

Comparison of Homeopathy, Placebo and Antibiotic Treatment of Clinical Mastitis in Dairy Cows – Methodological Issues and Results from a Randomized-clinical Trial

L. HEKTOEN^{1,2}, S. LARSEN¹, S. A. ØDEGAARD¹ and T. LØKEN¹

Address of authors: ¹Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Science, Pb. 8146 Dep., 0033 Oslo, Norway; ²Corresponding author: E-mail: lisbeth.hektoen@veths.no

With 5 tables

Received for publication March 11, 2004

Summary

Based on the widespread use of homeopathy in treatment of animal disease and the poor documentation of its possible effects and consequences, a clinical trial was carried out in order to evaluate the efficacy of homeopathy in treatment of clinical mastitis in dairy cows and a design for clinical studies on homeopathic treatment, taking into account the guidelines for randomized-clinical trials (RCT) as well as the basic principles of homeopathy. A three-armed, stratified, semi-crossover design comparing homeopathy, placebo and a standardized antibiotic treatment was used. Fifty-seven dairy cows were included. Evaluation was made by two score scales, with score I measuring acute symptoms and score II measuring chronic symptoms, and by recording the frequencies of responders to treatment based on four different responder definitions. Significant reductions in mastitis signs were observed in all treatment groups. Homeopathic treatment was not statistically different from either placebo or antibiotic treatment at day 7 ($P = 0.56$, $P = 0.09$) or at day 28 ($P = 0.07$, $P = 0.35$). The antibiotic treatment was significantly better than placebo measured by the reduction in score I ($P < 0.01$). Two-thirds of the cases both in the homeopathy and placebo groups responded clinically within 7 days. The outcome measured by frequencies of responders at day 28 was poor in all treatment groups. Evidence of efficacy of homeopathic treatment beyond placebo was not found in this study, but the design can be useful in subsequent larger trials on individualized homeopathic treatment.

Introduction

The use of alternative treatments is expanding in human medicine (Fisher and Ward, 1994; Eisenberg et al., 1998; Kessler et al., 2001), but information about the extension of such use to veterinary medicine is limited, as good records of the use of these treatments do not exist. In the treatment of farm animals, alternative therapies have been in focus mainly in relation to organic farming, because of the emphasis on natural methods and medicines in the organic standards and the general intention to reduce use of chemical substances (CEC, 1999; IFOAM, 2002). A Norwegian survey concluded that at least 15% of Norwegian organic farmers used homeopathy as a part of their herd health management (Henriksen, 2002). Studies in other countries have also found this use to be considerable (Krutzinna et al., 1996; Busato et al., 2000; Hovi and Roderick, 2000; Weller and Bowling, 2000). Hovi and Roderick (2000) found homeopathy to be

more frequently used on organic farms compared with conventional farms, but the use of homeopathy outside organic farming is generally little studied.

The homeopathic philosophy of health, disease and disease treatment was first described by Hahneman (1982) in the 19th century. The homeopathic remedies are of plant, mineral or animal origin, and are usually given to the patients in very high dilutions. These dilutions are claimed to be activated through a special dilution and shaking process called potentiation, and their use makes homeopathy a very controversial issue in medical science (Vickers, 2000). Clinical research evaluating homeopathic treatment is inconclusive regarding efficacy beyond placebo. However, several reviews and meta-analyses, evaluating clinical trials on homeopathy, claim that further research should be performed before conclusions can be drawn (Kleijnen et al., 1991; Vaarst, 1996; Linde et al., 1997; Waller et al., 1998; Cucherat et al., 2000).

The randomized-clinical trial (RCT) is generally accepted in conventional medicine as the gold standard for evaluating the effect of medical treatments (Pocock, 1983; Altman, 1991). Clinical studies of homeopathy are often criticized for their low scientific quality. These criticisms refer both to not using RCTs, and if using it, to the frequent lack of quality in the trials or reporting. The RCT has a rigid structure and strict guidelines exist for conducting such trials (Altman, 1991; EMEA, 2000). There is some discussion of whether interventions and therapies with a different understanding of health and disease can be evaluated in RCTs (Coulter, 1980; Walker and Anderson, 1999; Mason et al., 2002; Walach and Jonas, 2002). This discussion is particularly related to the implementation of individualized treatment in designs developed to evaluate standard treatments, and to the choice of relevant outcome measures (Hektoen, 2004). In homeopathy, as well as in most other alternative approaches, a holistic view of disease is emphasized, and individual judgement and treatment is important. This implies that patients with the same conventional medical diagnosis are treated with different homeopathic remedies, depending on the totality of symptoms expressed by the patient. When choosing the homeopathic remedy, attention is focused on the totality of the organism as a whole, including personality and behaviour, and not merely on symptoms related to the affected organ system. The investigation of efficacy of specific homeopathic remedies for specific medical diagnoses therefore meets criticism within the homeopathic profession (Oberbaum et al., 2003). These differences make it challenging to find research methods that are acceptable both from a scientific and a homeopathic view. The

use of generally accepted scientific methods, while simultaneously taking basic principles of the alternative therapy into account is, however, necessary if acceptance of results in both disciplines is the objective.

Because of the individualized treatment approach used in classical homeopathy, good information about the individual patient and the disease process is important. The availability of such information is usually good for dairy cows, when compared with other farm animals. Furthermore, mastitis is an important disease in dairy production, and known to be treated with homeopathy (Merck et al., 1989; Searcy et al., 1995; Spranger, 1998; Henriksen, 2002). These aspects imply that clinical mastitis in dairy cows is suitable for clinical trials on individualized homeopathic treatment, and that its investigation is also of practical relevance.

