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Evaluation of diagnostic methods and comparison of two treatment protocols of acute puerperal metritis in dairy cows

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1. INTRODUCTION

Acute puerperal metritis **(APM)** in dairy cows is a common disease with a prevalence ranging from 15.3 to 69 % (Mendelez et al., 2004; Urton et al., 2005; Goshen and Shpigel, 2006). APM appears as an acute systemic illness due to an infection of the uterus and is classified into 3 grades depending on the manifestation of clinical signs (Sheldon *et al.*, 2009). A cow showing an abnormally enlarged uterus and a purulent uterine discharge without any systemic signs of ill-health is classified as having grade 1 metritis. At grade 2 metritis, these signs are accompanied with fever >39.5 °C. A cow with grade 3 metritis shows signs of toxemia, having a poor prognosis (Sheldon *et al.*, 2009). In this study, grade 2 and 3 metritis were defined as cows, having APM, while grade 1 metritis was synonymous to clinical metritis (Sheldon *et al.*, 2006) and therefore was not considered in my thesis. The categorization is based on the appearance of fever together with abnormal vaginal discharge indicative of a generalized infection caused by interactions between the host immune system and bacterial endotoxins (Benzaquen *et al.*, 2007). Abnormal discharge without a fever could equally occur in healthy cows as a result of the opening of the cervix at day in milk **(DIM)** 7 to 10 (Wehrend *et al.*, 2003). In this thesis, the focus was set on APM (i.e. grade 2 and 3 metritis), because of the life-threatening character (Drillich *et al.*, 2007) and substantial economic losses caused by this disease (Overton & Fetrow, 2008). Therefore, strategies for effective prevention, accurate and early diagnosis, and efficacious treatment of APM are of great importance.

Up to the present day there is a lack of science-based evidence considering the diagnostic value of tools to identify cows with APM. The first part of this thesis includes a systematic literature search aimed to investigate how relevant research publications address the validity of diagnostic methods for APM in dairy cows. It gives us a general overview about the diagnostic methods used and their diagnostic implementation described by the author. The search revealed that the evidence supporting the use of diagnostic methods to identify cows with APM has either been not reported or is weak. More high quality research is necessary to improve diagnostic performance of the methods employed.

However, measuring sensitivity and specificity of clinical definitions is complicated since there is no "gold standard" for the diagnosis of uterine diseases (Sheldon *et al.* 2009). It is simply assumed that the diagnostic performance of these tests (i.e. sensitivity and specificity) is sufficient for field use. Until today the diagnosis of APM is elementary based on the sensorial assessment of colour, odour and viscosity of vaginal discharge **(VD).** There is science-based evidence both from accepted clinical (e.g. rectal palpation, temperature measurement) and advanced diagnostic methods (e.g. radiography, ultrasound) that the investigator is a relevant source of measurement errors (Andermann et al., 2007; Burfeind et al., 2010; Leutert et al., 2013). The scope of the second part of this thesis was to evaluate the reliability of the assessment of color, smell and viscosity from VD. Specifically, we set out to determine reliability (inter-, intraobserver and test-retest) of scoring VD regarding color, smell and viscosity, and the conclusions drawn from these criteria regarding the uterine health status of the cow. Our study revealed slight to moderate reliabilities concerning the assessment of vaginal discharge. Intraobserver reliability was highest followed by test-retest and interobserver reliability. It's a debatable point whether these results are satisfying for a treatment of such a frequent disease as APM with antibiotic drugs.

As we could see in the second experiment, the investigator is a relevant source of measurement errors. The third part of this work involves an experiment using an electronic nose device capable of detecting volatile organic compounds. The overall objective of this

study was to evaluate odor from VD of cows in the first 10 d after parturition by olfactory cognition and an electronic detection system ("electronic nose"). The study revealed a considerable subjectivity of the human nose concerning the classification into healthy and sick animals based on the assessment of vaginal discharge. The repeatability of the electronic nose was higher. With further research the electronic nose device might be an appropriate tool to detect cows with APM reproducibly.

The importance of a correct diagnosis of a cow with APM is based on the treatment, compromising the application of antibiotic agents (Drillich et al., 2001; LeBlanc et al., 2002; Sheldon et al., 2004). In the past few years serious concerns arise regarding resistance in zoonotic pathogens in cattle (Tragesser et al., 2006; CVMP 2009; Mann et al., 2011). Improved diagnostics to identify the aetiology of infections and help direct therapy belongs to the multifactorial interventions to prevent emergence and further spread of antibiotic resistance (Mann et al., 2011).

Therefore, the objective of the last part of this thesis was to compare two monitoring and treatment strategies of fresh calved cows for APM. Basically, cows were randomly assigned to one of two treatment groups and the effect of a delayed antibiotic treatment of cows, suffering of APM was tested. Considering this study as a proof of concept study, we hypothesize that there might not be a negative impact following a delayed screening and treatment protocol.

1.1. A critical evaluation of diagnostic methods used to identify dairy cows with acute post-partum metritis in the current literature

The most commonly described methods to detect cows with APM are the measurement of rectal temperature and the assessment of vaginal discharge. It is noteworthy, however, that there is a lack of science-based information on the value or significance of measuring the rectal temperature as a diagnostic tool to identify infectious diseases in the postpartum period (Benzaquen et al., 2007).The lacking "gold standard" Sheldon et al. (2009) for the diagnosis of uterine diseases complicates the measurement of sensitivity and specificity of clinical definitions. Improved and more reliable diagnostic methods are crucial, particularly in regard of the prevention of further spread of antibiotic resistance (Fishman, 2006).

Therefore, the overall objective of this study was to investigate how relevant research publications address the validity of diagnostic methods for APM in dairy cows.
Specifically we set out to determine the proportion of studies that 1) provide a concise definition for APM, 2) cite references to support the use of the selected diagnostic methods to identify cows with APM, and 3) discuss test characteristics of the diagnostic methods and possibility of errors.
The results of this study were published in the Journal of Dairy Research:
I. Sannmann, S. Arlt and W. Heuwieser. 2012. A critical evaluation of diagnostic methods used to identify dairy cows with acute postpartum metritis in the current literature. J. Dairy Res. 79(4): 436-4.

1.2. Intra,- interobserver and test-retest reliabilities of an assessment of vaginal discharge from cows with and without acute puerperal metritis

Acute puerperal metritis (APM) in dairy cows is a common disease occurring in the first 10 days after calving. According to a widely accepted definition the diagnosis is primarily based body temperature and on the sensorial assessment of vaginal discharge.

The scope of this study was to evaluate the reliability for color, smell, and viscositiy of vagnial discharge from healthy cows and cows with APM. A total of 15 investigators evaluated 6 vaginal discharge samples 10 times. Subsequently the investigators rated the health status of the cows and the diagnostic value of color, smell, and viscosity. In a final questionnaire, the investigators stated their own perception of their ability to diagnose APM correctly and the influence of experience. Reliability was tested using Cohen's kappa (K). Our study revealed slight to moderate reliabilities concerning the assessment of vaginal discharge. Overall interobserver reliability for color, smell, and viscosity was K= 0.15, 0.27 and 0.44, respectively. Overall intraobserver reliability for color, smell and viscosity were K= 0.35, 0.39 and 0.6, respectively. The overall personal expertise to detect cows suffering from APM correctly as such was estimated to be 59%. There was a discrepancy between reliability and the personal perception of the diagnostic value. Our study shows that the sensorial assessment of color, smell, and viscosity of vaginal discharge in cows postpartum is subjective.

The results of this study are prepared for publication: I.Sannmann and W. Heuwieser. 2014. Intra,- interobserver and test-retest reliabilities of an assessment of vaginal discharge from cows with and without acute puerperal metritis. J. Dairy Sci. *in Press*

1.3. Evaluation of Odor from Vaginal Discharge of Cows in the First 10 Days after Calving by Olfactory Cognition and an Electronic Device.

Acute puerperal metritis (**APM**) is an acute systemic illness due to an infection of the uterus, usually occurring within 10 days after parturition. It is characterized by an enlarged uterus and uterine discharge varying from watery red - brown to viscous and purulent fluid which often has a fetid odor. Recently a 3 grade classification has been suggested to improve diagnosis and therapy (Sheldon et al., 2009). A cow showing an abnormally enlarged uterus and a purulent uterine discharge without any systemic signs of ill health is classified as having grade 1 metritis while at grade 2 and 3 metritis, these signs are accompanied with fever > 39.5 °C and signs of toxemia and a fetid watery red-brown uterine discharge. The categorization is based on the appearance of fever together with abnormal vaginal discharge (**VD**) indicative of a generalized infection caused by interactions between the host immune system and bacterial endotoxins (Sheldon et al., 2009). Characteristics used in research and in the field to differentiate between a normal and abnormal VD include color, viscosity, and smell (Sheldon and Dobson, 2004; Benzaquen et al., 2007; Sheldon et al., 2009).

While VD and fever are plausible criteria and have been used in several research trials studying efficacy of different therapies there is a lack of science-based evidence for their diagnostic value (Sannmann et al., 2012). Most recent studies have demonstrated that body temperature can be measured with high repeatability (Burfeind et al., 2010) but is subject to certain variables such as time of day, parity, and ambient temperature (Burfeind et al., 2012). For a visual assessment of VD in cows suffering from clinical endometritis (**CE**) through vaginoscopic examination sensitivity and specificity ranged between 96.3 and 99.6% and 90.1 and 96.7%, respectively. Intra - ($\kappa = 0.55 - 0.60$) and interobserver ($\kappa = 0.44$) repeatability of a VD score on a scale from 0 to 3, however, was only moderate (Leutert et al., 2011). To our knowledge the sensorial assessments of viscosity and smell of VD from cows with APM or CE have not been studied yet.

Odor of VD is associated with the bacterial growth density of potential pathogens in the uterus (Williams et al., 2005) and seems intuitively to assess without the use of additional diagnostic tools. However, data on test characteristics for the evaluation of VD (intra- and interobserver agreement, sensitivity, specificity) are lacking (Sannmann et al., 2012). There is no evidence whether the sensorial assessment of odor of VD is reliable enough to draw sound conclusions concerning the health status of the animal and the necessity of treatments.

There is science-based evidence both from accepted clinical (e.g. rectal palpation, temperature measurement) and advanced diagnostic methods (e.g. radiography, ultrasound) that the investigator is a relevant source of measurement errors (Andermann et al., 2007; Burfeind et al., 2010; Leutert et al., 2013). Studies testing the repeatability of sensorial assessments of odors utilizing a graded solution of phenol in liquid paraffin and olfactometer threshold tests found a correlation between observers (n = 57 and 98, respectively) varying from r = 0.43 to 0.9 (Fordyce, 1960; Doty et al., 1995).

Electronic sensor devices, so called electronic noses (Gardner and Bartlett, 2004), have made possible several beneficial applications to a variety of commercial industries, including the agricultural, biomedical, cosmetics, environmental, food, manufacturing, military, pharmaceutical, regulatory, and various scientific research fields (Wilson and Baietto, 2009).

In dairy research, electronic devices have been used for the detection of substances indicating estrus or pathological conditions such as mastitis or respiratory infection in cattle (Eriksson et al., 2005; Knobloch et al., 2010; Wiegerinck et al., 2011). These electronic devices are capable of detecting different types and sources of chemical species and mixtures of compounds present in the headspace volatiles of sampled air. Volatile organic compounds

(**VOC**) are commonly produced and released from organic sources as living microbes and multicellular organisms (Barsan et al., 2007; Wilson and Baietto, 2011).

The overall objective of this study was to evaluate odor from VD of cows in the first 10 d postpartum by olfactory cognition and an electronic nose device. Specifically, we set out 1) to determine the intra- and interobserver variability of the human olfactory cognition, 2) to determine the repeatability of odor assessment conducted with an electronic nose and 3) to establish sensitivity and specificity of olfactory cognition and the electronic nose device.

The paper summarizing the results from this study has recently been published in the Journal of Dairy Science:

Sannmann I, Burfeind O, Suthar V, Bos A, Bruins M, Heuwieser W. Technical note: Evaluation of odor from vaginal discharge of cows in the first 10 days after calving by olfactory cognition and an electronic device. J Dairy Sci. 2013 Sep;96(9):5773-9.

1.4. Comparison of two Monitoring and Treatment Strategies for Cows with Acute Puerperal Metritis

Recently the systemic use of antimicrobial agents was proven to be an effective treatment strategy for cows with APM and thus recommended by several authors (Drillich et al., 2001; LeBlanc et al., 2002; Sheldon et al., 2004). Most frequently used drugs for treatment of APM are penicillin, oxytetracycline, ampicillin and ceftiofur (Smith et al., 1998; Drillich et al., 2001).

The use of antimicrobial agents is inherently associated with selective pressure for the emergence of resistant bacteria, which stresses the importance of their prudent use (Fishman, N. 2006). More recently, several publications have expressed serious concerns regarding resistance in zoonotic pathogens in cattle (Tragesser et al., 2006; Mann et al., 2011). Ceftiofur, a third generation cephalosporin has been demonstrated to be an efficacious treatment for APM in several research trials (Zhou et al., 2001; Chenault et al., 2004). However, third-generation cephalosporins are valued for treating serious infections in human medicine. Therefore, the use of ceftiofur in dairy cows could be a potential threat to the ability to cure life-threatening infections in humans (Allen et al., 2002).

For an early detection of APM a daily measurement of rectal body temperature during the first 4 to 13 days in milk **(DIM)** was recommended (Benzaquen et al., 2007; Drillich et al., 2007). Using rectal temperature as diagnostic criterion, frequencies of both type I (fever when the animal is actually healthy) and type II errors (no fever when the animal is actually sick) occur (Kristula et al., 2001; Sheldon et al., 2004; Wagner et al., 2007). Furthermore, body temperature of fresh cows in the first days after parturition can significantly increase physiologically under hot weather conditions (Suthar et al., 2012; Burfeind et al., 2012). Due to these limitations, diagnosing fever alone is less reliable than including an examination for abnormal uterine discharge because pyrexia is not consistently associated with pathogenic bacteria in the uterine lumen (Sheldon et al., 2004).

