



Invited review: Antibiotic treatment of metritis in dairy cows: A systematic approach

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Abstract

Acute puerperal metritis (APM) is an acute systemic illness with fever $\geq 39.5^{\circ}\text{C}$ and signs of toxemia due to an infection of the uterus occurring within 21 d after parturition. Because of the infectious nature of APM, antibiotics are considered beneficial for its treatment. Each use of an antimicrobial drug, however, is associated with selective pressure for the emergence of resistant bacteria. Hence, there is a significant need to encourage prudent use of antibiotics and alternative therapies to antibiotics. Therefore, the objective of this study was to systematically review the current literature on treatment of APM. A comprehensive and systematic literature search was conducted utilizing the PubMed and CAB Abstracts databases to identify literature focusing on the antibiotic therapy of puerperal metritis in the cow. After application of specific exclusion criteria, 21 publications comprising 23 trials remained for final evaluation. Data extraction revealed that the majority of the studies ($n = 19$) were attributable to the highest evidence level. Of 21 studies controlled, 11 had an untreated group and 3 a positive control group. The majority of the studies ($n = 17$) applied ceftiofur for the treatment of APM. Concerning the efficacy of ceftiofur, 7 studies observed clinical improvement, whereas none found improved reproductive performance. Fewer than half of the studies ($n = 10$) performed a bacteriological examination and only 4 implemented an antibiotic susceptibility test. Also, 3 studies (13.0%) described a self-cure rate per se. Little attention was given to the issue of bacterial resistance ($n = 3$), the need for reducing the application of antibiotics ($n = 2$), or guidelines for prudent use of antibiotics ($n = 1$). Our findings demonstrate that implementation of bacteriological examinations, sensitivity testing, and determination of minimum inhibitory concentrations, as well as reporting and discussion of critical issues (e.g., self-cure rates, resistance, prudent drug use), were suboptimal. On the other hand, the quality of studies on the treatment of

APM was good, as indicated by evidence level 1. Nevertheless, more high-quality research considering self-cure rates is necessary to address critical issues related to APM and crucial to the dairy industry, such as resistance, prudent use of antibiotics, animal welfare, and cost-benefit ratios.

Key words: acute metritis, antibiotic treatment, systematic review, dairy cow

INTRODUCTION

Definition and Diagnostic Challenges of Acute Puerperal Metritis

Acute puerperal metritis (APM) is an acute systemic illness with fever $\geq 39.5^{\circ}\text{C}$ and signs of toxemia due to an infection of the uterus, occurring within 21 d after parturition and characterized by an enlarged uterus and a watery red-brown fluid to viscous off-white purulent uterine discharge, which often has a fetid odor (Sheldon et al., 2006, 2009). This definition is well accepted and has been used widely in recent research trials (Dubuc et al., 2011; McLaughlin et al., 2013; Sannmann et al., 2013b). Signs of systemic illness include decreased milk yield, dullness or other signs of toxemia, decreased DMI, elevated heart rate, and dehydration (Sheldon et al., 2008). When applying this definition, the reported incidence rate of APM reaches 20% (Sannmann et al., 2012), but incidence rates up to 40% were reported in older studies (Markusfeld, 1987). Due to its severe detrimental effects on reproductive performance and economics, APM is one of the most important postpartum diseases in dairy cows (Azawi, 2008; Overton and Fetrow, 2008; McLaughlin et al., 2013).

In both research and practice, the most utilized diagnostic criteria to detect a case of APM are an elevated rectal temperature ($\geq 39.5^{\circ}\text{C}$) and fetid, watery, red vaginal discharge (VD). A more recent systematic review regarding diagnostic methods for APM demonstrated that these 2 criteria were described in 39 (fever) and 21 (fetid VD) of 48 peer-reviewed research papers, respectively, addressing APM (Sannmann et al., 2012). Therefore, this definition has to be considered the best

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available reference standard. We have emphasized, however, that this definition is empirically based and requires refinement (Burfeind et al., 2014a) as there is no gold standard available (Sheldon et al., 2006). Although evidence exists that VD and body temperature are related (Palenik et al., 2009; Burfeind et al., 2014b) and might therefore be useful to differentiate metritic from healthy cows, both criteria are also imperfect and could introduce bias. Several factors that affect body temperature in dairy cows have been described (Burfeind et al., 2010) and we have proposed increasing the fever threshold to 40.0°C for heat-stressed cows (Burfeind et al., 2012). The sensorial assessment of odor of VD is subjective and error prone (Sannmann et al., 2013a).

The role and potential diagnostic use of acute phase proteins such as haptoglobin (**Hp**), serum amyloid A, and lipopolysaccharide have been comprehensively reviewed (Cecilianani et al., 2012). The most intensively researched acute phase protein in relation to uterine health in dairy cattle is Hp. Although an early study could not find an elevation of plasma Hp concentrations in metritic cows, except for the 3 most severely affected (Hirvonen et al., 1999), an elevated serum Hp concentration (1.06 to 1.90 g/L) has been shown to indicate an acute infectious process in dairy cows (Drillich et al., 2007; Huzzey et al., 2009). This was also supported by Sheldon et al. (2001), who found that acute phase protein concentrations were increased by bacterial contamination.

Reported sensitivities for the detection of metritis (50 to 79%) and specificities (54 to 87%), however, were only moderate (Huzzey et al., 2009; Dubuc et al., 2010; Burfeind et al., 2014b). Furthermore, Smith et al. (1998b) showed a steadily declining Hp concentration during a 5-d antibiotic treatment period. Test characteristic of elevated Hp concentrations might be confounded by the fact that Hp concentration can increase in the first week after calving due to calving-related tissue damage or fatty liver syndrome (Humblet et al., 2006; Silvestre et al., 2011). Therefore, Hp as an indicator of infection should be used with caution in the days following parturition (Humblet et al., 2006).

