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Diagnosis and treatment of acute puerperal metritis in dairy cows

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To All Creatures Great and Small

Was immer du kannst, beginne es.

Kühnheit trägt Macht, Genius, Magie. Beginne jetzt.

(Johann Wolfgang von Goethe)

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1 INTRODUCTION

Acute puerperal metritis (APM) is an infection of the bacterially contaminated uterus of dairy cows with systemic signs of illness occurring usually within the first 10 days after parturition (Sheldon et al., 2006). This disease is common and of great importance in dairy cows as shown by an incidence of APM ranging between 14.8−40.9% (Chapinal et al., 2011; Sannmann et al., 2013a; Lima et al., 2014). As clinically defined by Sheldon et al. (2006), APM is characterized by reddish-brown, fetid, watery vaginal discharge in combination with an elevated rectal temperature of ≥ 39.5°C. Moreover it has been shown that metritis is painful as indicated by greater back arch during rectal palpation compared to healthy cows (Stojkov et al., 2015). Additionally, pain is illustrated by an increased sympathetic activity shown by measurement of heart rate variability compared to healthy cows (Stojkov et al. 2015). Furthermore, cows with APM have an impaired reproductive performance, are more likely to be culled and have less milk production in comparison to healthy cows (Fourichon et al., 2000; Wittrock et al., 2011; Machado et al., 2014). Risk factors for APM are retained placenta, dystocia, twins and negative energy balance ante partum (Benzaquen et al., 2007; Dubuc et al., 2010). Costs of APM include financial losses from decreased milk production, depression in pregnancy rate, increased culling risk and treatment costs and can be up to 358 $ per case (Overton and Fetrow, 2008). To sum up, animal welfare as well as economic reasons illustrate the need of an effective management and treatment.

In the infected uterus of cows with APM many different aerobic and anaerobic bacteria can be found including *Escherichia coli, Trueperella pyogenes, Prevotella ssp., Fusobacterium necrophorum, Fusobacterium nucleatum, Mannheimia haemolytica, Staphylococcus ssp. and Streptococcus ssp*. (Sheldon et al., 2004; Williams et al., 2005; Santos et al., 2011). *T. pyogenes* and *E.coli* are pathogens supposedly known to cause endometrial lesions and therefore are considered to be the main bacteria associated with APM (Sheldon et al., 2004; Santos et al., 2010; de Boer et al., 2015). Due to this wide range of involved bacteria it is reasonable to suggest the use of antimicrobials with broad spectrum activity as preferred treatment (Lima et al., 2014) and APM is commonly treated with 3rd generation cephalosporins (Machado et al., 2014). The reason for the widespread use of ceftiofur administered parenterally is on the on hand it´s high efficacy (Chenault et al., 2004; Sheldon et al., 2004). On the other hand, the amounts of residue appearing in milk are below the tolerance for human consumption and therefore there is no withdrawal time for milk (Scientific Advisory Group on Antimicrobials of the Committee for Medicinal Products for Veterinary Use, 2009; Lima et al., 2014). However, 3rd generation cephalosporins are very important antimicrobials for severe infections in humans and their use in food-producing animals could potentially lead to an increased prevalence of resistance (Scientific Advisory Group on Antimicrobials of the

Committee for Medicinal Products for Veterinary Use, 2009). As antibiotic resistance is recognized as a top public health challenge facing the 21st century (Machado et al., 2014), veterinarians should support the responsible use of antimicrobials to reduce the spreading of resistances and to create a positive public image of their profession.

Different approaches can be considered in order to minimize the use of antimicrobials in the treatment of cows with APM. On the one hand, potential risk factors should be detected, managed and prevented. The maternity pen for example should be kept clean and dry, dairy personnel needs to be trained on calving management, hygiene practice in calving assistance needs to be adopted, consumption of dietary energy above requirement in the “far-off” dry period should be prevented and unrestricted feed bunk access after calving should be provided (Cook and Nordlund, 2004; Dann et al., 2006; Janovick et al., 2011; Schuenemann et al., 2013).

On the other hand, a precise diagnosis is required to ensure that only cows truly suffering from APM are treated. Therefore, the specificity of different diagnostic criteria for the detection of sick cows should be as great as possible, resulting in a minimum of false positively treated cows. Moreover, sick cows have to be reliably identified. A high sensitivity to prevent false negatively diagnosed cows needs to be ensured due to animal welfare. Accordingly, the first two studies evaluated two different diagnostic measures for the detection of APM.

Finally, alternatives to the treatment with antimicrobials have to be evaluated. Thus, the use of nonsteroidal anti-inflammatory drugs for treatment of APM was assessed in a third study.

1.1 Fever detection by automatic milking systems

Currently, measurement of body temperature is one of the most common methods to monitor the fresh cow´s health status in the early puerperium in dairy cows (Smith and Risco, 2005; Burfeind et al., 2012). The earlier a sick cow is identified, the quicker the treatment can be applied resulting in a better chance of improved health, production and survival (Smith and Risco, 2005).

Besides vaginal discharge, fever (≥ 39.5°C) is also the second major symptom of APM (Sheldon et al., 2006). However, the daily measurement of rectal temperature is time consuming and a survey of fresh cow practices in Germany showed that only 34% of all farms monitored body temperature (Heuwieser et al., 2010).

Automatic milking systems (AMS) measure milk temperature for each cow individually during each milking event. Especially in North-Western Europe AMS have become more and more common (Svennersten-Sjaunja and Pettersson, 2008; de Koning, 2010). Reduced labour, a better social life for dairy farm families and increased milk yields due to more frequent milking were reported as benefits of AMS (de Koning, 2013). Previous studies found high correlations between milk and body temperatures of cows (Igono et al., 1987; Fordham et al., 1988; West et al., 1990; West et al., 1999) Hence, there is the potential of automatically detect febrile cows, which possibly leads to a reduction of undetected severe cases of infectious diseases and allows early treatment of sick animals.

Therefore, the objective of the first study was to evaluate if milk temperature measured by AMS is a reliable indicator of body temperature of dairy cows and if cows with fever could be detected.

The results of this study were recently published in the Journal of Dairy Science (impact factor 2.55):

A. Pohl, O. Burfeind, W. Heuwieser. 2014. Technical Note: Assessment of milk temperature measured by automatic milking systems as an indicator of body temperature and fever in dairy cows. 97:4333–4339

1.2 Puerperal inflammatory response indicated by haptoglobin

Even if simple diagnostic criteria (fever and vaginal discharge) are applied, the diagnosis of APM is not as trivial as it may seem and a lack of objectively measurable criteria has been described (Sannmann et al., 2013b; Burfeind et al., 2014b; Pohl et al., 2015). Therefore, different studies evaluated the association between haptoglobin, an objectively measurable criterion, and APM (Huzzey et al., 2009; Dubuc et al., 2010; Burfeind et al., 2014a). Haptoglobin is an acute phase protein synthesized in the liver in response to inflammation (Crawford et al., 2005) and can be measured in serum. Acute phase response is one defense mechanism of the body against infections (Schneider et al., 2013). Haptoglobin binds to free hemoglobin and thereby inhibits microbial proliferation by reducing the availability of iron (Huzzey et al., 2009). However, there is only moderate reported specificity and sensitivity in the detection of metritic cows by measuring serum haptoglobin concentrations (Huzzey et al., 2009; Dubuc et al., 2010; Burfeind et al., 2014a), because haptoglobin is an indicator of non-specific innate immune response activated by various inflammatory lesions (Eckersall, 2000; Huzzey et al., 2009; Ceciliani et al., 2012). The hypothesis of this study was, that there might be other events or disorders activating inflammatory response that are coinciding with the usual time period of APM.

Therefore, the objective of the second study was to evaluate the association between different variables occurring at the same period after calving as APM and haptoglobin concentration. The plausible factors parity, periparturient metabolic stress indicated by β-hydroxybutyric acid (BHBA) and nonesterified fatty acids (NEFA), calving difficulties, retained fetal membranes and APM were evaluated.

The results of this study were recently published in the Journal of Dairy Science (impact factor 2.55):

A. Pohl, O. Burfeind, W. Heuwieser. 2015. The association between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes and metritis. 98:4544-4551

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

1.3 Treatment of metritis with nonsteroidal anti-inflammatory drugs

The common treatment of APM with ceftiofur as a 3rd generation cephalosporin is a crucial issue in case of emerging antibiotic resistance, because the limited use as critically important antimicrobial is mandatory (Pyorala et al., 2014) and even a withdrawal from the market has been considered (Grove-White and Murray, 2009). Even though APM is a potential life threatening disease in dairy cows (Drillich et al., 2007), high self cure rates have been described (McLaughlin et al., 2012).

Nonsteroidal anti-inflammatory drugs (NSAID) have analgesic, anti-inflammatory, anti-pyretic and anti-endotoxic effects and thus can increase animal health and wellbeing (Fitzpatrick et al., 2004; Laven et al., 2012). Moreover, it is well known that NSAID are likely to be of value in diseases in which inflammation, endotoxaemia and pain are major components (Fitzpatrick et al., 2004; Laven et al., 2012). Anti-inflammatory and analgesic effects of NSAID in combination with remarkable self cure rates of APM make it plausible to study the efficacy of ketoprofen in treatment of APM.

Therefore, the objective of the third study was to evaluate the effect of the treatment of APM with ketoprofen on clinical cure, subsequent milk yield, prevalence of endometritis and reproductive performance compared to the treatment with ceftiofur. These data have not been published yet and are presented in section 3 “Additional unpublished data”.

2 RESEARCH PAPERS

2.1 Technical Note: Assessment of milk temperature measured by automatic milking systems as an indicator of body temperature and fever in dairy cows

2.2 The association between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes and metritis

2.1 Technical Note: Assessment of milk temperature measured by automatic milking systems as an indicator of body temperature and fever in dairy cows

Technical Note: Assessment of milk temperature measured by automatic milking systems as an indicator of body temperature and fever in dairy cows

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2.1.1 **Abstract**

The objective of this study was to evaluate whether milk temperature (MT) measured by automatic milking system (AMS) is a reliable indicator of body temperature of dairy cows and if cows with fever could be detected. Data loggers (Minilog 8, Vemco Ltd., Halifax, Canada) measuring the body temperature were inserted for 7 ± 1 days into the vaginal cavity of 31 dairy cows and programmed to take one reading/min. Milk temperature was recorded at each milking event by the AMS and values from the vaginal loggers were paired with the corresponding MT. The correlation (r) between vaginal temperature (VT) and MT was 0.52. Vaginal temperature was higher (39.1 ± 0.4°C) than MT (38.6 ± 0.7°C) with a mean difference of 0.5 ± 0.6°C. The ability of MT to identify cows with fever was assessed using 2 approaches. In the first approach, VT could indicate fever at any time of the day, whereas MT could display fever only during the milking events of a given day. Different definitions of fever based on thresholds of VT and duration exceeding these thresholds were constructed. Different thresholds of MT were tested to distinguish between cows with and without fever. The combination of 39.0°C as a threshold for MT and 39.5°C for at least 2 h/d as a threshold for VT resulted in the highest combination of sensitivity (0.65) and specificity (0.65). In the second approach, we evaluated whether MT could identify cows with fever at a given milking event. A threshold of MT > 38.7°C delivered the best combination of sensitivity (0.77) and specificity (0.66) when fever was defined as VT ≥ 39.5°C. Therefore, MT measured by AMS can be indicative of fever in dairy cows to a limited extent.

Keywords: automatic milking system, milk temperature, vaginal temperature, rectal temperature, fever

2.1.2 **Technical Note**

Automatic milking systems (AMS) have become increasingly common since their appearance in 1992 in the Netherlands, especially in North-Western Europe, where 90% of the world´s AMS farms are located (Svennersten-Sjaunja and Pettersson, 2008; de Koning, 2010). Dairy farms in North America and Japan are also increasingly utilizing AMS (de Koning, 2010). In 2012, more than 16,000 farms had adopted AMS for their daily milking routine worldwide (de Koning, 2013). The most common reasons for dairy farmers to invest in AMS are potential savings in labor, increased milk yields due to increased milking frequency, and a better social life for dairy farm families (Dijkhuizen et al., 1997; Wagner-Storch and Palmer, 2003; de Koning, 2010; Jacobs and Siegford, 2012). Using AMS, the focus of the traditional farm management shifts from the daily milking routine to a system reliant on new technology (Jacobs and Siegford, 2012).

One emerging challenge is to identify sick cows because the direct contact between personnel and cows during the daily milking routine is minimized. On the other hand, data of cows and milk (e.g., amount of milk, color, conductivity) generated during each milking event are promptly available and can be used for health assessments. In particular, the health status of postpartum cows should be monitored carefully because numerous diseases such as metritis, mastitis and metabolic disorders occur primarily in the postpartum period (Smith and Risco, 2005). In health monitoring programs, the most commonly used parameter to identify sick cows is the measurement of rectal temperature (RT; Smith and Risco, 2005).

One option the AMS provides is the measurement of the milk temperature (MT) for each cow during every milking event. In earlier studies, temperature sensors were integrated into the short piece of the milk tube for measurement of MT and comparison with body temperature (BT). The results showed that the correlation between MT and BT ranged from $r = 0.78$ to $r = 0.99$ (Igono et al., 1987; Fordham et al., 1988; West et al., 1990; West et al., 1999). Based on these findings it is plausible to use MT measured with the AMS to detect cows susceptible for diseases.

Therefore, the overall objective of this study was to investigate whether MT measured by an AMS is a reliable indicator of BT in dairy cows. Specifically, the objectives were 1) to determine the relationship (correlation and difference) between MT and BT and 2) to determine a threshold for MT to detect cows with fever considering different definitions of fever on a particular day and 3) to determine a threshold for MT to detect cows with fever considering different definitions of fever on a particular milking event.

Data collection

Between March and May 2013, 31 lactating early postpartum (DIM 5 to 15) Holstein dairy cows (parity 2.2 ± 1.0) were enrolled on a commercial dairy farm in Brandenburg, Germany, that housed a total of 315 cows with an average 305-d milk production of 7,269 kg (3.55% fat and 3.29% protein). Cows were housed indoors in a naturally ventilated freestall barn with cubicles equipped with rubber mats. Lactating cows were separated into 2 groups and each group was milked by 2 AMS (Lely, Astronaut 4, Maassluis, the Netherlands) in free cow traffic.

To measure BT of dairy cows continuously, data loggers inserted into the vaginal cavity have been used in several studies (Suthar et al., 2011; Burfeind et al., 2012; Suthar et al., 2012). This approach has been validated recently (Vickers et al., 2010; Suthar et al., 2013) and demonstrated that differences between RT and vaginal temperature (VT) were negligible. In this study, VT was recorded at 1-min intervals using microprocessor-controlled temperature loggers (Minilog 8, Vemco Ltd., Halifax, Canada) attached to modified vaginal controlled internal drug release (CIDR) inserts. Data loggers (size = 92 mm x 20 mm; weight = 40.5g) were inserted into the vaginal cavity of postpartum cows (DIM 5 to 15) for a 7 (±1)-d period as previously described (Vickers et al., 2010).

Milk temperatures were recorded during each milking event by the AMS. Sensors inside the arm of the robot measured MT of each quarter individually. The milk flowed through the teat cups and an approximately 20-cm-long milk tube before it reached the sensor. At the end of each milking event, the highest MT measured during the whole milking process, irrespective of the quarter, was recorded and stored in the database of the proprietary software of the AMS (T4C, Lely) for later use by the herdsman.

Three cows were excluded from the study due to the complete loss of loggers. Therefore, data from 28 cows were used for final analyses. Four cows lost their loggers before the end of the 7-d interval, but data of the first days could be used until temperature fell below 38.0°C, indicating the time of loss of the logger (Vickers et al., 2010). Overall, 1,037 of 240,664 measures of VT (0.4%) were < 38.0°C and excluded from analyses. In total, 418 milking events had paired values of MT and VT.

Because the results of previous studies were inconsistent in the difference between MT and BT, a sample size calculation could not be conducted in advance. Instead, a post hoc power analysis was performed using G*Power (Version 3.1.3, University of Düsseldorf, Düsseldorf, Germany) to verify the analysis of the difference between MT and VT in this study. The post hoc power analysis was applied with $\alpha = 0.05$, sample size = 418 and effect size = 0.83. The power of analysis ($1 - \beta$) for the difference between MT and VT was 1.0.

Relationship between MT and VT

Data from temperature loggers (VT) and the AMS software (MT) were downloaded into Excel spreadsheets (Office 2010, Microsoft Deutschland GmbH, Munich, Germany). Data included one MT for each milking event and several corresponding VT for the duration of each milking event. Values from the vaginal loggers were averaged for the minutes (7.1 ± 2.4) the cow was milked in the AMS and paired with the corresponding MT. Data were analyzed using IBM SPSS Statistics (Version 20.0, Munich, Germany) and Medcalc (Version 12.4.0.0, Mariakerke, Belgium). The difference and relationship between VT and MT was evaluated using a paired t-test and Pearson correlation. Furthermore, a Bland-Altman plot was generated displaying the average of VT and MT on the x-axis against the difference of VT and MT on the y-axis.

The correlation between VT and MT was $r = 0.52$ ($n = 418$; $P < 0.01$). Vaginal temperature was higher (39.1 ± 0.4°C, n = 418) than MT (38.6 ± 0.7°C, n = 418) with a mean difference of 0.5 ± 0.6°C ($n = 418$, $P < 0.01$, Figure 1).

Previous studies showed inconsistent results in the difference between MT and BT. In our study, MT was about 0.5°C lower than VT. This is in agreement with older findings (Fordham et al., 1988) that described a 0.34°C lower MT measured with a temperature sensor at each top of the short milk tube than VT measured with a temperature probe. We assume that the lower MT was caused by the passage through the teat cups and milk tubes before reaching the sensors located in the arm of the AMS. In this way, MT might have cooled before it was measured. In contrast, Bitman et al. (1984) found a high correlation of temperatures ($r = 0.98$ to 0.99) with negligible differences (body and udder temperature were 38.8°C ± 0.1°C). Those authors, however, implanted temperature sensors into the udder and the peritoneal cavity of lactating cows and thus excluded a cooling effect of the milk flowing from the udder to the sensor. West et al. (1999) recorded MT at each milking with a temperature sensor in the short piece of the milk tube of each milker and compared it with RT taken with a digital thermometer and found that MT was, on average, 0.15°C higher than RT. They did not, however, describe the penetration depth of the thermometer into the rectum, which has been shown to effect RT by as much as 0.4°C (Burfeind et al., 2010) or the distance between teat cup and temperature sensor in the milk tube.

Standard deviation of the mean difference of VT and MT was ± 0.6°C. The Bland-Altman-Plot (Figure 1) displays the distribution of differences between VT and MT. The 95% confidence interval reached from −0.58°C to +1.63°C, showing the lower and upper limit of difference. Considering this rather wide range makes interpretation of MT more difficult. One possible explanation could be that the milk yield per milking or flow rate of the milk and temperature of the tubes could have affected the temperature of the milk reaching the sensors.

The Pearson correlation coefficient between MT and VT in this study ($r = 0.52$; $P < 0.01$) was lower than reported in previous studies for traditional milking equipment and BT ranging from 0.78 to 0.89 (Fordham et al., 1988; West et al., 1990; West et al., 1999). There is, however, no scientific reference for MT measured by an AMS compared with BT of lactating dairy cows. Different amounts of milk and different temperature of teat cups and milk tubes depending on the ambient temperature could have affected the correlation between MT and VT. On the other hand, it is important to remember no ideal benchmark exists to measure BT (Bewley et al., 2008). Each method has its own errors, like insertion depth of the thermometer for RT, location of sensors for MT and the correct position of vaginal loggers for VT. Any comparison of these methods will reflect these errors on both sides of the equation (Bewley et al., 2008).