Several studies examined the diagnostic value of acute phase proteins as indicators for APM (Smith et al., 1998; Regassa and Noakes, 2007). Because serum Hp concentrations increased two days before clinical signs (i.e. VD and body temperature $\geq 39.5°$) of APM were diagnosed (Huzzey et al., 2009) and were related to the bacterial contamination of the uterus (Williams et al., 2007) they can support the diagnosis of APM (Sheldon et al., 2004; Huzzey et al., 2009).

Recent studies investigating treatments of APM measured body temperature for 3 to 14 days postpartum and utilized body temperature as part of the treatment decision. None of the studies, however, discussed the relationship between the frequencies of temperature measurements and type I errors (Sannmann et al., 2012). Therefore, the objectives of this study were to compare two strategies of monitoring fresh cows for APM differing in their screening intensity. Subsequently, I compared two treatment strategies and an untreated group of cows with APM.

The results of this study were recently published in Theriogenology:
I.Sannmann, O. Burfeind, R. Voigtsberger and W. Heuwieser. Comparison of two Monitoring and Treatment Strategies for Cows with Acute Puerperal Metritis. Theriogenology. 2013 Apr 1;79(6):961-9.

The papers are presented in the format outlined in the guide for authors of the respective journal.

2.   RESEARCH PAPERS

2.1. A critical evaluation of diagnostic methods used to identify dairy cows with acute post-partum metritis in the current literature

A CRITICAL EVALUATION OF DIAGNOSTIC METHODS

A Critical Evaluation of Diagnostic Methods Used to Identify Dairy Cows with Acute Postpartum Metritis in the Current Literature

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SUMMARY

The overall objective of this study was to investigate how relevant research publications address the validity of diagnostic methods for acute puerperal metritis (**APM**) in dairy cows; a disease commonly treated with antibiotic drugs. Therefore, a literature search was conducted in the Journal of Dairy Science, Theriogenology, Animal Reproduction Science and The Veterinary Journal utilizing the Sciencedirect database. The search revealed 259 articles addressing APM. After applying exclusion criteria, a total of 48 trials remained. It was determined whether the author gave a clear definition of APM, the time of diagnosis relative to calving, and the person who performed the diagnosis. Studies were checked for the presence of definitions of possible findings, thresholds, and test characteristics of the methods used.

Overall 9 different diagnostic methods were employed. On average 2.5 ± 1.75 diagnostic methods were used in a study. References to support the use of the diagnostic methods were provided in 10 of 48 articles (20.8%). Vaginal discharge, transrectal palpation and rectal temperature were examined in 39, 22, and 21 of the studies, respectively. Thresholds for diagnostic tests and test characteristics were mentioned in 6 and 3 of the 48 articles, respectively. Based on this systematic review of 48 research papers the evidence supporting the use of the diagnostic methods to identify cows with APM has either been not reported or is weak. In conclusion, the reporting of the diagnostic methods to identify cows with APM needs to be improved and further high quality research is necessary to improve diagnostic performance of the methods employed.

INTRODUCTION

Acute puerperal metritis (**APM**) is an acute systemic illness due to infection of the uterus, occurring within 21 days after parturition. The definition also includes an abnormally enlarged uterus and a fetid watery red-brown uterine discharge (Sheldon *et al.*, 2006). These symptoms are associated with fever (> 39.5°C) and signs of systemic illness such as decreased milk yield, dullness or other signs of toxaemia, decreased dry matter intake, elevated heart rate, dehydration (Sheldon *et al.*, 2009b). In studies applying these criteria, the incidence rate of APM was about 20 % (Drillich *et al.*, 2001; Benzaquen *et al.*, 2007; Dubuc *et al.*, 2010). An older study reported an incidence rate of 40 % (Markusfeld, 1987); however, the author did not provide a clear definition of APM.

It has been demonstrated that APM reduced feed intake, decreased long-term milk yield, and increased the chance of culling in multiparous but not primiparous cows (Dann *et al.*, 2005; Dubuc *et al.*, 2011; Wittrock *et al.*, 2011). Most recently the effect of APM on culling has been investigated in a study using 2178 cows in 6 herds in North America (Dubuc *et al.*, 2011). Metritis did not have a direct effect on culling risk at 30 and 63 DIM or on the cumulative culling hazard up to 300 DIM. Reproductive diseases, however, made pregnancy less likely, which was a substantial risk factor for culling (Dubuc *et al.*, 2011).

Substantial economic losses are incurred by reduced milk yield, culling, and treatment costs. The total costs per case of metritis have been calculated to approximate US $ 329 to US $ 386 (Overton & Fetrow, 2008). The reported incidence rates and the opportunity costs underline the importance of this disease. It is obvious that strategies for effective prevention, accurate and early diagnosis, and efficacious treatment of APM are essential. Antibiotic therapy has been adopted as a common treatment for metritis (Azawi, 2008). Today the systemic use of antibiotics is recommended by many authors (Drillich *et al.*, 2001; LeBlanc *et al.*, 2002; Sheldon & Dobson, 2004). Most frequently used drugs for treatment of APM are penicillin, oxytetracycline, ampicillin and ceftiofur (Smith *et al.*, 1998; Drillich *et al.*, 2001; Currin, 2010).

Antibiotic use is associated with selective pressure for the emergence of resistant bacteria, which underscores the importance of their prudent use (Fishman, 2006). More recently, several publications have addressed serious concerns regarding resistance in zoonotic pathogens (Tragesser et al., 2006; CVMP 2009; Mann et al., 2011). Ceftiofur, a third generation cephalosporin, approved for the treatment of APM has been widely used in research trials (Zhou et al., 2001; Chenault et al., 2004; Drillich et al., 2007) and is a common treatment on commercial dairy farms. Advantages include demonstrated efficacy, a zero day withdrawal period for milk, and fewer group changes which are disruptive (Cook & Nordlund, 2009; Schirmann et al., 2011). Multi-drug resistant *Salmonella* species have been associated with the usage of third generation chepalosporins in dairy cows and their function as a reservoir for these pathogens (Frye & Fedorka-Cray, 2007). Third-generation cephalosporins are valued for treating serious infections in human medicine. As a result, the use of ceftiofur in dairy cows could be a potential threat to the ability to cure life-threatening infections in humans (Allen & Poppe, 2002).

Particularly, rectal temperature and assessment of vaginal discharge have been recommended as screening tools to identify diseased animals. It is noteworthy, however, that there is a lack of science-based information on the value or significance of measuring the rectal temperature as a diagnostic tool to identify infectious diseases in the postpartum period (Benzaquen et al., 2007). Sheldon (2009a) pointed out that having no ''gold standard'' for the diagnosis of uterine diseases complicates the measurement of sensitivity and specificity of clinical definitions. It is simply assumed that the diagnostic performance of these tests (i.e. sensitivity and specificity) is sufficient for field use. Improved diagnostics to identify the aetiology of infections and help direct therapy belongs to the multifactorial interventions to prevent emergence and further spread of antibiotic resistance (Fishman, 2006).

Therefore, the overall objective of this study was to investigate how relevant research publications address the validity of diagnostic methods for APM in dairy cows.
Specifically we set out to determine the proportion of studies that 1) provide a concise definition for APM, 2) cite references to support the use of the selected diagnostic methods to identify cows with APM, and 3) discuss test characteristics of the diagnostic methods and possibility of errors.

MATERIALS AND METHODS

A systematic literature search was conducted on 02 April 2011 utilizing the Sciencedirect search engine (www.sciencedirect.com) to find articles involving the diagnosis of acute metritis. The search terms "acute AND metritis" OR "puerperal AND metritis" OR "postpartum AND metritis" AND "dairy cow" were used for the search in all fields. The search was conducted for the Journal of Dairy Science, Theriogenology, Animal Reproduction Science and The Veterinary Journal. The data range was set to all years. Retrieval and management of references was performed with Endnote (Version X4.0.2; Thomson Reuters EndNote, New York, NY, USA).

Using these criteria, the search revealed 259 articles published in the 4 journals specified. After exclusion of meta-analyses (n = 1), reviews (n = 12), personal experiences (n = 32), abstracts (n = 9), questionnaires (n = 2) and data analysis (n = 8) 195 articles remained. Of those, 40 publications were excluded because of other species than cattle (n = 11) and other diseases than acute metritis (n = 29) as defined previously (Sheldon et al., 2006). Post mortem and in vitro evaluations (n = 6) were likewise excluded. From the remaining 149 articles 48 were identified as addressing acute metritis as the main research topic and used for a systematic evaluation (Table 1). The other 102 publications did not address metritis in the primary research objective (e.g. considered metritis merely as a risk factor for another disease or reduced milk yield).

For the evaluation of the literature the focus was set on the diagnostic methods used to identify cows with acute puerperal metritis. Specifically, we determined whether the author gave a concise definition of APM and cited a reference for the definition. Also the investigator (i.e. author, veterinarian, farm personnel, not specified) and the time of diagnosis relative to calving were recorded.

Furthermore, the number and type of diagnostic methods used to identify cows with APM including references were determined considering the presence of references provided. Each diagnostic method was checked individually for 4 criteria (description of implementation, the definition of possible findings, thresholds, and test characteristics, such as sensitivity, specificity and accuracy) according to the STARD checklist which consists of 25 items to improve the accuracy and completeness of reporting of studies of diagnostic accuracy (Smidt, 2003). Presence and absence of such information was coded with yes (1) and no (0), respectively (Table 2).

When multiple methods were used each one was evaluated individually. Furthermore, it was evaluated if a certain combination of methods was defined and weighted in respect to the diagnostic performance.

Findings of the 48 assessed articles were documented with Excel (Microsoft Office 2010, Microsoft Deutschland GmbH, Munich, Germany) and statistically analysed with SPSS (Version 19.0, SPSS Inc., Munich, Germany). Frequency distributions were calculated for the criteria specified. Combinations of diagnostic methods were summarized in a cross table.

RESULTS

In the Journal of Dairy Science, Theriogenology, The Veterinary Journal and Animal Reproduction Science 162, 55, 21, and 21 papers were published, respectively. According to the exclusion criteria 211 publications had to be withdrawn (Table 1). Therefore, a total of 48 articles were eligible for further analysis (Table 3).

In 25 (52.1%) papers the author provided a definition of APM. References for the definition were cited in 9 of those 25 publications (36.0 %). Most often Sheldon et al. (2006) was cited (n = 7), whereas Olson et al. (1986), Roberts et al. (1986), Földi et al. (2006), Paisley et al. (1986) and Smith et al. (1998) were cited once each. In 3 publications (33.3%) multiple citations for the definition of APM were listed.

The diagnosis of APM was performed by one of the authors (n = 19) or not specified in 29 publications (60.4%). In 10, 7 and 2 studies diagnosis had been conducted by the farm veterinarian (20.8%), the farm personnel (14.6%), and by farm personnel and the investigator together (4.2%), respectively. The time of diagnosis relative to calving was specified in 33 (68.8%) of the articles. In 2, 10 and 1 of these papers APM was diagnosed between day 3 to 6, day 6 to 10 and on day 14 postpartum, respectively. In the remaining 20 papers the investigator conducted the diagnosis on multiple times from day 0 to 10 after parturition.

The total number of diagnostic methods counted in the 48 publications was 124 (mean 2.6 ± 1.6 SD; median 2.5 ± 1.75 IQR; minimum: 0; maximum: 7).

Overall 9 different diagnostic methods were used (Table 4). Most of the studies utilized a combination of 2 to 7 diagnostic methods to identify cows with APM. In 36 publications, more than 1 method had been used. In 23 (64%) and 9 (25%) publications it was described how the diagnostic methods were combined and the methods weighted within the combination, respectively. In the remaining 13 articles using more than 1 diagnostic criterion no information was provided on the combination or weighting of the diagnostic methods used to identify cows with APM.

In 34 of the 124 cases (27.4%) the implementation of the diagnostic methods was described. In 93 (75.0%) and 22 (17.7%) of the cases possible findings and precise thresholds were defined. Test characteristics have been presented in only 3 cases (2.4%).

The diagnostic methods were referenced in 10 articles (20.8%). Sheldon et al. (2006) and Dohmen et al. (1995) were cited 5 and 2 times, respectively (Figure 1). Studer and Morrow, 1978, Tennant et al (1986), Földi et al. (2006), Sheldon et al (2006), Urton et al. (2005) and Thomsen et al. (2007) were cited once in 4 articles. The remaining studies (n = 38; 79.2%) did not list any references for the diagnostic methods used.

Vaginal discharge has been assessed and classified in 39 (81.3%) of the studies. In 10 of these articles (25.6%) the authors provided specific information about their reasoning concerning the classification of vaginal discharge. In 31 of these studies (79.5%) specific findings indicative of a disease were defined. In 4 (10.2%) articles thresholds were quoted. Test characteristics were not mentioned in any of these studies.

Transrectal palpation has been used in 22 studies (45.8%) in combination with other methods and in 3 studies as the only diagnostic test. Diagnostic performance of transrectal examination in practice has been described in one of 22 articles. In 16 of those papers specific findings were described. Test characteristics were described in one article; information on thresholds was not given.

The rectal temperature has been used to diagnose cows with APM in 21 of the publications (43. 8%) in various combinations with other criteria. The implementation for measuring the rectal temperature has been described in 7 of the 21 publications (33.3%). In the majority of publications, findings (n = 17; 81.0%) and thresholds (n = 16; 76.0%) were provided. Data on test characteristics were completely missing.

The screening for vaginal discharge with a gloved hand, by vaginoscopy or the metricheck device has been conducted in 15 studies (31.3%) in combination with other methods and in one study as the only diagnostic test. The implementation of using the gloved hand, a speculum or the metricheck device was described in 8 articles (53.3%). Findings were specified in 10 out of 15 papers. A threshold for the diagnosis was described in one paper. Test characteristics were not stated.

The general condition of the cows examined has been used in 11 (22.9%) studies. A quantitative scoring system was not described, but 8 articles provided a description of possible findings. Thresholds and test characteristics for evaluating the general condition of cows were not given.

In 5 studies a bacteriological examination has been conducted. The implementation was specified in 3 of those. In 2 of them outcomes were defined. Thresholds were not specified but test characteristics provided in 1 publication.

A drop in milk yield in combination with other criteria was evaluated in 4 studies (8.3%). In all 4 publications the decrease has been specified. In none of the cases data on thresholds or test characteristics were provided.