Considerable self-cure rates (i.e., 15.6 to 55.0%) of cows diagnosed with APM using this definition have been reported (McLaughlin et al., 2012; Sannmann et al., 2013b). It is unclear whether low specificities of the diagnostic methods used to screen cows for APM with a consequently high prevalence of type I errors (i.e., high false-positive rate) might contribute to this self-cure rate.

After calving, the lumen of the uterus is usually contaminated by bacteria (Földi et al., 2006). Specific bacteria identified in the uterus of dairy cattle with

APM include *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, *Prevotella* spp., and *Bacteroides* spp. (Miller et al., 2007; Bicalho et al., 2010; Santos et al., 2011). According to Noakes et al. (1991), *Fusobacterium nucleatum* and *Proteus mirabilis* are also frequently observed in puerperal uteri. Furthermore, nonbacteriological agents such as bovine herpesvirus 4 can cause endometrial tissue damage (Sheldon et al., 2009). Because of the infectious nature, implications for animal well-being, and a potentially life-threatening course, antibiotics are considered beneficial for the treatment of APM (Beagley et al., 2010; Machado et al., 2012).

Antibiotic Treatment and Resistance

For the treatment of metritis, antibiotics are given by the intrauterine route, systemically, or both (Malinowski et al., 2011). Antibiotics such as penicillin, third-generation cephalosporins, and ampicillin have been systemically administered for the treatment of cows with APM. Furthermore, dairy cows with APM have been treated with systemic penicillin or ampicillin administrations in conjunction with intrauterine instillations of oxytetracycline or ampicillin and cloxacillin (Nak et al., 2011). As shown about 3 decades ago for oxytetracycline and chloramphenicol, no significant differences were observed concerning the distribution of the drug between various tissues or between healthy and infected postpartum cows (Bretzlaff et al., 1983, 1988). The efficacy of these antibiotic drugs, however, is impossible to evaluate conclusively from the literature as different routes, doses, and products were applied and different examinations were performed on farms by farm or study personnel or veterinarians. It is well known that not all infections are eliminated following antibacterial treatment (Malinowski et al., 2011), and definitions of self-cure and cure rates differ widely (Drillich et al., 2001; Galvão et al., 2009).

Each use of an antimicrobial drug is inherently associated with selective pressure for the emergence of resistant bacteria, which stresses the importance of their prudent use (Fishman, 2006; Ozawa et al., 2012). Recently, several publications have demonstrated emerging antimicrobial resistance of zoonotic organisms in food animals worldwide and expressed a potential threat to public health (Tragesser et al., 2006; CVMP, 2009; Mann et al., 2011; Ozawa et al., 2012). It is obvious that increasing antibiotic resistance is associated with decreasing clinical efficacy and could exacerbate animal welfare and economic consequences. It is noteworthy that the antibiotic sensitivity of bacteria from uterine discharges has not been frequently tested (Malinowski et al., 2011). Hence, there is a significant need

to encourage prudent use of antibiotics and alternative therapies to antibiotics (Ozawa et al., 2012). Such strategies involve selecting the most appropriate drug at its optimal dosage and duration to cure an infection while minimizing side effects and pressures for the selection of resistant strains (Fishman, 2006). Therefore, the objective of this study was to systematically review the current literature on antibiotic treatment of APM.

Evidence-Based Medicine and the Role of Systematic Reviews

In daily practice, veterinarians have to cope with an increasing amount of unstructured information (Smith, 1996; Eisend, 2004; Buchanan and Wooldridge, 2011). Nevertheless, they have to be able to critically judge information and to decide whether it can be adequately implemented for a given case. In this context, it is essential to base decisions on the latest and most sound scientific findings (Glasziou et al., 1998; Arlt and Heuwieser, 2005).

Reviews have long been used to summarize the body of literature on a given topic (Gordon et al., 2013). Especially in regard to the lack of time that today's doctors and scientists are confronted with (Vandeweerd et al., 2012), reviews can be very useful. Because systematic reviews follow a structured research protocol, they reduce sources of bias at all stages of the review (e.g., inclusion criteria for relevant literature, quality assessment of studies; Sargeant et al., 2006). When conducting a systematic review, it is important to consider the study designs of the articles as these determine the likelihood of bias (Karger et al., 2014). Consequently, studies concerning diagnostic tests should describe the methods of testing accurately to ensure reproducibility (Watson and Petrie, 2010). According to Kastelic (2006), randomized, controlled, double-blind studies represent the gold standard with regard to the evaluation of a certain treatment. However, there is a dearth of methodologically sound, rigorous, large-scale clinical studies in veterinary medicine, resulting in a lack of research results at the highest evidence level (Holmes and Cockcroft, 2004; Haimmerl et al., 2012; Simoneit et al., 2012).

Hypotheses

As a working hypothesis, we assumed that the frequent usage of antibiotic drugs for the treatment of APM is at least partly based on studies showing methodological flaws. Therefore, we hypothesize that there is a considerable number of cases in which the application of antibiotics could be omitted or replaced by an alternative treatment. Specifically, we set out to test the following hypotheses: (1) Most of the studies

are randomized and controlled; (2) fewer than 50% of the studies have an untreated control group; (3) fewer than 10% of the studies are blinded; (4) a bacteriological examination in the course of antibiotic treatment is conducted only by a minority of studies (<50%); (5) antibiotic susceptibility testing is done in fewer than one-fourth of the studies; (6) the majority of those studies having performed an antibiotic susceptibility test use a disk diffusion test; (7) self-cure rates are assessed by fewer than 25% of the studies; (8) an MIC regarding the antibiotic applied is reported in fewer than 5% of the studies; and (9) only a minority of the studies address the issues of bacterial resistance and prudent drug use guidelines.

