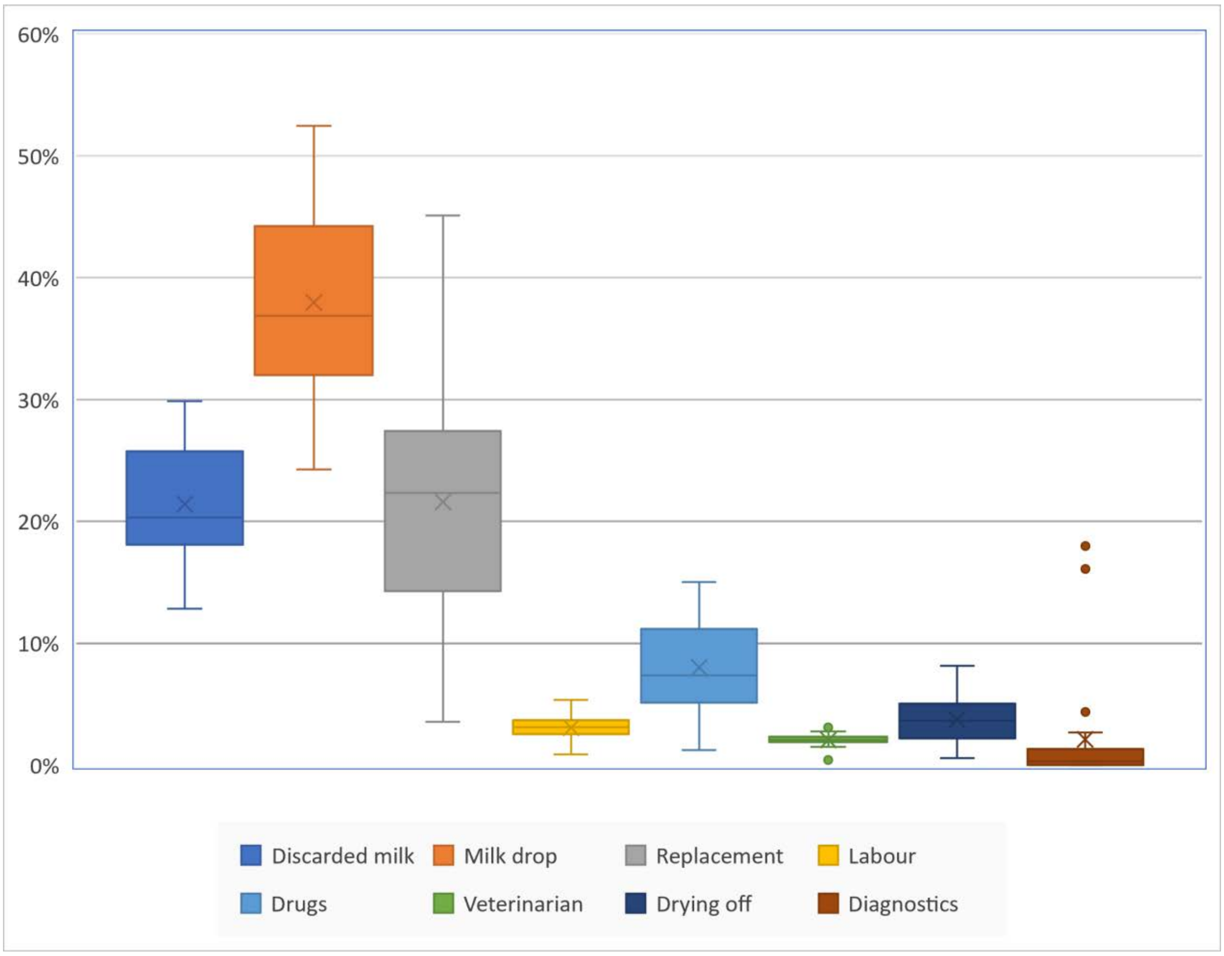

Figure 6: Percentage distribution of mastitis costs on 26 German dairy farms

A small portion of the costs was due to drug expenses (median 7%, 1% - 15%) and diagnostic milk samples (rated between 0% and 18%). The smallest portion of costs came from extra labour (1% - 5%, median 3%), dry cow treatment (1% - 8 %, median 4%) and the veterinary service (<1% to 3%, median 2%). The number of treatments per diseased cow (*e.g.*, those down to recurrences) was not connected to herd size (p=0.31; r=0.21). The potential amount each farm could invest was calculated for a possible scenario with a 5% decrease in mastitis cases. A total sum from € 1,268 (n =170 cows) up to € 30,058 (n=2339 cows) would be available for improvement of udder health depending on the farm, even without considering replacement and dry cow treatment. In relation to the herd size, an amount of € 5.25 (n=1413 cows) up to € 18.17 (n=3560 cows) per cow and year would be available.

3.4 Discussion

Antibiotic consumption

The study revealed that antibiotics were the main choice for treating mastitis. The portion of cows receiving antibiotic treatment during lactation (35%) reflects the incidence of clinical mastitis anticipated amongst herds in Germany (38.2%; Zoche-Golob et al., 2015) and Finland (33%;(Pitkälä et al., 2004) but was higher than the results of recent studies in Canada, at 23.7% (Levison et al., 2015) and in the Netherlands, 28.6% (Santman-Berends et al., 2016).

Considering that blanket dry-cow treatment has been so strongly promoted as a standard preventive practice over the last 20 years, the use of antibiotics during the dry period (49%) was comparably low. This low consumption of antibiotics may be due to the major push to implement selective dry cow treatment as standard practice, where only cows with a high SCC are treated, in order to reduce overall antibiotic consumption in dairy herds (Bundestierärztekammer, 2015; Scherpenzeel et al., 2018). Nevertheless, the study farms displayed even lower values than Dutch farms already implementing selective dry cow treatment, with 67% of the herd receiving antibiotic dry-cow treatment (Scherpenzeel et al., 2016). More probable however, is that during the study period a high percentage of cows did not receive antibiotic dry cow treatment because many of them were no longer on the farm due to premature culling/death (ranging from 3% to 37% between farms and due to mastitis) or a prolonged calving interval caused by fertility disorders.

