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Uterine bacterial flora in postpartum Danish Holstein dairy cows determined using DNA-based fingerprinting: Correlation to uterine condition and calving management

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ABSTRACT

The overall aim of this study was to describe uterine bacterial flora during the postpartum period in Danish Holstein cows using the Terminal Restriction Fragment Length Polymorphism (T-RFLP) method. This method produces a pattern of nucleic acid fragments from the microorganisms present, reflecting the "fingerprint" of the actual microbial flora. As well as characterizing changes in flora with time from calving and between herds, data were examined for strong relations between uterine bacterial flora, calving management and uterine condition. In total 125 Holstein cows from five herds were included, and for each cow calving management was recorded. Cows were clinically examined on average 8 (range 0–19) and 28 (range 22–38) days after calving, and a uterine sample was taken for bacterial identification using T-RFLP. Milk samples were taken weekly for progesterone analysis. Bacteria were found in all cows at both examinations, and the flora was composed of many species, including species not traditionally reported to be present in the bovine uterus. The bacterial composition differed according to days from calving and herd. In all five herds *Fusobacterium necrophorum*, *Pseudomonas/Acinetobacter* and *Bacteroides/Sphingobacterium/Prevotellaceae* were among the most common at both examinations. In four herds there was a percentage decrease of *F. necrophorum* from first to second examination, and in all herds there was a percentage increase of *Pseudomonas/Acinetobacter* from first to second examination. No differences in bacterial flora were found between cows with different uterine scores, which were influenced by herd, calving difficulty and retained placenta.

1. Introduction

In cows with a normal puerperium, prevalence and consequences of bacteria in the uterine lumen is debated. In some studies bacterial cultures were found in a high proportion of puerperal cows (Elliott et al., 1968; Griffin

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et al., 1974; Hussain et al., 1990) while in other studies they were not (Bekana et al., 1996; Fredriksson et al., 1985; Noakes et al., 1991). Prevalence rates of *Streptococci* spp. and *Staphylococci* spp. may be greater in cows with uncomplicated postpartum (p.p.) period, indicating a sort of 'normal' uterine flora (Huszenicza et al., 1999). From cows with abnormal uterine conditions, bacteria as *Escherichia coli*, *Arcanobacterium pyogenes* and gram negative anaerobes (e.g. *Bacteroides* spp. and *Fusobacterium* spp.) are more often isolated (Santos et al., 2011; Sheldon et al., 2008; Werner et al., 2012); such bacterial flora is characterized as "contamination" or "infection" (Sheldon et al., 2002). Among pathogenic bacteria, *A. pyogenes* is often related to a serious effect on reproduction (Griffin et al., 1974; Huszenicza et al., 1999; Williams et al., 2005). In some studies the bacterial flora in the uterus of cattle changes in composition and amount within the first weeks after calving (Bekana et al., 1996; Dolezel et al., 2010; Hussain et al., 1990). It remains to be shown if knowledge of the uterine bacterial status of a cow during the first two weeks p.p. can be used to indicate her risk of a prolonged p.p. anovulation.

Bacteria have traditionally been classified using bacterial culture. However, as some bacteria are easier to grow than others (Amann et al., 1995), and as some will be overwhelmed by others, a culture period can shift the flora from time of sampling to time of reading the culture result. Elliott et al. (1968) stated that six different growth-media should be used to detect all organisms in the bovine uterus at various times after calving, but this has rarely, if ever, been done in subsequent studies (Bekana et al., 1996; Griffin et al., 1974; Noakes et al., 1991). Several culture-independent methods for identifying and characterizing the microbiological communities of different environments are therefore now applied, also in studies of the bovine uterus (Santos et al., 2011; Sheldon et al., 2010; Silva et al., 2009). One such method is the Terminal Restriction Fragment Length Polymorphisms (T-RFLP) method that produces a pattern of nucleic acid fragments (TRFs) from all microorganisms in the sample, reflecting the "fingerprint" of the actual microbial flora (Kitts, 2001). If this does not result in a clear identification of the organism, DNA cloning and sequencing may be the next step. The final result is a picture of the bacteria present in the sample, including those difficult to grow (Amann et al., 1995; Kalmbach et al., 1997).

One important factor for infection risk may be hygienic conditions and cow management around calving, including cleanliness of the calving pen and routines regarding calving assistance. Very few studies have focused on this (Noakes et al., 1991; Potter et al., 2010), and how management practices affect uterine bacterial flora is not known for certain. Furthermore, the extent to which the uterine bacteria composition depends on the cow's herd has not been studied.

The overall aim of this study was to describe the uterine bacterial flora during the p.p. period in Danish Holstein cows using the T-RFLP method. The hypothesis was that a certain bacteria composition, assessed by the T-RFLP method, was associated with the appearance of the vaginal discharge. As well as characterizing changes in flora with time from calving and between herds, data were examined for strong relations between the uterine bacterial flora, calving management and uterine condition.

2. Materials and methods

2.1. Study farms

The trial was conducted from October 2008 to January 2009 in five herds with Danish Holstein cows. Four of the five herds were commercial farms, selected within the same veterinary practice with a standardized herd health agreement with weekly veterinary visits and routine examinations of fresh cows. The four commercial herds were chosen from a larger pool of herds according to occurrence of metritis (to obtain a variation in pathological picture), and based on the willingness of the herd owners to participate (to obtain the best possible registrations). Two herds (Herds 1 and 2) with a lesser incidence of metritis (7.9% and 12.6%, respectively) and two herds (Herds 3 and 4) with a greater incidence of metritis (21.4% and 20.9%, respectively) were chosen. The fifth herd was the research farm connected to Aarhus University and had a lesser metritis incidence at 6.0%. The herd size was 140–400 cows, and the milk yield was 9500–11,000 kg ECM/cow per year.

2.2. Animals and records

The first 25 cows that calved in each of the five herds after the start of the trial period were selected for this study, so a total of 125 cows of Parities 1–8 were included in the study. To standardize the clinical procedures and reporting of findings, all participants received standard instructions at the beginning of the study. All individual cow data were recorded directly for this study using a special protocol or through the Danish Central Cattle Database.

Calving management was evaluated by the farmer for every calving by the following parameters: use of calving pen, number of cows in the pen at calving (single or more), whether clean bedding was provided in the pen before calving, whether cleaning or disinfection of the pen was conducted before calving, and the time the cow spent in the calving pen before calving (coded as: 1 = moved to calving pen immediately before calving; 2 = moved 12 h before calving; 3 = moved 24 h before calving, 4 = moved more than 24 h before calving).

Calving date, calving difficulty and calf survival as well as time to fetal membrane detachment were recorded by the farmer. Calving difficulty is a mandatory registration for Danish farmers and is entered into the Danish Central Cattle Database. Farmers recorded calving difficulty using one of the five categories: 1 = easy calving with no assistance, 2 = easy calving, but with the farmer's assistance, 3 = difficult calving without veterinary assistance but with farmer's assistance, 4 = difficult calving with veterinary assistance, 5 = cesarean section. For evaluating calf survival two categories were used: 1 = living calf, 2 = stillborn or dead within the first 24 h after delivery. All cows gave birth to single calves and without any cesarean section.

Retention of the fetal membranes for more than 12 h was treated by manual removal of the placenta, and