MATERIALS AND METHODS

A comprehensive and systematic literature search was conducted on May 23, 2014, utilizing the databases PubMed (<http://www.pubmed.gov>) and CAB Abstracts (<http://ovidsp.tx.ovid.com/>) to identify literature focusing on the antibiotic therapy of puerperal metritis in the cow. The subject heading "bovine metritis AND antibiotics" was used to find all trials on this topic written in English or German. Specific exclusion criteria were defined to exclude articles that did not describe experimental studies; for example, descriptive or *in vitro* studies. Conference proceedings and review articles were also excluded. Furthermore, articles not meeting the inclusion criteria because of incorrect indexing, and those not obtainable through the Internet, bibliographies, or interlending services were excluded as well. If multiple publications were retrieved that described the same trial, those containing the least information were regarded as doublets and excluded. Retrieval and management of references was performed by using Endnote (version X4 for Windows, Thomson Reuters, New York, NY).

The remaining publications were evaluated according to various evidence parameters using a checklist developed and pretested in a previous step (Table 1). Relevant criteria of the study design such as sample size, the involvement of control groups, blinding, and randomization were considered. Furthermore, information concerning the drug(s) applied, bacteriological investigations and antibiotic sensitivity testing, self-cure rates, and the critical discussion of certain aspects were documented in a spreadsheet. Frequency distributions were compiled using SPSS for Windows (version 22.0; SPSS Inc., Munich, Germany).

RESULTS

In total, 53 publications (Figure 1) were retrieved through systematic database literature search (PubMed:

Table 1. Checklist used to evaluate publications on acute puerperal metritis in dairy cows considering the level of evidence

Bibliography	Possible input parameter
Paper identification	
Author	
Title	
Journal	
Year of publication	
Material and methods	
Sample size	Number
Stage of evidence	1 to 4 ¹
Blinding	Yes / No
Randomization	Yes / No
Control group	Yes / No
Use of antibiotic	Preventive / therapeutic / other ²
Size antibiotic treatment group	Number
Size reference group	Number
Size untreated control group	Number
Antibiotic agent	
Dosage	
Start and duration of treatment	DIM / days
Type of treatment	Systemic / local
Bacteriological examination	Yes / No
MIC	Yes / No
Susceptibility test	Yes / No
Disk diffusion method	Yes / No
Microdilution method	Yes / No
Reference treatment	
Dosage	
Start and duration of treatment	DIM / days
Efficacy of antibiotic treatment	
Efficacy of reference treatment	
Outcome variable(s)	
Self-cure rate	%
Topics discussed	
Applied antibiotic as “antibiotic of last resort”	Author’s statement / No
Antibiotic efficacy	Author’s statement / No
Bacterial load	Author’s statement / No
Bacteriological examination	Author’s statement / No
MIC	Author’s statement / No
Bacterial resistance	Author’s statement / No
Prudent use of antibiotics	Author’s statement / No
Prudent drug-use guidelines	Author’s statement / No
Alternative treatments / reference treatments	Author’s statement / No
Self-cure	Author’s statement / No
Is there an indication of financial support?	Name of company / No

¹Levels of research evidence according to Bassler and Antes (2000): 1 = meta-analyses of randomized, controlled trials (Ia)/randomized, controlled trials (Ib); 2 = controlled trials without randomization (IIa)/well-designed, quasi-experimental studies (IIb); 3 = well-designed, non-experimental descriptive studies (e.g., case-control studies); 4 = opinions of respected authorities, reports of expert committees, clinical experience.

²Studies describing other aspects such as pharmacokinetics, pharmacodynamics, pathogen load.

48; CAB: 4) and search by hand (n = 1). According to the exclusion criteria, 31 indexed articles had to be excluded. In addition, 1 duplicate was excluded, resulting in 21 remaining publications that comprised 23 individual trials (Table 2). Fourteen of the 23 evaluated studies (60.9%) had a sample size of ≥ 100 (Table 3), whereas 6 (26.1%) included fewer than 50 cows. According to the levels of evidence suggested by Bassler and Antes (2000), 19 of the studies (82.6%) included were attributable to the highest evidence level (i.e., randomized, controlled studies; Figure 2). Two (8.7%), 18 (78.3%), and 21 (91.3%) of the studies were fully blinded, ran-

domized, and controlled, respectively (Table 4). Of those 21 studies controlled, 11 had an untreated group and 3 a positive control group (applying a treatment other than an antibiotic). The majority of the studies (n = 13) pursued a therapeutic intervention and 5 a preventive approach. Another 5 studies described other aspects such as pharmacokinetics, pharmacodynamics, or pathogen load. Concerning the antibiotic chosen for treatment, within the 23 studies assessed, 10 different antibiotic agents were applied. Ceftiofur and oxytetracycline were used in 17 and 5 trials, respectively. Efficacy of the antibiotic applied was specified in almost every

study (22/23). Concerning the efficacy of ceftiofur (the most frequent drug investigated, with 17 trials), 12 and 6 assessed effects on clinical and reproductive parameters, respectively. Although 7 studies observed clinical improvement, none found improved reproductive performance after an application of ceftiofur compared with the reference group. Concerning the second most commonly applied antibiotic agent (oxytetracycline), only 1 of 5 studies evaluated the effect on clinical parameters. The other 4 studies focused on residues in various tissues or in milk. Fewer than half of the studies ($n = 10$) performed a bacteriological examination and only 4 implemented an antibiotic susceptibility test. Regarding the method applied, 3 of those 4 used disk diffusion antibiotic sensitivity testing. An MIC of the antibiotic drug applied was specified in 3 studies (13.0%). Also, 3 studies (13.0%) described a self-cure rate per se. Another 4 publications (17.4%) reported cure rates for the untreated control groups.