Fever detection by AMS on a particular day

To evaluate whether MT could identify cows with fever on a particular day using VT as gold standard, VT and MT did not have to measure fever simultaneously. Vaginal temperature could display fever at any time of the day, whereas MT could display fever only during the milking events of a given day. Different thresholds of fever to distinguish between healthy cows and cows suffering from an infectious disease have been described in the literature (Sheldon et al., 2006; Benzaquen et al., 2007; Wagner et al., 2007). Moreover, Burfeind et al. (2012) found that during hot periods, 28% of healthy cows exhibited RT ≥ 39.5°C, which might suggest that a higher threshold should be used in hot periods. Accordingly, 6 definitions of fever based on different thresholds of VT and duration exceeding these thresholds were constructed (≥ 39.5°C for ≥ 2h, ≥ 4h and ≥ 8h; ≥ 40.0°C for ≥ 2h, ≥ 4h and ≥ 8h). Using these definitions of fever as gold standard, the ability of 4 different values of MT (≥ 38.0°C, ≥ 38.5°C, ≥ 39.0°C, ≥ 39.5°C) were tested to distinguish between cows with fever and cows without fever. Based on hourly VT means calculated from sixty 1-min values, cows were identified as being within 1 of the 6 fever categories on a given day and classified as 0 (no fever) or 1 (fever), respectively. Values from the AMS software were checked for each cow and day for the occurrence of fever for the 4 different thresholds based on MT. If MT was higher than a fever threshold during at least one milking event per day, values for that day were classified as 1 (fever). If MT remained below the fever threshold, they were classified as 0 (no fever). These classifications of fever based on VT and MT were paired for each day, resulting in a total of 185 paired cow days. Values of VT were used as gold standard and combined with MT values in 2 x 2 tables. The ability to distinguish between fever and no fever was expressed as sensitivities (Se), specificities (Sp), positive predictive values (+PV) and negative predictive values (−PV). Cases where both MT and VT displayed fever were considered true positives (TP) and cases where MT failed to indicate fever were considered false negative (FN). True negatives (TN) represented occasions when both MT and VT did not indicate fever, and cases where cows without fever (VT) were classified as cows with fever using MT were considered false positives (FP). Sensitivity is the percentage of cows with fever that were classified correctly by MT [TP/(TP + FN) × 100]; Sp is the percentage of cows without fever that were classified correctly by MT [TN/(FP + TN) × 100]; +PV is the percentage cows that were correctly identified with fever by MT of the total of cows classified with fever by MT [TP/(TP + FP) ×100] and −PV is the percentage of cows correctly identified without fever by MT of the total of cows classified without fever by MT [TN/(TN + FN) × 100] (Norberg et al., 2004).

Sensitivities, Sp, +PV and −PV for the ability of MT to distinguish between cows with and without fever on a particular day (n = 185) defined by VT are given in Table 1. With an increasing threshold for MT, Se decreased and Sp increased for all definitions of fever. The combination of 39.0°C as a threshold for MT and 39.5°C for at least 2 h/d as a threshold for VT resulted in the highest combination of Se (0.65) and Sp (0.65).

This finding is plausible as the average MT was 0.5°C lower than VT, as mentioned above. This means that 65% of all cows with VT-registered fever were identified correctly by the AMS, and that 65% of all cows without fever were identified correctly. To explain the low Se, we evaluated how many cows with fever at any time of the day (VT ≥ 39.5°C, ≥ 2 h) also had fever (VT ≥ 39.5°C) during milking events. This was the case in only 36.5% of all milking events. Moreover, many studies showed that BT of dairy cattle exhibits a diurnal rhythm and is characterized by a minimum in the morning and a peak in the evening that lags 2 to 5 h behind peak ambient temperatures (Bitman et al., 1984; Kendall and Webster, 2009). Furthermore, Burfeind et al. (2012) found that VT is influenced by several factors such as DIM, time of day, and climate. In this study, cows chose freely when to go to the AMS and consequently BT was influenced by the time of day the cow was milked. On the other hand, cows showed fever only for at least 2 h/d to be classified as “cow with fever” for the whole day. Accordingly, the likelihood that the cow went to the AMS in a period of no fever was rather high. Further research is warranted to study the lying and standing behavior of cows with fever and to determine if cows prefer to lie down and not go to the AMS during a period of fever.

Fever detection by AMS during a particular milking event

To evaluate if MT could identify cows with fever during a particular milking event, VT and MT had to measure fever simultaneously at that particular milking event. Values from the vaginal loggers were averaged for the minutes the cow was milked in the AMS (7.1 ± 2.4) and paired with the corresponding MT. Values for VT were transformed into 1 (fever at that particular milking event) or 0 (no fever at that particular milking event) for 2 thresholds (≥ 39.5°C and ≥ 40.0°C). The ability of MT to identify cows with fever at each milking event was examined using receiver-operating characteristic (ROC) analysis for both VT thresholds. The continuous variable was MT and the classification variable was the occurrence of fever measured by VT for ≥ 39.5°C or ≥ 40.0°C. The ROC analysis involves calculating Se and Sp for different thresholds for MT and plots sensitivity on the x-axis against and (1 – specificity) on the y-axis. The closer the ROC curve is to the left corner, the greater the accuracy of differentiation between cows with or without fever (Greiner et al., 2000). The area under the ROC curve (AUC) is a measure of the overall diagnostic accuracy of the measure tested. The AUC would be 1 if MT could perfectly distinguish between cows with and without fever based on VT and 0.5 if MT could not distinguish at all between the 2 groups. A *P*-Value < 0.01 and AUC > 0.5 would demonstrate that MT can distinguish between cows with and without fever. The Youden Index is calculated as (Se + Sp – 1) for all thresholds of MT. The threshold of MT with highest Youden Index shows the value of MT with the best combination of Se and Sp.

The results of the ROC analysis to determine test characteristics to identify cows with fever on a particular milking event are given in Table 2. The Youden Index for both definitions of fever for VT (≥ 39.5°C, ≥ 40.0°C) was highest for MT > 38.7°C (Figure 2). The AUC for VT ≥ 39.5°C and ≥ 40.0°C were 0.79 and 0.90, respectively.

A threshold of MT > 38.7°C delivered the highest combination of Se (0.77) and Sp (0.66) when fever was defined as VT ≥ 39.5°C. For VT ≥ 40.0°C and MT > 38.7°C, Se was 1 and Sp was 0.63. Considering a VT above 40.0°C as fever, all cows were found correctly by the AMS; but FP rate was 37%. The result for the higher threshold should be interpreted with caution, because it is only based on 18 matched pairs of VT and MT.

In conclusion, MT measured by an AMS can be indicative of fever in dairy cows postpartum to a limited extent. Milk temperature thresholds of > 38.7°C or ≥ 39.0°C should be used to identify cows with fever at each milking event or for any time of day. Identified cows should be checked for other signs of systemic illness. Overall, MT should be interpreted with great caution because of high false positive and false negative rates.

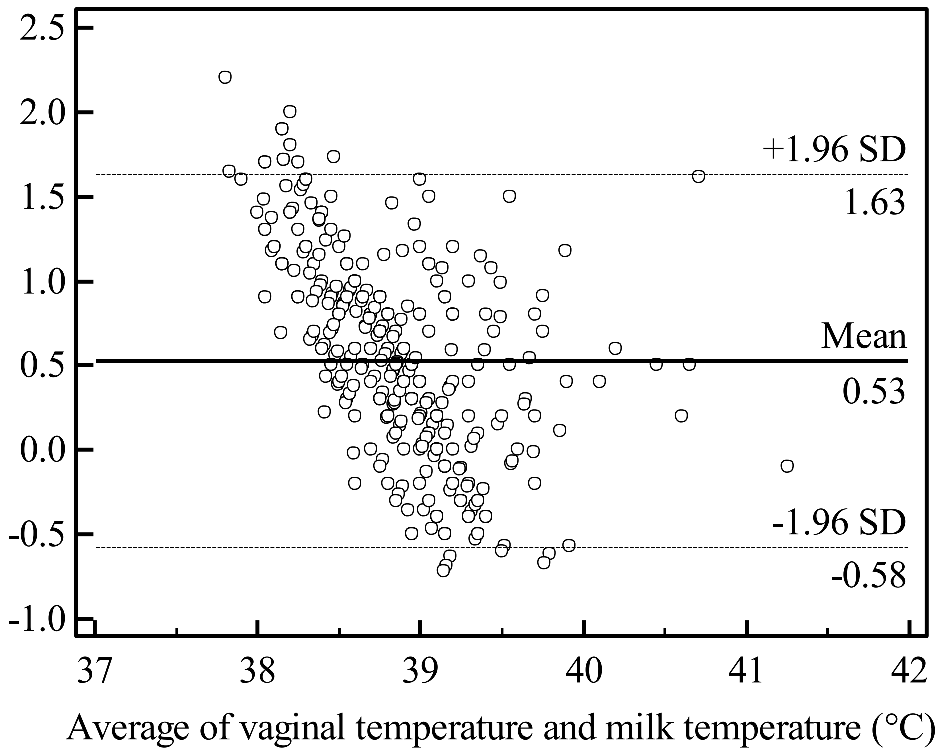

Figure 1. Bland-Altman Plot, displaying the average of vaginal temperature and milk temperature on the x-axis against the difference of vaginal and milk temperature on the y-axis. Vaginal temperature was higher (39.1 ± 0.4°C, n = 418) than milk temperature (38.6 ± 0.7°C, n = 418) with a mean difference of 0.5 ± 0.6°C (n = 418, $P < 0.01$).

Table 1. Test characteristics for different thresholds of milk temperature to identify cows with fever on a particular day (n = 185) considering different definitions of fever based on thresholds of VT and duration exceeding these thresholds considering vaginal temperature as gold standard

		Definition of fever (vaginal temperature)[3]					
Threshold of milk temperature[1]	Test charactaristics[2]	≥ 39.5°C 2 h n = 63	≥ 39.5°C 4 h n = 40	≥ 39.5°C 8 h n = 19	≥ 40.0°C 2 h n = 24	≥ 40.0°C 4 h n = 13	≥ 40.0°C 8 h n = 5
≥ 38.0°C n = 171	Se	0.95	0.98	0.95	0.92	0.92	1
	Sp	0.09	0.09	0.08	0.07	0.08	0.08
	+PV	0.35	0.23	0.11	0.13	0.07	0.03
	−PV	0.78	0.93	0.93	0.86	0.93	1
≥ 38.5°C n = 131	Se	0.83	0.88	0.95	0.79	0.92	1
	Sp	0.35	0.34	0.32	0.30	0.31	0.3
	+PV	0.40	0.27	0.14	0.15	0.09	0.04
	−PV	0.80	0.91	0.98	0.91	0.98	1
≥ 39.0°C n = 84	Se	0.65	0.73	0.89	0.79	0.92	1
	Sp	0.65	0.62	0.60	0.60	0.58	0.56
	+PV	0.49	0.35	0.20	0.23	0.14	0.06
	−PV	0.78	0.89	0.98	0.95	0.99	1
≥ 39.5°C n = 32	Se	0.27	0.4	0.53	0.5	0.69	0.8
	Sp	0.88	0.89	0.87	0.88	0.87	0.84
	+PV	0.53	0.5	0.31	0.38	0.28	0.13
	−PV	0.70	0.84	0.94	0.92	0.97	0.99

[1] Where n = number of days milk temperature reached a certain threshold in at least one milking event per day

[2] Se = sensitivity, Sp = specificity, +PV = positive predictive value, −PV = negative predictive value

[3] Where n = number of days vaginal temperature reached a certain definition of fever

Table 2. Test characteristics for different thresholds of milk temperature to identify cows with fever during a particular milking event. Different definitions of fever based on thresholds of vaginal temperature were used considering vaginal temperature at the time of milking as gold standard.

Threshold of milk temperature	Test characteristics[1]	Vaginal temperature[2] ≥ 39.5°C n = 57	Vaginal temperature[2] ≥ 40.0°C n = 18
> 38.0°C	Se	1.00	1.00
	Sp	0.28	0.19
	+PV	0.17	0.05
	−PV	1.00	1.00
> 38.7°C	Se	0.77	1.00
	Sp	0.66	0.63
	+PV	0.26	0.11
	−PV	0.95	1.00
> 39.0°C	Se	0.60	0.78
	Sp	0.81	0.78
	+PV	0.33	0.14
	−PV	0.93	0.99
	Area under the curve (95% CI) ($P < 0.01$)	0.79 (0.75-0.83)	0.90 (0.86-0.92)

[1] Se = sensitivity, Sp = specificity, +PV = positive predictive value, −PV = negative predictive value

[2] Where n = number of milkings with vaginal temperature displaying fever (≥ 39.5°C or ≥ 40.0°C)

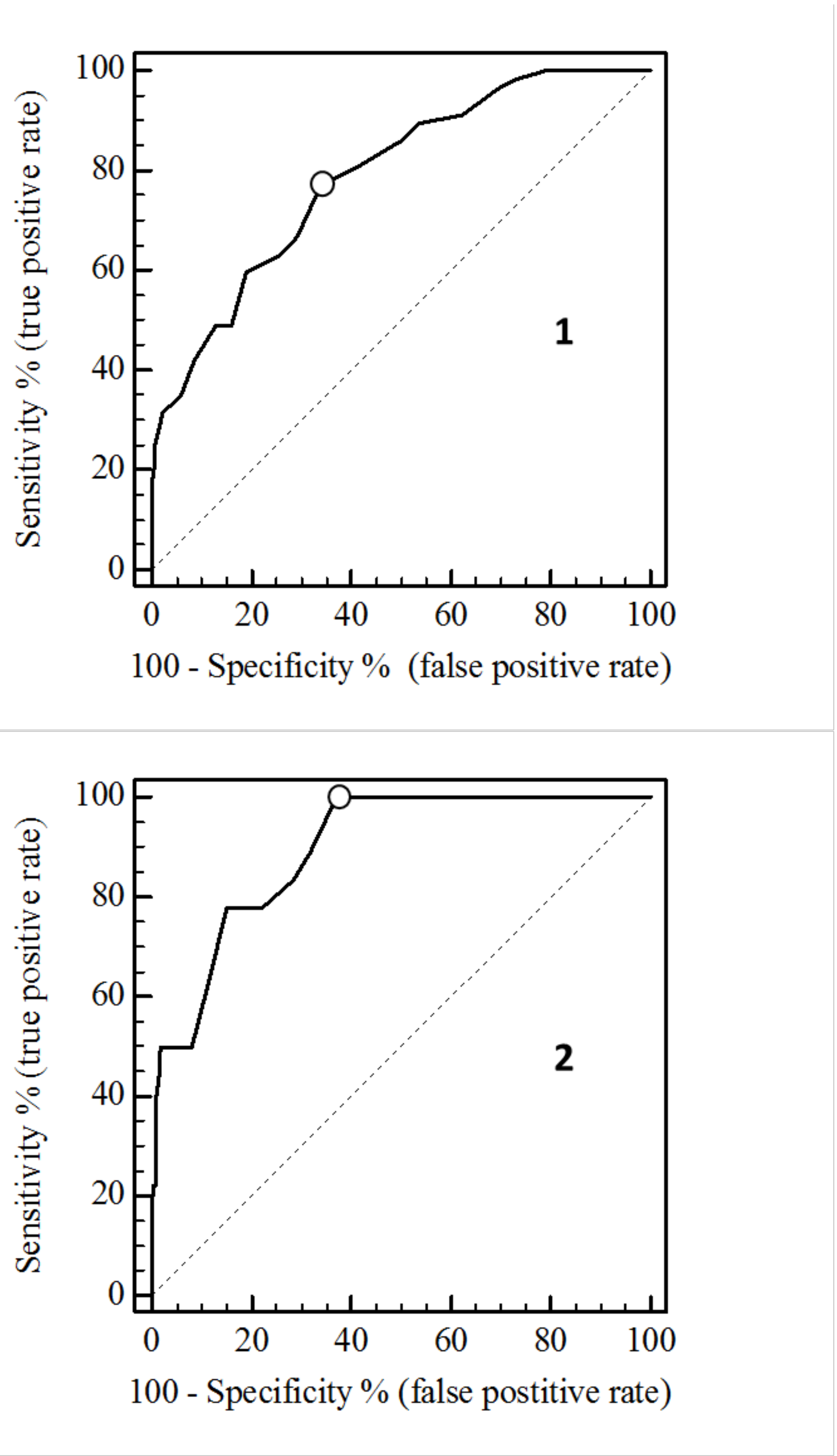

Figure 2. Receiver-operating characteristic (ROC) curve showing the false positive rate on the x-axis and the true positive rate on the y-axis for milk temperature (MT) as test parameter with vaginal temperature as gold standard defining fever as ≥ 39.5°C with area under the curve = 0.79 (1) and ≥ 40.0°C with area under the curve = 0.90 (2). Point with highest Youden Index (○) is shown in (1) and (2) with MT > 38.7°C for both vaginal temperature based thresholds.

2.1.3 **Acknowledgements**

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2.1.4 **References**

Benzaquen, M. E., C. A. Risco, L. F. Archbald, P. Melendez, M. J. Thatcher, and W. W. Thatcher. 2007. Rectal temperature, calving-related factors, and the incidence of puerperal metritis in postpartum dairy cows. J. Dairy Sci. 90:2804-2814.

Bewley, J. M., M. E. Einstein, M. W. Grott, and M. M. Schutz. 2008. Comparison of reticular and rectal core body temperatures in lactating dairy cows. J. Dairy Sci. 91:4661-4672.

Bitman, J., A. Lefcourt, D. L. Wood, and B. Stroud. 1984. Circadian and ultradian temperature rhythms of lactating dairy cows. J. Dairy Sci. 67:1014-1023.

Burfeind, O., V. S. Suthar, and W. Heuwieser. 2012. Effect of heat stress on body temperature in healthy early postpartum dairy cows. Theriogenology 78:2031-2038.

Burfeind, O., M. A. G. von Keyserlingk, D. M. Weary, D. M. Veira, and W. Heuwieser. 2010. Short communication: Repeatability of measures of rectal temperature in dairy cows. J. Dairy Sci. 93:624-627.

de Koning, K. 2010. Automatic milking - common practice on dairy farms. Pages V59 - V63 in Proc. Second North Am. Conf. on Robotic Milking, Toronto, Canada. Precision Dairy Operators, Elora, Canada.

de Koning, K. 2013. Developments in machine milking; past and future. Pages 5 - 9 in Proc. 4. Tänikoner Melktechniktagung, Switzerland, Agroscope, Ettenhausen, Switzerland.

Dijkhuizen, A. A., R. B. M. Huirne, S. B. Harsh, and R. W. Gardner. 1997. Economics of robot application. Computers and Electronics in Agriculture 17:111-121.

Fordham, D. P., P. Rowlinson, and T. T. McCarthy. 1988. Oestrus detection in dairy cows by milk temperature measurement. Res. Vet. Sci. 44:366-374.

Greiner, M., D. Pfeiffer, and R. D. Smith. 2000. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Preventive Veterinary Medicine 45:23-41.

Igono, M. O., H. D. Johnson, B. J. Steevens, G. F. Krause, and M. D. Shanklin. 1987. Physiological, productive, and economic benefits of shade, spray, and fan system versus shade for holstein cows during summer heat. J. Dairy Sci. 70:1069-1079.

Jacobs, J. A. and J. M. Siegford. 2012. Invited review: The impact of automatic milking systems on dairy cow management, behavior, health, and welfare. J. Dairy Sci. 95:2227-2247.

Kendall, P. E. and J. R. Webster. 2009. Season and physiological status affects the circadian body temperature rhythm of dairy cows. Livest. Sci. 125:155-160.

Norberg, E., H. Hogeveen, I. R. Korsgaard, N. C. Friggens, K. H. M. N. Sloth, and P. Løvendahl. 2004. Electrical conductivity of milk: Ability to predict mastitis status. J. Dairy Sci. 87:1099-1107.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516-1530.

Smith, B. I. and C. A. Risco. 2005. Management of periparturient disorders in dairy cattle. Veterinary Clinics of North America: Food Animal Practice 21:503-521.

Suthar, V., O. Burfeind, S. Bonk, R. Voigtsberger, C. Keane, and W. Heuwieser. 2012. Factors associated with body temperature of healthy holstein dairy cows during the first 10 days in milk. J. Dairy Res. 79:135-142.

Suthar, V., O. Burfeind, B. Maeder, and W. Heuwieser. 2013. Agreement between rectal and vaginal temperature measured with temperature loggers in dairy cows. J. Dairy Res. 80:240-245.

