Animal Reproduction Science 149 (2014) 145–151

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Receiver operating characteristic curve analysis to determine the diagnostic performance of serum haptoglobin concentration for the diagnosis of acute puerperal metritis in dairy cows

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ARTICLE INFO

Article history:
Received 27 February 2014
Received in revised form 11 July 2014
Accepted 17 July 2014
Available online 27 July 2014

Keywords:
Fever
Dairy cattle
Uterine disease
Diagnostic performance
Acute phase protein

ABSTRACT

Acute puerperal metritis (APM) in dairy cows is characterized by fever and fetid vaginal discharge within 21 days in milk (DIM). Increased serum haptoglobin concentration (Hp) can support the diagnosis of APM. However, there is a dearth of information of the test performance of Hp as a measure for APM with a consistent definition and considering parity. The objective of this trial was to study the test performance of Hp to distinguish healthy cows from cows with APM. A total of 33 of 60 (55.0%) primiparous cows and 43 of 133 (32.3%) multiparous cows developed APM. Primiparous cows with APM had the greatest Hp. However, in primiparous cows Hp did not significantly differ between healthy cows (DIM 2: 1.49 ± 0.64 mg/mL; DIM 5: 2.13 ± 0.66 mg/mL; DIM 10: 1.46 ± 0.85 mg/mL) and cows with APM (DIM 2: 1.78 ± 0.62 mg/mL; DIM 5: 2.48 ± 0.64 mg/mL; DIM 10: 1.60 ± 0.81 mg/mL). In multiparous cows, Hp was greater in cows with APM (DIM 2: 1.27 ± 0.68 mg/mL; DIM 5: 1.89 ± 0.94 mg/mL; DIM 10: 1.23 ± 0.78 mg/mL) than in healthy cows (DIM 2: 0.99 ± 0.68 mg/mL; DIM 5: 1.10 ± 0.80 mg/mL; DIM 10: 0.83 ± 0.68 mg/mL). Sensitivity and specificity of Hp to diagnose APM in multiparous cows ranged from 72% to 79% and 54% to 71% on DIM 2, 5 and 10, respectively.

1. Introduction

Postpartum uterine diseases in dairy cows affect reproductive performance (LeBlanc et al., 2002; Sheldon et al., 2006; Overton and Fetrow, 2008; Giuliodori et al., 2013). The prevalence of acute puerperal metritis (APM) varies from 15.3% to 69.0% (Melendez et al., 2004; Urton et al., 2005; Goshen and Shpigel, 2006). Metritis occurs within 21 d after parturition and is classified into three grades (Sheldon et al., 2009). A cow showing an abnormally enlarged uterus and a purulent uterine discharge without any systemic signs of ill-health is classified as having Grade 1 metritis. At Grade 2 metritis, these signs are accompanied with fever >39.5 °C, a cow with Grade 3 metritis shows signs of toxemia, having a poor prognosis (Sheldon et al., 2009). The categorization is based on the simultaneous appearance of fever and abnormal vaginal discharge (VD) indicative of a generalized infection (Benzaquen et al., 2007). Abnormal VD without fever could equally occur in healthy cows as a result of the opening of the cervix at DIM 7 to 10 (Wehrend et al., 2003). In the present study, there was a focus on APM (i.e. Grade 2 and 3 metritis) because of the life-threatening characteristics (Drillich et al., 2007)

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http://dx.doi.org/10.1016/j.anireprosci.2014.07.020
0378-4320/

and substantial economic losses caused by this disease (Overton and Fetrow, 2008), while cows without fever (Grade 1) were defined as healthy.

A serum haptoglobin concentration (Hp) between 1.06 and 1.90 g/L indicates an acute infectious process in dairy cows (Drillich et al., 2007; Huzzey et al., 2009). It has been described as valuable in the early diagnosis of APM, because the concentration of Hp is increased 2 d before clinical signs. However, several authors found elevated Hp in the first week after calving in healthy cows (Humblet et al., 2006; Huzzey et al., 2009; Silvestre et al., 2011). Therefore, Hp should be used with caution in the days following parturition as a marker for APM (Humblet et al., 2006).

Recent studies calculated a sensitivity of 83.3% and a specificity of 58.3% of Hp to detect animals with pathological processes within 1 week postpartum (Humblet et al., 2006). For the detection of metritis sensitivity and specificity were 50% and 87%, respectively (Huzzey et al., 2009). In this previous study, however, 32 cows with clinical metritis (Grade 1) and 12 cows suffering from APM (Grade 2 and 3) were included. Test characteristics might be confounded by the relatively large number of cows with lower grade metritis. Another study found similar sensitivity (51.6%) and specificity (79%) (Dubuc et al., 2010) diagnosing metritis as a systemic illness with fetid VD but body temperature was not assessed.