In 4 studies (8.3%) blood parameters have been determined [haptoglobin (n = 4), fibrinogen (n = 1), α1 Glycoprotein (n = 1), N-acetyl-β-D-glucosaminidase activity (n = 1)] in combination with an evaluation of vaginal discharge, rectal temperature, and transrectal palpation of the uterus. In all 4 studies the sampling and analytic procedures have been described. In one study the results are missing. Thresholds and test characteristics have been provided in one article.

Visual inspection of the tail and perianal area has been used in 3 studies (6.3%). In one of them the inspection, conducted by the farm's veterinarian served as the only criterion to diagnose APM. Data on the diagnostic performance of visual inspection of the tail and perianal area has not been described by any of the authors. Descriptions of findings were specified in 2 articles. Thresholds and test characteristics were not given.

In 4 studies diagnosis of APM has been mentioned but the authors did not specify the methods.

DISCUSSION

The overall objective of this study was to evaluate how relevant quality criteria of diagnostic methods to identify cows with APM are addressed in the current literature. Therefore we conducted a literature search in the ScienceDirect database considering the Journal of Dairy Science, Theriogenology, Animal Reproduction Science, and The Veterinary Journal. These Journals have a special section or focus on animal reproduction and have impact factors between 1.72 and 2.79 (Reuters, 2011). This procedure generated a convenience sample but does not give a representative overview of all the literature published. One should be aware that using such a selection of journals a potential bias is possible. Our goal, however, was to get a sample of research publications from peer reviewed and high quality journals that have a proven impact on management practices in the field of dairy reproduction and on future research. Our main research question was how intervention studies addressed quality criteria for diagnostic methods used during the trials. The focus was on clinical studies to generate information how the authors performed their diagnosis on APM in the field. Therefore, specific inclusion (e.g. cattle, metritis as main subject) and exclusion (e.g. meta-analyses, reviews, personal experiences) criteria were defined. Applying those criteria, 48 of 259 articles (18.5%) were eligible for final analyses.

Recently several attempts have been made in the field of equine and canine medicine to systematically assess the evidence of certain intervention strategies (Fahie & Shettko, 2007; Simoneit et al., 2011; Haimerl et al., 2012). Despite tremendous efforts the checklists used are prone to subjective interpretation and inter-observer repeatability was only moderate. Therefore, we assessed only distinct criteria that could be classified as present or absent (e.g. definition of APM, references for diagnostic procedures, test characteristics) or on a continuous scale (e.g. number of diagnostic methods used, days postpartum when diagnosis was conducted). Therefore, the results presented can be considered reproducible and objective.

In 4 of the 48 (8.3%) studies the diagnostic methods were not described at all. This constitutes a serious flaw that precludes valid conclusions. Authors of research publications should describe each intervention and diagnostic method thoroughly to allow a clinician wanting to use the intervention to know exactly how to perform the method used in the trial (Smidt, 2003; Glasziou et al., 2008). Only 10 articles (20.8%) cited references for the diagnostic methods utilized. According to the STARD checklist, the description should cover the full test protocol including the specification of materials and instruments together with their instructions for use, and specific measures in participants. If no citations are available, details must be provided in the text (Smidt, 2003). If this is not the case, it might be difficult to estimate the validity of the diagnostic methods used.

Frequently, the definition or characterization of the various manifestations of uterine disease either lack precision or definitions vary among research groups (Sheldon et al., 2006). Therefore, we evaluated whether the author gave a clear definition of the disease addressed in the study. Without a definition of a disease, it is hardly possible to distinguish between healthy and diseased cows, interpret test results, and provide recommendations for a larger population. Almost half of all articles, evaluated in our study did not provide such descriptions.

The diagnostic tests have been performed by one of the authors (n = 29), the farm veterinarian (n = 10), and by farm personnel (n = 7). One could speculate that the validity of a diagnosis is higher when performed by an animal health professional compared to farm personnel. At least we assume that the risk of an inaccuracy is higher, since the qualification of the farm personnel to diagnose APM can differ widely. It is noteworthy that none of the studies evaluated an intra- or in case of multiple investigators an inter-observer repeatability of findings indicative of APM. Only very recently, first data on intra- and inter-observer

variability on diagnostic methods relevant for the detection of APM were published (Burfeind *et al.*, 2010; Leutert *et al.*, 2012; Suthar *et al.*, 2011)

APM usually occurs within 14 days after calving (Sheldon *et al.*, 2006) and should therefore be diagnosed within this period. All of the authors, indicating the time of diagnosis (n = 33) were within this period. Since case definitions in the literature are highly confounded by diagnostic method and the interval postpartum at which diagnosis is made (LeBlanc, 2008) a failure to report the time of diagnosis of APM constitutes a flaw that limits the evidence of the conclusions drawn.

Despite its frequent use the assessment of vaginal discharge has been discussed controversially in current literature. Vaginal discharge can be both, a natural phenomenon after calving and a pathological condition (Lowder, 1993). Furthermore, the mere assessment of discharge without knowing where it comes from (i.e. vagina, cervix, and uterus) may not be reflective of endometrial inflammation. Cows with vaginal discharge might have cervicitis or vaginitis, but no endometrial inflammation (Dubuc *et al.*, 2010). This limitation applies for the diagnosis of APM as well. On the other hand, the assessment of vaginal discharge has been described as an effective, simple and non-invasive method, especially for field use (LeBlanc *et al.*, 2002; Sheldon *et al.*, 2006; Pleticha *et al.*, 2009). Also, a correlation between the type of discharge, the bacterial contamination of the uterus and the immune response of the diseased animal has been demonstrated (Williams *et al.*, 2005).

After all, vaginal discharge by itself is a useful and important criterion to incorporate into a clinical examination (LeBlanc *et al.*, 2002; Williams *et al.*, 2005; Sheldon *et al.*, 2006). Color, smell and viscosity of vaginal discharge should be assessed (Sheldon *et al.*, 2006). A description of these sensorial assessments was provided in 39 studies. Thresholds used to distinguish between healthy and diseased animals were not specified. Also, attempts were not described to objectify findings. In 4 articles, vaginal discharge was used as the only diagnostic criterion for APM without specifying how the discharge was obtained.

The second most commonly used diagnostic tool for APM was a transrectal palpation of the uterus (n = 22). Generally, this method is the most prevalent for the assessment of uterine infections (LeBlanc, 2008). It is well known, however, that transrectal palpation is a subjective method with limited sensitivity (Okano & Tomizuka, 1987). Frequently, rectal temperature was measured to diagnose APM. A high repeatability of rectal temperature measurements in dairy cows has been recently demonstrated (Burfeind *et al.*, 2010). However, when rectal temperature is used as a diagnostic criteria, frequencies of both type I (fever when the animal is actually healthy) and type II errors (no fever when the animal is actually sick) are significant (Kristula *et al.*, 2001; Sheldon & Dobson, 2004; Wagner *et al.*, 2007). A type I error causes financial losses to the producer since a healthy animal is unnecessarily treated. In case of APM an antibiotic drug would be used without need and selection pressure on pathogens exerted potentially contributing to the emergence of resistance. A type II error leaves a beneficial treatment effect on health and performance unrealized and might constitute an animal welfare issue, as the sick animal is not being treated and continues to suffer. None of the 48 studies addressed the issue of sensitivity or specificity of rectal temperatures for the diagnosis of APM. Plausible factors (e.g. ambient temperature, parity) that can influence body temperature were discussed only in 2 papers. Also the time of the temperature measurements relative to calving varied from 1 to 21 d postpartum.

Due to these limitations fever has been considered less reliable than including an examination for abnormal uterine discharge because pyrexia is not consistently associated with pathogenic bacteria in the uterine lumen (Sheldon & Dobson, 2004). In all 21 studies rectal temperature was used in combination with other diagnostic methods.

While the assessment of the general condition is recommended as part of a clinical examination (Jackson & Cockcroft, 2002) it is a subjective criterion that is difficult to

validate. External signs of toxemia, potentially occurring in APM, are lacking enough accuracy since they do not appear constantly (Paleniki *et al.*, 2009).

Four of 48 studies monitored milk yield as part of the APM diagnosis. A drop in milk yield can be observed from the first day of APM (Smith *et al.*, 1998). However, it has also been observed that milk yield corresponds poorly with mild or subclinical conditions (Urton *et al.*, 2005) and can even increase in cows with a mild fever (Rajala-Schultz *et al.*, 1999).

In the past few years several papers discussed the diagnostic value of acute phase proteins as an indicator for APM (Smith *et al.*, 1998; Regassa & Noakes, 1999). A haptoglobin concentration >10 mg/dl has been shown to indicate an acute infectious process in dairy cows and can support the diagnosis of APM (Hirvonen *et al.*, 1999). Haptoglobin is particularly valuable in the early diagnosis of APM, because the concentration already increased 2 d before the clinical signs of APM were diagnosed (Huzzey *et al.*, 2007). Furthermore, a relationship has been demonstrated between an elevated haptoglobin concentration and the bacterial contamination of the uterus (Williams *et al.*, 2007). Haptoglobin is not specific for uterine infections, however, and therefore should not be used as a single diagnostic criterion for APM (Smith *et al.*, 1998; Drillich *et al.*, 2007).

Overall, 9 different methods for the diagnosis of APM were reported. Interestingly, none of the publications provided evidence or discussed whether the combination of methods increased the overall diagnostic performance to identify cows with APM.

For an ideal combination of diagnostic methods it is of interest to estimate their sensitivity and specificity. Ideally, each diagnostic procedure has a different property, leading to an increased overall sensitivity and specificity of the screening or diagnosis program (Qin J, 2010). Because a gold standard to verify inflammation of the uterus is lacking (Drillich *et al.*, 2007), only few studies reported data on sensitivity and specificity and those that do reflect only a comparison of a new method with a reference method. Therefore it is impossible to determine a potential increase in sensitivity or specificity for a combination of methods. Only few authors (n = 7) discussed the diagnostic methods for APM and their possible limitations.

The most frequently cited paper has been authored by 4 internationally recognized experts in the field of uterine diseases (Sheldon *et al.*, 2006). This paper provides current and probably the best evidence to support diagnostic methods for the detection of APM in dairy cows. The author's advice is to perform the diagnosis on the basis of clinical signs of illness and the assessment of vaginal discharge. A scoring system for APM has been recently developed (Sheldon *et al.*, 2009b). Unlike the one for endometritis (Sheldon & Dobson, 2004) this has not been consistently adopted yet for APM. Overall, our results clearly demonstrate that more high quality research is necessary to better understand relationships between limitations of diagnostic methods and diagnostic and therapeutic errors.

Our findings encourage authors to explicitly describe implementation of diagnostic methods and to define possible findings, thresholds, and test characteristics or discuss the missing thereof. Practitioners and herd personnel should be aware of the potential of type I and type II errors. In some cases information on the magnitude of such errors is not available due to the lack of a gold standard. It is obvious that more high quality research is necessary to address issues related to APM and crucial to the dairy industry such as prudent use of antibiotics, animal welfare and financial costs.

Figure 1. Citation map showing the distribution of citations provided for the implementation of diagnostic methods for acute puerperal metritis.
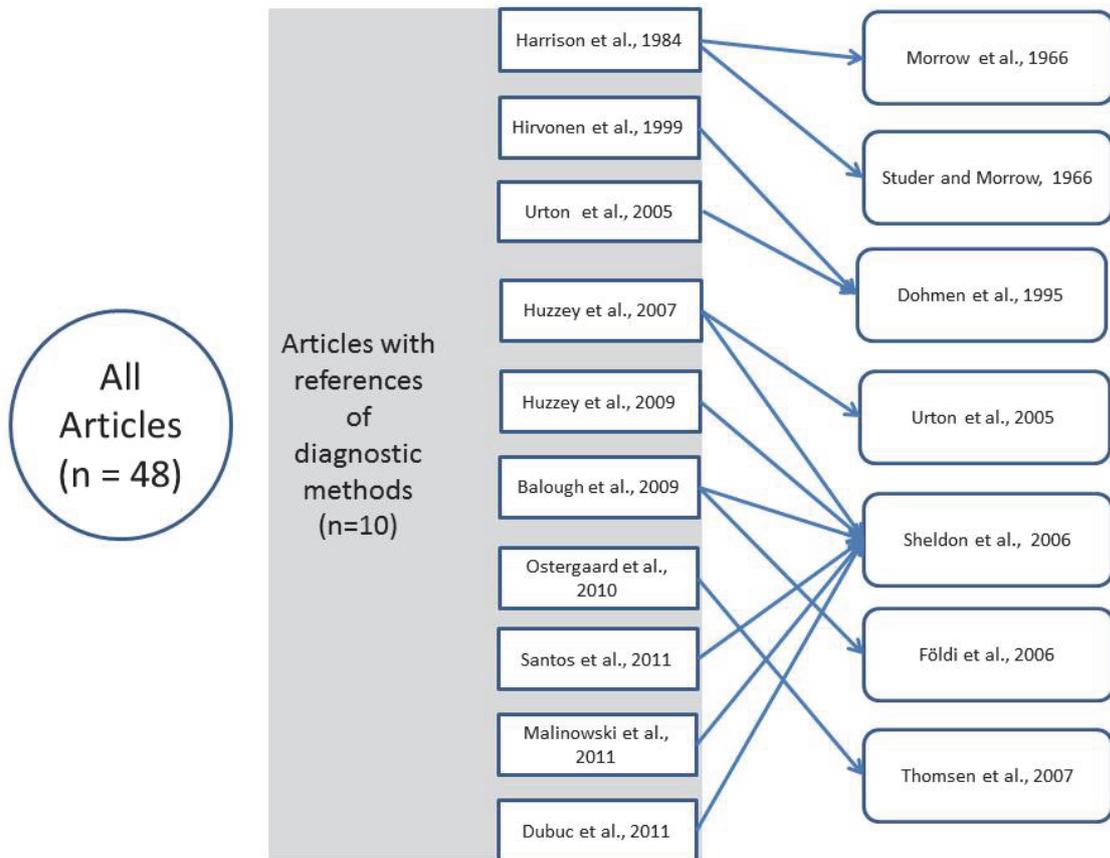
Table 1. Results of a literature search using the search criteria "acute AND metritis" OR "puerperal AND metritis" AND "dairy cow" conducted in 4 journals (Journal of Dairy Science, Theriogenology, The veterinary journal and Animal reproduction science) with an impact factor between 1.7 and 2.9 considering specific exclusion criteria

Criteria	Before 1971	1971-1980	1981-1990	1991-2000	2001-2010	2011	Total
Total found	9	14	32	45	149	10	259
Reviews	2	1	2	1	6		12
Personal experiences	3	3	3	6	17		32
Meta-analyses			4	4	2		10
Questionnaires					2		2
Abstracts	2			3	3	1	9
Other species than cattle			2		8	1	11
Other diseases than APM[1]			2	8	19	1	30
In vitro studies or post mortem evaluation				3		3	6
Acute puerperal metritis not primary research focus	2	9	13	14	63	1	102
Total excluded	9	13	26	36	123	4	211
Remaining articles		1	6	9	26	6	48

Table 2. Evaluation of the description of diagnostic methods in 48 research articles from Journal of Dairy Science, Theriogenology, The veterinary journal and Animal reproduction science according to the STARD-checklist

Presence[1] or absence[2] of description of			
Implementation	Possible findings	Thresholds	Test characteristics
Specification of materials and instruments together with their instructions for use and specific measures in participants	Expressions of test results are provided	Definition of category boundaries are provided	The statement of sensitivity and specify show, how well the test results corresponded with the presence or absence of the target condition

[1] = coded as yes
[2] = coded as no

Table 3. Research articles (n = 48) from Journal of Dairy Science, Theriogenology, Animal Reproduction Science and The Veterinary Journal used for final evaluation.

