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Diagnosis of acute puerperal metritis by electronic nose device analysis of vaginal discharge in dairy cows

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ABSTRACT

The objective of this study was to estimate the diagnostic accuracy of an electronic nose device using vaginal discharge samples to diagnose acute puerperal metritis (APM) in dairy cows. Uterine fluid was sampled manually with a gloved hand and under sterile conditions for electronic nose device analysis (day in milk (DIM) 2, 5, and 10) and bacteriologic examination (DIM 5), respectively, and on additional days, if APM was diagnosed during the daily clinical examinations. A dataset containing samples from 70 cows was used to create a model and to validate the APM status predicted by this model, respectively. Half of the dataset (n = 35; 14 healthy and 21 metritic cows) was provided with information regarding the APM diagnosis and contained all three measurements (DIM 2, 5, and 10) for each cow and was used as a training set whereas the second half was blinded (n = 35; 14 healthy and 21 metritic cows) and contained only the samples collected on DIM 5 of each cow and was used to validate the created prediction model. A receiver operating characteristic curve was calculated using the prediction results of the validation test. The best observed sensitivity was 100% with specificity of 91.6% when using a threshold value of 0.3. The calculated P-value for the receiver operating characteristic curve was less than 0.01. Overall, *Escherichia coli* was isolated in eight of 28 (28.6%) and 22 of 42 (52.4%) samples collected from healthy and metritic cows, respectively. *Trueperella pyogenes* and *Fusobacterium necrophorum* were isolated in 14 and six of 28 (50.0% and 21.4%) and 17 and 16 of 42 (40.5% and 38.1%) samples collected from healthy and metritic cows, respectively. The prevalence of *Escherichia coli* and *Trueperella pyogenes* was similar in the samples obtained from metritic cows used for the training set and the validation test. The results are promising especially because of the objective nature of the measurements obtained by the electronic nose device.

1. Introduction

Acute puerperal metritis (APM) is an acute systemic illness with fever of 39.5 °C or greater and signs of toxemia due to an infection of the uterus, usually occurring within 10 days after parturition characterized by an enlarged uterus and a watery red-brown fluid to viscous off-white purulent uterine discharge, which often has a fetid odor [1,2].

In the past several intervention studies [3–5], comparing efficacy of different therapies for APM has primarily used the appearance of fever together with abnormal vaginal discharge (VD) as inclusion criteria. These signs are indicative of a generalized infection caused by interactions between the host immune system and bacterial endotoxins [2]. Characteristics used in research and in the field to

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differentiate between normal and abnormal VD include color, viscosity, odor, and amount of VD [2,6,7]. Odor of VD is associated with the bacterial growth density of potential pathogens (i.e., *Escherichia* (*E.*) *coli*, *Trueperella* (*T.*) *pyogenes*, *Fusobacterium* (*F.*) *necrophorum*) in the uterus [8]. Therefore, abnormal VD is a plausible criterion, which is commonly used in the field because it is easy and intuitively assessable without the use of additional diagnostic tools. A most recent evidence-based review of diagnostic methods for APM, however, points out that information on the diagnostic value of the assessment of VD is lacking [4]. Furthermore, it has been demonstrated that sensorial assessments such as the olfactory evaluation of odor of VD or the visual evaluation of vaginal fluid through vaginoscopy can be confounded by only moderate intra- and interobserver repeatability [9,10].

In human medicine, electronic nose devices have been used for different purposes including diagnostics of urinary tract infection [11], wound infection [12], and tuberculosis [13]. These devices are capable of detecting different volatile organic compounds (VOCs) commonly produced and released from organic sources such as living microbes and multicellular organisms [14–16]. In bovine research, electronic nose devices were used for the detection of estrus, mastitis, or respiratory disease [17–19]. Data on sensitivity and specificity, however, were not described.

In the past, we have determined a moderate interobserver ($K = 0.43$) and intraobserver repeatability ($K = 0.52$) for the classification of healthy versus metritic animals based on the assessment of VD by olfactory cognition [10]. These data were generated in a laboratory setting evaluating identical frozen-thawed aliquots of five VD samples in 10 replicates and a panel of 16 observers. Interestingly, the intra-assay repeatability of an electronic nose device was higher (Cronbach's alpha = 0.97) compared with the human nose. Differences in the perception of odors between and within observers can be accounted to various factors such as age, experience, and environment [20–22].