Although, only one disease complex was considered, more than half of all cows (65%) received an antibiotic substance just for mastitis treatment; indicating that mastitis is one of the main causes of antibiotic use on farms. Last resort substances were used quite frequently in mastitis treatment on the study farms (> 32%) and the use clearly exceeded those found in other studies: In a study of 25 European countries, Briyne et al. (2014) revealed that an average 22% of all antibiotics which veterinarians prescribed for mastitis were HPCIAs. In Dutch dairy herds, HPCIAs were only applied for 18% of all medical disease treatments including mastitis (Kuipers et al., 2016). This may be due to different veterinary prescription customs, different frequency of administration (*e.g.,* long-acting formulations), differences in the availability of alternatives, lack of national policies to ban or restrict the use of HPCIAs *(e.g.*, as in Denmark, Netherlands). The results might also be related to a farmer's tendency to ask prescribing veterinarians for heavily advertised products associated with a shorter withdrawal period (Briyne et al., 2013; Benning, 2016). It may also be that many veterinarians and farmers are unaware which antibiotics are 'critically important', only recommended as a last resort, as there is currently no common definition of HPCIAs in veterinary medicine. Without a significant reduction in HPCIAs, it could be inevitable that these substances are forbidden for veterinary use as promoted by The European Consumer Organisation BEUC (2014). Identification of the pathogen, including susceptibility testing, followed by selecting an effective antibiotic is likely to reduce the risk of error and to increase the success rate of treatment. Costs would therefore decrease *e.g.,* via better recovery rates, reduction of overall use of antibiotics, minimizing discarded milk and a reduction in the risk of further infections (Cha et al., 2016).

Antibiotic consumption in animals is measured differently depending on the information available. Almost every European country collects sales figures for different antibiotics (in kg) to veterinary practices but differentiation of species, number of animals or diseases treated are not usually recorded. Antibiotic classes and formulations vary in their potency and pharmacokinetic properties; prescribers will thus administer a different dosage per kg body weight and treatment duration will vary. The number of daily doses (nDD_{ay}) thus appears to be a more suitable parameter than the current records of sales data in kg for active substances. The nDD_{ay} enables a more accurate comparison of antibiotic consumption between countries and farms and allows changes to be observed from year to year also within a farm. The daily dose administered may differ from the daily dose recommended by the pharmaceutical producer. However, it allows an estimation of antibiotic consumption in relation to herd size and treatment frequency.

The mean nDD_{ay} (3.7 days) exclusively for mastitis treatments during lactation identically calculated was consistent with previous data collected on 20 dairy farms in Wisconsin at 3.45 (Pol et al., 2007) but differed from results obtained on 47 dairy farms in Germany at 2.53 (Mollenhauer, 2010; Merle et al., 2012). In the latter, the number of different antibiotic substances (as found in combined drugs) was not considered separately for the calculation of the nDDay, thus underestimating the animals' true exposure to antibiotics. For all diseases and identical calculation, the nDD_{ay} was 2.75 (Merle *et al.* 2012) and 2.2 (Hemme et al., 2016) in German dairy cows and 4.2 or 5.86 in Dutch dairy cows (MARAN, 2012; Kuipers et al., 2016). This could be the result of individual treatment practices.

Although the study farms participated voluntarily, they represented a range of different farm sizes, high and low yielding dairy herds and different locations in Germany. However, the implementation of comprehensive, software-based documentation especially regarding treatments may not be the rule but the exception as we had huge difficulties to find a sufficient number of farms maintaining all records required for our evaluation. There may have been a bias regarding the representative antibiotic use on these farms because additional documentation (apart from the mandatory veterinary receipt of use and drugs dispensed) of medical treatment for each cow in a software system might sensitize farmers for their antibiotic consumption. Whether this might have led to reduced antibiotic consumption in comparison to other farms cannot be verified. The unexpectedly high consumption of antibiotics could have been due to various factors i.e. the continuous treatment documentation, an increased willingness to treat cows with higher milk yields, a high prevalence of resistant mastitis bacteria or a failure in treatment strategies or management measures.

Treatment effect

Amongst others, the probability of cure depends on individual cows (immune status, stage of lactation), causing pathogens, treatment factors (method of treatment, active agent, duration) and herd-specific factors (housing, stock density, hygiene, new-infection rate) (Degen et al. 2015). Cure rates decrease with increased age, a higher SCC count, increased duration of infection and bacterial load before treatment (Barkema et al., 2006). In contrast to randomized controlled trials, assessing 'cure' rate or the treatment outcome could only be done using the average of the 4-quarter SCC of milk records (in practice usually available) and served as an on-site indicator of the udder health situation, while the best measure of successful treatment would have been SCC and pathogens of single quarters from cyto-bacteriological results. Thus, the data are not comparable with experimental conditions where those results are available. On the other hand, the study time frame of one year was substantially longer and the selection of animals wider than in experimental studies assessing treatment outcomes on an individual cow level. By contrast, data collection over long time periods enables recurrences of mastitis to be considered. Furthermore, a high number of cases were evaluated, delivering more reliable results. The high inter-farm variations in SCC treatment outcomes may have been caused by different management practices *e.g.*, duration of treatment, appropriate choice of drug (with or without diagnostic tests) as well as by cow factors *e.g.*, immune status, teat lesions or the pathogenicity of the pathogen responsible (Barkema et al., 2006; Degen et al., 2015). The high average SCC of the herd (> 400,000 SCC/ml, not fulfilling the requirements for delivering milk to the dairies in Germany) on some farms indicated that a major share of cows suffered from mastitis.