Efficacy of the applied antibiotic was discussed in 20 (87.0%) of the publications. About two-thirds of the papers ($n = 15$) referred to the actual or possible bacterial load, whereas 4 papers (17.4%) discussed the issue of bacteriological examination. Minimum inhibitory concentrations were discussed in fewer than half of the studies ($n = 10$). Little attention was given to the issue of bacterial resistance ($n = 3$), the need to reduce the application of antibiotics ($n = 2$), or guidelines for prudent use of antibiotics ($n = 1$). The topic “antibiotics of last resort” was addressed in 3 of 23 papers. More than one-third ($n = 8$) of the studies referred to implemented or possible alternatives, whereas 8 of 23 studies (34.8%) discussed the possibility of a self-cure. A reference to the funding institution could be found in the majority of the papers ($n = 12$).

DISCUSSION

The literature search with the subject heading “bovine metritis AND antibiotics” in 2 well-recognized databases (i.e., PubMed and CAB) revealed only 52 publications. This result clearly shows a dearth of research on a highly prevalent topic, part of which (i.e., antibiotic resistance) has been recognized worldwide as a top public health challenge in the 21st century (Machado et al., 2014). This finding is in accordance with the outcome of the study conducted by Sannmann et al. (2012), who identified 48 publications related to APM in general. A similar shortage of research results has been described for other relevant veterinary topics by various authors (Silverlås et al., 2009; Haimerl et al., 2012; van Kneegsel et al., 2013; Karger et al., 2014). To ensure that we did not miss relevant literature based on the search strategy, we additionally searched the chosen

databases using the broader subject heading “metritis AND dairy cow.” Reviewing the retrieved literature (PubMed: 288; CAB: 67), however, revealed that the original search strategy had uncovered all relevant publications on antibiotic therapy of puerperal metritis in the cow. Nevertheless, we are aware that every literature search has its limitations. Selective inclusion by databases, various modes and different speeds of indexation, and partly incorrect indexation make it impossible to obtain every publication on a certain topic at a given time. Thus, we assume that every systematic review and meta-analysis runs the risk of overlooking studies with potential relevance.

While compiling the literature, we learned that 7 of the 52 publications found were not obtainable via the Internet. One could argue that we should have contacted the authors of publications not available online or through the interlibrary lending system. However, implementing the concepts of evidence-based veterinary medicine in practice requires obtaining high-quality scientific information in an adequate time period and with reasonable effort (Smith, 1996). Therefore, contacting the authors of publications not accessible online does not represent a practitioner’s or researcher’s reality.

Considering the current broad coverage of the Internet and future rapid technological advances (Nault and Baker, 2011), we postulate an online availability of every published paper. Otherwise, accessibility is not guaranteed.

In 6 of the 23 trials, the total sample size was <50 cows; however, 11 trials included >200 animals. More important than the absolute number of animals included is whether the sample size of each group was large enough to test the research hypotheses proposed. Only 6 of the 23 studies, however, described an a priori sample size calculation and 1 described a subsequent sample size calculation for the study. Therefore, a final evaluation of the adequacy of the sample size is not possible.

Surprisingly, assessment of the evidence level according to the stages suggested by Bassler and Antes (2000) revealed 19 publications (82.6%) attributable to the highest level. This is in contrast to several systematic literature evaluations on other veterinary topics that uncovered serious methodological flaws and a greater proportion of publications with lower evidence levels (Olivry and Mueller, 2003; Gordon et al., 2013; Haimerl et al., 2013; Karger et al., 2014). Besides the evidence level, accuracy and comprehensiveness of reporting are important indicators for the quality of a publication. For example, serious weaknesses concerning the reporting of the diagnostic methods to identify cows with APM (Sannmann et al., 2012) and reproductive tract disease (de Boer et al., 2014) have been described.