Suthar, V. S., O. Burfeind, J. S. Patel, A. J. Dhami, and W. Heuwieser. 2011. Body temperature around induced estrus in dairy cows. J. Dairy Sci. 94:2368-2373.

Svennersten-Sjaunja, K. M. and G. Pettersson. 2008. Pros and cons of automatic milking in europe. J. of Anim. Sci. 86:37-46.

Vickers, L. A., O. Burfeind, M. A. G. von Keyserlingk, D. M. Veira, D. M. Weary, and W. Heuwieser. 2010. Technical note: Comparison of rectal and vaginal temperatures in lactating dairy cows. J. Dairy Sci. 93:5246-5251.

Wagner-Storch, A. M. and R. W. Palmer. 2003. Feeding behavior, milking behavior, and milk yields of cows milked in a parlor versus an automatic milking system. J. Dairy Sci. 86:1494-1502.

Wagner, S. A., D. E. Schimek, and F. C. Cheng. 2007. Body temperature and white blood cell count in dairy cows during the first ten days after calving. Page 233. American Association of Bovine Practitioners, Stillwater.

West, J. W., G. M. Hill, J. M. Fernandez, P. Mandebvu, and B. G. Mullinix. 1999. Effects of dietary fiber on intake, milk yield, and digestion by lactating dairy cows during cool or hot, humid weather. J. Dairy Sci.82:2455-2465.

West, J. W., B. G. Mullinix, J. C. Johnson, K. A. Ash, and V. N. Taylor. 1990. Effects of bovine somatotropin on dry matter intake, milk yield and body temperature in holstein and jersey cows during heat stress. J. Dairy Sci. 73:2896-2906.

2.2 The association between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis

The association between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis

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2.2.1 Abstract

Measurement of serum haptoglobin (Hapto) concentrations results in only moderate reported specificity and sensitivity for the detection of metritic cows. The objective of this study was to evaluate the association between different variables and haptoglobin concentrations after calving. Parity, periparturient metabolic stress indicated by β-hydroxybutyric acid (BHBA) and nonesterified fatty acids (NEFA), calving difficulties, retained fetal membranes (RFM), and acute puerperal metritis (APM) were evaluated. A total of 443 Holstein Friesian cows were enrolled in this retrospective observational study. Acute puerperal metritis was diagnosed when a cow had fetid, reddish-brown, watery vaginal discharge in combination with rectal temperature ≥ 39.5°C. The retention of the fetal membranes has been defined as the failure to expel the fetal membranes within 12h after calving. Results of blood samples from 2 and 5 days in milk (DIM) were analysed for Hapto, BHBA and NEFA. Primiparous cows had a greater median Hapto concentration than multiparous cows at 5 DIM [primiparous: 2.25 g/L interquartile range (IQR) 1.45−2.50, n = 146; multiparous: 1.13 g/L IQR 0.52−2.22; n = 302; $P < 0.05$]. Therefore, different Hapto thresholds based on references from literature for all cows (1.4 g/L), primiparous cows (2.49 g/L), and multiparous cows (1.4 g/L) were used for further analysis. Periparturient metabolic stress indicated by elevated BHBA (≥ 1.2 mmol/L) at 5 DIM was associated with elevated Hapto (odds ratio: 2.39−2.87) regardless of parity. In contrast, elevated NEFA (≥ 0.6 mmol/L) at 2 DIM was not a risk factor for elevated Hapto. Multiparous cows with assisted calving had a 2.46 times higher risk for elevated Hapto, whereas primiparous cows with assisted calving had no elevated risk for elevated Hapto at 5 DIM. Moreover, multiparous cows with RFM were 5.51 times more likely to have elevated Hapto at 5 DIM than cows without RFM. Acute puerperal metritis within the first 5 DIM was associated with elevated Hapto (odds ratio: 2.74−5.01), regardless of parity. We speculate that the association of calving ease, RFM, and periparturient metabolic stress could explain the moderate sensitivity and specificity reported for the detection of metritic cows by measuring Hapto.

Keywords: haptoglobin, calving, retained fetal membranes, metabolic stress, acute puerperal metritis

2.2.2 Introduction

The transition period is associated with an elevated incidence of metabolic and infectious diseases because parturition and the onset of lactation represent huge challenges to the immune system and metabolism of the postpartum cow (Goff and Horst, 1997; Hammon et al., 2006; LeBlanc, 2008). In particular, cows with calving disorders such as dystocia, twins, and caesarian section are predisposed to develop metritis because human intervention is usually necessary, and increased contamination of the uterus during manual examination and extraction is hard to avoid (Dubuc et al., 2010). It is also known that cows with retained fetal membranes (RFM) are more likely to develop metritis than cows without RFM (Halpern et al., 1985; Bruun et al. 2002). Furthermore, metabolic diseases (e.g., ketosis-fatty liver complex) during early lactation are associated with a higher risk of metritis (Duffield et al., 2009). Within the first days of lactation, cows cannot obtain enough energy from dietary sources to meet their needs for body tissue and milk production, resulting in body fat mobilization as well as negative energy balance (Goff and Horst, 1997). The liver`s capacity to metabolize fatty acids during lipolysis is limited. When reaching this limit, NEFA and ketone bodies (e.g., acetoacetate, acetone, and BHBA) accumulate in the blood (Goff and Horst, 1997; Doepel et al., 2002). Because NEFA supply substrates for BHBA, this parameter is usually elevated earlier than BHBA (Doepel et al., 2002).

Negative energy balance has a suppressive effect on immune function. Hammon et al. (2006) found an association between impairment of neutrophil function and negative energy balance. Furthermore, it has been demonstrated that cows that developed uterine disease experience a greater degree of negative energy balance and have greater neutrophil impairment (Galvão et al., 2010). Neutrophils are the main leukocyte type involved in bacterial clearance after uterine infection, and cows with impaired neutrophil function are predisposed to uterine diseases (Hussain, 1989; Gilbert et al., 2007).

Acute phase response is one defense mechanism of the body against uterine infections (Schneider et al., 2013). Haptoglobin (Hapto) is an acute phase protein synthesized in the liver in response to inflammation (Crawford et al., 2005) and it can be measured in serum. The association between uterine infections and elevated Hapto concentrations is well recognized in cows (Huzzey et al., 2009; Dubuc et al., 2010; Burfeind et al., 2014a). Recently, however, our group has shown that the sensitivity to detect acute puerperal metritis (APM) by measuring Hapto in primiparous and multiparous cows was only 64% (threshold of 2.48 g/L) and 72% (threshold of 1.39 g/L) at 5 DIM, respectively (Burfeind et al., 2014a). Previous studies reported even lower sensitivities (50% and 51.6%) for thresholds of 1 g/L on 3 DIM (Huzzey et al., 2009) and 0.8 g/L within the first week postpartum (Dubuc et al., 2010), respectively.

Because Hapto is an indicator of non-specific innate immune response activated by various inflammatory lesions, different variables can influence Hapto concentrations and reduce sensitivity (Eckersall, 2000; Huzzey et al., 2009; Ceciliani et al., 2012). Experimentally induced fatty liver is associated with increased Hapto concentrations (Uchida et al., 1993), and one conclusion is that increased concentrations of Hapto around parturition could be caused by negative energy balance (Crawford et al., 2005). Little research has been conducted on the association between calving disorders and Hapto concentrations (Sabedra, 2012) but we hypothesized that vaginal and uterine lesions due to calving disorders could result in elevated Hapto. Moreover, RFM could increase inflammatory reactions due to bacterial contamination of the vagina and uterus (Bekana et al.; 1994). To evaluate whether antimicrobial treatment of APM is associated with Hapto, treatment as a variable was included in the analysis. Therefore, the objective of this study was to evaluate whether, in addition to APM, other variables occurring within the first 5 DIM are associated with elevated Hapto at 5 DIM. Specifically, we assessed metabolic stress indicated by elevated NEFA and BHBA serum concentrations, calving difficulties, antibiotic treatment of APM within the first 5 DIM, and RFM.

2.2.3 **Materials and Methods**

Animals

A total of 443 Holstein Friesian cows from a commercial dairy farm in Sachsen-Anhalt, Germany, were enrolled in this retrospective observational study. The study was part of 2 research projects involving a clinical trial on body temperature in 2010 (Burfeind et al., 2011; 2014b) and on therapy of APM in 2011 (Sannmann et al., 2013). Cows were managed according to the guidelines set by the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (Hellmann and Radeloff, 2000). The experimental procedures reported herein were conducted with the approval of the Institutional Animal Care and Use Committee of Freie Universität Berlin. The farm housed 1,200 dairy cows. Herd average milk yield was 10,124 kg per lactation (4.1% fat, 3.4% protein) in 2010 and 10,147 kg per lactation (4.0% fat, 3.3% protein) in 2011. All cows were housed indoors. Before calving, cows were housed in group pens with deep-straw bedding. Lactating cows were housed in a free-stall barn with cubicles equipped with rubber mats and slatted floors. The groups were separated into primiparous and multiparous cows. A TMR was distributed by conveyer belt system up to 10 times a day. Cows were milked 3 times a day (0600, 1400, and 2200 h).

Sample collection

Acute puerperal metritis was diagnosed when a cow had fetid, reddish-brown, watery vaginal discharge in combination with rectal temperature ≥ 39.5°C (Sheldon et al., 2006) within the first 10 DIM. Rectal temperature was measured and recorded daily by study personnel. Cows were examined for vaginal discharge by study personnel introducing a gloved hand into the vaginal cavity at 2, 5 and 10 DIM. Vaginal discharge was classified on a scale from 0 to 2 (0 = no discharge; 1 = normal lochial secretion, not smelly, viscous, reddish-brown; 2 = fetid, watery reddish-brown). If antibiotic treatment of APM was required, a single injection of ceftiofur crystalline free acid (Naxcel, Pfizer Limited, Kent, UK) was administered. In 2010, cows with APM were treated by farm personnel according to a standard operating procedure developed by the herd veterinarian. In 2011, cows were additionally examined for vaginal discharge on days with rectal temperature ≥ 39.5°C and treated either on the day of diagnosis (1 to 10 DIM) or only from 5 to 10 DIM (Sannmann et al., 2013). All cows that received antimicrobial drugs for purposes other than APM within the first 10 DIM (e.g., acute mastitis) were excluded from analysis. In this study, only metritis occurring before or at 5 DIM was analyzed, and antimicrobial treatment of APM before or at 5 DIM was included into analysis.

Retained fetal membranes were diagnosed by farm personnel if expulsion of fetal membranes occurred more than 12 hours after calving. Information on RFM was recorded by using herd management software (Herde, version 5.71, dsp Agrosoft GmbH, Ketzin, Germany).

All cows had blood collected from the coccygeal vein at 2, 5 and 10 DIM using sterile vacuum tubes (Venoject II, Termumo Europe N.V., Leuven, Belgium). In this study, only results of samples from 2 and 5 DIM were used for analysis. Immediately after blood collection, BHBA was measured with an electronic BHBA measuring system consisting of a handheld meter (Precision Xtra, Abbott Diabetes Care, Abingdon, UK) and electrochemical test strips (Precision Xtra β-ketone, Abbott Diabetes Care). After the test strip had been inserted into the meter, blood was applied to the sample chamber. After 10 s, the concentration of BHBA (mmol/L) was displayed on the meter. Iwersen et al. (2009) validated this method and found a highly significant correlation ($r = 0.95$, $P < 0.001$) between whole-blood BHBA concentrations measured by the handheld meter and serum BHBA concentrations measured photometrically in the laboratory. The incidence and prevalence of subclinical ketosis (SCK; BHBA ≥ 1.2 mmol/L) peaked at 5 DIM (Mc Ahrt et al., 2012) or within the first week postpartum (Duffield et al., 1998; LeBlanc, 2010). Therefore, concentrations of BHBA at 5 DIM were used for further analysis. Recent studies have recommended a threshold of 1.2 mmol/L, because it results in a good combination of sensitivity and specificity for metritis occurrence (Duffield et al., 2009; McArt et al., 2013; Suthar et al., 2013). Therefore, in this study, we defined SCK as a BHBA concentration ≥ 1.2 mmol/L.

After BHBA measurement, the blood was allowed to clot. Within 2 hours after sampling, blood samples were centrifuged at 1000 x g for 10 min at room temperature and serum stored at −22°C until analysis for serum Hapto and NEFA concentrations. Serum samples were sent to an accredited commercial laboratory (Synlab Laboratories, Berlin, Germany). Burfeind et al. (2014a) showed that Hapto at 5 DIM had the greatest combination of sensitivity and specificity to distinguish between healthy cows and cows with APM. Moreover, serum Hapto concentrations were highest between 3 and 6 DIM depending on the degree of uterine disease (Huzzey et al., 2009) and were elevated in cows with APM compared to a control group (Drillich et al., 2007). Therefore, Hapto was analyzed from samples collected at 5 DIM. For this analysis, an ELISA (Sunrice Reader; Tecan, Maennedorf, Switzerland) was used. The device has been calibrated by the laboratory using cattle serum with Hapto concentrations between 0.6 g/L and 1.3 g/L. The lower limit of detection was 0.31 g/L. The lower limit of detection was 0.31 g/L. All samples with concentrations < 0.31 g/L were set to 0.31 g/L. Upper limit of detection of samples from 2010 was 2.5 g/L, and all samples from 2010 with concentrations > 2.5 g/L were set to 2.5 g/L. There was no upper limit of detection of samples from 2011.

Because NEFA is usually elevated earlier than BHBA (Doepel et al., 2002), samples collected at 2 DIM were analyzed for NEFA by using a clinical chemistry analyser (AU 680, Beckman Coulter, Krefeld, Germany) and a testkit from Randox. Concentrations of NEFA were determined by photometric measurement. The lower limit of detection was 0.07 mmol/L. All samples with concentrations < 0.07 mmol/L were set to 0.00 mmol/L. For postpartum NEFA concentrations, a recent study showed that a threshold of 0.57 mmol/L is optimal to predict clinical ketosis, metritis or displaced abomasa (Ospina et al., 2010). Moreover, Hiss et al. (2009) showed an association between NEFA ≥ 0.6 mmol/L and Hapto concentration postpartum. Therefore, we chose a threshold of 0.6 mmol/L for NEFA.

Information about calving was recorded by farm personnel using the herd management software (Herde, version 5.71, dsp Agrosoft GmbH, Ketzin, Germany). Calving ease was scored on a 5-point scale (0 = calving not observed, 1 = calving without human intervention, 2 = calving with soft human pulling, 3 = dystocia, 4 = caeserian section or veterinary assisted calving). For analysis, the score was reduced to spontaneous calving (0 and 1) and assisted calving (2 to 4).

Statistical analysis

Data were analyzed using SPSS for Windows (Version 22, SPSS Inc. Munich, Germany). Median concentrations of Hapto and inter quartile ranges (IQR) were calculated for different variables. The IQR is the difference between the third and the first quartiles in a data set and is a measure of how the data spread around the median. Differences of the absolute concentrations of Hapto between cows with elevated NEFA (≥ 0.6 mmol/L) and without elevated NEFA concentrations (< 0.6 mmol/L) at 2 DIM, between cows with SCK (BHBA ≥ 1.2 mmol/L) and without SCK (BHBA < 1.2 mmol/L) at 5 DIM, between cows with spontaneous calving and assisted calving, between cows with and without RFM, between cows with and without antimicrobial treatment within the first 5 DIM, and between cows with or without metritis within the first 5 DIM were compared by using the Mann-Whitney U-Test. A *P*-Value < 0.05 represents significant differences between groups.

To determine the association between different variables and elevated Hapto at 5 DIM, we first tested the effect of the factors of interest on Hapto separately with Mann-Whitney U-tests to identify those factors that should be included in the binary logistic regression model. Therefore, parity (1 = primiparous, 2 = multiparous), NEFA (0 = < 0.6 mmol/L, 1 = ≥ 0.6 mmol/L), SCK (0 = BHBA < 1.2 mmol/L, 1 = BHBA ≥ 1.2 mmol/L), calving (0 = spontaneous, 1 = assisted), RFM (0 = no, 1 = yes), antimicrobial treatment (0 = no, 1= yes), and APM (0 = no, 1 = yes) were tested, and only variables with *P* < 0.20 were included in a binary logistic regression model. Furthermore, all variables were tested with Spearman´s correlation. If 2 variables showed a high, significant correlation (r > 0.5), only the one resulting in the Mann-Whitney U-test with the smaller *P*-Value was used in the final model (Bertulat et al. 2013). The binary regression model was constructed in a stepwise backwards manner such that in the final binary regression model variables with *P* > 0.05 were excluded. Odds ratios, confidence intervals and *P*-Values are reported. Confidence interval was set at 95%. To evaluate associations, three different approaches were investigated. To account for different Hapto thresholds in primiparous and multiparous cows to distinguish between healthy and metritic cows (Burfeind et al., 2014a), animals were classified according to their parity. In the first approach all cows were included and the Hapto threshold was 1.4 g/L. In the second approach, only primiparous cows were included and the threshold of Hapto was 2.49 g/L. In the third approach, only multiparous cows were included and the Hapto threshold was 1.4 g/L. Thresholds were based on a receiver operating characteristic curve analysis by Burfeind et al. (2014a), which demonstrated that different Hapto thresholds for metritis detection are optimal depending on parity, and that primiparous cows have higher Hapto concentrations than multiparous cows. Except for the variable parity, all mentioned variables were also evaluated in the second and third approach.

2.2.4 **Results**

Primiparous cows (2.25 g/L, IQR 1.45−2.50, n = 146) had a greater median Hapto concentration than multiparous cows (1.13, g/L IQR 0.52−2.22; n = 297; $P < 0.05$). Differences between Hapto concentrations considering different variables are presented in Table 1. We detected no difference in Hapto concentrations in primiparous cows between spontanous and assisted calving ($P = 0.40$), between primiparous cows with RFM and without RFM ($P = 0.37$), or between primiparous cows with and without antimicrobial treatment ($P = 0.10$). Concentrations of Hapto at 5 DIM differed depending on the occurrence of elevated NEFA at 2 DIM, SCK at 5 DIM, and APM in primiparous and multiparous cows ($P < 0.05$). Moreover, multiparous cows with assisted calving and antimicrobial treatment within 5 DIM had higher Hapto concentrations than those with spontaneous calving or without antimicrobial treatment ($P < 0.05$).

The binary logistic regression analysis was conducted to determine the association between different variables and elevated Hapto at 5 DIM. Calving ease, RFM, and NEFA at 2 DIM are variables occurring before Hapto measurement and therefore are possible risk factors for elevated Hapto. As BHBA was measured at 5 DIM and APM could occur within the first 5 DIM, increased odds ratios reflect associations instead of risks between these variables and elevated Hapto because the condition for causality is not given. Treatment before or at 5 DIM was highly correlated with APM within the first 5 DIM ($r > 0.5$, $P < 0.05$). Acute puerperal metritis was selected for inclusion into the final model because it was the variable of special interest in this study.

In the first approach, all cows were included into the final logistic regression model (Table 2). Remaining variables were parity, BHBA at 5 DIM, calving ease, RFM, and APM. Primiparous cows, cows with BHBA ≥ 1.2 mmol/L at 5 DIM, cows with assisted calving, cows with RFM, and cows with APM had 4.4 ($P < 0.05$), 2.87 ($P < 0.05$), 1.95 ($P < 0.05$), 3.39 ($P < 0.05$), and 4.7 (P < 0.05) times the odds of elevated Hapto (≥ 1.4 g/L) compared to multiparous cows, cows with BHBA at 5 DIM < 1.2 mmol/L, cows with spontaneous calving, cows without RFM, and cows without APM within 5 DIM, respectively.

In the second approach, only primiparous cows were included into the binary logistic regression model (Table 3), and the threshold for Hapto at 5 DIM was set at 2.49 g/L. Only SCK and APM remained significant in the final model. Cows with BHBA ≥ 1.2 mmol/L at 5 DIM and cows with APM within 5 DIM had 2.39 ($P < 0.05$) and 2.74 ($P < 0.05$) times the odds of elevated Hapto ($P < 0.05$).