Lead- author	Year	Journal*	Lead- author	Year	Journal*
Gustafsson	1976	Therio	Nocek	2006	JDS
Johnson	1981	JDS	Benzaquen	2007	JDS
Harrison	1984	JDS	Bobe	2007	ARS
Harrison	1986	JDS	Drillich	2007	JDS
Markusfeld	1986	JDS	Huzzey	2007	JDS
Carson	1988	Therio	Garcia- Ispierto	2007	Therio
El- Azab	1988	Therio	Watters	2008	JDS
Slama	1991	Therio	Lopez- Gatius	2008	Therio
Esteban	1994	JDS	Balough	2009	Therio
Barton	1996	JDS	Cerri	2009	JDS
Beckett	1998	JDS	Galvao	2009	JDS
Smith	1998	JDS	Huzzey	2009	JDS
Hirvonen	1999	Therio	Law	2009	JDS
Ostergaard	1999	JDS	Lima	2009	JDS
Loeffler	1999	JDS	Silvestre	2009	ARS
Jirrotsma	2000	Therio	Galvao	2010	JDS
Drillich	2001	JDS	Duboc	2010	JDS
Kaim	2003	JDS	Ostergaard	2010	JDS
Mateus	2003	ARS	Olson	2011	JDS
Reist	2003	Therio	Santos	2011	JDS
Mendelez	2004	JDS	Silvestre	2011	JDS
Gröhn	2004	JDS	Dubuc	2011	JDS
Dann	2004	JDS	Malinowski	2011	TVJ
Urton	2005	JDS	Toni	2011	JDS

*ARS = Animal reproduction science, JDS = Journal of dairy science, TVJ = The veterinary journal, Therio = Theriogenology

Table 4. Distribution of methods utilized alone or in combination to diagnose acute puerperal metritis in dairy cattle considering 48 peer reviewed publications of 4 journals (Journal of Dairy Science, Theriogenology, The veterinary journal and Animal reproduction science) with an impact factor between 1.7 and 2.9

Number	Method Type	Total used	Used alone	1	2	3	4	5	6	7	8	9
				Combined with Number								
1	Assesment of vaginal discharge	39	4		18	21	14	11	5	4	4	2
2	Transrectal palpation	22	3	18		10	9	5	3	2	3	1
3	Rectal temperature	21	0	21	10		6	10	3	4	4	0
4	Discharge by gloved hand, vaginoskopy or metricheck	15	1	14	9	6		3	4	1	2	0
5	General condition	11	0	11	5	10	3		2	3	2	0
6	Bacteriological examination	5	0	5	3	3	4	2		0	1	0
7	Milk yield	4	0	4	3	4	1	3	0		2	0
8	Blood parameters	4	0	4	3	4	2	2	1	2		0
9	Visual inspection	3	1	2	1	0	0	0	0	0	0	
		124	9	79	52	57	39	36	18	16	18	3

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2.2. Technical note: Intraobserver, interobserver and test-retest reliabilities of an assessment of vaginal discharge from cows with and without acute puerperal metritis.

EVALUATION OF VAGINAL DISCHARGE

Technical note: Intraobserver, interobserver and test-retest reliabilities of an assessment of vaginal discharge from cows with and without acute puerperal metritis

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2.3. Technical note: Evaluation of odor from vaginal discharge of cows in the first 10 days after calving by olfactory cognition and an electronic device

EVALUATION OF VAGINAL DISCHARGE BY OLFACTORY COGNITION AND AN ELECTRONIC NOSE

Technical note: Evaluation of odor from vaginal discharge of cows in the first 10 days after calving by olfactory cognition and an electronic device.

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2.4. Comparison of two Monitoring and Treatment Strategies for Cows with Acute Puerperal Metritis

COMPARISON OF TWO STRATEGIES FOR COWS WITH ACUTE PUERPERAL METRITIS

Comparison of two Monitoring and Treatment Strategies for Cows with Acute Puerperal Metritis

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Abstract

The objective of this study was to compare two strategies for screening and subsequent treatment of acute puerperal metritis (APM) in dairy cows. Therefore, we conducted a study with 79 cows, having APM (cows, having an enlarged uterus with fetid watery red-brown vaginal discharge and fever (> 39.5°C)) and 114 healthy control cows. Cows with APM were divided into two groups (treated n= 67 cows, not treated n= 12 cows). The treated animals were further subdivided into two groups (treated between D1 and 4post partum, n=12 and treated between D5-10, n= 55).

Serum haptoglobin concentrations, milk yield, cure rate, prevalence of endometritis and cervical diameter on DIM 21 to 27 were compared between the groups. Cows were defined as cured when their rectal temperature was < 39.5°C four days after treatment and did not rebound over 39.4°C until the end of the screening period which was DIM 10. The results of this study did not show any significant differences in cure rates, milk yield, serum haptoglobin concentrations on DIM 2, 5 and 10 and subsequent uterine health (DIM 21 to 27). Considering this study as a proof of concept study, we conclude that there might not be a negative impact following a screening and treatment protocol beginning at DIM 5 and leaving early APM cows untreated. This hypothesis needs to be confirmed by a larger field study.

Furthermore, antimicrobial therapy could be avoided in 12 of 55 (21.8 %) cows in group 2 due to the protocol implementing treatments after DIM 5. These cows did not show signs of APM during the following five days. Therefore, these animals were considered as self-recovered leading to a cure rate of at least 21.8 % (12 of 55 cows).

Keywords: acute puerperal metritis, treatment, haptoglobin, antibiotic drugs

1. Introduction

Acute puerperal metritis (**APM**) is an acute systemic illness due to infection of the uterus, occurring within 21 days after parturition [1]. A case of APM is diagnosed based on an abnormally enlarged uterus accompanied by fetid watery red-brown vaginal discharge (**VD**), fever (> 39.5°C) and signs of systemic illness (i.e. decreased milk yield, decreased dry matter intake, elevated heart rate and dehydration) [1]. In studies, applying these criteria the prevalence of APM in dairy cows ranged from 15.3 to 69 % [2-4].

Reduced milk yield, increased culling rate, and treatment costs in cases of APM result in substantial economic losses. The total costs per case of APM have been calculated to approximate US $ 329 to US $ 386 [5]. The prevalence rates and the reported costs underline the importance of this disease. Also considering the life-threatening character of APM [6] strategies for effective prevention, accurate and early diagnosis, and efficacious treatment of APM are of great importance.

Recently the systemic use of antimicrobial agents was proven to be an effective treatment strategy and thus recommended by several authors [7-9]. Most frequently used drugs for treatment of APM are penicillin, oxytetracycline, ampicillin and ceftiofur [7, 10].

The use of antimicrobial agents is inherently associated with selective pressure for the emergence of resistant bacteria, which stresses the importance of their prudent use [11]. More recently, several publications have expressed serious concerns regarding resistance in zoonotic pathogens in cattle [12-14]. Ceftiofur, a third generation cephalosporin has been demonstrated to be an efficacious treatment for APM in several research trials [6, 15, 16]. Advantages include demonstrated efficacy and a zero day withdrawal period for milk [17]. However, third-generation cephalosporins are valued for treating serious infections in human

medicine. Therefore, the use of ceftiofur in dairy cows could be a potential threat to the ability to cure life-threatening infections in humans [18].

For an early detection of APM a daily measurement of rectal body temperature during the first 4 to 13 DIM was recommended [6, 19]. Thresholds to define fever in dairy cows range from 39.4°C [19] to 39.7°C [9]. In the early postpartum period an increased body temperature most likely indicates a uterine infection [20].

Using rectal temperature as diagnostic criterion, frequencies of both type I (fever when the animal is actually healthy) and type II errors (no fever when the animal is actually sick) occur [9, 21, 22], although a high repeatability of rectal temperature measurements has been demonstrated [23]. Furthermore, two recent studies demonstrated that body temperature of fresh cows in the first days after parturition can significantly increase physiologically under hot weather conditions [24, 25]. Due to these limitations, diagnosing fever alone is less reliable than including an examination for abnormal uterine discharge because pyrexia is not consistently associated with pathogenic bacteria in the uterine lumen [9].

Several studies examined the diagnostic value of acute phase proteins as indicators for APM [10, 26]. Serum haptoglobin (**Hp**) concentrations between 1.06 and 1.9 g/L have been shown to indicate an acute infectious process in dairy cows. Because serum Hp concentrations increased two days before clinical signs (VD and body temperature $\geq 39.5°$) of APM were diagnosed [27] and were related to the bacterial contamination of the uterus [28] they can support the diagnosis of APM [9, 27].

Recent studies investigating treatments of APM measured body temperature for 3 to 14 days postpartum and utilized body temperature as part of the treatment decision. None of the studies, however, discussed the relationship between the frequencies of temperature measurements and type I errors [29]. Therefore, the objectives of this study were to compare two strategies of monitoring fresh cows for APM differing in their screening intensity. Subsequently we compared two treatment strategies and an untreated group of cows with APM.

2. Materials and Methods

2.1. Experimental animals and design

The study was conducted on a commercial dairy farm in Sachsen-Anhalt, Germany between October and December 2011 housing 1,200 Holstein dairy cows with an average 305 d milk production of 10,147 kg (3.98 % fat and 3.33 % protein). Cows were managed according to the guidelines set by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products [30].

Lactating cows were housed in a free-stall barn with cubicles equipped with rubber mats and slotted floors. Early-postpartum cows were fed a total mixed ration consisting of 34.1% corn silage, 20.5% grass silage, 4.2% barley straw, and 41.2% concentrate mineral mix on a dry matter basis distributed with a conveyer belt system up to 10 times per day. Cows were milked 3 times a day (06.00, 14.00, 22.00). Milk yield was recorded daily by using the parlor software (Fullexpert Software, 3.02, Lemmer Fullwood, Lohmar, Germany).

Cows entered the experiment one day after calving at 07.00. Cows that received anti-inflammatory drugs or antimicrobial drugs for purposes not related to the study (e.g. acute mastitis) or suffered from other inflammatory diseases than APM were excluded from the trial (n = 28).

Cows were randomly allocated to one of two groups according to the last digit of their ear tag. Cows with an odd number (1, 3, 5, 7, 9) were allocated to group 1 and cows with an even number (0, 2, 4, 6, 8) were allocated to group 2 (Figure 1). All cows underwent a general clinical examination including rectal temperature and VD daily between 08.00 and 11.00. All

examinations were performed by one of three investigators. To standardize the definition of disease, a scoring system has been used (0 = no discharge, 1 = normal lochial secretion; not smelly, viscous, reddish brown, 2 = fetid, watery, reddish-brown VD). Every Wednesday, the diagnostic procedures were performed by all three investigators jointly in order to assure a homogenous diagnostic classification. Cows having fetid, reddish-brown, watery vulvar discharge in combination with a rectal temperature ≥ 39.5°C (fever) were diagnosed as having APM. Cows that did not expel their fetal membranes within 24 h postpartum were defined as having retained fetal membranes (RFM). In group 1, cows suffering from APM (together with a rectal temperature ≥ 39.5°C and fetid discharge) received a systemic antimicrobial treatment of 6.6 mg/kg BW ceftiofur crystalline-free acid (Naxcel, Pfizer Limited, Kent, United Kingdom) on the day of diagnosis (independent of DIM; n = 28). Cows with APM in group 2 did not receive any treatment before DIM 5 (n = 50) even if they would have been diagnosed as suffering from APM before DIM 5. Therefore, it was possible, that cows diagnosed with APM at DIM 1 to 4 in group 2 (no treatment before DIM 5) self-recovered until DIM 5 and consequently did not meet our inclusion criterion (fever and abnormal VD) for an antimicrobial treatment thereafter (i.e. DIM 5 to 10). Therefore, these cows did not receive antimicrobial treatment (no treatment at the day of diagnosis of APM or later, **NoTx@D**, n = 12). If APM was diagnosed between DIM 5 to 10 cows were treated as described above (treatment at DIM 5 to 10, **Tx@D5 to 10**). Retrospectively, cows were categorized into 2 classes according to the time of treatment [i.e. treatment at DIM 1 to 4 (**Tx@D1 to 4**) vs. treatment DIM 5 to 10 (Tx@D5 to 10)] as shown in Figure 1. Considering the life-threatening character of APM [5] all cows diagnosed with APM after DIM 4 were treated. Cows were defined as cured when their rectal temperature was < 39.5°C four days after treatment and did not rebound over 39.4°C until the end of the screening period (DIM 10).

2.2. Methods of sampling

In all cows, rectal temperature was measured daily with a digital thermometer (Microlife AG, Heerbrugg, Switzerland) until DIM 10. Measurements were performed at the same time of the day (07.00 to 09.00) and at the same insertion depth (8 cm) to minimize any bias due to the measuring process [23]. Vaginal discharge was collected through manual vaginal examination with a gloved hand. To minimize contamination of the vagina the vulva and perineum were cleaned with dry paper towels before the discharge was collected. Manual vaginal examination has been validated and does not cause uterine bacterial contamination, provoke an acute phase protein response, or delay uterine involution [31]. For each sample of VD, quantity, color, proportion of pus, consistency and smell were evaluated by one of the performers. Therefore, cows with no discharge or physiologic lochia were classified as healthy and cows with fetid watery red-brown vaginal discharge and fever (> 39.5°C) were classified as having APM [1,6].