The question of efficacy beyond placebo dominates the discussion about homeopathy within conventional medicine. However, the clinical significance of such efficacy is also related to the difference between the homeopathic treatment and the conventional treatment. Inclusion of a conventional mastitis treatment as a third treatment arm facilitates the investigation of this difference. Additionally, the outcome in mastitis cases treated by placebo, when compared with conventional treatment, can be addressed using this trial design. The investigation of outcome in mastitis cases not treated with antibacterial drugs is of interest due to the general efforts to decrease utilization of antibacterial drugs within human as well as veterinary medicine (Norwegian Cattle Health Services, 1996; Huovinen, 1999; WHO, 2001).

The aim of this study was to compare the efficacy of homeopathy, placebo and a standardized antibacterial treatment in treatment of clinical mastitis in dairy cows, using a design taking into account the guidelines for RCTs as well as the basic principles of homeopathy.

Material and Methods

Study population

The study sample consisted of 57 lactating dairy cows in 39 different herds in eastern Norway, included in periods between October 2000 and December 2001. The herds were selected through the farmers' replies to an invitation to participate in the trial, sent out by letter to 500 dairy farmers in eastern Norway. Prior to the study, only three of the included farmers, represented by four of the included cows, had used homeopathic treatment in their herds. The cows were examined for inclusion when the project veterinarian (first author) was contacted by the farmers or their veterinarians about a mastitis case considered to fulfil the inclusion criteria. Cows suffering from other clinical diseases or prohibited from supplying milk commercially because of medical treatment were not included in the study, neither were severely affected cows with at least two of the symptoms: pulse $>100/\text{min}$, body temperature $>41^\circ\text{C}$ or considerably reduced thirst or appetite, evaluated by the farmer. Additional exclusion criteria were gangrenous mastitis, paresis, teat lesions affecting milking and planned drying off within the first month after inclusion. Four cows were evaluated for inclusion but not included in the study because of severe teat lesion (one cow) and subclinical mastitis (three cows).

The study was approved by the Norwegian Animal Research Authority.

Study design

The study was performed as a randomized, observer-blinded and placebo-controlled trial with a stratified, modified three-dimensional (3-D) semi-crossover design (Carlsen et al., 1993; Hektoen et al., 2003). In a 3-D semi-crossover design, patients defined as non-responders after a pre-defined period of time are re-randomized to one of the two other treatments. In this study, a modified semi-crossover design was used, crossing non-responders in the homeopathy and placebo groups to antibiotic treatment and non-responders in the antibiotic group to homeopathic treatment.

Lactation number and severity of mastitis were used as stratification factors. First lactation and second or later lactations in combination with mild, moderate and severe mastitis defined the six strata. The distribution of the stratification factors within the treatment groups is provided in Table 1. Mild mastitis was defined as a case with visible changes in the milk, but without other signs of inflammation in the udder. Moderate mastitis was defined as a case with acute inflammation signs in the udder without systemic signs, while severe mastitis was defined as a case with systemic signs. These definitions are in accordance with recommendations from the International Dairy Federation (IDF, 1999). In order to keep the number of patients in each treatment group closely balanced at all times, the patients were allocated to treatment by block randomization. A random block size between 3 and 12 was used in order to make the treatment sequences further unpredictable to the observer (Altman, 1991).

Clinical procedures

Clinical examinations and collection of milk samples were performed on days 0 (day of inclusion), 1, 7 and 28. Clinical examinations were also performed on day 2 in cows with systemic signs at day 1. If the farmers observed worsening of symptoms or new symptoms developed during the study period, additional examinations were performed. The project veterinarian, who was blinded to treatment, evaluated the cows for inclusion and performed all the clinical examinations and collected all milk samples. In cases with bacteria-negative samples at day 28, an additional milk sample was collected between days 35 and 42 to confirm the negative diagnosis. Laboratory examinations of milk samples, including identification of microorganisms, sensitivity testing and presence of antibacterial substances, were performed at the National Veterinary Institute, Oslo, using the official Norwegian procedure (State Veterinary Laboratories of Norway, 1993), which is in accordance with the recommendations of the IDF (1981).

The patients were treated according to a pre-randomized list made by a statistician who was not involved in the inclusion, evaluation or treatment of the patients. The patients were randomized to antibiotic treatment or to a letter from A to H. The letters referred to eight identical sets of homeopathic and placebo remedies: four sets of active homeopathic remedies and four sets of placebo remedies. A coordinator, not involved in the inclusion, evaluation or treatment of the cows, administered the randomization list. He contacted a local

Table 1. Lactation number, milk production and disease history in three treatment groups in a study of treatment of clinical mastitis in dairy cows

	Homeopathy (n = 21)	Placebo (n = 16)	Antibiotic treatment (n = 20)	All patients (N = 57)
Milk production before inclusion (kg/day)	23.1 (14–39)	25.4 (15–34)	21.8 (10–30)	23.3 (10–39)
Days from parturition to start of treatment	115.5 (1–320)	68.4 (1–203)	104.6 (1–305)	98.4 (1–320)
Lactation number				
1	4	2	4	10
> 1	17	14	16	47
Severity of mastitis				
Mild	6	3	6	15
Moderate	13	9	8	30
Severe	2	4	6	12
Days from first symptom to start of treatment				
0	13	7	9	29
1	6	7	8	21
2	1	2	1	4
3	1	0	2	3
Extra milking out before treatment				
Yes	4	5	2	11
No	17	11	18	46
Following procedure for milking out after start of treatment				
Yes	10	10	12	32
Partly	7	4	6	17
None	4	2	2	8
Previous mastitis this lactation				
Yes	8	4	7	19
No	13	12	13	38
Bacteria day 0				
Negative	6	3	4	13
<i>Staphylococcus aureus</i>	5	5	9	19
Others*	7	8	7	22
<i>Escherichia coli</i>	3	0	0	3

The results are given as mean values with maximum and minimum values for continuous variables and number of observations for categorical variables.