In the third approach, only multiparous cows were included in the binary logistic regression model (Table 4), and the threshold for Hapto at 5 DIM was set at 1.40 g/L. Remaining variables were SCK, calving ease, RFM, and APM. Cows with BHBA ≥ 1.2 mmol/L at 5 DIM, cows with assisted calving, cows with RFM, and cows with APM had 2.75 ($P < 0.05$), 2.46 ($P < 0.05$), 5.51 ($P < 0.05$) and 5.01 ($P < 0.05$) times the odds of elevated Hapto.

Table 1. Differences of median haptoglobin concentration (g/L) at 5 DIM differentiated by parity and considering NEFA at 2 DIM, BHBA at 5 DIM, calving ease, retained fetal membranes, antimicrobial treatment ≤ 5 DIM, and acute puerperal metritis ≤ 5 DIM

Variable	Parity[1]	Class	No. of cows	Median	IQR[2]	*P*-Value[3]
NEFA at 2 DIM	1	< 0.6 mmol/L	54	1.90	1.16−2.50	< 0.05
		≥ 0.6 mmol/L	68	2.48	1.94−2.50	
	2	< 0.6 mmol/L	124	0.96	0.45−2.00	< 0.05
		≥ 0.6 mmol/L	145	1.34	0.56−2.47	
BHBA at 5 DIM	1	< 1.2 mmol/L	109	2.02	1.23−2.50	< 0.05
		≥ 1.2 mmol/L	38	2.49	2.15−2.50	
	2	< 1.2 mmol/L	177	0.85	0.43−1.76	< 0.05
		≥ 1.2 mmol/L	120	1.65	0.68−2.50	
Calving	1	spontanous	67	2.14	1.24−2.50	0.40
		assisted	79	2.35	1.56−2.50	
	2	spontanous	234	0.99	0.49−2.02	< 0.05
		assisted	63	1.91	0.93−2.50	
Retained fetal membranes	1	no	139	2.27	1.53−2.50	0.37
		yes	7	1.47	0.89−2.50	
	2	no	255	0.97	0.47−2.01	< 0.05
		yes	42	2.40	1.76−2.50	
Treatment ≤ 5 DIM	1	no	125	2.19	1.35−2.50	0.10
		yes	21	2.49	1.87−2.54	
	2	no	274	1.02	0.49−2.15	< 0.05
		yes	23	2.47	1.66−2.66	
Acute puerperal metritis ≤ 5 DIM	1	no	107	2.15	1.20−2.50	< 0.05
		yes	39	2.50	1.66−2.52	
	2	no	254	0.98	0.49−2.04	< 0.05
		yes	43	2.40	1.65−2.50	

[1] Where 1 = primiparous, 2 = multiparous

[2] Interquartile range

[3] *P*-value from Mann-Whitney U-test for differences between haptoglobin concentrations depending on different variables and parities

Table 2. Final logistic regression model including all cows and showing odds ratios of parity, elevated BHBA at 5 DIM, calving ease, retained fetal membranes, and acute puerperal metritis ≤ 5 DIM for elevated serum haptoglobin concentration (≥ 1.4 g/L) at 5 DIM

Variable	Odds ratio	95% CI	*P*-Value
Parity			
multiparous	Referent		
primiparous	4.40	2.64−7.34	< 0.05
BHBA 5 DIM			
< 1.2 mmol/L	Referent		
≥ 1.2 mmol/L	2.87	1.80−4.56	< 0.05
Calving			
spontanous	Referent		
assisted	1.95	1.20−3.19	< 0.05
Retained fetal membranes			
no	Referent		
yes	3.39	1.51−7.61	< 0.05
Acute puerperal metritis ≤ 5 DIM			
no	Referent		
yes	4.70	2.33−9.48	< 0.05

Table 3. Final logistic regression model including primiparous cows and showing odds ratios of elevated BHBA at 5 DIM and acute puerperal metritis ≤ 5 DIM for elevated serum haptoglobin concentration (≥ 2.49 g/L) at 5 DIM

Variable	Odds ratio	95% Confidence interval	*P*-Value
BHBA 5 DIM			
< 1.2 mmol/L	Referent		
≥ 1.2 mmol/L	2.39	1.1−5.22	< 0.05
Acute puerperal metritis ≤ 5 DIM			
no	Referent		
yes	2.74	1.27−5.92	< 0.05

Table 4. Final logistic regression model including multiparous cows and showing odds ratios of elevated BHBA at 5 DIM, calving ease, retained fetal membranes, and acute puerperal metritis ≤ 5 DIM for elevated serum haptoglobin concentration (≥ 1.4 g/L) at 5 DIM

Variable	Odds ratio	95% Confidence interval	*P*-Value
BHBA 5 DIM			
< 1.2 mmol/L	Referent		
≥ 1.2 mmol/L	2.75	1.62−4.66	< 0.05
Calving			
spontanous	Referent		
assisted	2.46	1.3−4.66	< 0.05
Retained fetal membranes			
no	Referent		
yes	5.51	2.21−13.69	< 0.05
Acute puerperal metritis ≤ 5 DIM			
no	Referent		
yes	5.01	2.09−12.04	< 0.05

2.2.5 **Discussion**

The objective of this study was to evaluate whether, in addition to APM, other variables such as periparturient metabolic stress indicated by elevated NEFA and BHBA serum concentrations, calving difficulties, antibiotic treatment within 5 DIM, and RFM were associated with elevated Hapto at 5 DIM.

Primiparous cows had 4.4 times the odds of elevated Hapto at 5 DIM compared with multiparous cows. This is consistent with other findings (Crawford et al., 2005; Humblet et al., 2006; Schneider et al., 2013; Burfeind et al., 2014a) and it was speculated that primiparous cows may be more responsive to inflammatory stress associated with calving than multiparous cows (Schneider et al., 2013) because damage to the uterus, vagina, and vulva may be more severe during first parturition (Humblet et al., 2006). This could also explain why calving difficulties in primiparous cows did not result in increased odds of elevated Hapto at 5 DIM. Burfeind et al. (2014a) suggested that primiparous cows that calve spontaneously might be affected by mild cervical or vaginal trauma that remains undetected but stimulates an inflammatory response that could contribute to greater Hapto concentrations. Therefore, our analysis considered 3 parity groups (i.e., all cows, primiparous cows, multiparous cows) with higher thresholds of Hapto in primiparous cows. Multiparous cows with assisted calving had a 2.46-times-higher risk for elevated Hapto, which is consistent with previous findings (Sabedra, 2012).

Periparturient metabolic stress indicated by elevated BHBA at 5 DIM increased the odds (odds ratio: 2.39−2.87) of elevated Hapto for all 3 parity groups, which supports the results of Ospina et al. (2010). In contrast to our findings, Dubuc et al. (2010) did not find an association of postpartum BHBA concentrations and the risk of metritis. However, they did not evaluate the association between BHBA and Hapto per se.

In contrast to SCK, periparturient metabolic stress indicated by elevated NEFA at 2 DIM was not associated with a risk for elevated Hapto. This result is in line with the observation that concentrations of NEFA were similar between healthy cows and cows with uterine infections (Schneider et al., 2013) and that an association between postpartum NEFA and metritis was not found (Dubuc et al., 2010). Ospina et al. (2010), however, found elevated risk for metritis, displaced abomasum, and clinical ketosis in cows with elevated postpartum NEFA. Moreover, results from the literature are not consistent regarding an optimal threshold for NEFA and an optimal time for taking samples (Hiss et al., 2009; Dubuc et al., 2010; Ospina et al., 2010; Chapinal et al., 2012).

Multiparous cows with RFM were 5.51 times more likely to have elevated Hapto at 5 DIM than cows without RFM, and odds for RFM were even higher than for APM (odds ratio 5.01). The presence of RFM supports intrauterine bacterial growth and uterine tissue might be damaged due to potential manual removal (Bolinder et al., 1988), leading to inflammatory processes. Dubuc et al. (2010) also found strong association between RFM and metritis (odds ratio 6.25, $P < 0.01$). However, RFM was not a risk factor for elevated Hapto for primiparous cows. One reason for this could be the small number of primiparous cows with RFM (n = 7).

Acute puerperal metritis was highly associated (odds ratio: 2.74−5.01) with elevated Hapto in all parity groups. These results support previous studies in which a 3- to 7-times-higher risk for metritis was described in cows with elevated postpartum Hapto (Huzzey et al., 2009; Dubuc et al., 2010). It is noticeable that there was a greater association between elevated Hapto and multiparous cows with RFM than between Hapto and multiparous cows with APM.

To conclude, metabolic stress indicated as SCK as well as calving disorders and RFM in multiparous cows, is associated with Hapto at 5 DIM. This could explain the reduced sensitivity and specificity in the detection of metritic cows (Huzzey et al., 2009; Dubuc et al., 2010; Burfeind et al., 2014a) and has to be considered when using Hapto as a diagnostic tool for the detection of cows with APM. However, APM had high odds ratios, reflecting a strong association between APM and Hapto at 5 DIM.

2.2.6 **Acknowledgements**

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2.2.7 **References**

Bekana, M., P. Jonsson, T. Ekman, and H. Kindahl 1994. Intrauterine bacterial findings in postpartum cows with retained fetal membranes. J. Vet. Med. Ser. A 41:663-670

Bertulat, S., C. Fischer-Tenhagen, V. Suthar, E. Möstl, N. Isaka, and W. Heuwieser 2013. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yields. J. Dairy Sci. 96:377-3787.

Bolinder, A., B. Seguin, H. Kindahl, D. Bouley, and D. Otterby. 1988. Retained fetal membranes in cows; Manual removal versus nonremoval and its effect on reproductive performance. Theriogenology 30:45-56.

Bruun, J., A. K. Ersbøll, and L. Alban 2002. Risk factors for metritis in Danish dairy cows. Preventive Veterinary Medicine 54: 179-190.

Burfeind, O., I. Sannmann, R. Voigtsberger, and W. Heuwieser. 2014a. Receiver operating characteristic curve analysis to determine the diagnostic performance of serum haptoglobin concentration for the diagnosis of acute puerperal metritis in dairy cows. Anim. Reprod. Sci. 149:145-151.

Burfeind, O., V. S. Suthar, R. Voigtsberger, S. Bonk, and W. Heuwieser. 2011. Validity of prepartum changes in vaginal and rectal temperature to predict calving in dairy cows. J. Dairy Sci. 94:5053-5061.

Burfeind, O., V. S. Suthar, R. Voigtsberger, S. Bonk, and W. Heuwieser. 2014b. Body temperature in early postpartum dairy cows. Theriogenology 82:121-131.

Ceciliani, F., J. J. Ceron, P. D. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. Journal of Proteomics 75:4207-4231.

Chapinal, N., M. E. Carson, S. J. LeBlanc, K. E. Leslie, S. Godden, M. Capel, J. E. P. Santos, M. W. Overton, and T. F. Duffield. 2012. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J. Dairy Sci. 95:1301-1309.

Crawford, R. G., K. E. Leslie, R. Bagg, C. P. Dick, and T. F. Duffield. 2005. The impact of controlled release capsules of monensin on postcalving haptoglobin concentrations in dairy cattle. The Canadian Journal of Veterinary Research 69:208-214.

Doepel, L., H. Lapierre, and J. J. Kennelly. 2002. Peripartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. J. Dairy Sci. 85:2315-2334.

Drillich, M., D. Voigt, D. Forderung, and W. Heuwieser. 2007. Treatment of acute puerperal metritis with flunixin meglumine in addition to antibiotic treatment. J. Dairy Sci. 90:3758-3763.

Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2010. Risk factors for postpartum uterine diseases in dairy cows. J. Dairy Sci. 93:5764-5771.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571-580.

Duffield, T. F., D. Sandals, K. E. Leslie, K. Lissemore, B. W. McBride, J. H. Lumsden, P. Dick, and R. Bagg. 1998. Efficacy of monensin for the prevention of subclinical ketosis in lactating dairy cows. J. Dairy Sci. 81:2866-2873.

Eckersall, P. D. 2000. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. Revue de Méd. Toulouse Vét. 7:577-584.

Galvão, K. N., M. J. B. F. Flaminio, S. B. Brittin, R. Sper, M. Fraga, L. Caixeta, A. Ricci, C. L. Guard, W. R. Butler, and R. O. Gilbert. 2010. Association between uterine disease and indicators of neutrophil and systemic energy status in lactating holstein cows. J. Dairy Sci. 93:2926-2937.

Gilbert, R. O., N. R. Santos, K. N. Galvão, S. B. Brittin, and H. B. Roman. 2007. The relationship between postpartum uterine bacterial infection (bi) and subclinical endomatritis (se). J. Dairy Sci. 90 (Suppl. 1):469-470 (Abstr).

Goff, J. P. and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260-1268.

Halpern, N. E., H. N. Erb, R. David Smith 1985. Duration of retained fetal membranes and subsequent fertility in dairy cows. Theriogenology 23: 807-813.

Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. 2006. Neutrophil function and energy status in holstein cows with uterine health disorders. Veterinary Immunol. Immunopathol. 113:21-29.

Hellmann, K. and I. Radeloff. 2000. Guidance for industry: Good clinical practice. International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). VICH, Brussels, Belgium. http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052417.pdf

Hiss, S., C. Weinkauf, S. Hachenberg, and H. Sauerwein. 2009. Short communication: Relationship between metabolic status and the milk concentrations of haptoglobin and lactoferrin in dairy cows during early lactation. J. Dairy Sci. 92:4439-4443.

Humblet, M.-F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J.-M. Godeau. 2006. Relationship between haptoglobin, serum amyloid a, and clinical status in a survey of dairy herds during a 6-month period. Veterinary Clinical Pathology 35:188-193.

Hussain, A. M. 1989. Bovine uterine defense mechanisms: A review. J. Vet. Med. Ser. B 36:641-651.

Huzzey, J. M., T. F. Duffield, S. J. LeBlanc, D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Short communication: Haptoglobin as an early indicator of metritis. J. Dairy Sci. 92:621-625.

Iwersen, M., U. Falkenberg, R. Voigtsberger, D. Forderung, and W. Heuwieser. 2009. Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. J. Dairy Sci. 92:2618-2624.

LeBlanc, S. 2010. Monitoring metabolic health of dairy cattle in the transition period. J. Reprod. Dev. 56:S29-S35.

LeBlanc, S. J. 2008. Postpartum uterine disease and dairy herd reproductive performance: A review. Vet. J. 176:102-114.

McArt, J. A. A., D. V. Nydam, and G. R. Oetzel. 2012. Epidemiology of subclinical ketosis in early lactation dairy cattle. J. Dairy Sci. 95:5056-5066.

McArt, J. A. A., D. V. Nydam, G. R. Oetzel, T. R. Overton, and P. A. Ospina. 2013. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. Vet. J. 198:560-570.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern united states: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546-554.

Sabedra, D. A. 2012. Serum haptoglobin as an indicator for calving difficulties and postpartal diseases in transition dairy cows. Undergraduate Thesis. Oregon State University.

Sannmann, I., O. Burfeind, R. Voigtsberger, and W. Heuwieser. 2013. Comparison of two monitoring and treatment strategies for cows with acute puerperal metritis. Theriogenology 79:961-969.

Schneider, A., M. N. Correa, and W. R. Butler. 2013. Short communication: Acute phase proteins in holstein cows diagnosed with uterine infection. Res Vet Sci 95:269-271.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516-1530.

Suthar, V. S., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in european dairy cows. J. Dairy Sci. 96:2925-2938.

Uchida, E., N. Katoh, and K. Takahashi. 1993. Induction of serum haptoglobin by administration of ethionine to cows. J. Vet. Med. Sci. 55:501-502.

3 ADDITIONAL UNPUBLISHED DATA

3.1. Randomized, controlled clinical trial on the efficacy of nonsteroidal antiinflammatory drugs for the treatment of acute puerperal metritis

3.1 Randomized, controlled clinical trial on the efficacy of nonsteroidal antiinflammatory drugs for the treatment of acute puerperal metritis in dairy cows

Randomized, controlled clinical trial on the efficacy of nonsteroidal antiinflammatory drugs for the treatment of acute puerperal metritis in dairy cows

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3.1.1 Abstract

Acute puerperal metritis (APM) in dairy cows is often treated with antibiotics. An increasing antibiotic resistance is well documented and associated with decreasing clinical efficacy, animal welfare and economic consequences. Hence, there is the need to encourage prudent use of antibiotics and alternative therapies to antibiotics. The objective of this study was to compare the efficacies of ketoprofen and ceftiofur for the treatment of APM.

Between June 2013 and February 2015 a total of 610 dairy cows from 6 farms in Germany were enrolled. Inclusion criteria was a rectal temperature (RT) ≥ 39.5°C and a reddish-brown fetid vaginal discharge within the first 10 DIM. Cows meeting the inclusion criteria were randomly allocated to treatment with ketoprofen (3 mg/kg of body weight, n = 300) or treatment with ceftiofur (1 mg/kg of body weight, n = 310), both on 3 consecutive days. Rectal temperature was recorded daily for a period of 7 days after enrollment. Cows that showed RT ≥ 39.5°C on d 4 to 7 after inclusion received an extended treatment (extT) with ceftiofur for 3 (ketoprofen group) or 2 (ceftiofur group) more days. Between 21 and 40 DIM cows were examined with the metricheck device and vaginal discharge was categorized on a 5-point scale according to the presence of pus.

A total of 52 cows (35 from ketoprofen group, 17 from ceftiofur group) were excluded from analysis due to missing protocol compliance (n = 37) or concurrent disease (n = 15). Cows treated with ketoprofen had 3.47 [95% confidence interval (CI): 2.42−4.95; *P* < 0.001; n = 558] times the odds of extT compared to cows treated with ceftiofur. Occurrence of purulent vaginal discharge was similar among treatment groups (ketoprofen: 56%, ceftiofur: 53%). However, cows with extT had 2.00 (95% CI: 1.41−3.19; *P* = 0.001; n = 438) times the odds of endometritis compared to cows without extT. Treatment group did not affect mean 100 DIM milk yield (ketoprofen group 3531 ± 816 kg, ceftiofur group 3571 ± 743 kg; *P* = 0.58), first artificial insemination pregnancy risk (ketoprofen group: 20% vs. ceftiofur group: 25%; *P* = 0.25; n = 480), time to first artificial insemination (ketoprofen group: 75 d; 95% CI: 73−77 d vs. ceftiofur group: 73 d; 95% CI: 71−75 d; *P* = 0.36; n = 437) and time to pregnancy (ketoprofen group: 133 d; 95% CI: 120−147 d vs. ceftiofur group: 143 d; 95% CI: 132−154 d; *P* = 0.88; n = 437).

More than half of the cows initially treated with ketoprofen needed an extT with ceftiofur. However, there is potential of reducing antibiotic use by utilizing ketoprofen for the treatment of APM as up 39% of cows initially treated with ketoprofen did not need an extended treatment and the occurrence of purulent vaginal discharge, milk yield and reproductive performance was not negatively affected.

Key words: acute puerperal metritis, antibiotic, NSAID, reproduction, animal health

3.1.2 **Introduction**

Acute puerperal metritis (APM) is a potentially life threatening and painful disease (Drillich et al., 2007; Stojkov et al., 2015). The pain response is particularly obvious during transrectal palpation of the uterus (Stojkov et al., 2015). Diagnosis of acute puerperal metritis is based on reddish-brown, fetid, watery discharge and rectal temperature ≥ 39.5°C within the first 21 DIM (Sheldon et al., 2006). Cows with APM have signs of systemic illness, an impaired reproductive performance, are more likely to be culled and have less milk production in comparison to healthy cows (Fourichon et al., 2000; Wittrock et al., 2011; Machado et al., 2014). Because of these reasons related to animal health, animal welfare and economics, an efficacious treatment is indicated.

