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

I. Sannmann,* O. Burfeind,* V. Suthar,* A. Bos,† M. Bruins,† and W. Heuwieser*[1]
*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Königsweg 65, 14163 Berlin, Germany
†C-it, 7201 JB Zutphen, the Netherlands

ABSTRACT

The objective of this study was to determine test characteristics (i.e., intra- and interobserver variability, intraassay variability, sensitivity, and specificity) of an evaluation of odor from vaginal discharge (VD) of cows in the first 10 d postpartum conducted by olfactory cognition and an electronic device, respectively. In experiment 1, 16 investigators (9 veterinary students and 7 licensed veterinarians) evaluated 5 VD samples each on 10 different days. The kappa test revealed an agreement between investigators (interobserver) of $\kappa = 0.43$ with a Fleiss adjusted standard error of 0.0061. The overall agreement was the same for students ($\kappa = 0.28$) and veterinarians ($\kappa = 0.28$). Mean agreement within observers (intraobserver) was $\kappa = 0.52$ for all observers, and 0.49 and 0.62 for students and veterinarians, respectively. In experiment 2, the repeatability of an electronic device (DiagNose; C-it, Zutphen, the Netherlands) was tested. Therefore, 5 samples of VD from 5 cows were evaluated 10 times each. The repeatability was 0.97, determined by Cronbach's α. In experiment 3, 20 samples collected from healthy cows and 20 of cows with acute puerperal metritis were evaluated by the 16 investigators and the DiagNose using a dichotomous scale (1 = cow with acute puerperal metritis; 0 = healthy cow). Sensitivity and specificity of olfactory evaluation was 75.0 and 60.1% compared with 92.0 and 100%, respectively, for the electronic nose device. The study revealed a considerable subjectivity of the human nose concerning the classification into healthy and sick animals based on the assessment of vaginal discharge. The repeatability of the electronic nose was higher. In conclusion, the DiagNose system, although imperfect, is a reasonable tool to improve odor assessment of VD. The current system, however, is not suitable as a screening tool in the field. Further research is warranted to adapt such electronic devices to practical on-farm screening tools.

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[1]Corresponding author: w.heuwieser@fu-berlin.de

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Technical Note

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

Although VD and fever are plausible criteria and have been used in several research trials studying efficacy of different therapies, there is a lack of science-based evidence for their diagnostic value (Sannmann et al., 2012). Most recent studies have demonstrated that body temperature can be measured with high repeatability (Burfeind et al., 2010) but is subject to certain variables, such as time of day, parity, and ambient temperature (Burfeind et al., 2012). For a visual assessment of VD in cows suffering from clinical endometritis through vaginoscopic examination, sensitivity and specificity ranged between 96.3 and 99.6% and 90.1 and 96.7%, respectively. Intraobserver (κ = 0.55–0.60) and interobserver ($\kappa = 0.44$) repeatability of a VD score on a scale from 0 to 3, however, was only moderate (Leutert et al., 2012). To our knowledge, the

sensorial assessments of viscosity and smell of VD from cows with APM or clinical endometritis have not been studied yet.

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

Science-based evidence exists both from accepted clinical (e.g., rectal palpation and temperature measurement) and advanced diagnostic methods (e.g., radiography and ultrasound) that the investigator is a relevant source of measurement errors (Andermann et al., 2007; Burfeind et al., 2010; Leutert et al., 2013). Studies testing the repeatability of sensorial assessments of odors using a graded solution of phenol in liquid paraffin and olfactometer threshold tests found a correlation between observers (n = 57 and 98, respectively) varying from correlation coefficient = 0.43 to 0.9 (Fordyce, 1961; Doty et al., 1995).

Electronic sensor devices, so-called electronic noses (Gardner and Bartlett, 1994), have made possible several beneficial applications to a variety of commercial industries, including the agricultural, biomedical, cosmetics, environmental, food, manufacturing, military, pharmaceutical, and regulatory industries, and various scientific research fields (Wilson and Baietto, 2009).

In dairy research, electronic devices have been used for the detection of substances indicating estrus or pathological conditions such as mastitis or respiratory infection in cattle (Eriksson et al., 2005; Knobloch et al., 2010; Wiegerinck et al., 2011). These electronic devices are capable of detecting different types and sources of chemical species and mixtures of compounds present in the headspace volatiles of sampled air. Volatile organic compounds are commonly produced and released from organic sources as living microbes and multicellular organisms (Barsan et al., 2007; Wilson and Baietto, 2011).

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

Vaginal discharge samples were collected from a total of 45 cows (22 healthy and 23 with APM) in December 2011, held on a commercial dairy farm in Sachsen-Anhalt, Germany, housing 1,200 Holstein dairy cows. The cows were closely monitored after calving for 10 d postpartum by daily rectal temperature measurement and evaluation of VD (viscosity, color, and odor) according to Sheldon et al. (2009), at 2, 5, and 10 DIM. Cows having fetid, reddish-brown, watery vulvar discharge in combination with a rectal temperature ≥39.5°C (i.e., fever) were characterized as having APM and sampled. All VD samples were collected with a gloved hand from the vagina after cleaning of vulva and perineum with dry paper towels. Approximately 5 mL of VD were transferred into sterile vials (Sarstedt AG & Co., Nürnberg, Germany) and stored at −20°C for later analysis. For repeatability testing, 50 mL of VD from 5 cows each (2 healthy and 3 with APM) was collected and stored in 5-mL aliquots at −20°C.

Three experiments were conducted to determine (1) intra- and interobserver variability of olfactory cognition (i.e., human nose), (2) intraassay variation of an electronic device (i.e., DiagNose; C-it, Zutphen, the Netherlands), and (3) sensitivity and specificity by olfactory cognition and the electronic device, respectively, to diagnose APM based on odor alone.

Sixteen investigators were enrolled in experiment 1, including 9 veterinary students in their fourth year and 7 licensed veterinarians working at the Clinic of Animal Reproduction, Freie Universität Berlin (Berlin, Germany). All investigators consented to participate (informed consent) and had the same information about the study design (i.e., nature of substance under investigation and definition of APM) but no information about the health status of the cows.

A total of 10 appointments in 3- to 4-d intervals were made. All observers evaluated a set of 5 samples (2 healthy and 3 with APM) containing 5 mL of VD. The samples were newly randomized for each appointment with the random number function of Excel (Microsoft Office 2010; Microsoft Deutschland GmbH, Munich, Germany) and labeled with capital letters A to E. At each appointment, new 5-mL samples were incubated in a water bath at 38°C for 10 min before testing. To allow a semiquantitative assessment of odor, a 5-point scale (1 = penetrating, very fetid, 2 = fetid, 3 = slightly fetid, 4 = aromatic, and 5 = neutral) was used.

In experiment 2, intraassay variation of the DiagNose was determined according to the descriptions of the manufacturer (see below). The system uses an array of 12 micro-hotplate-type metal oxide-sensor modules (i.e., 6 different sensor types in duplicate). The sensors consist of a heating element and a sensor element (a sintered metal oxide). The sensors are temperature