*Others include *Streptococcus dysgalactiae* (n = 13), *Str. uberis* (n = 6), *Str. agalactiae* (n = 1) and coagulase negative staphylococci (CNS) (n = 1).

veterinarian or a homeopath about treatment of new cases and which set of homeopathic remedies to use. The randomization code was not broken until the initial data analyses had been performed.

The homeopathic and placebo remedies were identical with regard to packaging, physical appearance and labelling, with the exception of the letters A–H. Each set included 64 different sugar-based remedies for per oral use. These remedies were pre-selected by a homeopath, based on which remedies he experienced as most commonly used in treatment of mastitis. From the 64 remedies available in the trial, treatment could be individually selected for each patient. The homeopathic remedies were produced by Homeoden Heel, Gent, Belgium, in accordance with standards for Good Manufacturer Practice (GMP). A trained homeopath, qualified for membership in the Norwegian Homeopathic Association through a 5 years part-time education, examined the cows in the homeopathy and placebo groups, selected the homeopathic remedy and initiated treatment on day 0. Follow-up treatments were given by the farmers. The homeopathic remedies were given orally, dissolved in water. The homeopath was in contact with the farmers by phone and re-examined the cow if necessary. In homeopathic practice remedies are often changed, following the change in symptoms along the healing process. Change of homeopathic remedy within the same set was allowed as long as the cow was not classified as a non-responder to treatment and crossed over to a new treatment group. This classification was

made by the project veterinarian based on pre-defined criteria described in the following section.

Patients in the antibiotic group were treated by local veterinarians on day 0, following a standard treatment procedure commonly used in Norway (Plym Forshell and Østerås, 2001), consisting of one injection of benzylpenicillin procaine 15 mill IU/500 kg i.m. and oxytocin 10 IU i.v. followed by milking of the affected quarter(s) and local treatment, using intramammaries containing 300 000 IU benzylpenicillin procaine and 300 mg dihydrostreptomycin. Local treatment was administered by the farmers, once a day on days 0–3. In all treatment groups, the farmers performed extra milking of the affected quarter three times a day, on days 0–2, in addition to the regular milking two times a day. The farmers recorded the quantity of milk and milking intervals.

Cows with systemic signs at day 2, evaluated by body temperature and appetite, and patients with worsening of systemic or local signs of mastitis within the first 7 days were crossed over to new treatment. This procedure was used as it was seen as particularly important to ensure an ethically justifiable follow-up of non-responsive patients. Cows with mastitis signs corresponding to the definition of mild or moderate mastitis at day 7 were also crossed over to new treatment, based on the responsibility towards the owners to achieve an acceptable treatment result. All the patients crossed over to new treatment were classified as non-responders to the first treatment. These were not included as cases in the new treatment group.

Evaluation methods

Two score scales were used for evaluation (Hektoen et al., 2004). Score scales were chosen as outcome measures because such scales can be handled analytically as continuously distributed variables (Fenstad et al., 1977) and therefore generally allows fewer patients to be included in order to detect a difference of the same size, compared with binary variables. Score I was used to measure acute changes. This score scale included body temperature, appetite, acute inflammation symptoms in the affected quarter, visible changes in milk, California Mastitis Test (CMT) as an indirect measure of somatic cell count (SCC) and bacteriological findings. Score II was used to measure chronic changes. This score scale included atrophy, fibrosis and milk production in the affected quarter, in addition to visible changes in the milk, CMT and bacteriological findings. Scoring of atrophy and fibrosis was made by inspection and palpation of the udder after milking, comparing the included quarter to the corresponding quarter on the opposite side. Each factor and variable was scored on a scale from 1 to 5, and these scores added for each of the two score-scales. This gives both score scales a range from 6 to 30. A score of 6 indicates no systemic signs and a normal quarter. In cases with multiple affected quarters, only the most severely affected quarter was included in the analysis.

The frequency of responders at day 7 and three different responder definitions at day 28 were also used as outcome measures. The responder definitions are not related to specific scores on the two score-scales. However, score I at day 7 has been found to correspond to the classification of responders at day 7, and score II at day 28 to correspond to the classification of clinical responders at day 28 (Hektoen et al., 2004). The *responders at day 7* are the patients not defined as non-responders to the first treatment within 7 days. A *clinical responder at day 28* is defined as a patient with no chronic mastitis symptoms (atrophy, fibrosis or reduced milk production) and no visible changes in the milk. A *subclinical responder at day 28* is defined as a patient with negative bacteriology and CMT of 1 or 2, scored on a scale from 1 to 5 in accordance with the Scandinavian scoring system (Saloniemi, 1995). A *total responder at day 28* is defined as a patient fulfilling all the other responder definitions. The non-responders at day 28 were not crossed over to new treatment.