Different aerobic and anaerobic bacteria can be found in the infected uterus including *E.coli, T. pyogenes, Prevotella ssp., Fusobacterium necrophorum, Fusobacterium nucleatum, Mannheimia haemolytica, Staphylococcus ssp.* and *Streptococcus ssp.* (Sheldon, 2004; Williams et al., 2005; Santos et al., 2011). Due to this wide range of pathogens it is reasonable to suggest the use of antimicrobials with broad spectrum activity as preferred treatment (Lima et al., 2014). Therefore, APM is commonly treated with third-generation cephalosporins (Machado et al., 2014). The reason for the widespread use of ceftiofur administered parenterally is the high efficacy (Chenault et al., 2004; Sheldon, 2004) and the zero day withdrawal time for milk resulting in a major financial incentive for using it (Grove-White and Murray, 2009; Scientific Advisory Group on Antimicrobials of the Committee for Medicinal Products for Veterinary Use, 2009; Lima et al., 2014). However, third-generation cephalosporins are very important antimicrobials for severe infections in humans and its use in food-producing animals could potentially lead to an increased prevalence of resistance (Scientific Advisory Group on Antimicrobials of the Committee for Medicinal Products for Veterinary Use, 2009). As antibiotic resistance is recognized as a top public health challenge facing the 21st century (Machado et al., 2014), there is the need for a prudent use of antimicrobials and for the evaluation of alternative therapies.

In a clinical trial involving 1023 cows, McLaughlin et al. (2012) showed a self-cure rate of not treated cows with APM of 55.3% compared to a cure rate of 74.3% of cows with APM treated with the long-acting formulation of ceftiofur (ceftiofur crystalline free acid sterile suspension) twice. The results indicated that more than half of the cows from the saline treated group self-recovered. Moreover, Sannmann et al. (2013b) showed that there was no negative effect on cure rates, milk yield, serum haptoglobin concentration and subsequent uterine health when cows with APM prevalent within the first 5 DIM were left untreated. However, animal welfare has to be considered in cows without any treatment with regard to visceral pain of the uterus and systemic signs of illness (Sheldon et al., 2006; Stojkov et al., 2015).

Nonsteroidal anti-inflammatory drugs (NSAID) were described as a supportive treatment of APM in addition to antibiotics (Amiridis et al., 2001; Drillich et al., 2007). These drugs have an analgesic, anti-inflammatory, anti-pyretic and anti-endotoxic effects and thus can increase animal health and wellbeing (Fitzpatrick et al., 2004; Laven et al., 2012). Ketoprofen, a NSAID with zero day withdrawal time on milk, has been administered by Newby et al. (2013) after surgical correction of a left displaced abomasum to reduce pain, increase appetite and increase milk production. Anti-inflammatory and analgesic effects of NSAID in combination with a remarkable self cure rate of APM make it plausible to study the efficacy of ketoprofen in treatment of APM.

Considering this self cure rate and a necessary prudent use of antibiotic drugs, the objective of this study was to evaluate if a treatment of APM with ketoprofen is efficacious. Specifically, we set out to 1) compare cure rates of cows with APM treated with ceftiofur or ketoprofen, 2) to determine the incidence of endometritis indicated by purulent vaginal discharge (PVD) and the first 100 DIM milk yield and 3) to analyze reproductive performance (time to first artificial insemination (AI), first AI pregnancy risk and time to pregnancy) of cows treated with ketoprofen or ceftiofur.

3.1.3 **Materials and Methods**

The study was conducted as a multi-dairy randomized controlled design. An a priori sample size calculation was conducted for the two main outcome variables − cure rate based on the occurrence of extended treatment (extT) on day 4 after enrollment, and first AI pregnancy risk, respectively. For the comparison of cure rates Fisher´s exact test for two independent groups was applied using G*Power (version 3.1.5, University of Düsseldorf). Our assumption for differences in cure rates was based on a previous study (McLaughlin et al., 2012), which described clinical cure rates of 74.3% and 55.3% for cows treated with ceftiofur and untreated control cows. Using $\alpha = 0.05$, $\beta = 0.05$ and a two tailed study design, a sample size of 155 cows per treatment group would be necessary to verify our hypotheses.

Sample size considering first AI pregnancy risk was calculated using a power calculator for a binary outcome in non-inferiority trials (Sealed Envelope Ltd., London, United Kingdom). The non-inferiority limit was a difference of 10% in fist AI pregnancy risk between treatments ($\alpha = 0.05$; $\beta = 0.20$; one tailed). A first AI pregnancy risk of cows that previously suffered from metritis of 38% was assumed (Santos et al., 2010). Based on this assumptions, a total of 292 cows per treatment group was considered necessary.

Farms and Animals

The study was conducted on 6 commercial dairy farms in the northeastern part of Germany between June 2013 and February 2015. Participating farms were recruited based on convenience. Herd size ranged from 732 to 2244 with 677 to 1319 milking cows. A total of 610 Holstein Frisian cows (188 primiparous and 422 multiparous) were enrolled. In 2 herds only multiparous cows were enrolled due to specific housing practices for primiparous cows. Cows were managed according to the guidelines set by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medical Products (Hellmann and Radeloff, 2000). The experimental procedures reported herein were conducted with the approval of the Institutional Animal Care and Use Committee of Freie Universität Berlin. Cows were housed indoors in free stall barns with different bedding, e.g. rubber mats, straw or dried fermentation product from the on-site biogas plant and were fed total mixed ratios. The total mixed ration from close up and fresh cows was formulated to meet or exceed minimum nutritional requirements for high-producing dairy cows (NRC, 2001). Milk yield and milk quality was recorded and controlled monthly by the local dairy herd improvement association. Reproductive performance and medical treatment were recorded and stored by the herd management software (Herde, version 5.81, dsp Agrosoft GmbH, Ketzin, Germany).

The yearly 21 d pregnancy rates were 16, 16, 12, 17, 17 and 20% for farms 1, 2, 3, 4, 5, and 6, respectively. Voluntary waiting period was set at 40 DIM. Inseminations were performed based on estrus detection or timed artificial insemination (TAI). Inseminations based on TAI were conducted once per week. Ovulation was synchronized using GnRH (100 µg of Gonadorelin, Gonavet Veyx, Veyx Pharma Ltd., Germany) and $PGF_{2\alpha}$ (500 µg Cloprostenol, PGF Veyx forte, Veyx Pharma Ltd., Germany). For farms 1, 4, 5, and 6, all cows not detected in estrus at 90 ± 3 DIM were enrolled into TAI and given GnRH. Seven days later cows received $PGF_{2\alpha}$ and 56 hours later GnRH. Timed AI was performed 16 h after the second GnRH injection. Farm 2 was using a Presynch-Ovsynch protocol for the first insemination. Cows were presynchronized with two injections of $PGF_{2\alpha}$ at 36 ± 3 and 50 ± 3 DIM. Cows detected in estrus after $PGF_{2\alpha}$ injection were inseminated. The remaining cows received their first GnRH injection with 64 ± 3 DIM. Seven days later these cows received $PGF_{2\alpha}$ and 56 hours later GnRH. Timed AI was performed 16 h after the second GnRH injection at 74 ± 3 DIM. For farm 3, all cows not bred at 60 ± 3 DIM had their ovaries scanned by the herd veterinarian. Cows with a corpus luteum ≥ 20 mm received $PGF_{2\alpha}$, 56 h later GnRH, and TAI 16 h later. Cows without a luteal structure or a corpus luteum < 20 mm received GnRH, 7 days later $PGF_{2\alpha}$, 56 h later GnRH, and TAI 16 to 20 h later.

Study protocol

The farm personnel evaluated body temperature and vaginal discharge of all fresh cows by using a standardized definition of APM within the first 10 DIM. Inclusion criteria was rectal temperature ≥ 39.5°C in combination with reddish-brown fetid watery vaginal discharge. Every person involved in the examination and treatment of cows was trained in advance to minimize deviations in diagnoses. Cows meeting inclusion criteria were assigned to treatment from a random list generated with Excel (Office 2010, Microsoft Deutschland GmbH, Munich, Germany) created in advance to the study for each farm separately. Cows were either treated with ketoprofen (3 mg/kg of body weight, Dinalgen, 150 mg/ml, Bayer Vital GmbH, Leverkusen or Rifen 100mg/ml, Vétoquinol GmbH, Ravensburg or Romefen, 100 mg/ml, Merial GmbH, Hallbergmoos) or with ceftiofur (1 mg/kg of body weight, Excenel Flow, 50 mg/ml, Zoetis Deutschland GmbH, Berlin or Readycef 50 mg/ml, Virbac Tierarzneimittel GmbH, Bad Oldesloe), both for 3 consecutive days. Ketoprofen has been approved in Germany for the treatment of fever, pain and inflammation in dairy cows associated with respiratory tract infections, mastitis, lameness, arthritis, traumatic injuries and for relief of getting up after calving according to the specific product characteristics but not for APM. Therefore, the local veterinary authorities were notified about the experimental test use. Treatment group was not blinded to the attending person because duration of extT was different among treatment groups (Deluyker et al., 2005). Rectal temperature was recorded daily for a period of 7 d after enrollment. Cows not responding to initial treatment (i.e., rectal temperature ≥ 39.5°C on day 4 after inclusion) received an extT with ceftiofur for 3 days (ketoprofen group) or 2 additional days (ceftiofur group). Cows showing fever on day 5, 6 or 7 after enrollment were examined for further systemic signs of illness (i.e., dullness, loss of appetite) and received extT based on the decision of the herd manager or veterinarian. Clinical cure was defined as cows not receiving extT. During the trial each farm was visited biweekly by a veterinarian (author) and diagnosis of APM as well as protocol compliance were reviewed. Moreover, uterine bacteriological swap samples of untreated cows were collected from cows with APM, cows susceptible for APM (reddish-brown, fetid discharge or rectal temperature ≥ 39.5°C) and healthy cows. Sample collection was performed by the same veterinarian throughout the whole study. Samples were collected from the uterine body of each cow between 0 and 17 d postpartum using the cytobrush technique previously described (Sens and Heuwieser, 2013) in order to test the susceptibility of ceftiofur. Furthermore, cows between 21 and 40 DIM were examined for PVD as a sign for endometritis (Sheldon et al., 2006) with the metricheck device (Metricheck, Simcro, New Zealand). Vaginal discharge was categorized on a 5-point scale (0 = clear mucus; 1 = mucus containing flecks of pus; 2 = discharge containing less than 50% of pus; discharge containing more than 50% of pus; 4 = reddish-brown fetid discharge) as previously described (Williams et al., 2005; Sheldon et al., 2006; Chapinal et al., 2011). The follow-up period was 200 DIM and data of AI, pregnancy, milk yield and culling were generated from the herd´s management software (HERDE, version 5.81, dsp Agrosoft GmbH, Ketzin, Germany).

Statistical analysis

Data were analyzed using SPSS for Windows (version 22, SPSS Inc., Munich, Germany) and Medcalc (version 15.0., Mariakerke, Belgium). The significance level was set at $P \leq 0.05$. Homogeneity of the proportion of parity (i.e., 1st, 2nd or higher lactation) and treatment group (i.e., ceftiofur and ketoprofen) was evaluated with $\chi 2$ test. In order to determine the effect of treatment on cure rate based on the occurrence of extT on day 4 to 7, endometritis between 21 and 40 DIM and first AI pregnancy risk, a binary logistic regression model was used. All relevant factors were first tested in a univariable model in order to identify those factors that should be included in the multivariable, univariate binary logistic regression model. The following factors were considered for the models: treatment group (1=ceftiofur, 2 = ketoprofen), farm (1−6) and parity (1 = primiparous, 2 = multiparous). Calving season (1 = cold, 2 = warm) and extended treatment (1 = no, 2 = yes) were furthermore included in the model with endometritis and first AI pregnancy risk as outcome variables. Endometritis (1 = no, 2 = yes) was tested in the model covering first AI pregnancy risk. Only variables with $P < 0.20$ were included in the final models. The model was built in a stepwise backwards manner and in the final model variables with $P > 0.05$ were excluded. Odds ratios, confidence intervals and *P*-Values are reported. Confidence interval (CI) was set at 95%. Effects on 100 DIM milk yield was analyzed using linear mixed model ANOVA. The model was built according to the model building strategies provided by Dohoo et al., 2009. Factors tested in the univariable model were treatment, farm, parity, calving season, extended treatment and endometritis score. Survival analysis was conducted to evaluate the effect of treatment group on the hazard of AI within 100 DIM and on pregnancy by 200 DIM by utilizing a Cox semiparametric proportional hazard model. The outcome variables for the Cox model were DIM at first AI and DIM at which cows conceived for AI within 100 DIM and pregnancy by 200 DIM, respectively (Fricke et al., 2014). Right censoring occurred for cows that were not inseminated within 100 DIM or pregnant within 200 DIM or left the herd due to live culling, selling, ineligibility for breeding or death. Cows that left the herd within their voluntary waiting period (first 40 DIM) were excluded from analysis. The model for time to first AI as well as time to pregnancy included treatment group, extended treatment, parity, farm, calving season and endometritis as categorical variables. The model was built in a stepwise backwards manner by removing all variables with $P > 0.05$ from the model. Regardless of the significance level, treatment was forced to remain in the model.

3.1.4 **Results**

The overall incidence of metritis was 16.6%, ranging between 7.2% and 38.1% depending on farm (Table 1). A total of 610 cows (300 initially treated with ketoprofen and 310 treated with ceftiofur) were enrolled in this study. The distribution of parity was balanced among treatment groups (29% primiparous cows in ceftiofur group and 32% primiparous cows in ketoprofen group; $P = 0.26$). Fifty-two cows (35 from ketoprofen group, 17 from ceftiofur group) were excluded from analysis due to missing protocol compliance (n = 37) or concurrent disease (n = 15).

The proportion of cows that needed an extT between DIM 4 to 7 after enrollment differed between initial treatment groups and 61% of cows initially treated with ketoprofen needed extT compared to 31% of cows treated with ceftiofur. Cows treated with ketoprofen had 3.47 (95% CI: 2.42−4.95; $P < 0.001$; Table 2) times the odds of extT compared to cows treated with ceftiofur. Furthermore, multiparous cows had 0.62 (95% CI: 0.4−0.96; $P = 0.03$) times the odds of extT compared to primiparous cows. Moreover, there was an overall effect of farm ($P = 0.028$).

A total of 438 cows were examined for PVD. Cows without examination either left the herd before examination date, were not cooperative or undetectable during farm visit. The proportion of cows diagnosed with endometritis between DIM 21 and 40 did not differ between treatment groups (56% of ketoprofen group vs. 53% of ceftiofur group; $P = 0.64$). However, cows with extT had 2.00 (95% CI: 1.41−3.19; $P = 0.001$; Table 3) times the odds of endometritis compared to cows without extT. Moreover, cows that calved in in the warm season had 1.74 (95% CI: 1.05−2.87; $P = 0.03$) times the odds of endometritis compared to cows that calved in the cold season. Additionally there was an overall effect of farm ($P < 0.001$).

A total of 480 cows were analyzed for first AI pregnancy risk and only cows with at least one AI within the observational period of 200 DIM were included. Cows that left the herd before first AI or that were ineligible to breed were excluded from analysis. Treatment group did not affect first AI pregnancy risk (ketoprofen group: 20%, ceftiofur group: 25%; $P = 0.25$). Remaining variable in the final logistic regression model for first AI pregnancy risk was calving season. Cows that calved in the warm season had 1.57 (95% CI: 1.02−2.42; $P = 0.04$) times the odds of pregnancy at first AI compared to cows that calved in the cold season.

Milk yield of the first 100 DIM was available for 476 cows. Treatment group did not affect 100 DIM milk yield (mean ± SD: ketoprofen group: 3531 ± 816 kg vs. ceftiofur group: 3571 ± 743 kg; $P = 0.58$). Multiparous cows had a higher milk production than primiparous cows (3856 ± 686 kg vs. 2850 ± 462 kg; $P < 0.001$). Additionally there was an overall effect of farm ($P < 0.001$).

A total of 437 cows were analyzed for time to first AI within 100 DIM. Cox proportional analysis indicated that treatment did not affect the time until cows had their first AI (P = 0.36; Table 4 and Figure 1). Median days to first AI were 75 d (95% CI: 73−77) for cows initially treated with ketoprofen and 73 d (95% CI: 71−75) for cows initially treated with ceftiofur. The hazard ratio for first AI within 100 DIM was 0.71 (95% CI: 0.54−0.93; P = 0.01) for multiparous cows and 0.65 (95% CI: 0.53−0.80; P < 0.01) for cows with endometritis compared to primiparous cows and cows without endometritis. Furthermore there was an overall effect of farm (P = 0.002).

A total of 437 cows were analyzed for time to pregnancy within 200 DIM. Cox proportional analysis indicated that treatment did not affect the time the cows conceived (P = 0.88; Table 5 and Figure 1) and median days to pregnancy were 133 d (95% CI: 120−147) for cows initially treated with ketoprofen and 143 d (95% CI: 132−154) for cows initially treated with ceftiofur (see Table 1). The hazard ratio for pregnancy within 200 DIM was 0.71 (95% CI: 0.53−0.96; P = 0.03) for multiparous cows and 0.70 (95% CI: 0.55−0.88; P = 0.003) for cows with endometritis compared to primiparous cows and cows without endometritis. Additionally, there was an overall effect of farm (P = 0.001).

Table 1. Summary of enrollment on different farms

Item	No. of cows with acute puerperal metritis initially treated with		Total	Metritis incidence in %
	3 mg/kg Ketoprofen	1 mg/kg Ceftiofur		
Enrolled on dairy farm 1	26	30	56	15.6
Enrolled on dairy farm 2	60	59	119	19.4
Enrolled on dairy farm 3	96	96	192	38.1
Enrolled on dairy farm 4	44	44	88	7.2
Enrolled on dairy farm 5	36	37	73	33.3
Enrolled on dairy farm 6	38	44	82	10.8
Total enrollment	300	310	610	16.6

Table 2: Final logistic regression model showing odds ratios of treatment group, parity and farm for extended treatment in 558 Holstein cows

Variable	Odds ratio[1]	95% CI[2]	*P*-Value
Treatment group			< 0.001
ceftiofur	Referent		
ketoprofen	3.47	2.42−4.95	
Parity			0.03
primiparous	Referent		
multiparous	0.62	0.4−0.96	
Farm			0.028
1	Referent		
2	3.48	1.56−7.75	0.002
3	2.66	1.26−5.63	0.01
4	3.75	1.67−8.41	0.001
5	2.46	1.06−5.68	0.04
6	2.29	1.00−5.29	0.05

[1] Odds ratio < 1 indicates reduced odds of treatment extension, odds ratio > 1 indicates elevated odds of extended treatment

[2] Confidence interval for the odds ratio, lower and upper confidence limits

Table 3: Final logistic regression model showing odds ratios of extended treatment, season and farm for endometritis in 438 Holstein cows

Variable	Odds ratio[1]	95% CI[2]	*P*-Value
Extended treatment			0.001
no	Referent		
yes	2.00	1.41−3.19	
Season			0.03
cold	Referent		
warm	1.74	1.05−2.87	
Farm			< 0.001
1	Referent		
2	1.39	0.64−2.97	0.40
3	0.45	0.21−0.95	0.04
4	1.16	0.52−2.60	0.73
5	0.47	0.20−1.07	0.08
6	0.31	0.13−0.75	0.009

[1] Odds ratio < 1 indicates reduced odds of endometritis, odds ratio > 1 indicates elevated odds of endometritis

[2] Confidence interval for the odds ratio, lower and upper confidence limits

[3] Rectal temperature ≥39.5°C 4−7 d after initial treatment: 2 (cows initially treated with ceftiofur) or 3 (cows initially treated with ketoprofen) d treatment with ceftiofur

Table 4: Final Cox proportional hazard model showing hazard ratios of treatment group, parity, endometritis and farm for time to first artificial insemination (by 100 DIM) in 437 Holstein cows.

Variable		Hazard ratio[1]	95% CI[2]	*P*-Value
Treatment group				0.36
	ceftiofur	Referent		
	ketoprofen	1.10	0.90−1.35	
Parity				0.01
	primiparous	Referent		
	multiparous	0.71	0.54−0.93	
Endometritis				< 0.001
	no	Referent		
	yes	0.65	0.53−0.80	
Farm				0.002
	1	Referent		
	2	1.57	1.06−2.33	0.02
	3	0.77	0.52−1.12	0.17
	4	1.12	0.76−1.66	0.56
	5	1.00	0.66−1.50	1.00
	6	1.18	0.78−1.79	0.44

[1] Hazard ratio for getting inseminated within 100 DIM, hazard ratio < 1 indicates a reduced instantaneous relative risk of insemination within 100 DIM, hazard ratio > 1 indicates an elevated instantaneous relative risk.