The objective of this study was to determine sensitivity and specificity of an electronic nose device to diagnose APM in dairy cattle using VD samples collected from cows diagnosed by a veterinarian as metritic or healthy as reference and to evaluate the detection of pathogens.

2. Materials and methods

The study was conducted between October and December 2011 on a commercial dairy farm in Sachsen-Anhalt, Germany, housing 1200 Holstein dairy cows with an average 305-day milk production of 10,147 kg (3.98% fat and 3.33% protein). The main objective of the overall study was to compare the efficacy of two treatment protocols for APM in dairy cattle. Details of the study design have been described previously [5]. In brief, a total of 222 cows were enrolled, clinically examined by a graduate veterinarian affiliated with the Clinic of Animal Reproduction and diagnosed as having APM (i.e., fetid, reddish-brown, and watery vulvar discharge in combination with a rectal temperature $\geq$39.5 °C) or being healthy. Cows with APM received a systemic antimicrobial treatment of 6.6 mg/kg body weight ceftiofur crystalline-free acid (Naxcel; Pfizer Limited, Kent, UK) in two different treatment protocols as described elsewhere [5].

The cows were managed according to the guidelines set by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products [23]. The experimental procedures reported herein were conducted with the approval of the Institutional Animal Care and Use Committee. Lactating cows were housed in a free-stall barn with cubicles equipped with rubber mats and slotted floors. Early-postpartum cows were fed a total mixed ration consisting of 34.1% corn silage, 20.5% grass silage, 4.2% barley straw, and 41.2% concentrate mineral mix on a dry matter basis distributed with a conveyer belt system up to 10 times per day. Cows were milked three times a day (0600, 1400, and 2200 hours). Milk yield was recorded daily by using the parlor software (Fullexpert Software, Version 3.02; Lemmer Fullwood, Lohmar, Germany).

Cows entered the experiment 1 day after calving at 0700 hours. Cows that received antiinflammatory drugs or antimicrobial drugs for purposes not related to the study (e.g., acute mastitis and lameness) or suffered from other inflammatory diseases than APM were excluded from the trial ($n = 29$). Rectal temperature was measured daily with a digital thermometer (MT1831, Microlife AG, Heerbrugg, Switzerland) until day in milk (DIM) 10. Measurements were performed at the same time of the day (0700–0900 hours) and at the same insertion depth (8 cm) to minimize any bias due to the measuring process [24].

Vaginal discharge was collected through manual vaginal examination with a gloved hand. To minimize contamination of the vagina, the tails of the cows were held, and the vulva and perineum were cleaned with dry paper towels before the discharge was collected into screw-cap vials (Rotilabo; Carl Roth GmbH & Co. KG, Karlsruhe, Germany) and evaluated regarding color, proportion of pus, consistency, and smell by one of the investigators. Cows without VD or physiological lochia were classified as healthy and cows with fetid, red-brown, watery VD in combination with a rectal temperature of 39.5 °C or greater (fever) were classified as having APM [1,25]. Cows with fever or with fetid, red-brown, watery VD were not considered as metritic but further monitored to determine whether or not they developed APM.

2.1. Sampling

Sampling for electronic nose device analysis was performed immediately after routine clinical examination and diagnosis of APM and in all cows routinely on DIM 2, 5, and 10. Therefore, the vulva was cleaned with dry paper towels and VD collected manually with a gloved hand, and transferred into screw-cap vials. Samples were stored at −25 °C until analysis 3 months later. A bacteriologic examination of uterine fluid was conducted on DIM 5 in all cows and on the day of diagnosis of APM. The vulval lips were parted, and a sterile plastic catheter (insemination pipette for horses; Minitube GmbH, Tiefenbach, Germany), protected through a hygienic sheath (Minitube GmbH), was advanced under manual control into the vagina and through the cervix. Uterine fluid was aspirated using a