Nevertheless, using high levels of antibiotics for mastitis treatment (with 65% of cows receiving them) which only have limited effect on udder health status is questionable - not only with regard to responsible use of antibiotics in food-producing animals, but also in relation to animal welfare issues. Treatment outcomes (here 59% below 100,000 cells/ml milk for the best farm) should certainly be higher than spontaneous recovery (around 65% without treatment according to Wilson et al. (1999)) to justify the use of antimicrobials and the inevitable spread of antimicrobial resistance in bacteria. In cases where the use of antibiotics is not beneficial, aiding recovery i.e. via anti-inflammatory drugs to prevent a diseased animals' suffering should be ensured. The poor treatment outcomes could be a result of lack of awareness about the effectiveness of a treatment and thus how much capital is therefore wrongly invested in defective strategies, as well as the restraint to invest time and money in improvement. In addition, many different antimicrobial substances were administered as part of the same treatment. This gives rise to antimicrobial resistance on farms and beyond, as well as average treatment durations of only 1.19 days, can be seen as too short as the study of Pinzon-Sanchez et al. (2011) showed, that the probability of cure significantly increased with treatment duration.

Costs of mastitis

Mastitis is the disease which causes the highest costs in dairy production (Petrovski et al., 2006), whilst the overall milk yield usually provides the dairy farm's main income. The highest economic expenses came from loss of milk and culling and were comparable with results obtained in other studies (Seegers et al., 2003; Huijps et al., 2008; Steeneveld et al., 2011; van Soest et al., 2016). While the current calculations were more closer to real farm data, data from literature is mainly based on amalgamations derived from farmer's estimations (Seegers et al., 2003; Huijps et al., 2008). Comprehensive documentation via herd management software enabled a more accurate calculation, producing resilient data on costs especially connected to the number of cases, the individual diseased cow and herd size. Large differences between farms in the economic impact of mastitis confirmed earlier findings by Halasa et al. (2007), who recommended a farm-specific economic analysis. The average costs per lactating cow per year in this study (with € 289) were higher and ranged more wider (from € 158 and € 483) than results estimated by Hogeveen et al. (2011) with € 17 to € 198 or Van Soest et al. (2016; € 240). The latter showed that the costs can be strongly affected by different scenarios, such as a low/high milk price (€ 216 resp. € 264) or low/high labour costs (€ 204 resp. € 276). For the average costs per case and year, Van Soest et al. (2016) estimated an average of € 301, while Sørensen et al. (2010) found costs from € 149 to € 570, and Heikkilä et al. (2012) reported costs of € 458 compared to this study's results of € 474. The high variation in costs between the study farms was actually due to the more detailed data set that was available for each farm i.e. some farmers invested much more effort in diagnostic milk samples than others. The study authors expected that relatively high expenditure for diagnostics would lead to better treatment outcomes and therefore lower total costs of mastitis, but this was not observed in this study in the herd as a whole, but in individual mastitis cases. The great variations in costs support the need to base each analysis strongly on farm-specific data.

As economic considerations are an important reason for farmers to implement changes on their farm, a scenario showing the amount of money to be saved by decreasing clinical cases by 5% was calculated to support the farmer's decision-making on investing money in improvement measures. The next step would be to find effective preventive measures and estimate their costs, so that they could be compared with the farm-specific investment sum per cow per year, to calculate the potential net benefit. Four farms had to be excluded due to incomplete data sets, indicating that even if data recording is in use, it is essential to keep it complete, relying on a continuous documentation for 365 days, allowing accurate calculation. Other researchers also encountered a lack of comprehensive record keeping, so that antibiotic consumption could sometimes only be estimated based on what the farmer could recall from memory (Pol et al., 2007), further studies even failed to estimate the amounts of antimicrobials used due to poor on-farm record keeping (Sato et al., 2005). In a study on the treatment of mastitis in the USA, only 56% of the farms frequently or always maintained records of all cows treated (Kayitsinga et al., 2017). In Germany, recording each medical treatment (requiring a withdrawal time) is mandatory by law. However, documentation beyond the minimum requires extra time and a systemic analysis not only takes extra time, but also requires knowledge or external expertise (and thus additional costs), while benefits are not obvious for many farmers. Additionally, most of the farms do not employ electronic systems for storing this type of information making it substantially more difficult to perform a systemic analysis. Very little attention has been directed at studying the practice of record keeping, although it would provide farmers with the information necessary to assess the effectiveness of past decisions as well as to enable them to improve their future decision-making. An overview of the farm-specific situation would enable the farmer to make appropriate decisions and choose measures on different levels (cow level, herd level and farm level). By supporting the three steps necessary in establishing behavioural change - **awareness** by providing an overview of the udder health situation, aiding the **intention** to change by presenting the distribution and extent of costs and the **belief in efficacy** by supplying knowledge of measures proven to be effective and the investment sum available on the farm – farmers could be better motivated to undertake improvement measures (Ritter et al., 2017).

Ways to motivate farmers to change their behaviour and document health data, treatment outcomes and costs more thoroughly could evolve via different stimuli based on the **RESET** model (Lam et al., 2016): **R**egulation *e.g.*, by mandatory reporting and benchmarking, **E**ducation, **S**ocial pressure *e.g.*, for animal welfare and higher product quality, **E**conomic incentives and **T**ools *e.g.*, supporting monitoring and analysis of data with alerts and decision-supporting output. Furthermore, to satisfy consumer demands for more transparency in food production, tracking data on health and antibiotic consumption is likely to grow in importance. Likewise, producers who want their products to stand out in times of unstable markets will need records to verify their claims of higher quality and should provide assurances on good animal welfare in order to justify premium prices.