[2] Confidence interval for the hazard ratio, lower and upper confidence limits

Table 5: Final Cox proportional hazard model showing hazard ratios of treatment group, parity, endometritis and farm for time to pregnancy (by 200 DIM) in 437 Holstein cows

Variable	Hazard ratio[1]	95% CI[2]	*P*-Value
Treatment group			0.88
ceftiofur	Referent		
ketoprofen	0.98	0.78−1.24	
Parity			0.09
primiparous	Referent		
multiparous	0.71	0.53−0.96	0.03
Endometritis			
no	Referent		
yes	0.70	0.55−0.88	0.003
Farm			0.001
1	Referent		
2	1.37	0.86−2.17	0.18
3	0.77	0.49−1.22	0.26
4	1.01	0.63−1.62	0.96
5	1.43	0.88−2.33	0.15
6	1.79	1.10−2.90	0.02

[1] Hazard ratio for getting pregnant within 200 DIM, hazard ratio < 1 indicates a reduced instantaneous relative risk of pregnancy within 200 DIM, hazard ratio > 1 indicates an elevated instantaneous relative risk.

[2] Confidence interval for the hazard ratio, lower and upper confidence limits

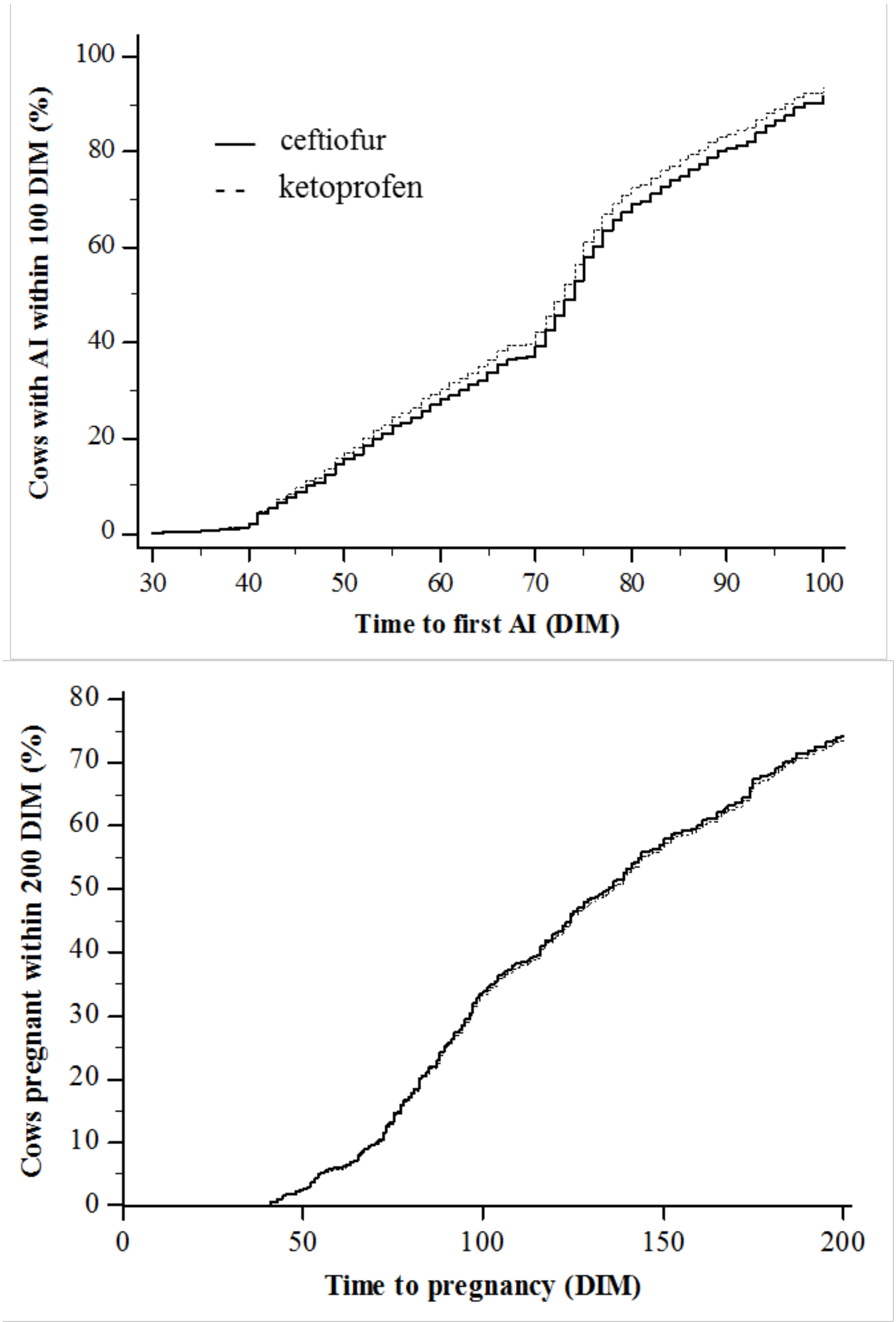

Figure 1. Cox proportional survival analysis illustrating the effect of treatment on time to first AI and time to pregnancy. Time to first AI and time to pregnancy was similar among treatments (*P* = 0.36 and *P* = 0.88).

3.1.5 **Discussion**

Postpartum uterine diseases are common and of great importance in dairy cows as shown by an incidence of APM between 14.8−40.9% (Chapinal et al., 2011; Sannmann et al., 2013b; Lima et al., 2014). This wide range is also reflected by our results which showed incidences of APM between 7.2 and 38.1% depending on farm.

The treatment of APM with ceftiofur as a new generation cephalosporin is a crucial issue in case of emerging antibiotic resistance, because the limited use as critically important antimicrobial is mandatory (Pyorala et al., 2014) and even a withdrawal from the market has been considered (Grove-White and Murray, 2009). As it is well known that NSAID are likely to be of value in diseases in which inflammation, endotoxaemia and pain are major components (Fitzpatrick et al., 2004; Laven et al., 2012), the objective of this study was to evaluate the efficacy of ketoprofen for the treatment of APM compared to ceftiofur. The effect on clinical cure, subsequent milk yield, prevalence of endometritis and reproductive performance were evaluated.

Cows initially treated with ketoprofen were more likely to have an extT than cows initially treated with ceftiofur. Clinical cure was considered as a rectal temperature < 39.5°C 4 days after first treatment and cows not receiving extT within the 7 days observation period. The quality of discharge was not regarded as criterion for extT at this point, because it has been shown that about 50% of cows with APM still have discharge even at d 6 after first treatment (Drillich et al., 2001). To our knowledge there is no evidence about cows with APM treated with NSAID alone. Amiridis et al. (2001), however, showed that cows treated with NSAID in combination with antibiotic treatment had reduced pyrexia, faster clinical improvement, a more rapid uterine involution and an earlier estrus compared to cows only treated antibiotically.

Compared to untreated controls, Chenault et al. (2004) and McLaughlin et al. (2012) found 15−19% higher cure rates for cows treated with ceftiofur, which is in accordance with our results. Because of concerns of animal welfare there was no untreated control group in this study and therefore spontaneous recovery cannot be determined (Drillich et al., 2001; Lima et al., 2014). If treatment expense would be expressed by total number of medical applications, cows initially treated with ketoprofen needed 4.83 doses and cows initially treated with ceftiofur needed 3.63 doses ($P < 0.001$). If the focus would be placed on the doses of antibiotic drugs, however, cows initially treated with ketoprofen needed 1.83 antibiotic doses and cows treated with ceftiofur needed 3.63 doses ($P < 0.001$). Consequently, there is considerable potential of reducing antimicrobial treatment by implementing this treatment protocol.

Treatment group did not affect the occurrence of PVD. Cows, however, that needed extT were more likely to develop endometritis than cows that did not need extT. It can be speculated that the immune system of the cows without extT in combination with the medication applied could cure APM effectively within 3 days of treatment and therefore a carryover effect in form of PVD could not be seen. On the other hand it is possible, that cows without extT were only mildly metritic or even false positively diagnosed due to potential confounders of diagnosis mentioned below.

Treatment group did not affect 100 DIM milk yield or reproductive performance indicating that an initial treatment with ketoprofen had no negative effects on subsequent performance. Other factors (i.e., parity, farm, season and endometritis) were associated with milk yield or fertility. Differences in breeding strategies on different farms might have influenced time to first AI as well as time to pregnancy due to timed AI according to synchronization protocol versus estrus detection. Farm, however, did not affect first AI pregnancy risk. This is in accordance with results of a meta-analysis which demonstrated that pregnancy rates for Ovsynch or natural breeding programs did not differ significantly (Rabiee et al., 2005).

Limitations of the study

The definition of APM in this study was based on rectal temperature > 39.4°C and a fetid, watery discharge. Even though this definition has been widely accepted in research and used practice (Mc Laughlin et al., 2012; Lima et al., 2014; Machado et al., 2014) it is suboptimal and both type I and type II errors can occur (Sannmann and Heuwieser, 2015).

An untreated or placebo treated control group was not included in the experiment as AMP is potentially life threatening (Drillich et al., 2007), associated with economic losses (Overton and Fetrow, 2008; Wittrock et al., 2011) and visceral pain (Stoikov et al., 2015). Also, a combination of ceftiofur and NSAID was not included as the primary scope was to study an alternative treatment to ceftiofur as a 3rd generation cephalosporin which has been considered critically.

Cows on farm 1 were less likely to have an extT than cows of all the other farms. Although diagnoses of APM were reviewed biweekly, individual influences of the examining personnel could not be entirely avoided despite a pretrial training, simple inclusion criteria, and the use of standard operating procedures. For vaginal discharge it has been shown that the interobserver agreement of olfactory assessment is only moderate (Sannmann et al., 2013a) and body temperature is influenced by several factors such as climate, time of day and insertion depth of the thermometer (Burfeind et al., 2010; Burfeind et al., 2012). Yet, the design of this study as a multi-centric clinical trial did not allow the examination of cows by only one examining person and a lack of objectively measurable criteria for the detection of APM has been described (Sannmann et al., 2013a; Burfeind et al., 2014; Pohl et al., 2015). Moreover, treatment group was not blinded to the attending person, which might be a limiting factor of the study. Most outcome variables, however, were objectively measurable e.g. time to insemination, time to pregnancy, and 100 DIM milk yield. Moreover this is the first study directly comparing the efficacy of ceftiofur and NSAID considering multiple dairy farms.

3.1.6 **Conclusions**

More than half of the cows initially treated with ketoprofen needed an extT with ceftiofur. The number of ceftiofur doses administered, however, could almost be cut in half as well without negative effects on the prevalence of PVD, milk yield, and reproductive performance.

3.1.7 **Ackknowledgements**

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3.1.8 References

Amiridis, G. S., L. Leontides, E. Tassos, P. Kostoulas, and G. C. Fthenakis. 2001. Flunixin meglumine accelerates uterine involution and shortens the calving-to-first-oestrus interval in cows with puerperal metritis. J. Vet. Pharmacol. Ther. 24:365-367.

Burfeind, O., M. Bruins, A. Bos, I. Sannmann, R. Voigtsberger, and W. Heuwieser. 2014. Diagnosis of acute puerperal metritis by electronic nose device analysis of vaginal discharge in dairy cows. Theriogenology 82:64-70.

Burfeind, O., V. S. Suthar, and W. Heuwieser. 2012. Effect of heat stress on body temperature in healthy early postpartum dairy cows. Theriogenology 78:2031-2038.

Burfeind, O., M. A. G. von Keyserlingk, D. M. Weary, D. M. Veira, and W. Heuwieser. 2010. Short communication: Repeatability of measures of rectal temperature in dairy cows. J. Dairy Sci. 93:624-627.

Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. P. Santos, and S. J. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. J. Dairy Sci. 94:4897-4903.

Chenault, J. R., J. F. McAllister, S. T. Chester, K. J. Dame, F. M. Kausche, and E. J. Robb. 2004. Efficacy of ceftiofur hydrochloride sterile suspension administered parenterally for the treatment of acute postpartum metritis in dairy cows. J. Am. Med. Assoc. 224:1634-1639.

Deluyker, H. A., S. N. Van Oye, and J. F. Boucher. 2005. Factors affecting cure and somatic cell count after pirlimycin treatment of subclinical mastitis in lactating cows. J. Dairy Sci. 88:604-614.

Drillich, M., O. Beetz, A. Pfützner, M. Sabin, H. J. Sabin, P. Kutzer, H. Nattermann, and W. Heuwieser. 2001. Evaluation of a systemic antibiotic treatment of toxic puerperal metritis in dairy cows. J. Dairy Sci. 84:2010-2017.

Drillich, M., D. Voigt, D. Forderung, and W. Heuwieser. 2007. Treatment of acute puerperal metritis with flunixin meglumine in addition to antibiotic treatment. J. Dairy Sci. 90:3758-3763.

Fitzpatrick, J. L., A. M. Nolan, P. Lees, and S. A. May. 2004. Inflammation and pain, Bovine medicine. (Second Ed.), Blackwell Publishing, Oxford, UK, 1045 – 1066.

Fourichon, C., H. Seegers, and X. Malher. 2000. Effect of disease on reproduction in the dairy cow: A meta-analysis. Theriogenology 53:1729-1759.

Fricke, P. M., J. O. Giordano, A. Valenza, G. Lopes Jr, M. C. Amundson, and P. D. Carvalho. 2014. Reproductive performance of lactating dairy cows managed for first service using timed artificial insemination with or without detection of estrus using an activity-monitoring system. J. Dairy Sci. 97:2771-2781.

Grove-White, D. and R. Murray. 2009. Use of antimicrobials. Vet. Rec. 164:727.

Hellmann, K. and I. Radeloff. 2000. International cooperation on harmonisation of technical requirements of veterinary medicinal products (vich). Brussels/Belgium.

Laven, R., P. Chambers, and K. Stafford. 2012. Using non-steroidal anti-inflammatory drugs around calving: Maximizing comfort, productivity and fertility. Vet. J. 192:8-12.

Lima, F. S., A. Vieira-Neto, G. S. Vasconcellos, R. D. Mingoti, E. Karakaya, E. Sole, R. S. Bisinotto, N. Martinez, C. A. Risco, K. N. Galvao, and J. E. Santos. 2014. Efficacy of ampicillin trihydrate or ceftiofur hydrochloride for treatment of metritis and subsequent fertility in dairy cows. J. Dairy Sci. 97:5401–5414.

Machado, V. S., M. L. de Souza Bicalho, E. B. d. S. M. Junior, R. Rossi, B. L. Ribeiro, S. Lima, T. Santos, A. Kussler, C. Foditsch, and E. K. Ganda. 2014. Subcutaneous immunization with inactivated bacterial components and purified protein of escherichia coli, fusobacterium necrophorum and trueperella pyogenes prevents puerperal metritis in holstein dairy cows. PloS One 9:e91734.

McLaughlin, C. L., E. Stanisiewski, M. J. Lucas, C. P. Cornell, J. Watkins, L. Bryson, J. K. S. Tena, J. Hallberg, and J. R. Chenault. 2012. Evaluation of two doses of ceftiofur crystalline free acid sterile suspension for treatment of metritis in lactating dairy cows. J. Dairy Sci. 95:4363-4371.

Newby, N. C., D. L. Pearl, S. J. LeBlanc, K. E. Leslie, M. A. G. von Keyserlingk, and T. F. Duffield. 2013. The effect of administering ketoprofen on the physiology and behavior of dairy cows following surgery to correct a left displaced abomasum. J. Dairy Sci. 96:1511-1520.

National Research Council (NRC). 2001. Nutrient requirements of dairy cattle. 7th Rev. Ed. National Academy Press. Washington, DC.

Overton, M, and J. Fetrow. 2008. Economics of postpartum uterine health. Pages 39–43 in Proc. Dairy Cattle Reprod. Council Conv.,Omaha, NE.

Pohl, A., O. Burfeind, and W. Heuwieser. 2015. The associations between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis. J. Dairy Sci. 98:4544-4551.

Pyorala S., Taponen J., Katila T. Use of antimicrobials in the treatment of reproductive diseases in cattle and horses. Reprod. Dom. Anim. 2014, 49 (Suppl 3), 16-26.

Rabiee, A. R., I. J. Lean, and M. A. Stevenson. 2005. Efficacy of ovsynch program on reproductive performance in dairy cattle: A meta-analysis. J. Dairy Sci. 88:2754-2770.

Sannmann, I., O. Burfeind, V. Suthar, A. Bos, M. Bruins, and W. Heuwieser. 2013a. 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. 96:5773-5779.

Sannmann, I., O. Burfeind, R. Voigtsberger, and W. Heuwieser. 2013b. Comparison of two monitoring and treatment strategies for cows with acute puerperal metritis. Theriogenology 79:961-969.

Sannmann, I. and W. Heuwieser. 2015. Technical note: Intraobserver, interobserver, and test-retest reliabilities of an assessment of vaginal discharge from cows with and without acute puerperal metritis. J. Dairy Sci. 98:5460–5466.

Santos, T. M. A., R. O. Gilbert, and R. C. Bicalho. 2011. Metagenomic analysis of the uterine bacterial microbiota in healthy and metritic postpartum dairy cows. J. Dairy Sci. 94:291-302.

Santos, J. E., Bisinotto, R. S., Ribeiro, E. S., Lima, F. S., Greco, L. F., Staples, C. R., Thatcher, W. W. 2010. Applying nutrition and physiology to improve reproduction in dairy cattle. Soc. Reprod. Fertil. Suppl. 67: 387-403.

Scientific Advisory Group on Antimicrobials of the Committee for Medicinal Products for Veterinary Use. 2009. Reflection paper on the use of third and fourth generation cephalosporins in food producing animals in the european union: Development of resistance and impact on human and animal health. J. Vet. Pharmacol. Ther. 32:515-533.

Sens, A. and W. Heuwieser. 2013. Presence of escherichia coli, trueperella pyogenes, α-hemolytic streptococci, and coagulase-negative staphylococci and prevalence of subclinical endometritis. J. Dairy Sci. 96:6347-6354.

Sheldon, I. M. 2004. The postpartum uterus. Vet. Clin. of North Am. Food Anim. Pract. 20:569-591.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516-1530.

Stojkov, J., M. A. G. von Keyserlingk, J. N. Marchant-Forde, and D. M. Weary. 2015. Assessment of visceral pain associated with metritis in dairy cows. J. Dairy Sci. 98:5352-5361.

Williams, E. J., D. P. Fischer, D. U. Pfeiffer, G. C. W. England, D. E. Noakes, H. Dobson, and I. M. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63:102-117.

Wittrock, J. M., K. L. Proudfoot, D. M. Weary, and M. A. G. von Keyserlingk. 2011. Short communication: Metritis affects milk production and cull rate of holstein multiparous and primiparous dairy cows differently. J. Dairy Sci. 94:2408

4 DISCUSSION

Postpartum uterine diseases of dairy cows negatively affect animal welfare and may result in early removal from the herd or impaired reproductive performance (Machado et al., 2014). Acute puerperal metritis in dairy cows is commonly treated with antibiotics and especially the established treatment with 3rd generation cephalosporins needs to be questioned due to the worldwide debate on emerging antibiotic restistances (Chenault et al., 2004; Lima et al., 2014; Machado et al., 2014). Therefore, the overall objective of this work was to evaluate and improve the diagnosis as well as the therapy of APM. A precise diagnosis combined with research on alternative treatment concepts could, on one hand, improve the cow´s health and wellbeing and on the other hand minimize the use of antibiotics in food producing animals. In this thesis automatic measurement of body temperature by AMS, the association between an acute phase protein and APM and the treatment of APM with NSAID were studied.

