



## Diversity and health status specific fluctuations of intrauterine microbial communities in postpartum dairy cows



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### ABSTRACT

For the interpretation of clinical findings of endometritis and the development of disease prevention and intervention strategies a better understanding of the dynamics and interactions within intrauterine bacterial communities in healthy and diseased cows is required. To gain deeper insights into fluctuations within the uterine microbiota, intrauterine samples were collected from 122 cows at the day of calving, days 3, 9, 15, 21 and 28 postpartum. A total of 2052 bacterial isolates were identified by Fourier-transform-infrared spectroscopy. This culturomics-based approach showed that the aerobic uterine microflora comprised a huge diversity of bacteria belonging to 202 different species, representing 76 genera, with members of the genus *Staphylococcus* (24.2%) being predominant. On species level the uterine microflora was dominated by *Trueperella pyogenes* (13.2%), *Escherichia coli* (11.2%), *Staphylococcus xylosum* (5.4%), *Bacillus pumilus* (5.2%) and *Streptococcus uberis* (4.9%). Comparative analysis of uterine bacteria from cows with different vaginal discharge scores (VDS) revealed health status specific temporal microbial diversifications. Although the intrauterine flora of all VDS groups was dominated by *T. pyogenes*, *E. coli* and *Staphylococcus* spp., the relative number of bacteria differed between VDS groups. The presence of *T. pyogenes* on days 15 and 21 significantly increased the risk of VDS 2 and 3 at day 21, whereas *Staphylococci* at day 9 reduced the likelihood of VDS 3 ( $P < 0.05$ ). This study demonstrates that intrauterine bacterial infections are highly dynamic processes and that bacterial species follow specific patterns of progression, which require further research to decipher their potential role in uterine disease development.

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## 1. Introduction

Bacterial uterine infections have a negative impact on uterine health and fertility in cows and consequently cause tremendous economic losses to dairy farmers. The uterus is

supposed to be sterile before birth but during or immediately after birth it becomes rapidly colonized through the open cervix with a broad diversity of bacteria (Földi et al., 2006). Within the first three weeks after calving 80–100% of the cows are positively tested for bacteria but not all animals show clinical symptoms of puerperal diseases such as metritis or clinical endometritis. Therefore, unspecific contamination and uterine infection must be distinguished (Földi et al., 2006). The severity of clinical endometritis is classified by the quality

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of vaginal discharge usually diagnosed from day 21 to 26 postpartum (LeBlanc et al., 2002; Williams et al., 2005; Sheldon et al., 2009; Leutert et al., 2012). Monitoring changes in the bacterial composition of the uterine fluid before the appearance of clinical signs of a disease is crucial not only for a critical interpretation of clinical findings but also for the development of optimal prevention and intervention strategies (Madoz et al., 2014). The natural defense mechanisms eliminate many of the microorganisms but the efficiency of elimination depends on the bacterial load and pathogenicity as well as on the immune status of the cow (Sheldon et al., 2002; LeBlanc et al., 2011). *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum* and *Prevotella* spp. are well known uterine pathogens. Members of the genus *Bacillus*, *Streptococcus*, *Enterococcus* and coagulase negative staphylococci (CNS), which are among the most frequently isolated intrauterine bacteria, are discussed as potential pathogens or opportunistic contaminants (Westermann et al., 2010; Werner et al., 2012). The diversity of the latter bacterial groups and their dynamics in the early postpartum period is poorly understood. Although uterine infection is a highly complex process with contamination, elimination and re-contamination (Földi et al., 2006), several studies have focused on the late puerperium (days 21 and 28) to identify bacteria and characterize uterine diseases (Williams et al., 2005; Westermann et al., 2010). Possible antagonistic interactions within the uterus have been reported for alpha hemolytic *Streptococci* and *T. pyogenes*. Preceding *E. coli* infections are assumed to facilitate subsequent persistent *T. pyogenes* infections (Bonnett et al., 1991; Dohmen et al., 2000). So far knowledge about the etiology of clinical endometritis derives primarily from studies focusing on known pathogens, such as *E. coli* and *T. pyogenes* (Sheldon et al., 2010; Amos et al., 2014), while detailed information on potential interactions between beneficial, opportunistic and pathogenic bacterial species is still lacking. Thus, we employed Fourier-transform infrared (FTIR) spectroscopy to explore the dynamics within the uterine microflora during the first four weeks postpartum. FTIR spectroscopy is a metabolome-based method that allows the identification and discrimination of bacteria down to species and subspecies level (Naumann et al., 1991; Ehling-Schulz et al., 2005; Wenning and Scherer, 2013) and it even can be used to identify hitherto unknown bacterial species and genera (Wagener et al., 2014a). Due to its high-throughput capacities and its high discriminatory power FTIR spectroscopy is also a suitable and effective tool for investigating complex bacterial communities (Fricker et al., 2011; Wagener et al., 2014b). It could be used to generate comprehensive collections of bacteria with known metabolic fingerprints for further downstream analyses and microbial interaction studies.