3.5 Conclusions

The study showed, that investing time and effort in comprehensive documentation and analysis enabled an objective and farm-specific assessment of the current state of efficacy and efficiency in disease treatment (here mastitis), as well as providing information on antimicrobial consumption and costs due to mastitis.

Antibiotics usage was very high on farms (especially in HCIAs) while poor treatment results signalled a need to improve treatment strategies. Accurate records of antibiotic consumption and frequent treatment follow-ups enables the level of consumption to be monitored and the comparison between the years on one farm and between farms supporting the increased necessity to lower antimicrobial consumption while improving animal health at the same time.

The analysis of systemic farm data allows the identification of areas where costs are high and where intervention might be most beneficial including possibilities for financial investments. Calculating the cost of mastitis and the improvement scenario provides knowledge on how much money would be saved by reducing mastitis and would thus be available for investment to reach this goal.

The high variations in antibiotic consumption, treatment outcome and cost of mastitis between the farms, showed that using average or incomplete figures risks giving the wrong impression of a farm. It can be concluded that the use of comprehensive, farm specific documentation and systemic analysis is both worthwhile and necessary. The benefit is undeniable and crucial for animal welfare, consumer safety and the economic viability of the dairy farm and can be expected to more than balance out any work put into documentation and analysis.

4. General discussion

The overall aim of the thesis is to contribute to an improvement of treatment success when using remedies in bovine mastitis. It focuses on treatment with homeopathy and antibiotics as they are the most common chosen therapies in mastitis. The discussion reflects on the efficacy of those remedies within scientific trials (paper 1 and 2) as well as the effectiveness under practice farm conditions (paper 2 and 3). In the beginning, difficulties when testing and comparing antibiotics and homeopathy are addressed, as they may bias/hamper the outcome of a study. This is followed by a comparison and discussion of the overall results and what can be done for improvement of mastitis treatment.

Principles of homeopathy and antibiotics

The mode of action concerning the impacts of homeopathic and antibiotic remedies on the course of diseases in farm animals is different. Thus, the treatment success depends on different factors. Antibiotics are ideally chosen not only on symptoms but in correspondence with the sensitivity towards the causing pathogen and its resistance pattern. This can be tested beforehand by cytobacteriological milk samples and antibiogram. Antibiotics have their main effect in bacteriostatic and bactericidal effects on pathogens, increasing the chances of the animal to recover because of a reduction or elimination of those pathogens. The success of mastitis treatment is evaluated by the absence of symptoms and pathogens as well as by a reduction of somatic cell counts. Antibiotics allow to treat cows infected by the same bacteria with a specific antibiotic. This means, in a scientific trial, the success of treatment can be compared with those within a control group without further considerations concerning the context of the individual cow (apart from chosen exclusion criteria).

In contrast, homeopathic treatment addresses the individual character of the disease in a specific animal. Among other things, no pre-test is available to select those remedy which can be expected to best fit to the current situation and individual. In general, one or several remedies are chosen according to the symptoms, behaviour, personality and constitution of the individual. Homeopathic remedies are suggested to have their main effect in stimulating the self-regulatory mechanism to heal the body (Hahnemann, 1869). This individualized approach means that many factors (time for proper observation, expertise to choose the most appropriate remedy and dilution, ability of reactivity of the individual) will have influence on the outcome and that the treatment is strongly context dependent.

Those factors make it difficult to fit trials on efficacy of homeopathy in the conventional standardized protocols for an RCT, which is mainly designed for testing a certain single remedy or drug for a certain disease, whereas in individualized homeopathy the remedy is chosen for each animal individually. The test of a single remedy could counteract the treatment procedure of homeopathy with selection of one or more remedies that might also change with alteration of symptoms, *e.g.,* by the so-called effect of “initial worsening” on a homeopathic remedy.

To overcome these difficulties, not a specific homeopathic remedy but the homeopathic treatment method (matching the remedy and the symptom picture) is tested in trials applying individualized homeopathy (Werner et al., 2010; Williamson et al., 2014; Ebert et al., 2017; Keller et al., 2018). Thereby, the problem of comparison and repeatability arises as there is seldom the same homeopathic symptom picture in comparably diseased animals. Additionally, each therapist might choose different homeopathic remedies according to her/his education and experiences which hampers the possibility to repeat trials and reproduce results. Thus, the efficacy of the treatment is influenced by the experience and knowledge of the treating person choosing the homeopathic remedy and the specific dilution in which it is applied.

Nevertheless, some studies have shown that these problems can be solved by anamnesis and examination by experts on homeopathy and standard protocols for selection of the homeopathic remedy and by collection of objective measures (cell counts, bacteriological milk samples including resistance test) apart from clinical symptoms and behavioural changes at different points of time before and after treatment (Werner et al., 2010; Keller et al., 2018).

Proof of efficacy by Randomized Controlled Trials

The initial basis for the use of a remedy in practice is that its efficacy could be proven in a scientific trial. Its validity depends on the scientific quality of the research. Currently, double blind Randomized Controlled Trials are the gold standard for providing evidence of efficacy of a certain intervention or medication. However, they are not seen as flawless. They are reliable to show cause-effect relationships between intervention and outcome. No other study design will opt out bias as good as an RCT. Drawbacks are the high costs in time and money, the problem of generalisability (not representative as only a small selected group of animals is tested under very specific and controlled conditions), the loss to follow up (consideration of a very small period of time results in possibly missing outcome data) and additionally the problem of selective outcome reporting, so-called reporting bias: not reporting insignificant effects or negative outcomes (Hariton et al., 2018). The short trial duration and no assessment of long-time development means that only short time effects can be displayed.

