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Risk factors for uterine diseases on small- and medium-sized dairy farms determined by clinical, bacteriological, and cytological examinations

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ABSTRACT

The involution process of the postpartum bovine uterus is usually accompanied by invasion of various bacteria. The objectives of this study were to identify the relationship between the postpartum findings as risk factors for clinical endometritis (CE) and subclinical endometritis (SE). Furthermore, the effects of CE or SE on reproductive performance in small- and medium-sized dairy herds were investigated. A total of 400 cows were examined by vaginoscopy for CE at 20 to 30 days postpartum, and samples were collected for cytological examinations for SE and for bacteriology by cytobrush technique. The vaginoscopic and cytological examinations showed that 27.3% and 21.0% of the cows were found with CE and SE, respectively. The bacterial community analyses revealed a large variety of bacteria. Overall, bacteria from the order *Actinomycetales, Lactobacillales, Bacillales, Burkholderiales, Caulobacteriales Enterobacteriales, Pasteurellales,* and *Pseudomonadales* were detected, whereas in 39.5% of the samples no bacterial growth was detectable. The uterine pathogens *Escherichia coli* and *Trueperella pyogenes* were found in 16.8% and 13.0% of the samples cultivated under aerobic conditions. Other frequently isolated bacteria were *Streptococcus* spp. (31.3%), *Staphylococcus* spp. (20.0%), *Corynebacterium* spp. (16.5%), and *Bacillus* spp. (10.5%). The infection with *T. pyogenes* was the most important bacteriological risk factor for the occurrence of CE (odds ratio (OR) = 5.72; 95% CI = 3.07–10.83) and had a detrimental effect on the hazard of nonpregnancy by 200 days postpartum (hazard ratio = 1.66; 95% CI = 1.12–2.46). Calving assistance (OR = 1.79; 95% CI = 1.16–2.98) and farm (OR = 1.11; 95% CI = 1.02–1.20) were indicated as further risk factors for CE and SE. Effects of CE and SE on reproductive performance parameters could not be demonstrated.

1. Introduction

After parturition, tremendous changes take place in the uterus. The process of involution is usually accompanied by invasion of different pathogenic and/or nonpathogenic bacteria into the uterine cavity [1–3]. A failure in adequate involution provokes the progression of pathogenic bacteria, impairs the uterine health, and results in metritis, clinical endometritis (CE), or subclinical endometritis (SE) [4,5].

Clinical endometritis is defined as an inflammation of the endometrium with the presence of purulent or mucopurulent vaginal discharge, detectable later than 21 days after calving and not accompanied by systemic signs of

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illness [5]. Some of the useful tools to diagnose CE are vaginoscopy, the metri-check device, and the gloved hand [6–8]. Vaginal discharge can be scored according to the classification by Williams et al. [2]. Barlund et al. [9] suggested that cytobrush cytology is the most reliable method for diagnosing CE in cattle. Clinical endometritis leads to impaired reproductive performance, not only during the present infection but also after resolution of clinical signs of disease [10,11]. The effect of different types of bacteria on the severity of the disease is not completely understood [12], but, for example, *Trueperella pyogenes* and other pathogenic bacteria are known to be associated with decreased reproductive performance [13,14].

The most important pathogens in the bovine uterus are *Escherichia coli*, *T. pyogenes*, *Fusobacterium necrophorum*, and *Prevotella* species [2,5]. In the early postpartum period, *E. coli* can be isolated frequently from the uterus, whereas infection with *T. pyogenes* occurs predominantly in the late postpartum period. It has been suggested that an infection with *E. coli* facilitates the infection with *T. pyogenes* [15]. In the last two decades, effects of the most common pathogens on uterine disease, that is, *E. coli* and *T. pyogenes,* have been described in detail [2,15,16].

