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

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### **ABSTRACT**

Acute puerperal metritis (APM) in dairy cows is a common disease occurring in the first 10 d after calving. According to a widely accepted definition, the diagnosis is primarily based on body temperature and sensorial assessment of vaginal discharge. The scope of this study was to evaluate the reliability for color, smell, and viscosity of vaginal discharge from healthy cows and cows with APM. Fifteen investigators evaluated 6 vaginal discharge samples 10 times. Subsequently, the investigators rated the health status of the cows and the diagnostic value of color, smell, and viscosity. In a final questionnaire, the investigators estimated their ability to diagnose APM correctly and the influence of experience. Reliability was tested using Cohen's kappa ( $\kappa$ ). Our study revealed slight to moderate reliabilities concerning the assessment of vaginal discharge. Overall interobserver reliability for color, smell, and viscosity was  $\kappa = 0.15, 0.27, \text{ and } 0.44$ , respectively. Overall intraobserver reliability for color, smell, and viscosity was  $\kappa = 0.35, 0.39, \text{ and } 0.6$ , respectively. By means of a questionnaire, overall personal expertise to detect cows suffering from APM correctly as such was estimated to be 59%, whereas the diagnostic value of a combination of color, smell, and viscosity to detect cows with APM correctly was estimated to be 91.1% perfect. We found a discrepancy between reliability and the personal perception of diagnostic value. Our study shows that the sensorial assessment of color, smell, and viscosity of vaginal discharge in cows postpartum is subjective.

**Key words:** vaginal discharge, color, smell, viscosity, acute metritis

### **Technical Note**

Acute puerperal metritis (APM) is an acute systemic illness caused by infection of the uterus, occurring

within 21 d after parturition. The definition includes an abnormally enlarged uterus and a fetid, watery, red-brown uterine discharge (Sheldon et al., 2006). The diagnosis of APM is usually based on assessment of rectal temperature and vaginal discharge (VD; Sheldon et al., 2009). Until now, color, smell, and to some degree viscosity have been judged through sensory appraisal by an observer (Sannmann et al., 2012). This diagnostic approach is subjective, however, and may not be adequate to address a significant disease with implications for animal health, animal welfare, and prudent antibiotic drug use.

Evidence suggests that the nature of VD changes with the invasion of pathologic agents (Sonnenburg et al., 2005). In general, the viscosity of mucus is contingent on mucus glycoproteins, which are capable of forming viscoelastic gels that can be adapted to local physiological requirements (Moran, 2009). The glycosylation of epithelial glycoproteins can be influenced and modified by factors such as hormonal status, inflammation, and microbial colonization (Corfield et al., 2001; Sonnenburg et al., 2005). Some bacteria can actively degrade mucins to access the underlying mucosal epithelium by utilizing secreted or cell-surface proteases and glycosidases (Moran, 2009). Anecdotal evidence shows that a low viscosity of VD of cows with APM is indicative of a poor uterine health status.

Odor of vaginal discharge is associated with the bacterial growth density of recognized and potential pathogens in the uterus (Williams et al., 2005). There is a dearth of information, however, on the significance of color changes of VD and potential diagnostic value.

Color, smell, and viscosity are easy to assess without the need for additional diagnostic tools. However, data on test characteristics (reproducibility, sensitivity, and specificity) are not available (Sannmann et al., 2012). Furthermore, science-based evidence both from accepted clinical (e.g., rectal palpation) and advanced diagnostic methods (e.g., radiography, ultrasound) indicates that the investigator is a relevant source of measurement error (Andermann et al., 2007; Burfeind et al., 2010; Leutert et al., 2012). Studies evaluating

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the reproducibility of odor tests found correlations between multiple observers ranging from  $r = 0.43$  to  $0.9$  (Fordyce, 1961; Doty et al., 1995; Lötsch et al., 2004). Overall, evidence is limited as to whether color, smell, and viscosity of vaginal discharge are reliable predictors for the health status of the animal.