Statistical analysis

All assumed continuously distributed variables are expressed by mean values with 95% confidence intervals (CIs) constructed using the Student procedure (Altman, 1991). The trapezian rule was used for calculation of area under the curve (AUC) (Altman, 1991). Frequencies of responders and

non-responders are expressed in percentage with 95% CIs evaluated by using the theory of simple binomial sequences (Agresti, 1990). All tests were performed two-tailed and differences considered significant if the P -values were $\leq 5\%$. Comparison of groups with regard to development of the assumed continuously distributed variables were performed by ANOVA with repeated measurements and the initial observed value as covariant. Comparisons of the percentage reduction in score I and score II, were performed by ANOVA with the initially observed value as covariant (Kleinbaum et al., 1998). Contingency table analysis was used for comparison of groups with regard to categorical variables. In patients crossed over to different treatment, the observations at time of crossover were carried forward and used in the subsequent comparisons of the treatment groups. All statistical analyses were carried out using JMP.5.0.1 (SAS Institute Inc., 2002, Cary, NC, USA).

In order to detect a difference between the treatments of one times the SD, with a power of 90% and a significance level of 5%, at least 18 patients in each group had to be included (Larsen et al., 1991). When accounting for the factors in the block-design the required number increased to 26.

Results

Twenty-one patients were allocated to homeopathic treatment by randomization, 16 to placebo and 20 to antibiotic treatment. Significant differences among the treatment groups in the initially observed factors and variables were not found (Table 1). The difference in bacteriological status at day 0 (Table 1) was not statistically significant ($P = 0.20$). None of the isolated bacteria were found to be resistant to penicillin, streptomycin or to any of the other tested substances except for naturally occurring resistance to penicillin in *Escherichia coli*. The homeopathic diagnoses (choice of remedy) made for the patients in the homeopathy and placebo groups were comparable (Table 2).

Score I

Score I was significantly reduced from day 0 to 7 in all treatment groups ($P < 0.05$) (Table 3). No significant difference in initial score I was detected among the groups ($P = 0.51$). The group treated by antibiotics showed the largest percentage reduction in score I from day 0 to 7, followed by the homeopathy group and the placebo group (Table 3). The differences in percentage reduction of score I between the homeopathy group and the placebo group and the homeopathy group and the antibiotic group were not statistically significant ($P = 0.56$, $P = 0.09$). The percentage reduction in score I was significantly larger in the antibiotic

Table 2. Homeopathic diagnoses for the cows in the homeopathy and placebo groups

Treatment group	Homeopathic diagnosis/remedy									
	<i>Aconitum</i>	<i>Apis mellifica</i>	<i>Arnica</i>	<i>Belladonna</i>	<i>Calcarea carbonica</i>	<i>Mercurius</i>	<i>Phosphorus</i>	<i>Phytolacca</i>	<i>Pulsatilla</i>	
Homeopathy	1	3	1	0	2	1	1	8	3	
Placebo	1	1	2	1	1	1	1	6	1	
Total	2	4	3	1	3	2	2	14	4	

The results are given as number of patients treated by the different homeopathic remedies on day 0. Nine of 64 available remedies were used.

Table 3. Score I at days 0, 1 and 7 and percentage reduction in score I from day 0 to day 7

Treatment	Score I				Score II			
	Day 0	Day 1	Day 7	Percentage reduction day 7	Day 0	Day 7	Day 28	Percentage reduction day 28
Homeopathy (n = 21)	17.9 (16.7–19.0)	15.7 (14.5–16.8)	12.2 (10.1–14.4)	31.0 (22.5–39.6)	16.0 (14.9–17.1)	14.5 (13.5–5.5)	11.6 (9.8–13.4)	27.6 (17.6–37.6)
Placebo (n = 16)	18.6 (16.5–20.6)	16.4 (14.1–18.6)	13.7 (11.3–16.1)	26.6 (16.8–36.3)	15.8 (14.4–17.1)	15.2 (13.4–17.0)	13.7 (11.4–16.0)	12.9 (0.06–25.7)
Antibiotic (n = 20)	19.2 (17.6–20.7)	16.6 (15.1–18.1)	11.0 (9.7–12.2)	42.0 (33.3–50.7)	16.1 (14.9–17.2)	15.2 (13.8–16.7)	13.0 (10.5–15.5)	20.1 (7.8–32.4)

Score II at days 0, 7 and 28 and percentage reduction of score II from day 0 to day 28. The results are expressed as mean values with 95% confidence intervals.

Table 4. Effect of treatment expressed by area under the curve (AUC) for score I and score II, from day 0 to day 28

Treatment	Score I	Score II
Homeopathy (n = 21)	348.0 (294.7–401.2)	350.1 (294.7–401.2)
Placebo (n = 16)	393.1 (323.0–463.2)	392.1 (331.5–452.6)
Antibiotic (n = 20)	325.8 (212.0–553.5)	378.8 (321.1–436.5)

The results are expressed as mean values with 95% confidence intervals.

compared with the placebo group ($P < 0.01$). Comparison of effect expressed by AUC of score I from day 0 to 28, detected the lowest AUC in the antibiotic group, followed by the homeopathy group and the placebo group (Table 4). Neither the difference between the homeopathy and placebo groups ($P = 0.15$), nor the difference between the homeopathy and antibiotic groups ($P = 0.66$) was found to be significant. The AUC of the antibiotic group was significantly smaller than in the placebo group ($P = 0.05$).