The first study was designed to evaluate if milk temperature measured by an AMS is a reliable indicator of body temperature of dairy cows and if cows with fever could be detected automatically. The results of this study showed that the correlation ($r = 0.52$) between vaginal temperature and milk temperature was lower than reported in previous studies with traditional milking equipment ($r \geq 0.78$, Fordham et al., 1988; West et al., 1990, 1999). Moreover, vaginal temperature was higher than milk temperature. This difference might have been caused by the passage through teat cups and milk tubes before reaching the sensors inside of the arm of the AMS and might be influenced by ambient temperature. The ability to identify cows with fever by milk temperature was assessed using 2 approaches. In the first approach, the combination of 39.0°C as a threshold for the milk temperature and 39.5°C for at least 2 h per day as a threshold for vaginal temperature resulted in the highest combination of sensitivity (0.65) and specificity (0.65). In the second approach, it was evaluated if measurement of milk temperature could identify cows with fever at a given milking event. A threshold of milk temperature > 38.7°C delivered the best combination of sensitivity (0.77) and specificity (0.66) when fever was defined as vaginal temperature ≥ 39.5°C. These results indicate that the automatic detection of febrile cows with AMS is only possible to a very limited extend. Depending on the chosen approach, the false positive rate ranged between 23 and 35%. Moreover, 34−35% of febrile cows could not be found with the AMS. On the one hand, re-measurement of the cows identified by the AMS would be necessary due to high false positive rates, which might lead to a reduced compliance of the performing farm personnel. On the other hand, considering that APM is a potentially life threatening (Drillich et al., 2007) and painful (Stoikov et al., 2015) disease, the high rate of false negatively identified cows make it unjustifiable to use milk temperature measured by AMS as an alternative to daily measurement of rectal temperature.

Therefore, measurement of milk temperature by AMS is no alternative option to daily monitoring of rectal temperature within fresh cow protocols.

However, it has also been shown that body temperature is influenced by several factors such as DIM, time of day, climate and insertion depth of the thermometer (Burfeind et al., 2010; Burfeind et al., 2012), and that there is no ideal benchmark to measure body temperature (Bewley et al., 2008). For vaginal discharge, the second major symptom of APM, it has been shown that the interobserver agreement of olfactory assessment is only moderate (Sannmann et al., 2013a). This clearly illustrates the lack of objectively measurable criteria for the diagnosis of APM (Sannmann et al., 2013b; Burfeind et al., 2014b). Therefore, the second study of my thesis evaluated the relationship between an objectively measurable criterion of inflammation and APM. Haptoglobin is an acute phase protein and elevated in serum during inflammation (Crawford et al., 2005). Because there is only moderate reported specificity and sensitivity in the detection of metritic cows by measuring serum haptoglobin concentrations (Huzzey et al., 2009; Dubuc et al., 2010; Burfeind et al., 2014a), the association between different variables and haptoglobin concentrations after calving was assessed.

Therefore, the objective of my second study was to evaluate the association between haptoglobin concentrations and parity, periparturient metabolic stress indicated by BHBA and NEFA, calving difficulties, retained fetal membranes and APM. The results showed that primiparous cows had a greater median haptoglobin concentration than multiparous cows. This is in line with other findings (Crawford et al., 2005; Humblet et al., 2006; Schneider et al., 2013; Burfeind et al., 2014a). It is hypothesized that damage to the uterus and vagina might be more severe during the first parturition and therefore, primiparous cows may be more responsive to inflammatory stress than multiparous cows (Humblet et al., 2006; Schneider et al., 2013). Periparturient metabolic stress indicated by elevated BHBA was also associated with elevated haptoglobin regardless of parity. It has been demonstrated, that cows that developed uterine diseases, experienced a greater degree of negative energy balance and had greater neutrophil impairment (Galvão et al., 2010). Moreover, negative energy balance has a suppressive effect on immune function, which might increase the risk for inflammatory diseases after calving (Hussain, 1989; Gilbert et al., 2007). In addition to subclinical ketosis, multiparous cows with assisted calving had a 2.46 times higher risk for elevated Hapto whereas primiparous cows with assisted calving had no elevated risk for elevated haptoglobin. Moreover, multiparous cows with retained fetal membranes were 5.51 times as likely to have elevated haptoglobin than cows without retained fetal membranes. An explanatory approach could be that the presence of retained fetal membranes supports intrauterine bacterial growth and uterine tissue might be damaged due to potential manual removal (Bolinder et al., 1988), leading to inflammatory processes. However, retained fetal membranes was not a risk factor for elevated haptoglobin for primiparous cows, potentially due to the small number of primiparous cows with retained fetal membranes in this study. Finally, cows with APM had 2.74−5.01 times the odds of elevated haptoglobin depending on parity. These results support previous studies in which a 3 to 7 times higher risk for metritis was described in cows with elevated postpartum haptoglobin (Huzzey et al., 2009; Dubuc et al., 2010). To sum up, different variables occurring at the same time period as APM were associated with elevated haptoglobin at DIM 5. It is speculated that the association of calving ease, retained fetal membranes and periparturient metabolic stress could explain the reported moderate sensitivity and specificity in the detection of metritic cows by measuring haptoglobin. When using haptoglobin as a diagnostic tool for the detection of APM, these potential confounders have to be considered. It becomes clear once again, that there is no ideal measure for the diagnosis of APM underlining the importance of research in this field combined with the critical assessment of the own examination practice.

However, these results also show a great association between APM and haptoglobin illustrating a strong inflammatory reaction of the body. In combination with the demonstrated painfulness of the inflamed uterus (Stojkov et al., 2015), it is plausible to study the effects of an antiinflammatory drug as a treatment of APM.

Therefore, the objective of my third study was to compare the efficacies of ketoprofen and ceftiofur for the treatment of APM. The effect on clinical cure, subsequent milk yield, prevalence of endometritis and reproductive performance were evaluated. To the best of my knowledge, this is the first controlled, randomized study on the treatment of APM with NSAID considering reproductive performance and milk production. Reproductive status as well as milk yield are the most important influences on culling decisions (Gröhn et al., 2003) and therefore clearly illustrate the importance of investigation in clinical studies. The results of the study showed that 61% of cows initially treated with ketoprofen needed an extended treatment with ceftiofur compared to 31% of cows initially treated with ceftiofur. In other words more than half of the cows initially treated with ketoprofen needed an antimicrobial treatment with ceftiofur. However, there is huge potential of reducing antibiotic use by utilizing ketoprofen for the treatment of APM as up 39% of cows initially treated with ketoprofen did not need an extended treatment. It is speculated that recovery without antibiotic treatment could occur either due to reported high self cure rates of APM (Chenault et al., 2004; McLaughlin et al., 2012) or due to false positive diagnosis (Burfeind et al., 2010; Burfeind et al., 2012; Sannmann et al., 2013a).

Previous studies showed that cows with APM have an increased risk to develop endometritis (Galvão et al., 2009; Martinez et al., 2012). In turn, cows with endometritis have an impaired reproductive performance (LeBlanc et al., 2002) highlighting the need of an evaluation of differences in the prevalence of endometritis between treatment groups. In my study, the occurrence of purulent vaginal discharge between DIM 21 and 40 was similar among treatment groups. However, cows with extended treatment had 2.00 times the odds of endometritis compared to cows without extended treatment. Treatment group did not affect mean 100 DIM milk yield, first artificial insemination pregnancy risk, time to first artificial insemination and time to pregnancy. Hence, there is considerable potential of reducing antimicrobial treatment by implementing this treatment protocol without negative effects on the prevalence of endometritis, milk yield, and reproductive performance.

5 SUMMARY

5.1 Diagnosis and treatment of acute puerperal metritis in dairy cows

Acute puerperal metritis is characterized by reddish-brown, fetid, watery vaginal discharge in combination with an elevated rectal temperature of ≥ 39.5°C. Cows with acute puerperal metritis have an impaired reproductive performance, are more likely to be culled and have less milk production in comparison to healthy cows (Fourichon et al., 2000; Wittrock et al., 2011; Machado et al., 2014). Moreover, animal welfare is impaired due to painfulness of the inflamed uterus (Stojkov et al., 2015). The widespread use of 3rd generation cephalosporins for the treatment of acute puerperal needs to be re-evaluated due to the worldwide debate on emerging antibiotic resistances (Chenault et al., 2004; Lima et al., 2014; Machado et al., 2014). Hence, there is the need to encourage prudent use of antibiotics and alternative therapies to antibiotics. Therefore, the overall objective of this work was 1) to evaluate the diagnosis of acute puerperal metritis in order to reduce false positively treated cows and 2) to evaluate an alternative treatment of acute puerperal metritis.

Even though measurement of rectal temperature is part of most fresh cow protocols (Smith and Risco, 2005), this time consuming procedure is only implemented by 34% of farms in Germany (Heuwieser et al., 2010). Therefore, the first study investigated if milk temperature measured by an automatic milking system is a reliable indicator of body temperature of dairy cows and if cows with fever could be detected automatically. The results showed a moderate correlation between vaginal temperature and milk temperature (r = 0.52). Moreover, vaginal temperature was higher (39.1 ± 0.4°C, n = 418) than milk temperature (38.6 ± 0.7°C, n = 418) with a mean difference of 0.5 ± 0.6°C, which might have been caused by cooling effects of the milk tubes. Two different approaches assessed the ability to identify cows with fever by milk temperature. In the first approach, the combination of 39.0°C as a threshold for the milk temperature and 39.5°C for at least 2 h per day as a threshold for vaginal temperature resulted in the highest combination of sensitivity (0.65) and specificity (0.65). In the second approach it was evaluated, if measurement of milk temperature could identify cows with fever at a given milking event. A threshold of milk temperature > 38.7°C delivered the best combination of sensitivity (0.77) and specificity (0.66) when fever was defined as vaginal temperature ≥ 39.5°C. In conclusion, milk temperature should be interpreted with great caution because of high false positive and false negative rates and therefore cannot be recommended as an alternative to measurement of rectal temperature.

Because a lack of objectively measurable criteria for the diagnosis of acute puerperal metritis has been reported (Sannmann et al., 2013a; Burfeind et al., 2014b), the second study evaluated the association between an acute phase protein and acute puerperal metritis.

Specifically, it was evaluated if, in addition to acute puerperal metritis, other variables occurring within the first 5 DIM are associated with elevated haptoglobin at 5 DIM. The results showed that primiparous cows had a greater median haptoglobin concentration (2.25 g/L, IQR 1.45−2.50, n = 146) than multiparous cows (1.13 g/L IQR 0.52−2.22; n = 302). Therefore, different haptoglobin thresholds based on references from literature for all cows (1.4 g/L), primiparous (2.49 g/L) and multiparous cows (1.4 g/L) were used for further analysis. Cows with elevated BHBA (≥ 1.2 mmol/L) at 5 DIM had 2.39−2.87 times the odds of elevated haptoglobin depending on parity. Multiparous cows with assisted calving had a 2.46 times higher risk for elevated haptoglobin whereas primiparous cows with assisted calving had no elevated risk for elevated haptoglobin at 5 DIM. Moreover, multiparous cows with retained fetal membranes were 5.51 times as likely to have elevated haptoglobin as cows without retained fetal membranes. Finally, acute puerperal metritis within the first 5 DIM was highly associated with elevated haptoglobin (odds ratio: 2.74−5.01) depending on parity. In summary, different variables occurring at the same time period as acute puerperal metritis were associated with elevated haptoglobin at DIM 5. It is speculated, that the association of calving ease, retained fetal membranes and periparturient metabolic stress could explain the reported moderate sensitivity and specificity (Huzzey et al., 2009; Dubuc et al., 2010; Burfeind et al., 2014a) in the detection of metritic cows by measuring serum haptoglobin concentrations. When using haptoglobin as a diagnostic tool for the detection of acute puerperal metritis, these potential confounders have to be considered.

The third study was designed in order to compare the efficacies of ketoprofen and ceftiofur for the treatment of acute puerperal metritis. The effect on clinical cure, subsequent milk yield, prevalence of endometritis and reproductive performance were evaluated. Cows with acute puerperal metritis were allocated to treatment with ketoprofen or treatment with ceftiofur and cows that showed fever on d 4 to 7 after inclusion received an extended treatment with ceftiofur. Cows treated with ketoprofen had 3.47 times the odds (n = 558) of extended treatment compared to cows treated with ceftiofur. Occurrence of purulent vaginal discharge was similar among treatment groups (ketoprofen: 56%, ceftiofur: 53%). However, cows with extended treatment had 2.00 times the odds (n = 438) of endometritis compared to cows without extended treatment. Treatment group did not affect mean 100 DIM milk yield (ketoprofen group 3531 ± 816 kg, ceftiofur group 3571 ± 743 kg), first artificial insemination pregnancy risk (ketoprofen group: 20% vs. ceftiofur group: 25%; n = 480), time to first artificial insemination (ketoprofen group: 75 d; 95% CI: 73−77 d vs. ceftiofur group: 73 d; 95% CI: 71−75 d; n = 437) and time to pregnancy (ketoprofen group: 133 d; 95% CI: 120−147 d vs. ceftiofur group: 143 d; 95% CI: 132−154 d; n = 437). In conclusion, more than half of the cows initially treated with ketoprofen needed an extended treatment with ceftiofur (61%). However, there is potential of reducing antibiotic use by utilizing ketoprofen for the treatment of acute puerperal

metritis as up 39% of cows initially treated with ketoprofen did not need an extended treatment and the occurrence of purulent vaginal discharge, milk yield and reproductive performance was not negatively affected.

Overall, the first two studies on the diagnosis of acute puerperal metritis clearly demonstrated that further research for an improvement of the diagnosis of acute puerperal metritis is still warranted. Measurement of milk temperature by automatic milking systems to detect febrile cows is insufficient, because of high false positive and false negative rates. Although a high association between acute puerperal metritis and serum haptoglobin could be shown in the second study, also other variables impacting the same postpartum period as acute puerperal metritis were associated with elevated haptoglobin and therefore have to be considered when haptoglobin is used as a diagnostic measure. The third study showed that in terms of alternative treatment of acute puerperal metritis, ketoprofen could be used as an initial treatment without negative effects on reproductive performance or milk production. Finally, antibiotic usage could be minimized and moreover, the cow´s welfare could potentially be improved due to the analgesic effect of ketoprofen.

6 ZUSAMMENFASSUNG

6.1 Diagnose und Therapie der akuten Metritis beim Rind

Die akute Metritis beim Rind ist durch übelriechenden, wässrigen, rot-braunen Ausfluss und eine erhöhte Körpertemperatur ≥ 39,5°C innerhalb der ersten 10 Tage nach dem Abkalben charakterisiert (Sheldon et al., 2006). Erkrankte Kühe haben schlechtere Fruchtbarkeitsleistungen, ein erhöhtes Abgangsrisiko und eine geringere Milchleistung im Vergleich zu gesunden Kühen (Fourichon et al., 2000; Wittrock et al., 2011; Machado et al., 2014). Außerdem ist das Wohlbefinden der Tiere durch die erst kürzlich nachgewiesene Schmerzhaftigkeit des entzündeten Uterus beeinträchtigt (Stojkov et al., 2015). Die Behandlung der akuten puerperalen Metritis umfasst in der Regel eine systemische Antibiose mit einem Cephalosporin der dritten Generation (Ceftiofur). Die Anwendung von Antibiotika in der Nutztiermedizin gerät jedoch mehr und mehr in den Mittelpunkt der Kritik und die Verhinderung der Verbreitung von Resistenzen sollte angestrebt werden (Chenault et al., 2004; Lima et al., 2014; Machado et al., 2014). Deshalb ist es umso wichtiger Alternativen zur antibiotischen Therapie unter Berücksichtigung des Tierwohls, sowie wirtschaftlicher Fruchtbarkeitsleistungen zu finden. Durch eine präzise und tiernahe Diagnostik lassen sich außerdem Tiere identifizieren, die von einer frühzeitigen Therapie profitieren und solche, die keiner therapeutischer Maßnahmen bedürfen. In dieser Doktorarbeit wird deshalb die Diagnose und Therapie der akuten Metritis bei Milchkühen bearbeitet. Speziell wird dafür die Möglichkeit des automatisierten Fiebermessens evaluiert, Einflussfaktoren auf einen häufig angewandten Entzündungsparameter zur Metritisdetektion untersucht und die Möglichkeit einer alternativen Therapie getestet.

Das tägliche Messen der Körpertemperatur ist Bestandteil vieler Protokolle zur Überwachung der Gesundheit von Frischabkalbern (Smith and Risco, 2005). Diese Maßnahme ist allerdings zeitintensiv und wird laut einer Umfrage von nur 34% der befragten Betriebe in Deutschland routinemäßig durchgeführt (Heuwieser et al., 2010).

Seit Ende der neunziger Jahre werden immer mehr Automatische Melksysteme in landwirtschaftlichen Milchviehbetrieben genutzt. Weltweit waren es 2012 bereits 16.000 Betriebe (de Koning, 2013). Aus diesem Grund wurde in der ersten Studie untersucht, ob die von Automatischen Melksystemen erfasste Milchtemperatur mit der Körpertemperatur von Milchkühen vergleichbar ist und ob fieberhafte Kühe zuverlässig erkannt werden können. Zusammenfassend war die mittlere Vaginaltemperatur höher (39,1°C ± 0,4°C, n = 418) als die mittlere Milchtemperatur (38,6°C ± 0,7°C, n = 418) mit einer mittleren Differenz von 0,5°C ± 0,6°C. Dieser Unterschied lässt sich eventuell durch ein Abkühlen der Milch in Melkbecher und

Milchschlauch in Abhängigkeit von der Umgebungstemperatur erklären. Der Korrelationskoeffizient (r) von Milch- und Vaginaltemperatur betrug 0,52.

Um zu untersuchen, wie zuverlässig fieberhafte Kühe anhand der Milchtemperatur identifiziert werden können, wurden zwei verschiedene Ansätze gewählt. Dabei wurden jeweils unterschiedliche Definitionen von Fieber evaluiert. Eine Kombination aus Körpertemperatur ≥ 39,5°C für mindestens 2 Stunden am Tag und ein Grenzwert von mindestens 39,0°C für die Milchtemperatur stellte im ersten Ansatz die höchst mögliche Kombination von Sensitivität (0,65) und Spezifität (0,65) dar. Im zweiten Ansatz wurde überprüft, ob sich höhere Übereinstimmungen zeigen lassen, wenn die Milchtemperatur mit der Körpertemperatur während eines konkreten Melkvorganges miteinander verglichen werden. Die höchste Kombination von Sensitivität (0,77) und Spezifität (0,66) ergab sich hierbei durch eine Kombination aus Milchtemperatur > 38,7°C und Körpertemperatur ≥ 39,5°C. Zusammenfassend lässt sich schließen, dass die von Automatischen Melksystemen gemessene Milchtemperatur aufgrund einer hohen Falsch-Positiv-Rate, wie auch einer hohen Falsch-Negativ-Rate mit großer Vorsicht zu interpretieren ist und deshalb nicht als alternative Messmethode zur Rektaltemperaturmessung verwendet werden sollte.

Allerdings wird auch das Messen der Körpertemperatur von verschiedenen Faktoren beeinflusst wird und objektiv messbare Kriterien um eine akute Metritis mit ausreichend hoher Sicherheit zu identifizieren fehlen derzeit (Sannmann et al., 2013a; Burfeind et al., 2014b). Deshalb wurde in einer zweiten Studie der Zusammenhang eines Akute-Phase-Proteins und der Erkrankung an akuter Metritis untersucht. Speziell wurde der Einfluss von metabolischem Stress, Geburtsstörungen, Nachgeburtsverhaltung und Metritis auf die Haptoglobinkonzentration untersucht. Die Ergebnisse zeigten, dass Erstkalbinnen höhere Haptoglobinkonzentrationen hatten (2,25 g/L, IQR 1,45−2,50, n = 146), als multipare Tiere (1,13 g/L, IQR 0,52−2,22, n = 302). Deswegen basierten die folgenden Analysen auf unterschiedlichen Grenzwerten für alle Kühe (1,4 g/L), primipare Kühe (2,49 g/L und multipare Kühe (1,4 g/L). Kühe, die an Tag 5 postpartum eine erhöhte Konzentration von β-Hydroxybutyrat (≥ 1,2 mmol/L) zeigten, hatten 2,39−2,87-mal die Chance auch eine erhöhte Haptoglobinkonzentration an Tag 5 zu haben. Multipare Kühe mit Geburtsstörungen hatten außerdem 2,46-mal die Chance für erhöhte Haptoglobinwerte an Tag 5, wobei primipare Kühe mit Geburtsstörungen keinen Einfluss auf die Haptoglobinkonzentration hatten. Des Weiteren hatten Kühe mit Nachgeburstverhalten 5,51-mal so wahrscheinlich erhöhte Haptoglobinwerte, wie Kühe ohne Nachgeburtsverhalten. Schlussendlich hatten Kühe, die an einer akuten Metritis erkrankt waren 2,74−5,01-mal so wahrscheinlich eine erhöhte Haptoglobinkonzentration wie Kühe ohne akute Metritis, abhängig von der Parität. Der Zusammenhang von Geburtsstörungen, Nachgeburtsverhalten, peripartalem metabolischem Stress und Haptoglobin kann eine mögliche Erklärung für die begrenzte Sensitivität und

Spezifität von Haptoglobin für die Metritisdiagnostik sein. Wenn dieser Parameter für die Metritisdiagnostik eingesetzt werden soll, müssen die genannten potentiellen Störgrößen berücksichtigt werden.