There is evidence that cows with APM have a greater probability of recovering from the condition without treatment (McLaughlin et al., 2012). It seems logical that the incidence of recovery without treatment is greater in cows with Grade 1 (i.e. less severe) metritis not accompanied by fever. Therefore, it is questionable to include these cows in the calculation of an optimal Hp threshold. Overall, there is a dearth of information of the test performance of Hp as a measure for APM on a larger scale (Huzzey et al., 2009) with a consistent definition of APM. Furthermore, in the previous studies optimal Hp thresholds were calculated regardless of parity.

The objective of the present study was to evaluate the test performance of Hp to distinguish healthy cows from cows with APM consistently diagnosed by fever (≥39.5 °C) and fetid, reddish-brown, watery VD and considering parity. Specifically, in the present study, the objective was the determination of the sensitivity, specificity, positive and negative predictive values of Hp towards the presence or absence of APM in the first 10 DIM for both primiparous and multiparous dairy cows. After identifying the optimal critical thresholds of Hp its relationship to rectal temperature was determined.

2. Materials and methods

The present study was conducted on a commercial dairy farm in Sachsen-Anhalt, Germany between October 2011 and January 2012 housing 1,200 Holstein dairy cows with an average 305 days milk production (DIM) of 10,147 kg (3.98% fat and 3.33% protein). Cows were managed according to the guidelines set by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (Hellmann and Radeloff, 2000). Lactating cows were housed in a free-stall barn with cubicles equipped with rubber mats and slotted floors. Early postpartum cows were fed a TMR consisting of 34.2% corn silage, 20.7% grass silage, 4.2% barley straw, and 41.6% concentrate mineral mix on a dry matter basis distributed with a conveyer belt system up to 10 times per day. Cows were milked three times a day (06:00, 14:00, 22:00). Milk yield was recorded daily by using the parlor software (Fullexpert Software, Lemmer Fullwood, Lohmar, Germany). During the experimental period a total of 222 cows calved and were enrolled. Cows were assigned to the experiment one day after calving. Cows that received anti-inflammatory drugs or antibiotic drugs for other diseases than APM (e.g. acute mastitis) or suffered from other inflammatory diseases than APM were excluded from the trial. All cows were examined daily between 08:00 and 11:00 during the first 10 DIM. All examinations were performed by one of three investigators. The examination included the measurement of rectal temperature using a digital thermometer (MT1831; Microlife AG, Widnau, Switzerland) inserted with a constant penetration depth of 8.4 cm (up to the display) to minimize variation (Burfeind et al., 2010). In terms of a rectal temperature ≥39.5 °C and on DIM 2, 5 and 10 VD was evaluated inserting a gloved-hand in the vagina after cleaning the vulva (Pleticha et al., 2009). To standardize the definition of disease, a scoring system had been used (0 = no discharge, 1 = normal lochial secretion; not fetid, viscous, reddish brown, 2 = fetid, watery, reddish-brown VD). Once a week, the diagnosis was performed by all three investigators jointly to standardize the diagnostic classification.

Acute puerperal metritis was diagnosed when a cow had fetid, reddish-brown, watery VD in combination with a rectal temperature ≥39.5 °C (Sheldon et al., 2006). Cows suffering from APM were treated using a protocol described elsewhere (Sannmann et al., 2013). In brief, 66 cows with APM were treated with 6.6 mg/kg ceftiofur (ceftiofur crystalline free acid, Naxcel, Pfizer Limited, Kent, UK) and 10 cows remained untreated due to the treatment protocol. Blood samples were collected at DIM 2, 5 and 10 from coccygeal vessels using sterile vacuum tubes (Venoject II, Terumo Europe N.V., Leuven, Belgium). Within 2 h after sampling, blood samples were centrifuged at 1000 × *g* for 10 min at room temperature and serum stored at −20 °C until further analysis. At the end of the study, serum samples were sent to a commercial laboratory (Synlab laboratories, EU accreditation number: D-PL-14016-01-00, accreditation body: DAkkS, Berlin, Germany) and analysed for Hp using an ELISA (Sunrice reader, Tecan, Maennedorf, Switzerland). The lower limit of detection was 0.31 mg/mL. All samples with values less than 0.31 mg/mL were set to 0.31 mg/mL. Intra-assay coefficients of variation for the low (0.6 mg/mL) and high (1.3 mg/mL) Hp was 5.3% and 6.3% while the inter-assay coefficients of variation were and 4.1% and 5.7%, respectively.

Data were analyzed using SPSS for Windows (Version 19.0, SPSS Inc. Munich, Germany) and MedCalc (Version 12.0.3.0., MedCalc Software bvba, Mariakerke, Belgium). Serum haptoglobin concentration at DIM 2, 5 and 10 were compared using a repeated-measures ANOVA. Furthermore, rectal temperature during the first 10 DIM was compared using a repeated-measures ANOVA separately