Score II

Score II was significantly reduced from day 0 to day 28 in all groups ($P < 0.05$) (Table 3). No significant difference in initial score II was detected among the groups ($P = 0.92$). The homeopathy group showed the largest percentage reduction in score II from day 0 to 28, followed by the antibiotic group and the placebo group (Table 3). The difference between the homeopathy and placebo groups in percentage reduction of score II did not reach the 5% significance level ($P = 0.07$). Neither did the difference between the homeopathy and antibiotic groups ($P = 0.35$) nor the antibiotic and placebo groups ($P = 0.42$). Comparison of effect expressed by AUC of score II from day 0 to 28, detected the smallest AUC in the homeopathy group, followed by the antibiotic group and the placebo group (Table 4). No significant differences were found in AUC of score II when comparing the homeopathy and placebo groups ($P = 0.15$), the homeopathy and antibiotic groups ($P = 0.66$) or the antibiotic and the placebo groups ($P = 0.78$).

Responders and non-responders

A total of 14 patients were classified as non-responders on day 7 (Table 5). Seven of these were initially given

Table 5. Number of responders to three different treatments of clinical mastitis in dairy cows, based on four different responder-definitions

Treatment	Responders (day 7)	Clinical responders (day 28)	Subclinical responders (day 28)	Total responders (day 28)
Homeopathy (n = 21)	14	10	6	4
Placebo (n = 16)	11	9	2	1
Antibiotic (n = 20)	18	9	7	4
Total (N = 57)	43	28	15	9

homeopathic treatment, five were given placebo and two antibiotic treatment. The frequency of non-responders was 33.3% (95% CI: 14.6–57.0) in the homeopathy group, 31.3% (95% CI: 11.5–58.7) in the placebo group and 10% (95% CI: 1.2–31.7) in the antibiotic group. The antibiotic treatment generated fewer non-responders to treatment at day 7 than both homeopathy ($P = 0.06$) and placebo ($P = 0.08$). The differences in frequencies of responders based on the three definitions of responders at day 28 (Table 5) were not significant.

Discussion

Methodological issues

Evaluation of individualized homeopathic treatment in RCTs

The principle of individualized homeopathic treatment is often claimed to be a major obstacle in the evaluation of classical homeopathy in clinical trials. In this study, patients given individualized homeopathic treatment composed one treatment group, which was compared with two other treatment groups. Individualized homeopathic treatment and not single remedies was thus evaluated. As there are no standard treatments in classical homeopathy, the accuracy or precision of the treatments cannot be verified. It can therefore be asserted that the outcome might be affected by the skills of the homeopaths. However, giving trained homeopaths free choice of remedy seems to be as close as one can come to applying the principle of individualized homeopathic treatment in a clinical trial. If the effect of homeopathy should depend on a match of remedy beyond the skills of trained homeopaths, the therapy must be

regarded as too difficult to implement under practical on-farm conditions. Using individualized homeopathic treatment as one treatment group must thus be considered as a reasonable way to implement the principle of individualized treatment in clinical trials on homeopathy.

Outcome measures

Differences in definitions of success and outcome measures between academic medicine and alternative therapies, is a matter of discussion (Coulter, 1980; Walker and Anderson, 1999; Mason et al., 2002; Walach and Jonas, 2002). In treatment of mastitis, disappearance of symptoms, elimination of infection, good milk quality and minimal reduction in milk production are important results (Craven, 1987; Pyörälä and Syväjärvi, 1987). These factors are important for animal welfare, and have practical and economic implications. A treatment with no measurable effect on these practical relevant factors can hardly be seen as an interesting strategy in disease handling in animal husbandry, even if there should be other effects not being observed and evaluated. Clinical effects of homeopathic treatments therefore should be evaluated using outcome measures based on the same factors as used in the evaluation of conventional treatments.

Principles of RCTs

Strictly defined inclusion and exclusion criteria, comparable treatment groups, randomization, blinding and statistical testing of hypotheses are important factors in RCTs (Pocock, 1983). These factors were applied in the present study. The trial was performed double-blind for the comparison of the homeopathy and placebo groups and observer blind for the antibiotic group. Four sets of homeopathic remedies and four sets of placebo were used to facilitate blinding of these treatments and make the treatment allocation less predictable. In the antibiotic group, the farmers were aware that the patients were given this treatment. A possible consequence could have been that the standard procedure for extra milking the first 3 days was applied to a lesser extent in these patients, because they had been given 'a well-known effective treatment'. However, no differences among the treatment groups regarding milking procedures, as recorded by the farmers, were detected. The described procedures for randomization and blinding were applied to keep treatment allocation blinded, but no assessment of the success of blinding was carried out, by means of questioning the farmers, homeopaths or the observer, which treatments they assumed had been given. The assessment of blinding procedures is not an issue related to the investigation of homeopathy in particular (Fergusson et al., 2004), but should be included in subsequent trials.

The applied treatment procedures

There were no reports on cases in the homeopathy and placebo groups, for which homeopathic remedies other than those available would have been preferred. However, in retrospect, a closer follow-up of the homeopathic treatment, by means of more clinical examinations carried out by the homeopath instead of using phone calls as the routine procedure, would have been preferred by the participating homeopaths.

The standard antibiotic treatment used in the study is commonly used in treatment of clinical mastitis in Norway (Plym Forshell and Østerås, 2001). Except in the three cases of *E. coli* infection, no bacteria resistant to the used antibiotics were detected. The applied antibacterial treatment therefore seems appropriate for the study. Bacteria-negative cases are not possible to detect from clinical symptoms, and such cases are treated in practice without bacteriological diagnosis. As the trial was a comparison of treatments as applied in practice, the bacteria-negative cases were not excluded from the analysis.