An RCT strongly depends on its design and procedure, thus a poor methodology can either over- or underestimate effects of treatment. Kaptchuk (2001) points out biases that are only rarely discussed and arise through the procedure of selection i.e. by definition of in- and exclusion criteria or of specific outcome effects that both might result in deviations from outcomes as in an ordinary practice. The author also describes the effect of a "masking bias" in which the knowledge of participating in a double-blind RCT and the doubt whether to receive a placebo can diminish the effect of a drug, while "informed" patients believing they received solely the experimental treatment show increased effects with the drug and still with only receiving placebo treatment. Hence, the expectations of the patients changed the outcome so that blinding might not be a neutral device. This might also affect farmers or practitioners in veterinary medicine participating in a double-blinded placebo controlled RCT in that they influence the outcome by their judgement/expectation.

Even if the risk of bias of a RCT is low, there are still reasons for false-positive effects: With p-value ≤ 0,05 (random effect 5% or lower) to show a significant difference within the groups, five percent of the trials could result in a positive effect and this is why it is crucial to test study outcomes by repetition. In addition scientists are sometimes tempted to use hypothesizing after results are known (Kerr, 1998) to receive an interesting result by changing the endpoints of the study (Mukerji et al., 2022).

Paper I and II showing that there is much room for improvement of published research as a high number of publications do not fully apply with all requirements for acceptable and repeatable scientific trials. Similar findings were criticized by Krauss (2018) who found RCTs to be only partially blinded or unblinded, poor distribution regarding balance of outcome influencing factors and the limitation to "simple treatment at individual level" where no complex questions on general herd health or topics related to genetics, behaviour, practices are possible. He emphasized the need of observation studies to complement RCTs to fully consider the CONSORT statement for higher quality in trials (Schulz et al., 2010) and to find out if patients in everyday practice would receive similar outcomes. Also, Ioannidis (2014) demanded that published research must diminish biases, conflicts of interest and fragmentation of efforts in favour of unbiased, transparent, collaborative research with greater standardization.

If RCT guidelines are fully considered, scientific validity will improve substantially. Additionally, a standard protocol for scientific proof of efficacy of a treatment method or a remedy could not only consider rules on how to conduct a double-blind randomized controlled trial but should also consider specific outcome measures for the disease in question (*e.g.,* for mastitis: absence of pathogen, reduced somatic cell count post treatment beside improvement of clinical symptoms) to facilitate repetition and comparison.

Efficacy of homeopathy according to publications

The first and second paper shows that studies on homeopathy were often better fulfilling scientific demands than studies on antibiotic treatment. Despite a higher level of methodological quality, the review (paper I) and paper II provide no clear evidence of efficacy of homeopathic remedies due to a high risk of bias and a high heterogeneity. Similar findings were published for the impact of study quality of research on efficacy of homeopathic treatment in humans by Linde et al. (1999) and also by Bergh at al. (2021) for complementary medicine including homeopathy in cats, dogs and horses.

Many publications are available, examining efficacy of single remedies as well as of the homeopathic treatment method. However, the efficacy in farm animals (and especially in bovine mastitis – paper I and II) could not be proven by the current publications. Single positive or negative studies are not sufficient to provide evidence of efficacy. Other reviews on efficacy of homeopathy ended with similar results regarding a lack of efficacy for homeopathy (Linde et al., 1997; Hektoen, 2005; Rijnberk et al., 2007; Francoz et al., 2017; Lees et al., 2017a, 2017b). In contrast, some reviews found limited evidence for efficacy of homeopathy beyond placebo (Mathie et al., 2015; Mathie et al., 2017; Weiermayer et al., 2020). The outcome of a review depends on several factors, in particular on the studies published (and thereby available) and which of them are selected by the reviewer, which may lead to different results, when "cherry-picking" studies supporting homeopathy (Mukerji et al., 2022).

It can be assumed, that also the presented results of paper I and II may be biased by unpublished work as there is a tendency to report only significant effects or a desired effect (reporting or publication bias).

Reporting bias in trials dealing with homeopathic remedies in human medicine (Gartlehner et al., 2022) was found to be a high risk. Although a prospective registration for each clinical study in human medicine (Declaration of Helsinki) with subsequent publication is required (World Medical Association, 2018) and supported by the International Committee of Medical Journals Editors (ICMJE) by publishing only prospectively registered trials, 38% of the studies registered from 2002 to April 2019 remained unpublished (until April 2021). Only 47% of 193 published RCTs on homeopathy had been registered (52% of them retrospectively) and 53% remained unregistered. Primary outcomes according to the registration were modified in 25% of the studies. Unregistered RCTs showed substantially larger treatment effects as registered studies. Unfortunately, this selected reporting is not restricted to homeopathy. Similar results were found in a German Study evaluating 2.132 medicinal trials registered between 2009 and 2013, in which 26% of them were not published more than 6 years after study completion (Wieschowski et al., 2019).

The effect of this reporting bias, was further examined by Gartlehner et al. (2022) choosing the popular review of Mathie et al. (2017) enclosing studies on homeopathy in humans between 1976 to 2014 as an example. Studies with possibility for registration (between 2002 and 2014 published) were separately evaluated according to their registration status, providing only a significant difference for homeopathy compared to placebo in unregistered studies, but not in the registered.

Subsequently, also in the present analysis of trials, the influence by reporting bias might be high and the proof of effect for homeopathy and antibiotics might be even more deficient. Searching for registration schemes in European countries, trials involving animals must be registered and authorized by an ethical commission before the trial can be carried out in most countries. When searching for trials registered in Germany on homeopathy not restricted to species or time (starting at 2012 till 2022), only one trial on bovine mastitis (Keller et al., 2018) could be found (BfR, 2022), although at least one other study was found to be performed in Germany and published on homeopathy (Ebert et al., 2017). The gap between registration and publication suggests a lack of scientific and ethical standards and can be expected to have a substantial impact on the assessment of the efficacy of a treatment and might lead to overestimation of the true efficacy.