Body condition score was determined at DIM 2 and 10 [32]. Blood samples were collected at DIM 2, 5 and 10 from coccygeal vessels using sterile vacuum tubes (Venoject II, Termumo Europe N.V., Leuven, Belgium). Within 2 h after sampling, blood samples were centrifuged at $1,000 \times g$ for 10 min at room temperature and serum stored at −18°C until their analysis for serum Hp concentration.

Sampling for bacteriological examination was performed immediately after clinical examination and diagnosis of APM and in all cows at DIM 5, respectively. The vulva was cleaned with dry paper towels and the vulvar lips parted. A sterile, disposable insemination pipette for horses (75 cm, Minitube GmbH, Tiefenbach, Germany), protected through a hygienic sheath (Minitube GmbH, Tiefenbach, Germany) from vaginal contamination, was advanced under manual control into the vagina and fixed in the internal opening of the cervix. Uterine fluid was aspirated using a 20 ml syringe (Soft-Ject, Luer, Henke- Sass, Wolf GmbH,

Tuttlingen, Germany). The fluid was then transferred into sterile test tubes (Sarstedt AG & Co, Nürnberg, Germany), soaked into a sterile cotton swap and inoculated into a tube containing a reduced transport medium to maintain the viability of anaerobic, facultative and aerobic microorganisms during transport from the patient to the laboratory within 2 h of sampling (BD BBL Prepared culture medium; BBL Port-A-Cul Tubes, Becton, Dickson and Company, Sparks, MD 21152 USA).

All cows were examined with the metricheck device (Metricheck, Simcro, New Zealand) for (muco-) purulent VD as a sign for endometritis between 21 to 27 DIM [33]. Vaginal discharge was categorized on a 4-point classification system (0 = clear mucus, 1 = mucus containing flecks of pus, 2 = discharge containing less than 50 % of pus, 3 = discharge containing more than 50 % of pus) as described previously [34]. Cows with a vaginal discharge score (**VDS**) of 0 were classified as healthy, whereas cows with a VDS of 1 to 3 were classified as suffering from endometritis. The cervical diameter was measured with an ultrasonic scanner equipped with a 7.5 MHz linear rectal transducer (Easi-scan; BCFtm Technology Ltd., Livingston, UK). Cervical diameters were measured by placing the transducer over the middle of the cervix [35]. The images were stored and later analyzed on the computer screen. The distance from serosa to serosa was considered to correspond to the cervical diameter. Diameters were measured in power point 2010 (Microsoft Corporation, Redmont, USA) utilizing a ruler with mm increments.

2.3. Analytical Methods

The microbiological analyses were performed by a commercial laboratory (Synlab.vet GmbH, Leipzig, Germany). Swabs were cultured aerobically on columbia blood agar, columbia blood agar with colistin and nalidixinacid (CNA), endo agar and sabouraud- glucose agar. Bacteria were identified after 24 h (pro re nata 48 h) of incubation at 35 ± 2 °C. For the differential diagnosis of *Arcanobacterium pyogenes,* swabs were cultured under CO_2 atmosphere (6 - 8%) for 48 h at 35 ± 2 °C on columbia blood agar with CNA. For anaerobic bacterial growth, swabs were cultured on Schaedler agar and Schaedler KV agar for 48 h at 35 ± 2 °C and subsequently for another five days (overall seven days). Identification was based on biochemical recognition using Api consisting of a strip of biochemical tests combined with a database (BioMerieux SA, Marcy L'Étoile, France) for anaerobes and gram stain, as well as mass-spectrometry through MALDI-TOF (Synlab.vet, Leverkusen, Germany) for anaerobes. An antibiogram of isolated strains was performed through MICRONAUT-S, a software-supported antimicrobial sensitivity test (Merlin, Bornheim-Hersel, Germany). Bacterial sensitivity was tested for tetracycline and cefotaxim (a human cephalosporin analogous to Ceftiofur). Serum samples were sent to a commercial lab (Synlab laboratories, Berlin, Germany) and analysed for Hp concentrations by an ELISA (Sunrice reader, Tecan, Maennedorf, Switzerland). The device has been validated by using cattle serum with Hp concentrations between 0.6 and 1.3 mg/ml. The lower limit of detection was 0.31 mg/ml. All samples with concentrations less than 0.31 mg/ml were set to 0.31 mg/ml. Intra-and interassay coefficient of variation was 5.3 to 6.3 % and 4.1 to 5.7 %, respectively.

2.4. Statistical analysis

Data were analyzed using SPSS for Windows (Version 19.0, SPSS Inc. Munich, Germany). Proportion of cows with fever and with endometritis, as well as cure rates between classes (Tx@D1 to 4, NoTx@D, Tx@D5 to 10 and healthy cows) were compared using chi-square analysis.

Haptoglobin concentrations at DIM 2, 5 and 10 were compared in a general linear model for repeated measurements, using time of treatment as a fixed factor, cow as random

factor and DIM as the repeated factor. The analysis of cervical diameter between the groups was performed by oneway ANOVA. The effects of parity, presence or absence of APM earlier in lactation and presence or absence of endometritis (VDS of 0: healthy, VDS of 1 to 3: chronic endometritis) were tested by the Mann-Whitney U Test.

The effect of degree of endometritis was tested by using the Kruskall Wallis Test. Level of significance for all statistical analysis was $P= 0.05$. Correlations between serum Hp concentration and fever (rectal temperature $\geq 39.5°$) were calculated using Spearmen's rank correlation coefficient.

The relationship between the variables (parity, DIM, group, treatment class, state of health, fever and serum Hp concentration) and milk yield was analyzed in a linear mixed model with DIM as a repeated factor and cow as random factor. First, the relationship of each variable with milk yield was evaluated in a univariate analysis. All variables with a $P < 0.2$ were included in the final model which was constructed in a backward stepwise manner. Pearson's correlation between the parameters was determined and if two variables were highly correlated (r > 0.6) only the variable with the smallest P-value was included in the final model.

3. Results

3.1. Acute puerperal metritis, endometritis and cervical diameter

Twenty-eight of 221 cows were withdrawn from the analyses because of treatments with anti-inflammatory or antimicrobial drugs not related to the study (n = 13) (e.g. acute mastitis) or culling before the end of the observation period (n = 15). The allocation to our study groups was 78 for group 1 and 115 for group 2 (Figure 1).

In group 1 and 2, 29 and 38 cows were treated with a single injection of 6.6 mg/kg BW ceftiofur crystalline-free acid (Naxcel, Pfizer Limited, Kent, United Kingdom), respectively. Twelve cows (41.4 %) were diagnosed with APM in group 1 and 19 cows in group 2 (34.5 %) during 1 to 4 DIM ($P = 0.66$). According to the trial protocol those were treated only in group 1 while they remained untreated in group 2. Twelve of 19 (63.1 %) cows in group 2 recovered without any treatment (NoTx@D) and were analyzed separately.

Cows, that did not develop APM during DIM 1 to 10 were used as healthy controls (n = 114). Figure 2 and Table 1 show the number of cows in the different classes showing fever during the first 10 DIM.

The overall incidence of APM was 40.9%. The incidence in multiparous and primiparous cows was 33.6% (44 of 134) and 61% (35 of 59), respectively ($P = 0.03$). First signs of APM (watery, fetid, red- brown VD and fever) were evident 5 ± 3 (median ± SD) postpartum. Table 2 shows the occurrence of fever and abnormal VD (fetid, watery, red-brown) for healthy cows and cows with APM.

The overall incidence of RFM was 11.9 % (n = 23). Fourteen of these cows (six in Tx@D1 to 4 and eight in Tx@D5 to 10) subsequently developed APM at day 3 ± 3 (median ± IQR). Five cows did not develop APM and four were culled. Reasons for culling were mastitis (n = 2) and ketosis (n = 2) between DIM 13 and 24.

Incidence of endometritis at DIM 21 to 27 for all cows (n = 193) was 65.3%. The incidence for Tx@D1 to 4, NoTx@D, Tx@D5 to 10 and healthy cows was 58.3%, 70.0%, 75.5% and 67.3%, respectively ($P > 0.05$).

Mean cervical diameter at DIM 21 to 27 was 4.2 ± 0.7 cm, 4.0 ± 0.5 cm, 3.7 ± 0.4 cm and 3.5 ± 0.5 cm for Tx@D1 to 4, NoTx@D, Tx@D5 to 10 and healthy cows, respectively. There were no significant differences between parity ($P = 0.28$) and the presence or absence of APM earlier in lactation ($P = 0.07$) between those classes. Cows suffering from grade 3 endometritis (i.e. purulent) had greater cervical diameters (3.9 ± 0.5 cm) than cows with lower

grades (grade 1: 3.6 ± 0.5 cm and grade 2: 3.5 ± 0.5 cm) ($P < 0.05$). There was no difference in cervical diameters for healthy cows and cows with endometritis (grade 1, 2 and 3, $P > 0.05$).

Cure rates were 75 % (9/12) and 85.4 % (47/55) for Tx@D 1 to 4 and Tx@D5 to 10 cows, respectively ($P = 0.78$).

3.2. Haptoglobin

Mean Hp concentration regardless of DIM was 1.2 ± 0.8 mg/ml and 1.7 ± 0.8 mg/ml for healthy cows and cows suffering from APM, respectively ($P = 0.02$). Mean Hp concentrations on DIM 2, 5 and 10 are shown in Figure 3. The Mann-Whitney U Test revealed significantly higher serum Hp concentrations for cows with APM than for healthy cows for all 3 collection times (DIM 2, 5 and 10) ($P < 0.05$).

The general linear model revealed a significant influence for DIM. Serum Hp concentrations were higher for DIM 5 than at DIM 2 and 10 ($P < 0.05$) while there was no difference between serum Hp concentrations at DIM 2 and 10 ($P = 0.06$). Mean Hp concentration was higher for Tx@D5 to 10 cows compared to healthy cows at DIM 5 ($P < 0.05$). No differences were found within the classes Tx@D1 to 4, NoTx@D, healthy cows ($P = 0.11$) and Tx@D1 to 4, NoTx@D, Tx@D5 to 10 ($P = 0.89$) for DIM 2, 5 and 10 (Figure 3). Correlation between the serum Hp concentration and the appearance of fever at DIM 2, 5 and 10 was r = 0.43, 0.41, 0.45, respectively ($P < 0.01$).

3.3. Bacteriology

The most prevalent pathogen from uteri of cows with APM were *Escherichia coli* (53%, n = 37), *Arcanobacterium pyogenes*, (47%, n= 32), *Fusobacterium necrophorum* (30%, n = 20) and *Prevotella spp.* (20%, n = 12). Bacterial sensitivity of all considered species was 75.4% and 100% for Tetracyclin and Cefotaxim, respectively.

3.4. Milk yield

The linear mixed model revealed a significant influence for DIM with an increasing milk yield depending on DIM and parity on milk yield with a lower milk yield in primiparous cows. Not surprisingly, our analysis revealed a high correlation between treatment class and the presence or absence of APM (r = 0.85), therefore treatment class has been excluded from our model. There was no influence of the presence or absence of APM ($P = 0.97$), presence or absence of fever (P = 0.66) and serum Hp concentrations ($P = 0.59$) on milk yield.

4. Discussion

The objective of this study was to compare two strategies for screening and subsequent treatment of APM. In the first group, cows with APM were treated immediately when imminent signs of APM were present, while cows in group 2 were not treated before DIM 5 even if they suffered from APM. Retrospectively, cows were categorized into 2 classes according to the time of treatment [i.e. treatment at DIM1 to 4 (Tx@D1 to 4) vs. DIM5 to 10 (Tx@D5 to 10)].

In this study, a cow with APM was defined as having fetid, reddish-brown, watery vaginal discharge in combination with a rectal temperature $\geq 39.5°C$ [19, 36]. The 40.9 % overall incidence of APM in our study was within the range of reported incidence rates of 15.3 to 69 % of previous studies [2-4]. First signs of APM were diagnosed at DIM 5 ± 3 which is also consistent with other studies; showing ranges from 5.3 to 7 days postpartum [37-39]. In cows with RFM, APM was prevalent at DIM 3 ± 3 postpartum. The difference of

the day of diagnosis of APM between cows with and without RFM was not significant (P = 0.12).

Haptoglobin has been shown to reflect inflammatory processes in the early postpartum period [6, 27]. The absolute concentrations as well as the differences between APM (1.7 ± 0.8 mg/ml) and healthy (1.1 ± 0.78 mg/ml) cows agree with previous findings [6, 27]. The increase in the concentration of serum Hp in cows with APM on DIM 5 and the considerable difference to healthy control cows (P < 0.05) confirmed the presence of an inflammatory process in cows with APM. Furthermore, serum Hp concentrations were positively correlated with fever at each sampling time (DIM 2, 5, and 10). The results show that serum Hp concentrations were significantly higher for Tx@D5 to 10 cows compared to healthy cows, which indicates that these cows were actually sick according to previous studies [6, 27]. Serum Hp concentrations for Tx@D1 to 4 and NoTx@D did not differ (P = 0.11). This may be due to the early treatment of Tx@D1 to 4 preventing worsening of the inflammation, an increase of serum Hp concentration and causing a more rapid decrease. These findings might support the conclusion that an early treatment would be advantageous. Another possible explanation could be that NoTx@D cows were only mildly metritic [27] which was not classified as such with the implemented diagnostic criteria allowing them to self-cure. Furthermore, they might have been misdiagnosed false positively healthy (type I error).

It has been demonstrated, that fever alone is not a reliable sign for infections in the first days after parturition [9, 19, 24]. Furthermore, the threshold for fever (39.3 to 39.7°C) has been discussed controversially in the literature [19, 24, 40]. Recent studies have identified factors that might considerably increase physiological rectal temperature such as parity and ambient temperature [22, 24, 25]. Our data showed that 36.8% of all healthy cows enrolled had a rectal temperature ≥ 39.5°C during the first 4 DIM which is consistent with previous findings [24]. Most recently a systematic review of clinical studies addressing APM revealed that rectal temperature, in combination with other criteria (such as abnormal discharge) was a reason for treating cows with APM for 43.8% of the authors (n = 48) [29]. When rectal temperature is used as single diagnostic criterion, frequencies of both type I (fever when the animal is actually healthy) and type II errors (no fever when the animal is actually sick) are significant [9, 21, 22]. To reduce the frequency of these type I errors, the evaluation of VD was included in our diagnosis.