The objective of this study was to monitor the fluctuation in the intrauterine bacterial community in the postpartum period by means of FTIR spectroscopy. To gain a deep insight into the bacterial community dynamics six sampling points during the first 28 days postpartum were defined and included in the analysis. Furthermore, the results from the microbial survey were evaluated in the context of vaginal discharge character of each cow to

decipher the impact of microbiota on clinical signs of endometritis.

## 2. Materials and methods

The study was approved by the institutional ethics committee and the national authority according to § 8 of Law for Animal Experiments (Tierversuchsgesetz-TVG BMWF-68.205/246-II/3b/2010).

### 2.1. Study farm

The study was conducted on the Teaching and Research Farm of the University of Veterinary Medicine Vienna between February 2011 and February 2013. The herd consisted of 70 lactating dairy cows (60% Simmental, 15% Holstein Friesian, and 25% Brown Swiss), housed in free-stall barns with straw-bedded cubicles. The average milk yield was 8279 kg per lactation. The ration consisted of grass and maize silage, hay and was supplemented with minerals. Concentrates were fed by an automatic transponder station according to the individual milk yield. Cows were bred by artificial insemination and calved throughout the year in a straw bedded group maternity pen.

### 2.2. Study population and study design

A total of 170 calvings were enrolled, including 46 (27.1%) primiparous cows. Cows with cesarean section, vaginal lacerations, downer cows and animals that received an antibiotic treatment were excluded. Intrauterine samples were taken within 12 h after calving (day 0), and on days 3, 9, 15, 21 and 28 after parturition. Prior to each sampling procedure, the rectal temperature was measured and a short general clinical examination was performed. Cows with  $>39.5^{\circ}\text{C}$ , fetid red-brown watery uterine discharge and systemic signs of illness were classified as having metritis according to Sheldon et al. (2006). The latter animals ( $n=15$ ) received a systemic antibiotic treatment with 1 mg/kg of Ceftiofur and were consequently excluded from further sampling. Other reasons for antibiotic treatment were mastitis ( $n=10$ ), orthopedic diseases ( $n=7$ ), and others ( $n=10$ ). From 6 cows no complete dataset was available because sampling was not possible. Thus, the final study population included in the analysis comprised 122 animals.

### 2.3. Gynecological examination

On day 21 postpartum, a gynecological examination consisting of a vaginal inspection and rectal palpation of the uterus was performed. Vaginoscopic examination was carried out with a speculum and a torch as described by Westermann et al. (2010). Vaginal discharge was classified on a 4-point scale according to Williams et al. (2005). In brief, cows with clear discharge on day 21 were assigned to vaginal discharge score (VDS) 0, discharge with flecks of pus was defined as VDS 1 and animals with discharge containing  $<50\%$  pus and  $>50\%$  pus were classified as VDS 2 and 3.

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