Therefore, the objective of this study was to evaluate the reliability of the assessment of color, smell, and viscosity from vaginal discharge. Specifically, we set out to determine reliability (interobserver, intraobserver, and test-retest) of scoring vaginal discharge regarding color, smell, and viscosity, and the conclusions drawn from these criteria regarding the uterine health status of the cow. The study was conducted from December 2011 to January 2012 at the Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin (Germany).

### Vaginal Discharge Collection

Material for the evaluation of VD was collected in December 2011 on a commercial dairy farm in Sachsen-Anhalt, Germany, that housed 1,200 Holstein dairy cows. The cows were closely monitored from calving until d 10 postpartum by means of daily temperature measurement, blood sampling for measurement of haptoglobin concentration, and evaluation of VD on d 2, 5, and 10. Cows having fetid, reddish-brown, watery vulva discharge in combination with a rectal temperature  $\geq 39.5^\circ\text{C}$  (fever) were characterized as having APM (Sheldon et al., 2006). Serum haptoglobin concentrations were measured for each cow at d 2, 5, and 10 after parturition. Healthy cows showed an overall serum haptoglobin concentration of  $0.6\text{ mg/mL}$ , whereas the overall serum haptoglobin concentration for cows with metritis was  $1.7\text{ mg/mL}$ . Serum haptoglobin concentrations between  $1.6$  and  $1.9\text{ g/L}$  are indicative of acute infectious processes in cows during the early postpartum period (Huzzey et al., 2009; Burfeind et al., 2014b).

Samples of VD were collected through a gloved hand from the vagina after cleansing of the vulva and perineum with dry paper towels. From each cow,  $50\text{ mL}$  of VD was sampled and stored at  $-18^\circ\text{C}$  in 10 sterile vials (Sarstedt AG & Co., Nürnberg, Germany), each containing  $5\text{-mL}$  aliquots for later use. To include a

broad spectrum of discharge, VD samples from 5 cows representing various states of uterine health (3 healthy, 2 with APM) were selected for the study. One of the samples collected from a cow with APM was used twice to evaluate test-retest reliability.

### Sensorial Evaluation

Fifteen investigators consented to participate in the study and were enrolled in the experiment, including 9 veterinary students in their fifth year and 6 licensed veterinarians working at our clinic. All investigators had the same information about the study design but no information about the health status of the cows.

The investigators were invited to 10 sessions held at intervals of 3 to 4 d. In each session, the investigator evaluated 6 VD samples (i.e., 5 different samples and 1 doublet) of  $5\text{ mL}$  each, presented in clear  $10\text{-mL}$  plastic vials labeled A, B, C, D, E, and F. The investigators were blinded to the samples and samples were newly randomized for each session by using the random number function by Excel for Windows (Microsoft Office 2010, Microsoft Deutschland GmbH, Munich, Germany). The assessment of color, smell, and viscosity was conducted after unfreezing for 10 min in a  $38^\circ\text{C}$  water bath. The investigators used a checklist with predetermined 5-point scales for the evaluation of color, smell, and viscosity of the samples (Table 1). For the evaluation of color, the investigators used a standardized color scheme with a number for each color, as shown in Figure 1.

Subsequently, the investigators classified the presumed health status of the animal belonging to each sample using a 5-point scale (i.e., 1 = healthy to 5 = very sick) and ranked the diagnostic value of the 3 criteria used (i.e., color, smell, viscosity) to reach a diagnosis. At the end of the experiment, the investigators answered a final questionnaire (Table 2), comprising 5 questions regarding their own perceptions of their ability to diagnose APM correctly and their experience. Furthermore, the diagnostic performance of odor assessment to detect animals with APM was compared with the diagnostic performance of a combination of odor with color and viscosity.

**Table 1.** Five-point scales for the sensory assessment of odor and viscosity of vaginal discharge

Criterion	Score				
	1	2	3	4	5
Odor	Penetrating, very fetid	Fetid	Slightly fetid	Aromatic	Neutral
Viscosity	Very high, gelatinous	High (mucous)	Watery to mucous	Low (watery)	Very low (aqueous)

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