Efficacy of treatment

Comparability between the treatment groups

The three treatment groups were similar in lactation number, daily milk production and disease history, and the homeopathy and the placebo groups were comparable in homeopathic diagnosis. These factors are therefore assumed not to influence differences in outcome between the groups. There was an overrepresentation of *Staphylococcus aureus* cases in the antibiotic group because of the randomized allocation. Cases with *S. aureus* on the day of inclusion have been found to have a poorer outcome than bacteria-negative cases measured by both score scales (Hektoen et al., 2004). This may have affected the results for the antibiotic group negatively compared with the other groups. The homeopathy group had more bacteria-negative cases, which might have affected the results in this group positively. However, the homeopathy group also included all three cases in which *E. coli* was detected. The latter showed poor results in this study. It is difficult to conclude to what degree the bacterial findings affected the differences in outcome. In total, the bacterial findings probably cause some disfavour in score II for the antibiotic group.

Score I

In all groups, a significant reduction in score I was found. This indicates that most clinical mastitis cases show some spontaneous improvement even if not treated with antibiotics. More severe cases were randomly included in the antibiotic group compared with the homeopathy group because of the relatively small number of patients. This is reflected in the initial level of score I, although statistically significant differences were not detected. The severity of the mastitis has been shown to influence the reduction in the scores (Hektoen et al., 2004). Severe cases show a larger percentage reduction of score I than mild cases. A potential effect of skewness in severity would thus be in favour of the antibiotic group for score I. However, initial level of score I was corrected for in the analysis.

The antibiotic treatment was found to have a significantly better effect than placebo regarding score I, but the homeopathic treatment could not be concluded to be different from either of these two. This result could mean that homeopathic treatment is not different from placebo, the observed tendencies in favour of homeopathy randomly occurring because of factors distributed unequally among the groups. The result could also be due to real differences between the groups that were not detected because of considerable variation within the groups, small differences among the groups and the relatively small number of patients included. Power is defined as the ability of a study to detect an effect of a specified size (Altman,

1991). If setting this specified size to be the observed difference, the study had a power of 58% to detect a difference between the homeopathy and placebo groups in the comparison of total effect (*AUC*) measured by score I. To detect the observed difference between these two groups with a power of 80% at the 95% significance level, at least 31 patients in each group are needed. The corresponding values in the comparison with the homeopathy and the antibiotic groups are a calculated power of 27% and 85 patients in each group.

Score II

Severe cases show the smallest reduction in score II (Hektoen et al., 2004). This could disfavour the antibiotic group regarding score II. However, no differences between the groups were detected either when correcting for severity of mastitis or for the initial score II. The homeopathy group showed the best results for score II followed by the antibiotic group and the placebo group. There are, however, no statistically significant differences between any of the three treatment groups using this outcome measure. Comparing total effect (*AUC*) measured by score II, the study had a power of 64% to detect the observed difference between the homeopathy and placebo groups. In order to detect the observed difference between these two groups with a power of 80% at the 95% significance level, at least 27 patients in each group are needed. The corresponding values in the comparison of the homeopathy and the antibiotic groups, is a calculated power of 36% and 62 patients in each group.

Responders and non-responders

The frequency of responders at day 7 is comparable with clinical cure rate. The rate of clinical cure of clinical mastitis is generally reported to be high after antibacterial treatment (Craven, 1987). Clinical cure rate in mastitis cases not treated by antibiotics have been reported to be from 0 to 87% (Chamings, 1984; Morin et al., 1998; Hillerton and Kliem, 2002). In this study, the frequency of clinical responders at day 7 is clearly higher in the antibiotic group compared with the homeopathy and placebo groups even if not significant at the 5% level. From this result and the changes in score I, the antibiotic treatment can be concluded to be the best for improvement of acute mastitis symptoms. However, it is noteworthy that two of three patients not treated by antibiotics could be classified as clinically cured at day 7.

About one-half of the patients in all groups were classified as clinical responders at day 28. The frequency of bacteriological cure and normalized cell count (subclinical responders) were even lower, with in total only 15 patients reaching this level of cure. In the antibiotic group, the frequency of subclinical responders at day 28 was found to be 35%. This is somewhat lower than the frequencies of mastitis cases with normal CMT and no evidence of bacteria reported by Jarp et al. (1989) to be 46.7% 3 weeks after comparable antibiotic treatment and Waage (1997) who found a frequency of healthy cows of 57.8% 4 weeks after treatment. None of the treatments in this study can be claimed to show particularly good effect regarding outcome at day 28. Along with the high clinical cure rates at day 7, also in the homeopathy and placebo groups, the poor outcome at day 28 in all treatment groups calls for further focus on the current use of antibacterial drugs

in mastitis treatment and a more target-oriented use of such drugs.

Conclusions

It is reasonable to believe that the applied design took into account the basic principles of both clinical trials and individualized homeopathy, and that it did not favour any of the treatments. Homeopathic treatment was not found to be different from either placebo or from the standard antibiotic treatment. However, the number of patients did not reach the number of patients required to give the study a sufficient power. It did not prove practically possible to include a higher number of patients in this study. Repeated studies with a large number of patients are needed in order to provide conclusions regarding possible differences between homeopathy and placebo. Further, evaluation of efficacy and consequences of homeopathic treatment is important as this treatment approach is widely used in treatment of diseased animals. For subsequent clinical trials on individualized homeopathic treatment, the design used in this study will be useful.