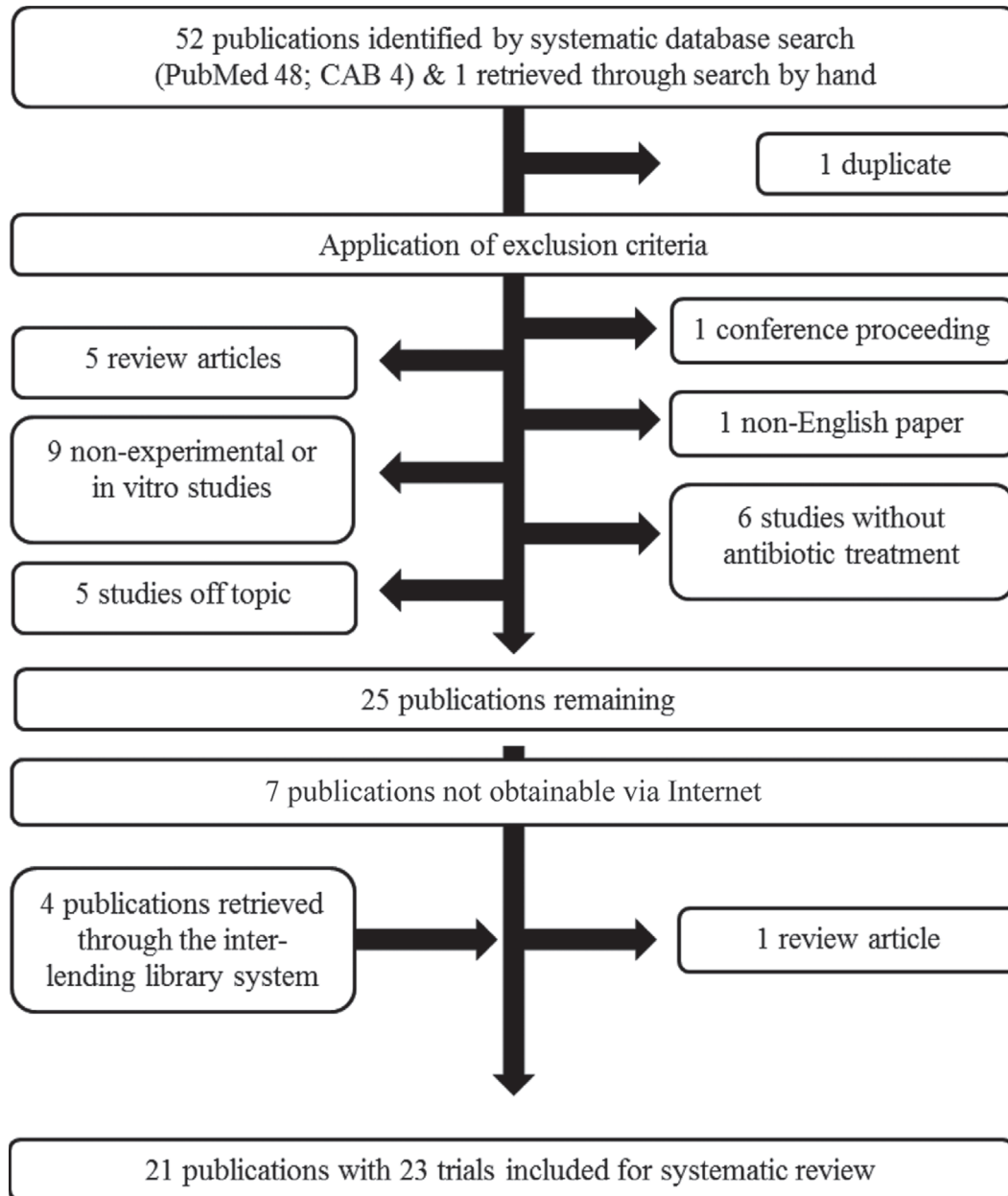


Figure 1. Publications on antibiotic treatment of acute puerperal metritis in dairy cows retrieved and systematically evaluated after applying preset exclusion criteria.

Concerning the antibiotic drug chosen for treatment, data extraction revealed that more than two-thirds (73.9%) of the studies used ceftiofur, a third-generation cephalosporin approved for the treatment of APM, pneumonia, and lameness in cattle. This result, however, is not surprising because Sannmann et al. (2012) stated that ceftiofur is widely used in research trials. In human medicine, third-generation cephalosporins are valued for treating serious or life-threatening infections. Therefore, the use of ceftiofur in dairy cows is

seen as a potential threat to its ability to cure a range of life-threatening infections in people. Specifically, it has been hypothesized that therapeutic use of ceftiofur in food-producing animals may be responsible for the emergence of ceftriaxone resistance (Allen and Poppe, 2002). Only 3 papers, however, addressed the critical issue of “antibiotics of last resort.” Despite steadily increasing antimicrobial resistance (Ozawa et al., 2012), fewer than half of the investigated studies ($n = 10$) performed a bacteriological examination and even fewer

Table 2. Research articles (n = 21) on the antibiotic therapy of puerperal metritis in dairy cows chosen for systematic evaluation