In einer dritten kontrollierten, randomisierten Studie wurde eine alternative Therapiemethode zur antibiotischen Behandlung der akuten Metritis evaluiert. Dafür wurden Kühe mit fieberhafter Metritis nach einem von zwei Protokollen (Ceftiofur oder nicht-steroidales Antiphlogistikum) behandelt und der Effekt auf Heilungsrate, Milchleistung, Endometritisprävalenz und Fruchtbarkeit untersucht. Die antiphlogistische, antipyretische und analgetische Wirkung von Ketoprofen sollte den erkrankten Kühen während der Behandlung die Möglichkeit zur Selbstheilung geben. Kühe aus der Ketoprofengruppe wurden 3,47-mal so wahrscheinlich mit Ceftiofur nachbehandelt, wie Kühe aus der Ceftiofurgruppe. Die Endometritisprävalenz unterschied sich nicht zwischen den beiden Behandlungsgruppen (Ketoprofengruppe: 56%, Ceftiofurgruppe: 53%). Allerdings erkrankten Kühe, die nachbehandelt werden mussten 2-mal so wahrscheinlich an Endometritis, wie Kühe ohne Nachbehandlung. Die Behandlungsgruppe hatte keinen Einfluss auf die Milchleistung, den Erstbesamungserfolg und die Güstzeit. Zusammenfassend lässt sich sagen, dass mehr als die Hälfte der Kühe, die initial mit Ketoprofen behandelt worden waren, eine Nachbehandlung mit Ceftiofur benötigten. Andererseits mussten 39% der Kühe mit akuter Metritis nicht nachbehandelt werden. Somit kann der Einsatz von Antibiotika in der Nutztiermedizin deutlich verringert werden, denn sowohl Milchleistung wie auch Fruchtbarkeitsleistung wurden nicht negativ durch die Behandlung mit Ketoprofen beeinflusst.

Insgesamt zeigen die ersten beiden Studien, dass weitere Untersuchungen zur Verbesserung der Diagnose der akuten Metritis nötig sind. Die Messung der Milchtemperatur mit Automatischen Melksystemen zur Detektion von fieberhaften Kühen ist ungenügend. Obwohl in der zweiten Studie ein hoher Zusammenhang zwischen akuter Metritis und Haptoglobinkonzentration bestätigt werden konnte, wurde darüber hinaus auch gezeigt, dass andere Faktoren, die zeitgleich mit der akuten Metritis auftreten, ebenfalls im Zusammenhang mit der Haptoglobinkonzentration stehen und berücksichtigt werden müssen. Die dritte Studie zeigt, dass eine Initialtherapie mit Ketoprofen keine nachteilige Wirkung auf Milchleistung und Fruchtbarkeitsergebnisse hat und daher als Alternative zur antibiotischen Therapie verwendet und als effektiver Ansatz zur Reduktion des Antibiotikaeinsatzes angesehen werden kann.

7 REFERENCES FOR INTRODUCTION AND DISCUSSION

Benzaquen, M. E., C. A. Risco, L. F. Archbald, P. Melendez, M. J. Thatcher, and W. W. Thatcher. 2007. Rectal temperature, calving-related factors, and the incidence of puerperal metritis in postpartum dairy cows. J. Dairy Sci. 90:2804-2814.

Bewley, J. M., M. E. Einstein, M. W. Grott, and M. M. Schutz. 2008. Comparison of reticular and rectal core body temperatures in lactating dairy cows. J. Dairy Sci. 91:4661-4672.

Bolinder, A., B. Seguin, H. Kindahl, D. Bouley, and D. Otterby. 1988. Retained fetal membranes in cows: Manual removal versus nonremoval and its effect on reproductive performance. Theriogenology 30:45-56.

Burfeind, O., I. Sannmann, R. Voigtsberger, and W. Heuwieser. 2014a. Receiver operating characteristic curve analysis to determine the diagnostic performance of serum haptoglobin concentration for the diagnosis of acute puerperal metritis in dairy cows. Anim. Repr. Sci. 149:145-151.

Burfeind, O., V. S. Suthar, and W. Heuwieser. 2012. Effect of heat stress on body temperature in healthy early postpartum dairy cows. Theriogenology 78:2031-2038.

Burfeind, O., V. S. Suthar, R. Voigtsberger, S. Bonk, and W. Heuwieser. 2014b. Body temperature in early postpartum dairy cows. Theriogenology 82:121-131.

Burfeind, O., M. A. G. von Keyserlingk, D. M. Weary, D. M. Veira, and W. Heuwieser. 2010. Short communication: Repeatability of measures of rectal temperature in dairy cows. J. Dairy Sci. 93:624-627.

Ceciliani, F., J. J. Ceron, P. D. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. J. Proteomics 75:4207-4231.

Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. P. Santos, and S. J. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. J. Dairy Sci. 94:4897-4903.

Chenault, J. R., J. F. McAllister, S. T. Chester, K. J. Dame, F. M. Kausche, and E. J. Robb. 2004. Efficacy of ceftiofur hydrochloride sterile suspension administered parenterally for the treatment of acute postpartum metritis in dairy cows. J. Am. Vet. Med. Assoc. 224:1634-1639.

Cook, N. B. and K. V. Nordlund. 2004. Behavioral needs of the transition cow and considerations for special needs facility design. Vet. Clin. North Am. Food Anim. Pract. 20:495-520.

Crawford, R. G., K. E. Leslie, R. Bagg, C. P. Dick, and T. F. Duffield. 2005. The impact of controlled release capsules of monensin on postcalving haptoglobin concentrations in dairy cattle. Can. J. Vet. Res. 69:208-214.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D'Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows1. J. Dairy Sci. 89:3563-3577.

de Boer, M., C. Heuer, H. Hussein, and S. McDougall. 2015. Minimum inhibitory concentrations of selected antimicrobials against escherichia coli and trueperella pyogenes of bovine uterine origin. J. Dairy Sci. 98:4427-4438.

de Koning, K. 2010. Automatic milking - common practice on dairy farms. Pages V59 - V63 in Proc. Second North Am. Conf. on Robotic Milking, Toronto, Canada. Precision Dairy Operators, Elora, Canada.

de Koning, K. 2013. Developments in machine milking; past and future. Pages 5 - 9 in Proc. 4. Tänikoner Melktechniktagung, Switzerland, Agroscope, Ettenhausen, Switzerland.

Drillich, M., D. Voigt, D. Forderung, and W. Heuwieser. 2007. Treatment of acute puerperal metritis with flunixin meglumine in addition to antibiotic treatment. J. Dairy Sci. 90:3758-3763.

Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2010. Risk factors for postpartum uterine diseases in dairy cows. J. Dairy Sci. 93:5764-5771.

Eckersall, P. D. 2000. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. Rev. Med. Vet. 7:577-584.

Fitzpatrick, J. L., A. M. Nolan, P. Lees, and S. A. May. 2004. Inflammation and pain, Bovine medicine. (Second Ed.), Blackwell Publishing, Oxford, UK, 1045 – 1066.

Fordham, D. P., P. Rowlinson, and T. T. McCarthy. 1988. Oestrus detection in dairy cows by milk temperature measurement. Res. Vet. Sci. 44:366-374.

Fourichon, C., H. Seegers, and X. Malher. 2000. Effect of disease on reproduction in the dairy cow: A meta-analysis. Theriogenology 53:1729-1759.

Galvão, K. N., M. J. B. F. Flaminio, S. B. Brittin, R. Sper, M. Fraga, L. Caixeta, A. Ricci, C. L. Guard, W. R. Butler, and R. O. Gilbert. 2010. Association between uterine disease and indicators of neutrophil and systemic energy status in lactating holstein cows. J. Dairy Sci. 93:2926-2937.

Galvão, K. N., L. F. Greco, J. M. Vilela, M. F. Sá Filho, and J. E. P. Santos. 2009. Effect of intrauterine infusion of ceftiofur on uterine health and fertility in dairy cows. J. Dairy Sci. 92:1532-1542.

Gilbert, R. O., N. R. Santos, K. N. Galvão, S. B. Brittin, and H. B. Roman. 2007. The relationship between postpartum uterine bacterial infection (bi) and subclinical endometritis (se). J. Dairy Sci. 90(Suppl. 1):469.

Gröhn, Y. T., P. J. Rajala-Schultz, H. G. Allore, M. A. DeLorenzo, J. A. Hertl, and D. T. Galligan. 2003. Optimizing replacement of dairy cows: Modeling the effects of diseases. Rev. Vet. Med. 61:27-43.

Grove-White, D. and R. Murray. 2009. Use of antimicrobials. Vet. Rec. 164:727.

Heuwieser, W., M. Iwersen, J. Gossellin, and M. Drillich. 2010. Short communication: Survey of fresh cow management practices of dairy cattle on small and large commercial farms. J. Dairy Sci. 93:1065-1068.

Humblet, M.-F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J.-M. Godeau. 2006. Relationship between haptoglobin, serum amyloid a, and clinical status in a survey of dairy herds during a 6-month period. Vet. Clin. Path. 35:188-193.

Hussain, A. M. 1989. Bovine uterine defense mechanisms: A review. J. Vet. Med B. 36:641-651.

Huzzey, J. M., T. F. Duffield, S. J. LeBlanc, D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Short communication: Haptoglobin as an early indicator of metritis. J. Dairy Sci. 92:621-625.

Igono, M. O., H. D. Johnson, B. J. Steevens, G. F. Krause, and M. D. Shanklin. 1987. Physiological, productive, and economic benefits of shade, spray, and fan system versus shade for holstein cows during summer heat. J. Dairy Sci. 70:1069-1079.

Janovick, N. A., Y. R. Boisclair, and J. K. Drackley. 2011. Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous holstein cows1. J. Dairy Sci. 94:1385-1400.

Laven, R., P. Chambers, and K. Stafford. 2012. Using non-steroidal anti-inflammatory drugs around calving: Maximizing comfort, productivity and fertility. Vet. J. 192:8-12.

LeBlanc, S. J., T. F. Duffield, K. E. Leslie, K. G. Bateman, G. P. Keefe, J. S. Walton, and W. H. Johnson. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci. 85:2223-2236.

Lima, F. S., A. Vieira-Neto, G. S. Vasconcellos, R. D. Mingoti, E. Karakaya, E. Sole, R. S. Bisinotto, N. Martinez, C. A. Risco, K. N. Galvao, and J. E. Santos. 2014. Efficacy of ampicillin trihydrate or ceftiofur hydrochloride for treatment of metritis and subsequent fertility in dairy cows. J. Dairy Sci. 97:5401–5414.

Machado, V. S., M. L. de Souza Bicalho, E. B. d. S. M. Junior, R. Rossi, B. L. Ribeiro, S. Lima, T. Santos, A. Kussler, C. Foditsch, and E. K. Ganda. 2014. Subcutaneous immunization with inactivated bacterial components and purified protein of escherichia coli, fusobacterium necrophorum and trueperella pyogenes prevents puerperal metritis in holstein dairy cows. PloS one 9:e91734.

Martinez, N., C. A. Risco, F. S. Lima, R. S. Bisinotto, L. F. Greco, E. S. Ribeiro, F. Maunsell, K. Galvão, and J. E. P. Santos. 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J. Dairy Sci. 95:7158-7172.

McLaughlin, C. L., E. Stanisiewski, M. J. Lucas, C. P. Cornell, J. Watkins, L. Bryson, J. K. S. Tena, J. Hallberg, and J. R. Chenault. 2012. Evaluation of two doses of ceftiofur crystalline free acid sterile suspension for treatment of metritis in lactating dairy cows. J. Dairy Sci. 95:4363-4371.

Overton, M. W. and J. Fetrow. 2008. Economics of postpartum uterine health. Dairy Cattle Reproduction Council Convention, Omaha, Nebraska. 39 – 44.

Pohl, A., O. Burfeind, and W. Heuwieser. 2015. The associations between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis. J. Dairy Sci. 98:4544-4551.

Pyorala, S., J. Taponen, and T. Katila. 2014. Use of antimicrobials in the treatment of reproductive diseases in cattle and horses. Repro. Domest. Anim. 49 Suppl. 3:16-26.

Sannmann, I., O. Burfeind, R. Voigtsberger, and W. Heuwieser. 2013a. Comparison of two monitoring and treatment strategies for cows with acute puerperal metritis. Theriogenology 79:961-969.

Sannmann, I., O. Burfeind, V. Suthar, A. Bos, M. Bruins, and W. Heuwieser. 2013b. 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. 96:5773-5779.

Santos, T. M. A., R. O. Gilbert, and R. C. Bicalho. 2011. Metagenomic analysis of the uterine bacterial microbiota in healthy and metritic postpartum dairy cows. J. Dairy Sci. 94:291-302.

Santos, T. M. A., R. O. Gilbert, L. S. Caixeta, V. S. Machado, L. M. Teixeira, and R. C. Bicalho. 2010. Susceptibility of escherichia coli isolated from uteri of postpartum dairy cows to antibiotic and environmental bacteriophages. Part ii: In vitro antimicrobial activity evaluation of a bacteriophage cocktail and several antibiotics. J. Dairy Sci. 93:105-114.

Schneider, A., M. N. Correa, and W. R. Butler. 2013. Short communication: Acute phase proteins in holstein cows diagnosed with uterine infection. Res. Vet. Sci. 95:269-271.

Schuenemann, G. M., S. Bas, E. Gordon, and J. D. Workman. 2013. Dairy calving management: Description and assessment of a training program for dairy personnel. J. Dairy Sci. 96:2671-2680.

Scientific Advisory Group on Antimicrobials of the Committee for Medicinal Products for Veterinary Use. 2009. Reflection paper on the use of third and fourth generation cephalosporins in food producing animals in the european union: Development of resistance and impact on human and animal health. J. Vet. Pharmacol. Ther. 32:515-533.

Sheldon, I. M., M. Bushnell, J. Montgomery, and A. N. Rycroft. 2004. Minimum inhibitory concentrations of some antimicrobial drugs against bacteria causing uterine infections in cattle. Vet. Rec. 155:383-387.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516-1530.

Smith, B. I. and C. A. Risco. 2005. Management of periparturient disorders in dairy cattle. Vet. Clin. North Am. Food. Anim. Pract. 21:503-521.

Stojkov, J., M. A. G. von Keyserlingk, J. N. Marchant-Forde, and D. M. Weary. 2015. Assessment of visceral pain associated with metritis in dairy cows. J. Dairy Sci. 98:5352-5361.

Svennersten-Sjaunja, K. M. and G. Pettersson. 2008. Pros and cons of automatic milking in europe. J. Anim. Sci. 86:37-46.

West, J. W., G. M. Hill, J. M. Fernandez, P. Mandebvu, and B. G. Mullinix. 1999. Effects of dietary fiber on intake, milk yield, and digestion by lactating dairy cows during cool or hot, humid weather. J. Dairy Sci. 82:2455-2465.

West, J. W., B. G. Mullinix, J. C. Johnson, K. A. Ash, and V. N. Taylor. 1990. Effects of bovine somatotropin on dry matter intake, milk yield and body temperature in holstein and jersey cows during heat stress. J. Dairy Sci. 73:2896-2906.

Williams, E. J., D. P. Fischer, D. U. Pfeiffer, G. C. W. England, D. E. Noakes, H. Dobson, and I. M. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63:102-117.

Wittrock, J. M., K. L. Proudfoot, D. M. Weary, and M. A. G. von Keyserlingk. 2011. Short communication: Metritis affects milk production and cull rate of holstein multiparous and primiparous dairy cows differently. J. Dairy Sci. 94:2408-2412.

8 PUBLICATIONS

8.1 Research articles

Pohl, A.; W. Heuwieser and O. Burfeind (2014):

Technical Note: Assessment of milk temperature measured by automatic milking systems as an indicator of body temperature and fever in dairy cows. Journal of Dairy Science. 97:4333-4339.

Pohl, A.; W. Heuwieser and O. Burfeind (2015):

The association between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes and metritis. Journal of Dairy Science. 98:4544-4551.

8.2 Oral presentations at conferences

Pohl, A.; Burfeind, O.; Heuwieser, W. (2015):

Haptoglobin − Faktoren, die die Haptoglobinkonzentration beeinflussen können. DVG Vet-Congress Berlin, Germany, 12.−15.11.2015. In: 5. Jahrestagung der DVG-Fachgruppe Dt. buiatrische Gesellschaft (DbG) in Zusammenarbeit mit der DVG-Fachgruppe Fortpflanzung und ihre Störungen, p 20-21.

Heuwieser, W.; Pohl. A. (2015):

Efficacy of nonsteroidal antiinflammatory drugs for the treatment of acute puerperal metritis in dairy cows. ADSA Joint annual meeting, Orlando, Florida, USA, 12.−16.07.2015. In J. Anim. Sci. Vol. 93, Suppl. s3/ J. Dairy Sci. Vol. 98, Suppl. 2, p 191.

Pohl, A.; Burfeind, O.; Heuwieser, W. (2015):

The association between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes and metritis. XV. Middle European Buiatric Congress, Maribor, Slowenia 10.−13.06.2015. In: Proceedings of the XV. Middle European Buiatric Congress, p 83.

Pohl, A. (2013):

Aktuelle Forschung für die Praxis: wie aussagekräftig ist die Milchtemperatur? 13. Modul FTA Rind "Eutergesundheit und Mastitis", Berlin, Germany 29.−30.11.2013. In: Eutergesundheit und Mastitis, p 24–26.

8.3 Poster presentations at conferences

Pohl, A.; Heuwieser, W.; Burfeind, O. (2014):

Milchtemperatur versus Körpertemperatur: Erkennung von fieberhaften Kühen mit Hilfe von automatischen Melksystemen? 10. Berlin-Brandenburgischer Rindertag, 02.−04.10.2014, Berlin, Germany. In: 10. Berlin-Brandenburgischer Rindertag Tagungsband, p 224–226.

Pohl, A.; Heuwieser, W.; Burfeind, O. (2014):

Comparison of vaginal and milk temperature in lactating dairy cows using data loggers and automatic milking systems. Congreso International ANEMBE de Medicina Bovina, 9. ECBHM-Symposium, 25.−27.06.2014. Oviedo, Spain. In: Presentations, oral presentations and poster book, p 353–355.

Pohl, A.; Heuwieser, W.; Burfeind, O. (2013):

Comparsion of vaginal and milk temperatures in lactating dairy cows using data loggers and automatic milking systems. 8th PhD-Symposium − bringing future scientists togehter & DRS presentation seminar, Berlin, Germany 15.07.2013. In: Program and Abstracts − Freie Universität Berlin, Fachbereich Veterinärmedizin, p 60.

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10 DECLARATION OF INDEPENDENCE

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

Tabelle 1. Eigener Anteil[1] an den Forschungsprojekten der vorliegenden Dissertation

	Studie 1[a]	Studie 2[b]	Studie 3[c]
Studienplanung	+++	+++	+++
Datenerhebung	+++	+++	+++
Datenanalyse	+++	+++	+++
Verfassen des Manuskripts	+++	+++	+++
Editieren des Manuskripts	++	++	++

[1]Legende: +++: > 70%

++: 50−70%

+: < 50%

[a] *Technical Note:* Assessment of milk temperature measured by automatic milking systems as an indicator of body temperature and fever in dairy cows

[b] The association between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes and metritis

[c] Randomized, controlled clinical trial on the efficacy of nonsteroidal antiinflammatory drugs for the treatment of acute puerperal metritis in dairy cows

Berlin, den 02.02.2016

Alina Pohl