Acknowledgements

The authors thank the farmers who kindly let their animals be included in the study and the homeopaths and the veterinary practitioners doing the fieldwork. The study was funded by the Research Council of Norway, Oslo, Norway. Homeopathic remedies and placebo remedies were provided by Norges Naturmedisin Sentral, Drammen, Norway and produced by Homeoden Heel, Gent, Belgium.

References

- Agresti, A., 1990: Categorical Data Analysis. Wiley, New York, USA.
- Altman, D. G., 1991: Practical Statistics for Medical Research. Chapman & Hall/CRC, London, UK.
- Busato, A., P. Trachsel, M. Schällibaum, and J. W. Blum, 2000: Udder health and risk factors for subclinical mastitis in organic dairy farms in Switzerland. *Prev. Vet. Med.* **44**, 205–220.
- Carlsen, K. H., J. Kramer, H. E. Fagertun, and S. Larsen, 1993: Loratadine and terfenadine in perennial allergic rhinitis. Treatment of nonresponders to the one drug with the other drug. *Allergy* **48**, 431–436.
- CEC, 1999: Council Regulation No. 1804/1999 supplementing Regulation No. 2092/91 on organic production. *Official J. Eur. Communities* **42 L 222**, 1–28.
- Chamings, R. J., 1984: The effect of not treating mild cases of clinical mastitis in a dairy herd. *Vet. Rec.* **115**, 499–500.
- Coulter, H. L., 1980: The question of clinical trials in homeopathy and allopathy. In: *Homeopathic Science and Modern Medicine*, pp. 154–168. North Atlantic Books, Berkeley.
- Craven, N., 1987: Efficacy and financial value of antibiotic treatment of bovine clinical mastitis during lactation – a review. *Br. Vet. J.* **143**, 410–422.
- Cucherat, M., M. Haugh, M. Gooch, and J. P. Boissel, 2000: Evidence of clinical efficacy of homeopathy. A meta-analysis of clinical trials. *Eur. J. Clin. Pharmacol.* **56**, 27–33.
- Eisenberg, D. M., R. B. Davis, and S. L. Ettner, 1998: Trends in alternative medicine use in the United States, 1990–1997. *JAMA* **280**, 1569–1575.
- EMA, 2000: Guidelines to Good Clinical Practice VICH Topic GL9. The European Agency for the Evaluation of Medicinal Products, London, UK.