As has been previously described [19, 41] abnormal VD, however, was not always associated with rectal temperature ≥ 39.5°C. In class Tx@D5 to 10, 19 of 55 cows with a fever in combination with abnormal VD (three cows with RFM) were detected during DIM 1 to 4. At DIM 5 twelve of those cows (21.8%) had a rectal temperature below 39.5°C but an abnormal VD. Because these animals were allocated in class Tx@D5 to 10 and our criteria for APM were fever and abnormal VD on the same day, they were not treated (NoTx@D). Furthermore, these cows did not show signs of APM during the following five days. Therefore, these animals were considered as self-recovered leading to a cure rate of at least 21.8 % (12 of 55 cows in Tx@D5 to 10). The cure rate might have been even higher, but due to the study protocol the remaining 39 cows of group 2 were treated and therefore it was not possible to determine the self-cure rate in these cows. Most recently a study with 1023 cows showed a self-cure rate of 55.3% in cows, suffering from APM using a treatment with saline [42]. Implementing a combination of abnormal VD and fever, we avoided the categorization of 42 of 114 (36.8%) healthy cows with a rectal temperature ≥ 39.5° as having APM. This would have led to an antibiotic treatment of another 42 cows and an unnecessary treatment for 21.8% (12 of 55) cows in class Tx@D5 to 10 due to self-recovery.

In total, 50 cows with APM had a temperature ≥ 39.5°C at DIM 1 to 4. Ten of these cows (20.0 %) had a body temperature < 39.5°C at DIM 5 and therefore would have been overlooked in the Tx@D5 to 10 treatment protocol. Indeed, fever reappeared in the following

days and these cows were finally treated for APM. It remains questionable, whether these cows may have benefited from an earlier treatment and could be considered as type 2 errors. We assume that these cows might have had a mild APM [37] and developed a more serious condition after DIM 5. Cure rates ($P = 0.78$) and serum Hp concentrations ($P = 0.89$) however, did not differ between the two treatment classes (Tx@D1 to 4 and NoTx@D). Also, we did not find any noteworthy differences in the incidence of endometritis and cervical diameter, possibly due to the lack of power in our study. Compared to other studies [33, 43, 44] the incidence of endometritis was high in our study. This could be due to the small number of animals enrolled in our study. We decided to dichotomize the VD scores for endometritis for reasons of clarity as has been recommended previously [34, 44, 45].

Mean cervical diameter for healthy and APM cows was 3.6 ± 0.5 cm and 3.7 ± 0.5 cm, respectiely. The measurement of cervical diameter has been demonstrated to be repeatable [46] and agrees well with previous studies which described a range from 3.11 to 4 cm [35, 47, 48]. Cervical diameters did not differ between treatment classes, cows and heifers, or healthy and APM cows, respectively. The only statistically significant difference in cervical diameter was found between grade 3 endometritis and cows with lower grades (i.e. grade 1 and 2). It is questionable whether a difference of 0.4 and 0.3 cm may be clinically relevant. There was no difference between healthy cows and cows with endometritis (grade 1, 2 and 3) ($P > 0.05$). However, a large cervix is supposed to be indicative for cows at risk of a significantly prolonged time to pregnancy [8].

The results of the bacteriological examination revealed a similar distribution of isolates as described by other authors [34, 48, 49]. Cure rates did not differ between classes (NoTx@D, Tx@D1 to 4, Tx@D5 to 10). The definition of a cure was based on rectal temperature alone, without considering the nature of VD. Abnormal discharge without a fever can occur in both, diseased and healthy cows as a result of the opening of the cervix at DIM 7 to 10 [41] or as a sign of clinical metritis defined as abnormal VD with a rectal temperature $< 39.5°C$ [9], which was not the scope of our study. The cut point for a cured animal (day four after treatment) was chosen for reasons of practicability. Other authors chose later cut-points such as day six [7] and day 14 [17] after treatment. Since we followed up cows in our study only until DIM 10 (or until DIM 14 when treated at DIM 10,) the interval for the definition of a cow as being cured or not was shorter. Cure rates might be even higher when considering longer observation intervals. Nevertheless, cure rates of our study agree well with those of others describing a range of 74.3 to 87.4 % [7, 17 42].

Overall, our data on cure rates, milk yield and subsequent uterine health did not reveal any indication of a negative impact of a screening and treatment protocol beginning at DIM 5 and leaving cows with APM earlier than DIM 5 untreated. Ceftiofur crystalline-free acid has been approved for the treatment of APM with a single administration. However, a recent study described a treatment protocol using 2 injections of 6.6 mg/kg BW Ceftiofur crystalline-free acid 3 days apart [42]. It is speculative that a single injection of 6.6 mg/kg BW Ceftiofur crystalline-free acid might not be sufficient to treat cows with APM successfully. Our treatment protocol and the limited sample size in this study might be a reason for the uniformity of results between the groups.

It is noteworthy, however, that other experiments studying new concepts related to APM such as the relationship between prepartum dry matter intake and APM [37], the efficacy of bacterial microbiota in healthy and metric postpartum cows [50], and a comparison of various antibiotic treatments of cows with APM [10] used similar sample sizes (n = 51 to 101). Because of the life-threatening character of APM [6], we decided to test our hypothesis (i.e. delayed antibiotic treatments) in a small study first.

Reproductive performance was not the scope of this study and such measures were not analyzed due to limited numbers of animals. Further studies with an adequate sample size are

warranted to investigate the effect of protocols addressing different treatment intensities on reproductive performance

5. Conclusions

The objective of our study was a proof of concept that a delayed antibiotic treatment of cows with APM did not have profound negative effects on animal health and to provide first results on self-cure rates.

The results of our study show that the implementation of diagnostic performance for APM is crucial to avoid type I and type II errors. Abnormal VD should be considered together with an elevated rectal temperature ($\geq$ 39.5°C). Using the combination of abnormal VD and fever we avoided the categorization of 54 healthy cows (36.8%) with a rectal temperature $\geq$ 39.5°C as having APM leading to antibiotic treatment. Furthermore, antimicrobial therapy could be avoided in 12 cows (i.e. 21.8 % of Tx@D5 to 10) due to the protocol implementing antibiotic treatments after DIM 5. Our data support the use of a close screening protocol of fresh cows for APM beginning at DIM 5, including daily measurement of rectal temperature and the assessment of VD.

As animal welfare and economics are important considerations for commercial dairy farms the scale of the trial was intentionally narrow and the number of animals limited (Tx@D1to4 = 12, Tx@D5to10 = 55, untreated cows n = 12). Further research is necessary to determine if a delayed treatment of APM can contribute to a reduction of antibiotic drug use without jeopardizing animal welfare and reproductive performance. Based on this proof of concept study a field trial with a larger sample size can be conducted to confirm our findings and to study reproductive performance.

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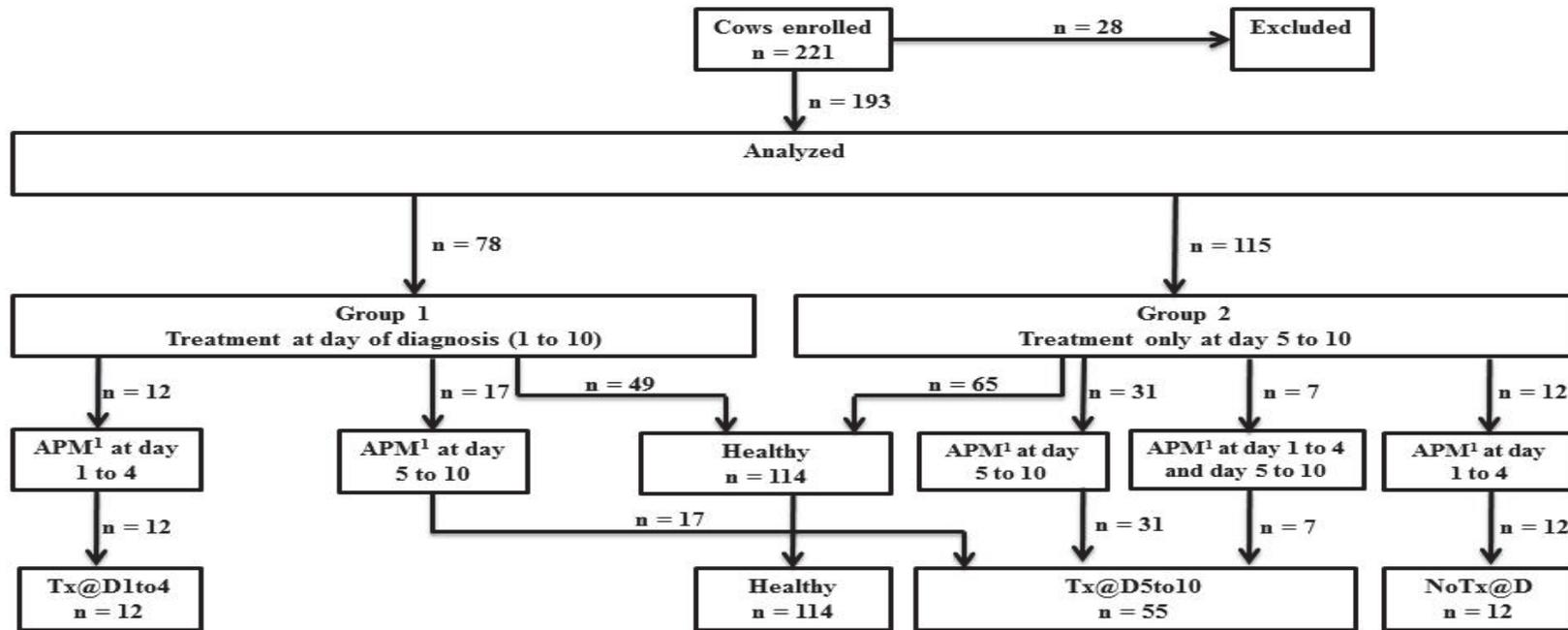
Tables and figures

Figure 1. Allocation of cows enrolled to two different treatment groups and to four different classes.
APM: acute puerperal metritis; NoTx@D: no treatment at diagnosis of APM or later; Tx@D1 to 4 post partum: treatment at days in milk 1 to 4; Tx@D5 to 10: treatment at days in milk 5 to 10.
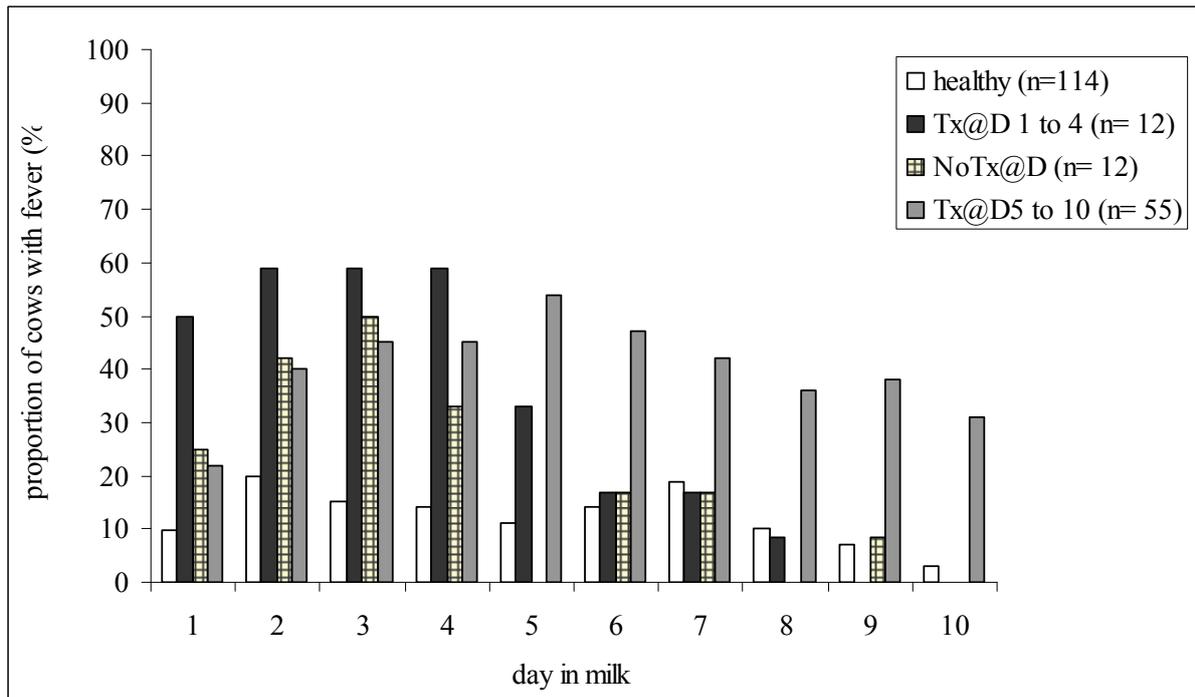
Figure 2. Proportion of cows in the different classes showing fever during the first 10 days postpartum.