Reference	Title	Journal/source
Bretzlaff et al. (1983)	Distribution of oxytetracycline in genital tract tissues of postpartum cows given the drug by intravenous and intrauterine routes	<i>American Journal of Veterinary Research</i>
Chenault et al. (2004)	Efficacy of ceftiofur hydrochloride sterile suspension administered parenterally for the treatment of acute postpartum metritis in dairy cows	<i>Journal of the American Veterinary Medical Association</i>
Drillich et al. (2001)	Evaluation of a systemic antibiotic treatment of toxic puerperal metritis in dairy cows	<i>Journal of Dairy Science</i>
Drillich et al. (2007)	Treatment of acute puerperal metritis with flunixin meglumine in addition to antibiotic treatment	<i>Journal of Dairy Science</i>
Dubuc et al. (2011)	Randomized clinical trial of antibiotic and prostaglandin treatments for uterine health and reproductive performance in dairy cows	<i>Journal of Dairy Science</i>
Goshen and Shpigel (2006)	Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membranes in dairy cows	<i>Theriogenology</i>
Giuliodori et al. (2013)	Metritis in dairy cows: Risk factors and reproductive performance	<i>Journal of Dairy Science</i>
Jeremejeva et al. (2010)	Bacteriological and cytological findings during the late puerperal period after two different treatments of retained placenta followed by acute puerperal metritis	<i>Acta Veterinaria Scandinavica</i>
Jeremejeva et al. (2012)	Treatment of dairy cows with PGF _{2α} or NSAID, in combination with antibiotics, in cases of postpartum uterine inflammation	<i>Acta Veterinaria Scandinavica</i>
Kaneene et al. (1986)	Drug residues in milk after intrauterine injection of oxytetracycline, lincomycin-spectinomycin, and povidone-iodine in cows with metritis	<i>American Journal of Veterinary Research</i>
Lima et al. (2014)	Efficacy of ampicillin trihydrate or ceftiofur hydrochloride for treatment of metritis and subsequent fertility in dairy cows	<i>Journal of Dairy Science</i>
Mann et al. (2011)	Antimicrobial susceptibility of fecal <i>Escherichia coli</i> isolates in dairy cows following systemic treatment with ceftiofur or penicillin.	<i>Foodborne Pathogens and Disease</i>
McLaughlin et al. (2012)	Evaluation of two doses of ceftiofur crystalline free acid sterile suspension for treatment of metritis in lactating dairy cows	<i>Journal of Dairy Science</i>
McLaughlin et al. (2013) ¹	Evaluation of ceftiofur crystalline free acid sterile suspension for control of metritis in high-risk lactating dairy cows	<i>Theriogenology</i>
Moore et al. (1984)	On the use of oxytetracycline in reducing the incidence of metritis in dairy cows	<i>Journal of the South African Veterinary Association</i>
Nak et al. (2011)	Course and severity of postpartum metritis cases following antibiotic and PGF _{2α} administration in postpartum metritis cows infected with BoHV-4	<i>Transboundary and Emerging Diseases</i>
Risco et al. (2003)	Comparison of ceftiofur hydrochloride and estradiol cypionate for metritis prevention and reproductive performance in dairy cows affected with retained fetal membranes	<i>Theriogenology</i>
Sannmann et al. (2013b)	Comparison of two monitoring and treatment strategies for cows with acute puerperal metritis	<i>Theriogenology</i>
Smith et al. (1998a)	Comparison of various antibiotic treatments for cows diagnosed with toxic puerperal metritis	<i>Journal of Dairy Science</i>
Steffan et al. (1984)	Treatment of metritis with antibiotics or prostaglandin F ₂ alpha and influence of ovarian cyclicity in dairy cows	<i>American Journal of Veterinary Research</i>
von Krueger et al. (2013) ¹	Determination of ceftiofur derivatives in serum, endometrial tissue, and lochia in puerperal dairy cows with fever or acute puerperal metritis after subcutaneous administration of ceftiofur crystalline free acid	<i>Journal of Dairy Science</i>

¹Journal articles comprising 2 experiments.

(n = 4) an antibiotic susceptibility test. Sound diagnostics to identify the etiology of infections, however, are an essential prerequisite for prudent use of antibiotics

and to prevent emergence and further spread of antibiotic resistance (Fishman, 2006). Furthermore, only 4 and 3 of 23 studies discussed issues of bacteriologi-

Table 3. Sample sizes of the assessed studies (n = 23) considering study group

Group	Sample size		
	<100	≥100	Not specified
Total (n = 23)	9	14	0
Antibiotic group (n = 23)	13	9	1
Untreated control (n = 17)	9	7	1
Reference group (n = 6)	4	2	0

cal examination and bacterial resistance, respectively. With regard to the different objectives addressed by the different studies, one might argue whether every type of study should be obligated to discuss the issue of bacterial resistance in depth. However, due to the multi-layer nature of this problem (i.e., decreasing efficacy of antimicrobials, major public health concern, perception by the public, and potential consequences for the industry), we believe that at least every current study on antibiotics should mention this issue. Concerning the method of antibiotic sensitivity testing, 3 of the 4 studies conducting sensitivity testing used a disk diffusion test. Despite the fact that the disk diffusion method is widely used in routine clinical laboratories and in some research laboratories, unreliable test results have been shown for different bacteria species, such as *Campylobacter* spp. (Lehtopolku et al., 2012). Major disadvantages of the disk diffusion test are its qualitative nature (i.e., susceptible, intermediate, or resistant) rather than providing a MIC (Jorgensen and Ferraro, 2009) and the lack of mechanization or automation of the test. According to Rocksinn et al. (2007), the

broth microdilution method is considered the method of choice for in vitro susceptibility testing of bacterial pathogens, which also provides data concerning the MIC (Pierce-Hendry and Dennis, 2010). Irrespective of the test applied, appropriate breakpoints are a prerequisite to differentiate susceptible from resistant bacteria and to guide a therapeutic approach based on clinical breakpoints (Supré et al., 2014). However, microbiological breakpoints derived from the MIC do not necessarily equate to clinical breakpoints (Dalhoff et al., 2009). In addition, as different research groups use different breakpoints, data on susceptibility are rarely comparable (Supré et al., 2014).

Surprisingly, in spite of the frequent usage of ceftiofur (n = 17), none of those studies provided evidence of improved reproductive performance after treatment compared with a reference group. Although clinical improvement, as demonstrated in 7 studies, is important concerning animal welfare and animal health, it is reproductive performance that has a long-term effect on profitability because it affects milk production, reproductive culling rates, and animal sales (Plaizier et al., 1998). Furthermore, the different approaches (therapeutic, preventive, or those focusing on pharmacokinetics, pharmacodynamics, or pathogen load) decrease comparability of the studies and create a challenge for synoptically assessing the efficacy of a certain treatment.