Most of the studies used the classical culture–based diagnostic as a method of choice for the identification of bacteria. In recent years, however, the polymerase chain reaction has been used increasingly for the identification of pathogens. An alternative method, combining the advantages of classical culture–based identification with the higher resolution capacities of molecular methods, is the Fourier-transform infrared (FTIR) spectroscopy, which classifies the microorganisms according to their metabolic profiles [17,18]. This physicochemical technique is a whole-organism fingerprint method and generates complex pictures of the total chemical composition of the cell. Every microbial cell comprises a huge variety of different components, for example, fatty acids, proteins, or polysaccharides [17], which are expressed in different extents depending on the type of bacteria [19,20]. These fingerprints can be compared with the reference data sets to identify the microorganism under investigation [17]. Fourier-transform infrared spectroscopy is a fast, cheap, and easy to perform method [17,18,20]. It is well established in microbiology for the identification of bacteria and for the analyses of microbial population [21,22]. Fourier-transform infrared spectroscopy allows differentiating bacteria without any preselection of strains by other taxonomic criteria and is suitable for the identification at the serogroup, species, and genus level [22,23].

In the last 10 years, the definition of “subclinical endometritis” has been established. Subclinical endometritis is characterized as an endometrial inflammation with no systemic signs of illness and no signs of CE [4]. The usually applied technique for the diagnosis of SE is uterine cytology. Endometrial samples are collected either with the cytobrush technique [24] or by low-volume flushing of the uterus [4]. The diagnosis is on the basis of the proportion of polymorphonuclear neutrophils (PMNs) and epithelial cells in the sample obtained from the endometrium [5]. The threshold of PMNs for the diagnosis of SE is still under discussion and ranges from 4% to 18% depending on, for example, days postpartum (dpp) [4,9,24,25]. In many studies, SE was associated with an impaired reproductive performance of affected cows, that is, lower conception rates, longer days open, and lower pregnancy rates [24,26,27]. Further studies have also demonstrated the effects of SE on embryo development [28,29] and revealed that cows with SE had greater mRNA expression of proinflammatory factors, for example, interleukins and tumor necrosis factors [30,31]. Some studies, however, did not find an effect of SE on reproductive performance [10].

Most of the published data concerning uterine health were analyzed in countries with large agricultural structures, for example, United States, Canada, and Germany [4,29,32,33]. The information from countries with smaller agricultural structures is limited. The objectives of this study were to identify the relationship between the postpartum findings as risk factors for CE and SE. Furthermore, the effects of CE or SE on reproductive performance in small- and medium-sized dairy herds were investigated.

2. Materials and methods

This study was approved by the Institutional Ethics Committee and the National Authority according to Section 8 of Law for Animal Experiments, Tierversuchsgesetz-TVG (BMWF-68.205/0105-II/3 b/2011).

2.1. Study farms

The study was conducted on 10 commercial dairy farms in Lower Austria between June 2011 and April 2012. The number of milking cows was 31 to 223 cows per farm (median herd size 39 cows). The predominant breed was Simmental, and Holstein Frisian and Brown Swiss were also present. The herd average milk yield was 8568 kg per lactation (range 6264 kg to 10,826 kg). On all the participating farms, lactating cows were milked twice daily. The cows were kept in free-stall facilities with cubicles and slotted floors (eight farms) or concrete floors (two farms). Before parturition, the cows were brought to calving pens (eight farms) or tie stalls (two farms). Calving occurred all year round. The farms were visited by two veterinarians (authors IP and HP) together every second week. During the visit, the farmers were interviewed about calving assistance (no intervention *vs*. assisted calving) of examined cows. Feeding was based on grass silage, maize silage, and hay, supplemented with minerals. Concentrates were fed *via* an automatic feeding station (eight farms) or by hand (two farms) according to the individual milk yield of the cows. The voluntary waiting period was not defined on some farms or was set at 40 to 50 dpp. In all herds, cows were bred by artificial insemination (AI) after observed estrous. No timed breeding protocols (e.g., Ovsynch) were used. Pregnancy diagnosis was performed by transrectal palpation and ultrasonography of the uterus and its contents by the local veterinarians. Cows not pregnant or not observed in estrous received a treatment according to the clinical diagnosis, for example, prostaglandins in cows with a corpus luteum, GnRH in cows with ovarian cysts, or no corpus luteum.