Table1. Number and proportion of cows having fever during the first 10 days postpartum

Day in milk	Cows treated for acute puerperal metritis[1]				No Tx@D			Healthy cows		
	[2] n	fever absolute	%	treated	n	fever absolute	%	n	fever absolute	%
1	67	18	26.8	1	12	3	25	114	11	9.6
2	66	29	43.9	4	12	5	41.7	114	23	20.2
3	62	32	51.6	2	12	6	50	114	16	12.3
4	60	32	53.3	5	12	4	33.3	114	16	12.3
5	55	35	63.6	27	12	0	0	114	13	11.4
6	28	26	92.8	10	12	2	16.7	114	16	12.3
7	18	18	100	6	12	2	16.7	114	22	19.3
8	12	12	100	3	12	0	0	114	13	11.4
9	9	9	100	5	12	1	8.3	114	8	7
10	4	4	100	4	12	0	0	114	2	1.7

[1] includes Tx@D 1 to 4 and Tx@D 5 to 10

[2] after treatment cows with fever on the subsequent days are not included
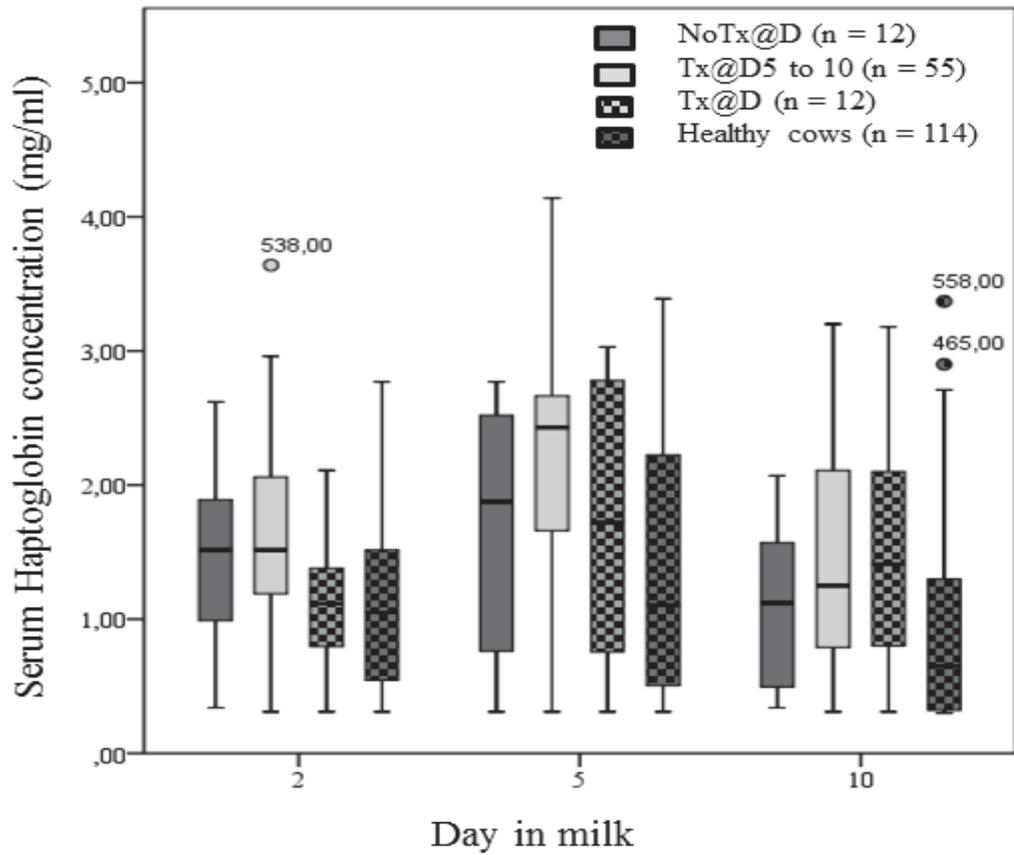
Figure 3. Concentrations of haptoglobin in plasma (mg/ml) for NoTx@D (dark grey boxplot, n = 12), Tx@D 1 to 4 (grey boxplot, n = 12), Tx@D5 to 10 (grey squared boxplot, n = 55) and healthy cows (dark grey squared boxplot, n = 114) at DIM 2, 5 and 10.

Table 2. Occurrence of fever (≥ 39.5) and abnormal vaginal discharge (VD) during the first 10 days postpartum for cows, suffering from APM, compared to healthy control cows

Finding and time of treatment	treated [1] (n = 67)		not treated [2] (n= 12)		healthy [3] (n = 114)	
	n	%	n	%	n	%
Fever	67	100	12	100	66	57.9
No fever	0	0	0	0	48	38.1
Fever @ DIM 1 to 4	50	74.6	12	100	42	36.8
Fever @ DIM 5 to 10	55	82	3	25	47	41.2
Fever and abnormal VD	67	100	12	100	0	0
Fever @ DIM 1 to 4 but not after DIM 5	10	14.9	9	75	35	30.7
Abnormal VD without fever	0	0	0	0	22	17.2
Abnormal VD and fever on different days	42	62.7	3	25	51	44.7

[1] treated cows include Tx@D 1 to 4 and Tx@D5 to 10 (n = 67)
[2] not treated include NoTx@D (n = 12)
[3] cows not affected from APM (n = 114)

3. DISCUSSION

The objective of this thesis was to investigate how relevant research publications address the validity of diagnostic methods for APM as well as the assessment of our sensory diagnostic capabilities, followed by a comparison with an electronic nose. Finally, two monitoring and treatment strategies of fresh cows for APM were compared.

In the first part of my theses, I investigated how intervention studies addressed quality criteria for diagnostic methods used in their experiments. The results show that the evidence supporting the use of various diagnostic methods to identify cows with APM has either been not reported or is weak. Specifically, the investigation was focused on a clear description of the diagnostic methods used to determine cows with APM. Only a small proportion of articles (20.8%) cited references for the diagnostic methods utilized. Schmidt (2003) developed a STARD checklist for authors setting basic points for the construction of the materials and methods section in scientific papers. He specified therein that the description of a diagnostic method should cover a full test protocol including the specification of materials and instruments together with their instructions for use, and specific measures in participants. If this is not the case, it might be difficult to estimate the validity of the diagnostic methods used. However, not only the methods described in the articles were incomplete, our research revealed, that in 23 (47.9%) of the studies the authors gave no clear definition of the disease addressed in their study. Without a definition of a disease, however, it is hardly possible to distinguish between healthy and diseased cows, interpret test results, and provide recommendations for a larger population. It is noteworthy that none of the studies evaluated an intra- or in case of multiple investigators an inter-observer repeatability of findings indicative of APM. Only very recently, first data on intra- and inter-observer variability on diagnostic methods relevant for the detection of APM were published (Burfeind et al., 2010; Leutert et al., 2011).

Our results clearly demonstrate that more high quality research is necessary to better understand relationships between limitations of diagnostic methods and diagnostic and therapeutic errors.

Therefore, the second part of my thesis covers the validity of the most important diagnostic tool to identify cows with APM which is until today the evaluation of vaginal discharge during the first 10 days post partum (Sheldon et al., 2009; Sannmann et al., 2012).

In this part fifteen investigators (including 9 veterinary students in their 5th year and 6 licensed veterinarians working at our clinic) evaluated the same 5 vaginal discharge samples (i.e. 2 samples from cows with APM and 3 samples of healthy cows) on 10 different dates. The samples were blind to the investigators and newly randomized at each evaluation date. One of the samples was doublet for the evaluation of test- retest reliability.

Color, smell and viscosity were evaluated by sensory assessment. Based on these criteria the investigators were held to decide whether the sample came from a healthy cow or a cow with APM. At the end of the experiment all investigators answered a small questionnaire compromising their own perception about the diagnostic value of color, smell and viscosity and their certainty to diagnose a cow with APM correctly.

Our study demonstrates the highest reliabilities within observers (intraobserver reliability) followed by test-retest and interobserver reliabilities. Interestingly, test-retest reliabilities were only slight (K = 0.19 for color) to moderate (K = 0.48 for the diagnostic value) whereas the intraobserver reliabilities ranged from fair (K= 0.32 for the diagnostic value) to moderate (K= 0.6 for viscosity). The finding that intraobserver reliability was higher compared to test-retest reliability provides further evidence that the assessment of VD is

highly subjective. One limitation of our study was the limited number of participants. Our results are in line, however, with previous reports from human and veterinary medicine demonstrating higher within than between observer reliability for estimations such as classification of ankle fractures (Malek et al., 2006), thyroid volume measurements (Andermann et al., 2007) and evaluation of vaginoscopy (Leutert et al., 2011).

For the majority of observer the criterion with the highest diagnostic value to detect a cow with APM was odor of vaginal discharge (49.9%), followed by color (13.9%), and viscosity (23.6%). The remaining 15% did not specify a diagnostic value for the criteria. Interestingly, viscosity has the highest inter- intra and test- retest reliabilities so just the diametrically opposite of the subjective grading by the observer. The highest diagnostic value for the assessment of vaginal discharge was stated for odor (49.9%), the criterion with the lowest reliability. Obviously, there is a discrepancy between reliability and the personal perception of the diagnostic value. This observation is important as it demonstrates the lack of an appropriate clinical foundation to justify the option of antibiotic intervention.

In the field of course we do not evaluate color, smell and viscosity of VD separately but as a combination together with the general appearance of the cow and body temperature. On the other hand, we have various confounders in the field such as lighting, humidity, temperature and distracting odors, exacerbating a correct diagnosis.

The diagnostic value of a combination of color, smell and viscosity to detect cows with APM correctly was estimated to be 91.1% (students 88.4%, veterinarians 94.7%) perfect. This observation is in agreement with the generic recommendation to use of a combination of several imperfect diagnostic tests to create a better reference standard (Todd et al., 1999). As there is no gold standard for the diagnosis of APM (Sheldon et al., 2009) the current combination of assessment of vaginal discharge and measurement of rectal temperature remains the method of choice (Földi et al., 2006; Benzaquen et al., 2007; Sheldon et al., 2009).

Although the overall personal expertise to detect cows suffering from APM correctly as such was estimated to be 59% on a 100% scale. Students felt less confident to diagnose a cow with APM correctly than licensed veterinarians. Students and veterinarians rated their expertise to detect cows with APM correctly with 47% and 75%, respectively. However, we could not confirm an influence of experience in our experiment. While there were significant differences between the two types of observer reliabilities were not consistently higher for the veterinarians.

In conclusion, the evaluation of vaginal discharge by the subjective assessment of human senses remains subject to a range of errors prohibiting a reliable allocation to healthy and sick cows. Recently, attempts have been made to utilize new diagnostic methods for APM such as blood haptoglobin concentrations or electronic nose devices (Huzzey et al., 2009; Sannmann et al., 2013a, Burfeind et al., 2014). Further research is warranted to determine if these methodologies can help improve diagnostic accuracy in the field.

The third part of my thesis aimed to find out whether a technical device would be capable to classify healthy and sick cows more precisely than human senses. Specifically, an electronic nose device was used to evaluate the diagnostic performance of APM based on odor alone and compared with human olfaction. In 3 experiments intra- and interobserver variability of olfactory cognition (i.e., human nose), intraassay variation of the electronic device (i.e., DiagNose; C-it, Zutphen, the Netherlands), and sensitivity and specificity by olfactory cognition and the electronic device, respectively was evaluated.

The result showed a moderate overall agreement between 16 investigators (9 Students and 7 Veterinarians) for the olfactory assessment. Differences in the perception of odors between and within observers can be accounted for by various factors such as age, experience, and environment (Doty et al., 1995; Hadley et al., 2004; Orhan et al., 2012). Intraobserver agreement was proportional to the experience of the observers (veterinarians vs. students) in

diagnosing cows with APM while interobserver agreement was not influenced by experience. As we could notice in the previous part of this thesis, the ability to distinguish healthy cows from cows with APM (sensitivity and specificity) did not differ between students and veterinarians considering presence of fever and abnormal VD at the day of VD collection as a reference.

When testing the electronic Nose for the ability to differentiate between VD of healthy cows and cows with APM we obtained first evidence that the DiagNose is able to detect sick and healthy cows. Therefore, the device could be used as a screening tool to identify cows with APM based on an electronic assessment of odor from VD.

Finally, the results of the human observers were compared with those obtained from the DiagNose. Although a direct comparison was challenging because of the different types of data (ordinal vs. continuous) the sensitivity and specificity of the electronic nose device were higher than those of olfactory assessment. Furthermore, repeatability of odor assessment using the electronic nose was high, whereas the olfactory assessment with multiple observers showed considerable variability.

Despite the promising results, there are some points in the study design we should consider. Because a sensory evaluation of odor of VD was part of the cow side diagnosis (i.e., healthy vs. APM), the sensitivity and specificity determined in the laboratory was potentially biased by the inherent limitations of olfactory cognition by an investigator conducting the examination. As of this writing, no gold standard exists for APM (Sheldon et al., 2006). Therefore, confounding through type I and type II errors cannot be fully excluded.

A confounding factor could be the nature of VD itself, since it even varies in healthy cows up to a certain degree. Furthermore, abnormal VD without a fever could occur in healthy cows as a result of the opening of the cervix at 7 to 10 DIM (Wehrend et al., 2003) and cause type I errors by the assessment of VD alone.

To minimize the risk of such errors, only VD samples collected at DIM 5 were used to avoid a potential bias caused by this natural phenomenon for the evaluation of sensitivity and specificity.

The relatively low sensitivity (75.0%) and specificity (60.1%) for the olfactory assessment might be explained by the variations in color and viscosity of the VD samples distracting the investigators. All observers were familiar with the definition of APM, which includes odor, color, consistency, and a rectal temperature $\geq39.5°C$ and, therefore, may have unconsciously included these attributes into their conclusion. Disregarding color and consistency may be an advantage of the DiagNose system measuring only the concentration of odorous gases. Furthermore, investigators did not have any information on rectal temperature of these cows, which might have biased the diagnosis as well.

This study was designed as a diagnostic accuracy cohort study, which is appropriate for the early investigation of an experimental diagnostic test to determine if further research is warranted, but such studies provide only relative accuracy of an experimental diagnostic test compared with a reference standard (Rodger et al., 2012).

However, this is the first validation of a measuring system to electronically assess odor of VD from cows, with the objective to distinguish between healthy and metric cows. Our data provide the first evidence that the DiagNose system, although imperfect, is a useful tool to improve odor assessment of VD. The current system, however, is not suitable as a screening tool in the field. Further efforts are warranted to (1) adapt such electronic devices to on-farm screening tools, providing just-in-time findings and (2) to determine test characteristics, considering fertility as a gold standard.

While the first three parts of my thesis considered the diagnostic methods, the last part is focused on the management and therapy of cows with APM. As part two and three showed, the sensorial assessment of color, smell, and viscosity of VD of cows postpartum is subjective and needs refinement. This diagnostic limitation requires attention considering the

observation that in most cases of APM antibiotic drugs are used (Drillich et al., 2007; von Krueger et al., 2013). Practitioners are constrained to a prudent antibiotic drug use enclosing the prevention of type I and type II errors while diagnosing a cow with APM. Therefore, in this part a special focus was set on the implementation of diagnostic performance, following a screening and treatment protocol beginning at DIM 5 and leaving cows with an early case of APM untreated. Using the combination of abnormal VD and fever for the diagnosis of APM we avoided the categorization of 54 healthy cows with a rectal temperature $\geq$ 39.5°C as having APM leading to antibiotic treatment. Furthermore, antimicrobial therapy could be avoided in 12 cows due to the protocol implementing antibiotic treatments after DIM 5. These 12 cows self- cured until DIM 5 and did not need any antibiotic treatment. In conclusion, we could avoid an unnecessary treatment for 21.8% (12 of 55) of cows due to self- cure. Most recently a larger study with 1023 cows showed a remarkable self-cure rate of 55.3% in cows, suffering from APM using a treatment with saline (McLaughlin et al., 2012).