It is well known that the MIC of the pathogen is relevant for selection of an appropriate antibiotic drug (Sheldon et al., 2004). None of the studies included in this systematic review determined an MIC for relevant

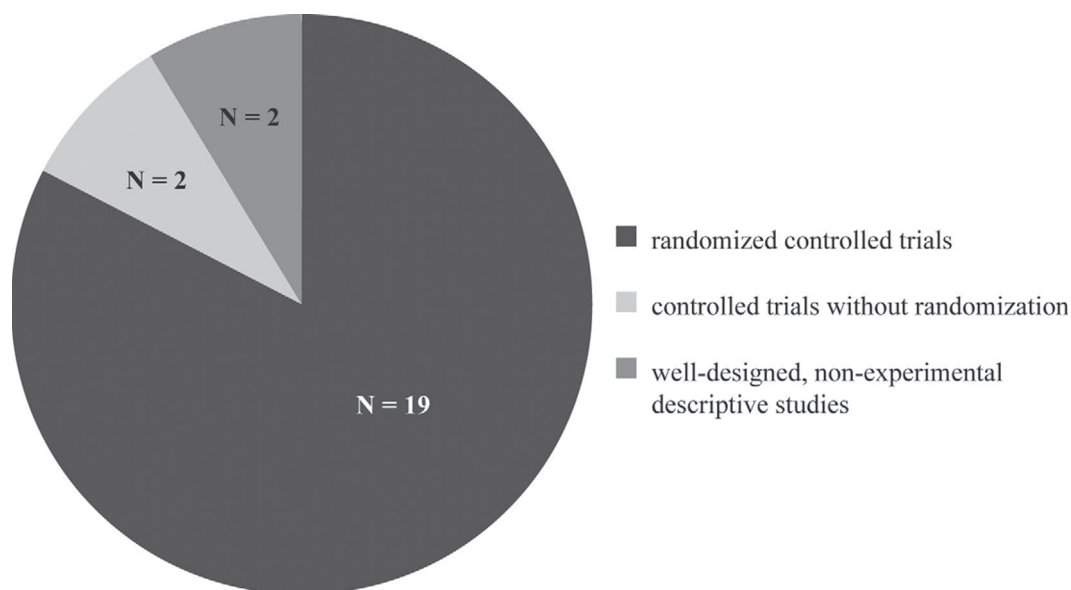
**Figure 2.** Levels of evidence of 23 studies addressing antibiotic treatment of acute puerperal metritis in dairy cows.

Table 4. Results of data extraction considering quality criteria and topics discussed of 23 trials

Criterion	Yes	No
Materials and Methods		
Blinding ¹	2	16
Randomization	18	5
Control group, overall	21	2
Control group, untreated	17	6
Reference group ²	11	12
Sample size calculation	7	16
Efficacy of antibiotic reported	22	1
Bacteriological examination	10	13
MIC	3	20
Antibiotic susceptibility testing	4	19
Self-cure rate reported	3	20
Financial support indicated	12	11
Topics discussed		
Applied antibiotic as “antibiotic of last resort”	3	20
Antibiotic efficacy	20	3
Bacterial load	15	8
Bacteriological examination	4	19
MIC	10	13
Bacterial resistance	3	20
Prudent use of antibiotics	2	21
Prudent drug-use guidelines	1	22
Alternative treatments/reference treatments	8	15
Self-cure	8	15

¹Five studies partly blinded.

²Including 3 studies with a reference group treated with drugs other than antibiotics.

pathogens. Three studies specified an MIC for the chosen antibiotic drug and certain pathogens based on findings of other researchers. Interestingly, all 3 authors referred to one paper by Sheldon et al. (2004). Nevertheless, a discussion of MIC was included in more than one-third of the studies ($n = 9$).

The majority of the studies ($n = 19$) discussed the efficacy of the antibiotic applied based on clinical parameters such as cure rates as defined by fever, discharge, and, in some cases, Hp concentrations ($n = 3$) or considering reproductive performance parameters. Only 1 of the studies, however, evaluated bacteriological cure rates as frequently conducted in mastitis research (Kalmus et al., 2014). A total of 7 studies mentioned self-cure rates ($n = 3$) or reported cure rates for their untreated control groups ($n = 4$). The average cure rate without any treatment of those 7 studies was 36.9% (minimum: 16%, maximum: 62%). A quantitative representation summarizing clinical cure rates from different studies by means of a forest plot as used in meta-analysis (Barker and Carter, 2005) was not possible because the parameters to define clinical cure were inconsistent.

Ceftiofur has been approved for the treatment of APM in the United States and Europe, specifically for a treatment for 5 consecutive days. Management practices associated with antibiotic use on dairy farms vary considerably and compliance is suboptimal (Sawant et al., 2005). Furthermore, anecdotal evidence from the

field suggests that, on average, only 2 to 3 treatments are administered for treatment of feverish metritis. It is unknown if a shortened time above MIC causes suboptimal cure rates, poses a risk for subclinical endometritis, or reduces reproductive performance (Witte et al., 2011). Furthermore, an abbreviated treatment (<3 d) can be considered off-label drug use and, due to suboptimal pharmacokinetics, exacerbate emergence of resistant bacteria.

Only 2 papers referred to the need to reduce the application of antibiotics and only 1 to the implementation of existing guidelines concerning prudent antibiotic drug use for the treatment of APM. Certainly, one explanation for this finding is the age of the studies included. The majority of the studies ($n = 18$) were published after the year 2000. On the one hand, public concern regarding the emergence of resistance has emerged more broadly in the last 10 years. Therefore, it is plausible that a significant part of the studies evaluated did not address prudent antibiotic drug use or resistance. On the other hand, the World Health Organisation (OIE) demanded prudent use of antibiotic agents in 1997, followed by the introduction of various guidelines (Teale and Moulin, 2012). Therefore, critical consideration of the topic of bacterial resistance might have been expected by those 18 studies conducted after the year 2000. This was true, however, in only 3 cases.