- Fenstad, G., L. Walle, and S. O. Wille, 1977: 3 tests for regression compared by stochastic simulation under normal and heavy tailed distribution of errors. *Scand J. Stat.* **4**, 31–34.
- Fergusson, D., K. C. Glass, D. Waring, and S. Shapiro, 2004: Turning a blind eye: the success of blinding reported in a random sample of randomised, placebo controlled trials. *BMJ* **328**, 432.
- Fisher, P., and A. Ward, 1994: Complementary medicine in Europe. *BMJ* **309**, 107–111.
- Hahneman, S., 1982: *Organon of Medicine – a New Translation*. J.P. Tarcher, Los Angeles.
- Hektoen, L., 2004: Review of the current involvement of homeopathy in veterinary practice and research. *Vet. Rec.* (in press).
- Hektoen, L., T. Løken, and S. Larsen, 2003: A stratified semi-cross-over design used in evaluation of clinical mastitis in dairy cows. In: *Proceedings from the 10th International Symposium on Veterinary Epidemiology and Economics (ISVEEX)*. ISVEE, Vina Del Mar, Chile.
- Hektoen, L., S. A. Ødegaard, T. Løken, and S. Larsen, 2004: Evaluation of stratification factors and score scales in clinical trials of treatment of clinical mastitis in dairy cows. *J. Vet. Med. A* **51**, 196–202.
- Henriksen, B. I. F., 2002: Stor interesse for homeopati og urtemedisin hos norske økobønder (Huge interest in homeopathy and herbal medicine among Norwegian organic farmers). *Praksisnytt* **7**, 55–58.
- Hillerton, J. E., and K. E. Kliem, 2002: Effective treatment of *Streptococcus uberis* clinical mastitis to minimize the use of antibiotics. *J. Dairy Sci.* **85**, 1009–1014.
- Hovi, M., and S. Roderick, 2000: Mastitis and mastitis control strategies in organic milking. *Cattle Pract.* **8**, 259–264.
- Huovinen, P., 1999: Bacterial resistance: an emerging health problem. *Acta Vet. Scand. Suppl.* **92**, 7–13.
- IDF, 1981: *Laboratory Methods for Use in Mastitis Work*. International Dairy Federation Bulletin No. 132. IDF, Brüssel.
- IDF, 1999: *Suggested Interpretation of Mastitis Terminology*. International Dairy Federation Bulletin No. 338. IDF, Brüssel. pp. 3–26.
- IFOAM, 2002: *The IFOAM Basic Standards for Organic Production and Processing*. International Federation for Organic Agriculture Movements, Tholey-Theley, Germany.
- Jarp, J., H. P. Bugge, and S. Larsen, 1989: Clinical trial of three therapeutic regimens for bovine mastitis. *Vet. Rec.* **124**, 630–634.
- Kessler, R. C., R. B. Davis, D. F. Foster, M. I. Van Rompay, E. E. Walters, S. A. Wilkey, T. J. Kaptchuk, and D. M. Eisenberg, 2001: Long-term trends in the use of complementary and alternative medical therapies in the United States. *Ann. Intern. Med.* **135**, 262–268.
- Kleijnen, J., P. Knipschild, and G. ter Riet, 1991: Clinical trials of homeopathy. *BMJ* **302**, 316–323.
- Kleinbaum, D. G., L. L. Kupper, K. E. Muller, and A. Nizam, 1998: *Applied Regression Analysis and other Multivariable Methods*, 3rd edn. Duxbury Press, Pacific Grove.
- Krutzinna, C., E. Boehncke, and H. J. Hermann, 1996: Organic milk production in Germany. *Biol. Agric. Hort.* **13**, 351–358.
- Larsen, S., L. Sandvik, and M. Osnes, 1991: The sample size in multicentre and stratified-designed trials. *Pharm. Med.* **5**, 97–105.
- Linde, K., N. Clausius, G. Ramirez, D. Melchart, F. Eitel, L. V. Hedges, and W. B. Jonas, 1997: Are the clinical effects of homeopathy placebo effects? A meta-analysis of placebo-controlled trials. *Lancet* **350**, 834–843.
- Mason, S., P. Tovey, and A. F. Long, 2002: Evaluating complementary medicine: methodological challenges of randomised controlled trials. *BMJ* **325**, 832–834.
- Merck, C. C., B. Sonnenwald, and H. Rollwage, 1989: Untersuchungen über den Einsatz homöopathischer Arzneimittel zur Behandlung akuter Mastitiden beim Rind. *Berl. Münch Tierärztl. Wschr.* **102**, 266–272.
- Morin, D. E., R. D. Shanks, and G. C. McCoy, 1998: Comparison of antibiotic administration in conjunction with supportive measures versus supportive measures alone for treatment of dairy cows with clinical mastitis. *JAVMA* **213**, 676–684.
- Norwegian Cattle Health Services, 1996: *Strategier for å redusere forbruket av antibakterielle midler i storfeproduksjonen – med særlig vekt på behandling av mastitt (Strategies Aiming at a Reduction in Antibacterial Drug Use in Cattle in Norway with Special Emphasis on Mastitis Therapy)*. Report. The Norwegian Cattle Health Services, Ås, Norway.
- Oberbaum, M., G. Vithoulkas, and R. VanHaselen, 2003: Clinical trials of classical homeopathy: reflections on appropriate research designs. *J. Altern. Complement. Med.* **9**, 105–111.
- Plym Forshell, K., and O. Østerås, 2001: Trends in Norwegian udder health data during 1975 through 2000. In: *Proceedings of the 2nd International Symposium on Mastitis and Milk Quality*, pp. 321–325. National Mastitis Council, Verona, USA.
- Pocock, S. J., 1983: *Clinical Trials: a Practical Approach*. John Wiley & Sons, New York, USA.
- Pyörälä, S., and J. Syväjärvi, 1987: Bovine acute mastitis: Part II. Effect of mastitis pathogen, initial inflammatory reaction and therapy on the outcome of the disease. *J. Vet. Med. B* **34**, 629–639.
- Saloniemi, H., 1995: Use of somatic cell count in udder health work. In: Sandholm, M., T. Honkanen-Buzalski, L. Kaartinen, and S. Pyörälä (eds), *The Bovine Udder and Mastitis*, pp. 105–110. University of Helsinki, Helsinki.
- Searcy, R., O. Reyes, and G. Guajardo, 1995: Control of subclinical bovine mastitis – utilization of a homeopathic combination. *Br. Homeopathic J.* **84**, 67–70.
- Spranger, J., 1998: *Richtliniengemässe, Prävention und Therapie in der Tierhaltung des Ökolandbaus am Beispiel der Mastitis der Kuh*. *Dtsch. Tierärztl. Wschr.* **105**, 285–332.
- State Veterinary Laboratories of Norway, 1993: *Routines for Mastitis Diagnostics*. State Vet Lab, Oslo, Norway.
- Vaarst, M., 1996: *Veterinær homøopati: Baggrund, principper og anvendelse med speciel fokus på økologiske malkekvægbesætninger – et litteraturreview (Veterinary Homeopathy: Background, Principles and use with Emphasis on Organic Dairy Herds – a Literature Review)*. Report No. 731. Danish Institute of Agricultural Science, Foulum, Denmark.
- Vickers, A., 2000: Clinical trials of homeopathy and placebo: analysis of the scientific debate. *J. Altern. Complement. Med.* **6**, 49–56.
- Waage, S., 1997: Comparison of two regimens for the treatment of clinical bovine mastitis caused by bacteria sensitive to penicillin. *Vet. Rec.* **141**, 616–620.
- Walach, H., and W. B. Jonas, 2002: Homeopathy. In: Lewith, G., W. B. Jonas, and H. Walach (eds), *Clinical Research in Complementary Therapies*, pp. 229–246. Churchill Livingstone, London, UK.
- Walker, L. G., and J. Anderson, 1999: Testing complementary and alternative therapies within a research protocol. *Eur. J. Cancer* **35**, 1614–1618.
- Waller, K. P., Beyer, W., and Ekman, T., 1998: *Veterinär homeopati till lantbrukets djur – en sammanställning av vetenskapliga försök (Veterinary Homeopathy for Farm Animals – an Overview of Scientific Studies)*. Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Weller, R. F., and P. J. Bowling, 2000: Health status of dairy herds in organic farming. *Vet. Rec.* **146**, 80–81.
- WHO, 2001: *WHO Global Principles for the Containment of Antimicrobial Resistance in Animal Intended for Food*. http://www.who.int/emc/diseases/zoo/who_global_principles.html [Accessed 11 November 2004]