Overall, our data on cure rates, milk yield and subsequent uterine health did not reveal any indication of a negative impact of a screening and treatment protocol beginning at DIM 5 and leaving cows with APM earlier than DIM 5 untreated.

Further research is necessary to determine if a delayed treatment of APM can contribute to a reduction of antibiotic drug use without jeopardizing animal welfare and reproductive performance. Based on this proof of concept study a field trial with a larger sample size can be conducted to confirm our findings and to study reproductive performance.

4. SUMMARY

Ines Sannmann: Evaluation of diagnostic methods and comparison of two treatment protocols of acute puerperal metritis in dairy cows

The importance of acute puerperal metritis **(APM)** and an efficacious treatment has been outlined by many authors (Benzaquen et al., 2007; Drillich et al., 2007; Overton and Fetrow 2008). However, there is a lack of science-based evidence for the diagnostic values of methods to identify cows with APM.

Therefore, the objective of this thesis was to investigate how relevant research publications address the validity of diagnostic methods for APM, to determine the test characteristics of an evaluation of vaginal discharge by human senses and an electronic device and to compare two monitoring and treatment strategies of fresh cows for APM.

In the first study, a literature search was conducted in four relevant scientific Journals utilizing the Sciencedirect database. Studies were checked for definitions of possible findings, thresholds, and test characteristics of the diagnostic methods used to identify a cow with APM. On average 2.5 ± 1.75 diagnostic methods were used in a study. References to support the use of the diagnostic methods were provided in 10 of 48 articles (20.8%). Thresholds for diagnostic tests and test characteristics were mentioned in 6 and 3 of the 48 articles, respectively. Based on this systematic review of 48 research papers the evidence supporting the use of the diagnostic methods to identify cows with APM has either been not reported or is weak. In conclusion, the reporting of diagnostic methods to identify cows with APM needs to be improved and further research is necessary to improve diagnostic performance of the methods employed.

The objective of the second part of my thesis was to evaluate the reliability of a sensory assessment for color, smell, and viscosity of vaginal discharge from healthy cows and cows with APM. A total of 15 investigators evaluated 6 vaginal discharge samples 10 times each. Subsequently the investigators rated the health status of the cows and the diagnostic value of color, smell, and viscosity. In a final questionnaire, the investigators estimated their ability to diagnose APM correctly and the influence of experience. Reliability was tested using Cohen's kappa (Κ). Our study revealed slight to moderate reliabilities concerning the assessment of vaginal discharge.

Overall interobserver reliability for color, smell, and viscosity was Κ= 0.15, 0.27 and 0.44, respectively. Overall intraobserver reliability for color, smell and viscosity were Κ= 0.35, 0.39 and 0.6, respectively. The overall personal expertise to detect cows suffering from APM correctly as such was estimated to be 59%. Our study shows that the sensorial assessment of color, smell, and viscosity of vaginal discharge in cows postpartum is subjective.

Therefore, the third part of this thesis involves the use of an electronic nose device to test whether the diagnosis of APM can be refined by technical means. This part includes the definition of test characteristics of an evaluation of odor from vaginal discharge (VD) of cows in the first 10 d postpartum conducted by olfactory cognition and an electronic device, respectively. In experiment 1, 16 investigators (9 veterinary students and 7 licensed veterinarians) evaluated 5 VD samples each on 10 different days. The kappa test revealed an agreement between investigators (interobserver) of $\kappa = 0.43$. Mean agreement within observers (intraobserver) was $\kappa = 0.52$. In experiment 2, the repeatability of an electronic device (DiagNose; C-it, Zutphen, the Netherlands) was tested. Therefore, 5 samples of VD from 5 cows were evaluated 10 times each. The repeatability was 0.97, determined by

Cronbach's α. In experiment 3, 20 samples collected from healthy cows and 20 of cows with acute puerperal metritis were evaluated by the 16 investigators and the DiagNose using a dichotomous scale (1 = cow with acute puerperal metritis; 0 = healthy cow). Sensitivity and specificity of olfactory evaluation was 75.0 and 60.1% compared with 92.0 and 100%, respectively, for the electronic nose device.

The study confirms a considerable subjectivity of the human nose concerning the classification into healthy and sick animals based on the assessment of vaginal discharge. The repeatability of the electronic nose was higher. In conclusion, the DiagNose system, although imperfect, is a reasonable tool to improve odor assessment of VD. Further research is warranted to adapt such electronic devices to practical on-farm screening tools.

In the forth part of my thesis, two strategies for screening and subsequent treatment of acute puerperal metritis (APM) in dairy cows have been compared. Therefore, a study with 79 cows, having APM and 114 healthy control cows was conducted. Cows with APM were divided into two groups (treated n= 67 cows, not treated n= 12 cows). The treated animals were further subdivided into two groups (treated between D1-4, n=12 and treated between D5-10, n= 55).

The results of this study did not show any significant differences in cure rates, milk yield, serum haptoglobin concentrations on DIM 2, 5 and 10 and subsequent uterine health (DIM 21 to 27). Considering this study as a proof of concept study, we conclude that there might not be a negative impact following a screening and treatment protocol beginning at DIM 5 and leaving early APM cows untreated. This hypothesis needs to be confirmed by a larger field study.

Overall, in the course of the four studies a systematic review of current literature considering cows with APM revealed that the evidence of diagnostic methods to identify cows with APM is weak. My second study was implemented to evaluate the diagnostic performance of color, smell and viscosity of cows with and without APM evaluated by human senses. The third part involves the use of an electronic nose device, revealing a better diagnostic performance than by sensorial assessment of VD. The aim of the forth part of this theses was to find out the most efficient strategy to detect cows, suffering from APM and cope with the prudent antibiotic drug use. The results show that a delayed treatment beginning at DIM 5 can lead to a considerable reduction of antibiotic drug use in cows with APM.

5.   ZUSAMMENFASSUNG

Ines Sannmann: Analyse der diagnostischen Methoden und Vergleich von zwei Behandlungsprotokollen bei Kühen mit akuter puerperaler Metritis.

Die Bedeutung, die dem Krankheitskomplexes der akuten puerperalen Metritis (APM) in Milchviehbeständen beigemessen wird, wurde bereits in verschiedenen Publikationen hervorgehoben (Benzaquen et al., 2007; Drillich et al., 2007; Overton and Fetrow 2008).

Bis heute besteht jedoch ein Mangel an wissenschaftlich fundierter Evidenz zum diagnostischen Wert der Methoden, Kühe mit und ohne APM korrekt voneinander zu unterscheiden.

Der erste Teil meiner Doktorarbeit umfasst eine systematische Aufarbeitung der diagnostischen Methoden für Kühe mit APM in anerkannten wissenschaftlichen Zeitschriften.

Insgesamt wurden 9 verschiedene Diagnostikmethoden aufgezählt, im Mittel 2.5 ± 1.75 pro Artikel. Referenzen für diese Methoden waren in 10 von 48 Artikeln (20.8%) angegeben. Grenzwerte und Testcharakteristika der Methoden wurden in 6 und 3 der 48 Artikel erwähnt. Basierend auf dieser systematischen Untersuchung ist die Evidenz, auf der die Diagnostik von Kühen mit APM beruht entweder gering oder nicht beschrieben. Es besteht weiterhin Forschungsbedarf um die Diagnostik für Kühe mit APM zu plausibel und reproduzierbar zu machen.

Aufgrund dieser Erkenntnisse umfasst der zweite Teil dieser Arbeit die Bewertung der Diagnostikmethoden, die aktuell im Feld angewendet werden. In einem Versuch mit 15 Probanden wurden bei 6 Proben von vaginalem Ausfluss gesunder und kranker Kühe Farbe, Geruch und die Viskosität beurteilt. In einem sich anschließendem Fragebogen wurden die Probanden gebeten ihre eigene Fähigkeit zur Korrekten Diagnose einer Kuh mit APM einzuschätzen.

Die Reliabilitäten wurden mittels Cohen's kappa (Κ) berechnet und ergaben nur schwach bis moderate Reliabilitätswerte für die sensorische Diagnostik von APM. Die Interobserver- Reliabilitäten für Farbe, Geruch und Viskosität waren Κ= 0.15, 0.27 und 0.44. Die Intraobserver Reliabilitäten lagen bei Κ= 0.35, 0.39 und 0.6. Insgesamt schätzten Probanden ihre Fähigkeit zur Korrekten Diagnose von APM als zu 59% korrekt ein. Somit zeigt nicht nur die subjektive Einschätzung der Probanden sondern auch die errechnete Reliabilität, dass die sensorische Beurteilung von vaginalem Ausfluss bezüglich der Diagnostik von APM insgesamt schlecht wiederholbar ist.

Diese Ergebnisse führten zu meiner dritten Studie in welcher eine elektronische Nase (DiagNose; C-it, Zutphen, the Netherlands) mit der menschlichen Nase bezüglich der Diagnostik von APM verglichen wurde. Im ersten Teil bewerteten 16 Probanden (9 Studenten und 7 Tierärzte) 5 Proben von vaginalem Ausfluss an je 10 verschiedenen Zeitpunkten. Der Kappa- Test ergab eine Interobserver Reliabilität von κ = 0.43 und eine Intraobserver Reliabilität von κ = 0.52 .

Im zweiten Teil wurde die Wiederholbarkeit der Messung mit der DiagNose getestet. Dafür wurden 5 Proben von vaginalem Ausfluss von 5 verschiedenen Kühen 10-mal hintereinander gemessen. Die Wiederholbarkeit wurde mittels Cronbach's α bestimmt und ergab einen Wert von 0.97.

Im dritten Teil der Studie wurden je 20 Proben von Gesunden und Kühen mit APM von 16 Probanden und der DiagNose analysiert. Die Sensitität und Spezifität der menschlichen Nase ergab 75.0 and 60.1%, die der DiagNose 92.0 und 100%.

Diese Studie bestätigt die hohe Subjektivität des menschlichen Geruchssinnes bezüglich der Beurteilung von vaginalem Ausfluss. Die elektronische Nase hat in den Versuchen deutlich besser abgeschnitten. Zwar ist dieses System bisher noch nicht im Feld

anwendbar, bietet aber vielversprechendes Optimierungspotential für die objektive Diagnose von Kühen mit APM.

Während der Schwerpunkt der ersten drei Teile dieser Arbeit vorwiegend in den diagnostischen Methoden liegt, geht es im vierten Teil um Management und Therapie von Kühen mit APM.

In einer Studie mit 193 Kühen (79 mit APM, 114 Gesunde) wurden die erkrankten Tiere zunächst in zwei Gruppen aufgeteilt (antibiotisch behandelt n= 67, unbehandelt n= 12). Die behandelten Tiere wurden anschließend in zwei weitere Gruppen unterteilt (Behandlung an Tag 1-4, n =12 und Behandlung an Tag 5-10, n = 55).

Die Daten zu Heilungsraten, Milchleistung und Uterusgesundheit zwischen Tag 21 und 27 in Milch zeigen keinen negativen Einfluss eines Beobachtungs- und Behandlungsprotokolls, welches ab Tag 5 post partum beginnt und Kühe, die vor diesem Tag erkrankten, zunächst unbehandelt lässt.

Ziel dieser Dissertation ist, in einem ersten Teil durch die Aufarbeitung von aktueller Literatur einen Überblick über die Evidenz der Diagnostik von Kühen mit APM zu gewinnen. Es zeigt sich eine schwache Evidenz sowie dass Diagnostikmethoden zurzeit nur unzureichend beschrieben werden.

Im zweiten Teil dieser Arbeit wird der vaginale Ausfluss von an APM erkrankten und gesunden Kühen untersucht. Im Ergebnis weist die sensorische Beurteilung von Farbe, Geruch und Viskosität durch die dem Menschen zur Verfügung stehende Sinne gewisse Schwächen auf und kann als Entscheidung für eine antibiotische Behandlung im Grunde nicht solide genug sein.

Der dritte Teil umfasst den Vergleich des menschlichen Geruchssinnes mit einem elektronischen Gerät welches imstande ist bestimmte flüchtige organische Komponenten zu messen. Im Zuge dessen wurden die Test- Charakteristika der menschlichen Nase und dieses Gerätes bestimmt und miteinander verglichen. Das Ergebnis zeigte eine relativ hohe Subjektivität für die menschliche Nase und eine im Vergleich recht gute Wiederholbarkeit des elektrischen Gerätes. Weitere Studien sind jedoch von Nöten um dieses Gerät auch im Feld einsetzen zu können.

Der vierte Teil dieser Dissertation beinhaltet eine Feldstudie, im Zuge derer zwei verschiedene Behandlungsprotokolle für Kühe mit APM verglichen wurden. Diese ergab, dass eine Behandlung ab Tag 5 post partum zu relevanten Einsparungen von Antibiotika bei Kühen mit APM führen kann.

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8.   ACKNOWLEDGEMENTS

9. DECLARATION OF INDEPENDENCE

Hiermit erkläre ich, dass ich alle Studien selbständig durchgeführt und die vorliegende Arbeit selbstständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

This is to declare that I conducted all of the studies described herein myself and the manuscripts were produced independently. I confirm that I have used only the specified resources and tools to complete this thesis. My personal contributions to the research projects presented under this cumulative doctoral thesis are summarized in the following table.

Contribution	Res. Project 1	Res. Project 2	Res. Project 3	Res. Project 4
Study design	++[1]	+++	+++	++
Data collection	+++	+++	+++	++
Data analyses	+++	+++	++	+++
Manuscript writing	+++	+++	+++	+++
Manuscript editing	++	++	++	++

[1]Score: + = < 50%; ++ = 50 to 70%; +++ = > 70%

Ines Sannmann   Berlin, 11.04.2015