Only little is known about the share of farms using homeopathy on a regular basis (ECCH, 2017). How it is actually used and which knowledge/expertise on homeopathy is available on each farm was topic of a broader study on use of homeopathy on dairy farms in France, Germany and Spain. It revealed that only limited knowledge, no uniform treatment procedures nor a state-of-the-art treatment for the use of homeopathy existed on the farms visited (Keller et al., 2019).

With missing evidence of efficacy of homeopathy and an on-farm treatment without expertise a responsible use of homeopathy in a diseased animal is highly questionable as they may lead to low cure rates and prolonged suffering.

Efficacy of antibiotics according to publications

Considering the amount of different registered antibiotic remedies for veterinary use in bovine mastitis (in Germany around 39 different drugs for intramammary use and 76 drugs for parenteral use in cows according to "*vetidata"*, a German information service on veterinary drugs supported by the government), the number of trials found, are relatively low. Although scientific trials have to be performed for authorisation of a medicinal product (EMEA, 2003), those studies don't have to be published by the pharmaceutical company producing the remedy.

The studies on antibiotic efficacy in bovine mastitis available by publications (paper II) were not only low in quantity but were also alarmingly low in quality. Most trials were performed without blinding and lacking appropriate control, like placebo or untreated group, as many of them were performed as non-inferiority trials comparing the experimental treatment solely with a standard treatment. The performance as non-inferiority trials of antibiotics studies was also criticized by Ruegg (2021) as trials without a negative control makes it impossible to separate spontaneous cure from a possible treatment effect.

Although most of the antibiotic studies took cyto-bacteriological milk samples, only two (of 17) tested the susceptibility of the pathogen found, although its crucial for the selection of the appropriate antibiotic. Thus, culture-negative and gram-negative infections with a good cure rate without antibiotic treatment skews the results towards positive outcomes (Ruegg, 2021).

Similar outcome regarding the quality of scientific trials had a systematic review and network meta-analysis on antibiotic treatment in bovine clinical mastitis performed by Winder et al. (2019) showing that no conclusions regarding efficacy were possible due to lack of sufficient scientific study quality (mainly due to lack of blinding, high risk of bias by insufficient randomization and measurement of outcome). The authors also emphasized the need for full consideration of reporting guidelines to improve study quality as well as the importance of information on the mastitis causing pathogen. However, it should be considered that a lack of reporting may lead to exaggerated treatment effects as mainly positive and significant results will be submitted and published.

Proof of effectiveness or on-farm efficacy

How effective a certain treatment or remedy in farming practice under real-life condition is depends on various factors. This encloses a state-of-the-art treatment with early detection of symptoms, appropriate diagnostic of the cause, selection of a remedy (shown to be effective under study conditions), administration in the right dose, interval, duration and under hygienic conditions and a close follow-up on improvement or worsening of symptoms and in case of mastitis, control of cyto-bacterial milk samples to ensure full recovery. Apart from the treatment, cure also depends on farm-, cow-, herd- or pathogen-specific factors like housing conditions, management, milking hygiene, the diseased individual (general condition, immune status, age, reproduction status, additional diseases, teat lesions and resilience) and the prevalent bacteria as well as their resistance towards the remedies applied.

Effectiveness or "real-world evidence" can be examined by observational studies (*e.g.*, generated by routine practice using medical or health records), sometimes also accompanied by interviews of stakeholders or by pragmatic clinical trials. They do not include randomisation, seldom in-/exclusion criteria or blinding and focusses more on the correlation between treatment and outcomes than on proving causative explanations for outcomes.

Currently, research on actual effectiveness of antibiotic or homeopathic treatments under farming conditions is scarce and the existing studies show low healing rates (Manglai, 2016; Keller et al., 2018) and high antibiotic consumption for mastitis treatments depending on the farm (paper III).

The main strategy in practice to treat mastitis is by using antibiotics. However, indiscriminate use of antibiotics often fail to be effective due to various reasons *e.g.*, resistance of bacteria, hygiene, errors in administration etc. (Hossain et al., 2017; Cheng et al., 2020). While antibiotic mastitis treatment has on average a higher bacteriological cure rate compared to homeopathic treatment, in the case of certain pathogens, *e.g.*, *E. coli*, cure rates for antibiotic treatment are similarly low or even comparable to placebo or untreated control. In those cases, when no effective treatment exists, the use of antibiotics should be replaced by supportive treatment or measures such as non-steroidal anti-inflammatory drugs, frequent milk out and fluid therapy (Suojala et al., 2013).

It should be viewed very critically, that antibiotic treatment success on the evaluated farms (paper III) was low (18 – 59%) while antibiotic consumption was high (65% of all cows received antibiotics due to mastitis), enclosing a considerable percentage of *highly critically important antimicrobials* (> 32%) and the use of up to 17 different antibiotic agents within the same treatment.

A reduction in antibiotic use in food-producing animals was found to reduce antibiotic resistant bacteria prevalence by 10 – 15% within these animals and with a smaller evidence for humans in direct exposure to these animals, but not for the human population in general (Tang et al., 2017). Therefore, reducing antibiotic consumption is necessary in both, humans and animals, to avoid the risk to arrive a post-antibiotic era, where antibiotics are no longer effective (Laxminarayan et al., 2013).

A constant decrease in antibiotic sales in food-producing animals in 31 European countries could be shown from 2010 to 2020 (European Medicines Agency, 2021) and also in Canada (Nikky et al., 2022) by legislation changes restricting the antibiotic use but without consideration of changes in disease incidence. Only a decrease in antibiotic consumption without an increase in disease incidence is an acceptable scenario and should be considered as well. This makes a control of disease incidences and treatment success on farm level necessary.