As pointed out by Ozawa et al. (2012), antimicrobial resistance is steadily increasing, which might lead to

decreased clinical efficacy. However, it seems unlikely that new antibiotic drugs will become available soon. Absurdly, the rapid advance of resistance and the consequent need to use these drugs sparingly has convinced pharmaceutical companies that antibiotics are not worth the investment (McKenna, 2013). Hence, there is a significant need to encourage the use of alternative therapies. Only 8 of 23 studies, however, discussed implemented or possible alternatives to antibiotics. Six of the studies actually determined the efficacy of a drug other than an antibiotic. However, 3 of those (Drillich et al., 2007; Nak et al., 2011; Jeremejeva et al., 2012) applied a treatment consisting of a prostaglandin $F_{2\alpha}$ analog or flunixin in combination with an antibiotic drug and hence did not aim to reduce the amount of applied antibiotics. According to Malinowski et al. (2011), possible alternatives to the treatment of APM include prostaglandin $F_{2\alpha}$ analogs and nonsteroidal antiinflammatory drugs (NSAID). The efficacy of NSAID (e.g., flunixin meglumine) for the treatment of APM has not yet been demonstrated, and the need for further research has been described by Drillich et al. (2007). Most recently, vaccination against the predominant bacteria to prevent postpartum uterine diseases has been described as another promising alternative to an antibiotic treatment (Machado et al., 2014). Finally, there is some evidence that suggests that leaving early APM cows untreated for 2 d is a justified approach to restrict antibiotic treatment without negative effects (Sannmann et al., 2013b). Considerable self-cure rates of 15.5 and 55.3% have been calculated within the first 5 d and 14 d postpartum, respectively (McLaughlin et al., 2012; Sannmann et al., 2013b). Despite these interesting findings, only 8 of the 23 studies discussed the possibility of a self-cure. Also, 7 studies calculated self-cure rates or reported cure rates for their untreated control groups. The latter studies, however, were not identical to those studies discussing the possibility of a self-cure, which means that some of the studies discussing a possible self-cure rate did not report one, and vice versa.

It is noteworthy that the majority of papers ($n = 12$) included a reference to financial support. Financial relationships among industry, scientific investigators, and academic institutions are widespread, and conflicts of interest arising from these ties can influence biomedical research in important ways (Bekelman et al., 2003). Many authors have expressed concern about financial interests threatening not only research integrity but also independence in decision making, communication between researchers, public trust in the research establishment, and patient safety (Okike et al., 2008). Furthermore, it has been pointed out that studies sponsored by pharmaceutical companies are approximately

4 times more likely to have outcomes favoring the sponsor's product than studies with other funding (Lexchin et al., 2003). This might be due to biased choices of comparator drugs (seriously inferior or at unusually low doses), or to decisions not to publish less favorable results, or both. Needless to say, financial conflicts of interest threaten patient care, especially when new drugs are promoted on the basis of flawed research, and taint medical information (Okike et al., 2008). Nevertheless, according to Blumenthal (1994), some evidence suggests that academic-industry relationships may have scientific and educational benefits in addition to their health and economic payoffs. Life science faculty with industrial research support publish more peer-reviewed articles (controlling for level of support from all sources) than faculty without industry funding. Furthermore, it is obvious that a considerable amount of data must have been generated by pharmaceutical companies for their submissions to regulatory bodies. Although these studies are usually of very high standard, the data are not readily accessible.

In summary, through systematic literature assessment, we confirmed the majority of our hypotheses (i.e., nos. 1, 3, 4, 5, 6, 7, and 9). On the other hand, we had to reject our hypotheses that fewer than 50% of the studies have an untreated control group (no. 2) and that an MIC is reported in fewer than 5% of the studies (no. 8).

SUMMARY AND CONCLUSIONS

Antibiotic resistance worldwide is recognized already as a top public health challenge in the 21st century (Machado et al., 2014). While conducting this systematic review with the objective to elucidate the evidence behind antibiotic treatment and to evaluate indicators of cure after antibiotic treatment of APM in dairy cattle, particularly considering ceftiofur, certain limitations but also certain strengths of the available literature became obvious. It has been demonstrated that the diagnostic value of methods used to identify APM is weak (Sannmann et al., 2012) and a gold standard does not exist (Sheldon et al., 2006), which makes refinement of diagnostic procedures difficult. Considering the growing concern regarding the potential impact of extensive use of antibiotics in food animals, including later-generation cephalosporins (Dolejska et al., 2011), potential type I errors (i.e., treatment of a healthy animal) are particularly critical. The magnitude of such errors, however, is not available because of the lack of a gold standard. Furthermore, reported cure rates for treatment groups can be confounded by low sensitivities of the diagnostic tests employed. Our findings demonstrate that implementation of bacteriological

examinations, sensitivity testing, and determination of MIC, as well as reporting and discussion of critical issues (e.g., self-cure rates, resistance, prudent drug use), was suboptimal. On the other hand, quality of studies on the treatment of APM was surprisingly good, as indicated by evidence level 1 (i.e., randomized, controlled trials) in 19 of 23 studies. It is also noteworthy that one antibiotic drug (ceftiofur) was predominantly used (17 of 23 studies) for the treatment of APM and can be considered well researched. Efficacy of this antibiotic drug concerning clinical and reproductive parameters was demonstrated by 13 (7 showed improvement) and 6 (0 showed improvement) studies, respectively. Nevertheless, more high-quality research considering self-cure rates is necessary to address critical issues related to APM and crucial to the dairy industry such as accuracy of diagnostic methods, resistance, prudent use of antibiotics, animal welfare, and cost-benefit ratios.

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