Diagnostics, keeping records and use of health and milk record data are of high importance to gain an overview on treatment success and enable research to a broader scope.

Data are available to some degree as medical treatments on farms must be recorded by national and European law to protect public health and prevent residues of active ingredients entering the food chain by animal products like milk or meat (European Parliament and of the Council, 2019). Nevertheless, the documentation of outcome or effectiveness of applied treatments is neither obligatory nor comprehensively monitored and thus currently unknown. Falkenberg et al. (2019) described that only 29.4% of dairy farms in Germany had good documentation on mastitis and treatment while records often were inaccurate or incomplete (Pucken et al., 2021). A lack of record keeping regarding the outcome of any treatment hampers the assessment of owner and veterinarian to improve and promote the best possible treatment, although positive effects of record keeping and benchmarking are well-known in animal farming

(Manglai, 2016). Monitoring of each treatment and its outcome combined with regular available data (*e.g.,* milk records, milk samples for mastitis) would allow a target-oriented and reduced use of antimicrobials, higher cure rates (Kayitsinga et al., 2017) and therefore more healthy cows within a herd. Additionally, other preventive or therapeutic measures applied can be evaluated for their benefit by ongoing records on disease prevalence and performance on the individual farm.

Obligation to record treatment outcomes and an evaluation of those data would allow to formulate treatment recommendations for the individual farm as well as for evidence-based therapy in general *e.g.,* when a certain treatment shows a very low or no effectiveness at all.

Improvement of treatment success

Incidence rates of clinical mastitis on dairy farms are reported between 30 – 40% (Kossaibati et al., 1998; Zoche-Golob et al., 2015; Jamali et al., 2018), which is in accordance with the average 37% of the cows receiving an antibiotic treatment due to clinical mastitis on the farms under examination (paper III). Despite increasing knowledge and comprehensive research on bovine mastitis, influencing factors and options for improvements, no clear tendency for a positive development has taken place.

1. <u>Early detection of mastitis followed by determination of pathogen</u>

A first step towards improvement is an early detection of mastitis. Apart from clinical symptoms, various diagnostic techniques can be used to detect bovine mastitis (*e.g*., somatic cell count, California-Mastitis-test, electric conductivity, modified white side test, trypsin inhibition test, pH of milk, NAGase and quantification of lactate dehydrogenase activity). The use of those indicators should always be followed by bacterial culturing including sensitivity test. It allows to identify the causing pathogen and to choose the most effective antibiotic (targeted and selective use). Additionally, the identification of the causing pathogen allows to classify them as contagious (*e.g*., *Staphylococcus aureus, Streptococcus agalactiae* etc.) or environmental pathogens (*Escherichia coli, Streptococcus uberis* etc.) and therefore determines the best control measures/interventions and the prospect of cure (Cheng und Han 2020). After treatment, it allows to confirm bacterial cure. Without control, bacterially contaminated milk is at risk of transmitting disease to other animals and humans, allowing possibly resistant strains to enter the food chain, causing food poisoning or impeding the food manufacturing process (Hossain et al., 2017).

Conventional treatment on farms is usually performed without knowledge of the pathogen (due to extra costs for diagnostic, additional efforts for taking milk samples and delay of treatment, as results of laboratory samples need extra time) and the treatment chosen by clinical symptoms and knowledge of the therapist (Ouweltjes et al., 2008). A possible solution is the use of rapid on-farm culture systems like 3M Petrifilm®/mastDecide® (differentiation of gram-negative/-positive bacteria or no growth within 12 to 24hours) or the SpeedMamColor® (differentiation of pathogen group and a restricted susceptibility test) to decide whether antibiotic use is required and to find the most effective treatment. Recent studies showed that implementation of an evidence-based mastitis therapy concept enclosing clinical symptoms, individual cow characteristics (*e.g*., stage of lactation, age, days-in-milk) (J. Kock et al., 2018) and on-farm testing of the pathogen followed by treatment according to current scientific knowledge resulted in significantly better cure rates and reduction in antibiotic use (Mansion-de Vries et al., 2016; Krömker et al., 2017; Preine et al., 2022). Up to 73% of antibiotics for treatment of mastitis could be saved by applying on-farm culture supported decisions in a study of Schmenger et al. (2020b).The costs for diagnostic and extra work were balanced out by the higher cure success, reduced costs for medication and lower milk losses compared to conventional blanked antibiotic treatment.

2. <u>Control and documentation of treatment outcome</u>

Without monitoring treatment success beyond clinical symptoms (*e.g.,* somatic cell counts still high, causing pathogen still detectable) no awareness of an absent treatment success exists, accompanied by the associated costs and losses (Hogeveen; van Soest et al., 2016).

With knowledge on costs and financial losses for each case of mastitis, it becomes clear to what amount financial investments and extra work are possible/reasonable for the farm by a reduction of mastitis (as described in paper III). Even if awareness on this is present on a farm, it may be a financial drawback to invest extra working time, costs for diagnostics, veterinarian, remedies, documentation effort and milk losses due to withdrawal time and it seems more lucrative to wait for possible self-healing and accept a certain level of subclinically diseased animals (Sundrum, 2020). In most countries the dairies contribute to this by accepting an average somatic cell count of bulk milk of up to 400.000 cells/ml and higher, although a healthy udder is defined to have a somatic cell count below 100.000 (DVG, 2012) or 200.000 cells/ml (IDF, 2022).

It could be shown (paper III), that investing time and money for diagnostics, documentation and preventive measures can have a better cost-benefit relation than to "wait and see" because improved treatment strategies lead to better cure rates, reduced antibiotic consumption, lower disease rates and an increased milk production. On the other hand, the lower the treatment success and the higher the disease incidence and costs, the smaller the cost-benefit – and thus trade-offs are strongly depending on the individual farm (Halasa et al., 2007) and incentives by dairies (*e.g*., higher prices for bulk milk below 200.000 cells/ml) or public subsidies by animal health service might be necessary in some situations (*e.g.,* high prevalence of multi-resistant bacteria within the herd) to support animal welfare and "one health".

3. <u>Improvement of predisposing factors for mastitis</u>

Treatment success is a complex topic as multiple factors, apart the optimal treatment, influence the cure of mastitis *e.g.,* herd factors, cow factors, attitude of the farmer etc. – and must be considered (Jamali et al. 2018; Degen et al. 2015). Each farm should take measures to control if there might be improvements necessary regarding management, hygiene, nutrition supply, stress, genetics (udder/teat form, resilience), housing conditions or other stressors, which promote infections. In the end the specific conditions on the farm and the individual cow will determine the treatment success.

4. <u>Improvement by framework conditions and legislation</u>

a. Farm-specific

Farmers and also their veterinarians have usually no overview of the overall success of their treatment strategies or the cost caused by mastitis. As shown in paper III, an improvement of treatment will lead to economic advantages by reduced losses, expenditures as well as overall better herd health (Hoischen-Taubner et al., 2021) and investments to improve treatment and udder health will become available. A farm-specific monitoring of disease incidence, cure rate and costs of mastitis would show where improvement is necessary and also allow a comparison between farms by benchmarking to motivate farmers and their veterinarians to implement new schemes (Ruegg, 2022).

b. External incentives

It should also be a common goal for external stakeholders (*e.g.*, politics, dairies, retail companies) to support improvement of treatment as well as cow and herd health for several reasons:

Animal welfare:

Mastitis is a serious, painful disease that should be treated immediately with selective and effective treatment (Hossain et al., 2017). A delayed or/and ineffective treatment will lead to prolonged suffering and decrease in cure, causing chronic infections and a decline in herd health due to spreading of the disease.

Consumer protection:

Irresponsible use of antibiotics carries the risk for more antibiotic resistant bacteria in food chain and environment (Fair et al., 2014), as they will be selected by choosing an ineffective antibiotic agent (Chantziaras et al., 2014).

Improvement of product quality:

Only a SCC below 100.000 cells/ml (DVG, 2012) resp. 200.000 cells/ml milk (IDF, 2022) indicates that the milk is from a healthy udder, but the currently of dairies accepted SCC up to 400.000 cells/ml (depending on the country) means that a considerable part of the milk is produced by cows with a subclinical or clinical mastitis (Preine et al., 2022). In order to compete with an increasing market of plant-based milk products, cow milk has to provide a higher quality in terms of animal health and welfare, i.e. milk of healthy cows, which are kept under good husbandry conditions in respect to their needs.

Benchmarking:

In responsibility towards animals and humans, public policies should reward investments in improved husbandry and good management defined by control of disease prevalence, cure rates, antibiotic treatment frequency (by daily dose per animal) and cost of disease on the individual farm as well as benchmarking between farms to identify farms that require improvement by external expertise. The Netherlands impose a penalty for exceeding a certain amount of antibiotic consumption, which may be a strong motivator for a more reflected use of antibiotics (Valeeva et al., 2007), but should not be done at the expense of animal welfare.

5. General conclusions

The research work has shown that current available publications provide only insufficient evidence of efficacy for homeopathic and antibiotic treatment in mastitis due to insufficient scientific quality or lack repetition. Reliable, transparent, repeatable high-quality studies are necessary to provide meaningful results on research questions.

As the treatment success of antibiotic treatment is highly variable in practice, on some farms alarmingly low with use of up to 17 different antibiotics within one case, every treatment should be documented including its outcome. Unknown treatment success in practice and insufficient control may result in an antibiotic overconsumption and responsible use is not provided without on-going record keeping and utilizing real-world data including milk sample analysis (externally or for on-farm diagnostics). Only then treatment strategies can be optimized, while the increased effort may be balanced out by reduction of costs of mastitis and improvement of animal health and welfare.

Internal and external validation are not fully performed, leaving practitioners with mainly results from trials under optimized conditions that are not comparable with actual practice conditions and might result in reduced effectiveness. Requirements for the evaluation of efficacy and effectiveness of a treatment should therefore be:

1. Full assessment of efficacy under optimal, standardized conditions within repeatable scientific trials (blinded RCTs) including placebo/ untreated control and appropriate outcome measures for the disease in focus (internal validity).
2. Full assessment of effectiveness under various practice conditions (external validity).
3. On-going monitoring of all treatments (farm- and cow-specific) including cure rate, treatment frequency (*e.g.,* daily defined doses for antibiotic consumption) and disease incidence are necessary to optimize farm-specific treatment strategies according to the needs and at the same time reduce costs as well as justify use in practice independent of the kind of treatment.

Monitoring results of treatments when gathered and evaluated nationwide would allow to put general effectiveness in practice to the test. A remedy or treatment method which is not effective on any dairy farm could be banned while effective treatment could be supported and may change first-line selection of a remedy/treatment for a specific disease and thus improve animal health and welfare. Furthermore, with low profit margins in dairy business, incentives might be necessary to help farms decrease antibiotic consumption by improved cure rates, supporting diagnostic measures and rewarding low disease incidence rates.

The use of homeopathy cannot be recommended according to the current scientific knowledge as it does not provide efficacy under optimized conditions and effectiveness might even be lower under real-world conditions.

Finally, antibiotic use needs to be justified by officially registered and published results of high-quality studies (efficacy in science) AND on-farm treatment monitoring with outcome control (effectiveness in practice) on individual farms but also for comparison between farms (benchmarking) to motivate and support stakeholders (farmers, veterinarians, politicians) to improve treatments and define treatment guidelines or protocols to reduce unnecessary antibiotic consumption.

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