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Associations between intrauterine bacterial infection, reproductive tract inflammation, and reproductive performance in pasture-based dairy cows

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ABSTRACT

Reproductive tract bacterial infections, particularly those caused by *Escherichia coli* and *Trueperella pyogenes*, can have a negative impact on reproductive performance. It has been hypothesized that the presence of *E coli* early postpartum may increase the risk of isolation of *T pyogenes* later postpartum. The objective of the present study was to examine associations between intrauterine bacterial infections with *E coli* and *T pyogenes* and any bacterial growth (irrespective of bacterial species), purulent vaginal discharge (PVD), cytologic evidence of endometritis (an increased proportion of polymorphonuclear cells [PMNs]), and reproductive performance. Dairy cows (n = 272) from six herds were examined at Days 0 (median, 2 days in milk), 21 and 42 postpartum. From each cow two intrauterine samples were collected *via* triple-guarded cytobrush at Days 0 and 21. The first cytobrush was used for bacteriologic culture. *Escherichia coli* and *T pyogenes* were isolated by culture, and *E coli* isolates were assigned to one of four phylogenetic groups using a two-step triplex polymerase chain reaction. In addition, *T pyogenes* was confirmed by polymerase chain reaction. The second cytobrush was used to prepare a cytology slide. Nucleated cells (n = 200) were categorized as epithelial cells, PMNs, or macrophages. Cows were also assessed for body condition score, PVD score, the presence of a CL, and pregnancy. Statistical analysis was performed using multivariable models. There was no association between the presence of *E coli* at Day 0 and probability of isolation of *T pyogenes* 3 weeks later; however, *E coli* positive cows at Day 0 were more likely to be diagnosed with *E coli* at Day 21 (relative risk [RR] = 2.0, P < 0.01). *Escherichia coli* at Day 0 or *T pyogenes* at Day 21 increased the risk of PVD diagnosis 3 weeks later (RR = 1.9; P = 0.04 and RR = 3.0; P = 0.05, respectively). Cows with any bacterial growth at Day 21, irrespective of species, were less likely to conceive within 3 weeks after the start of the seasonal breeding program (RR = 0.8; P = 0.05). Interestingly, cows with 25% PMNs or greater at Day 0 had shorter time to pregnancy (hazard ratio = 1.32; P = 0.05). Intrauterine bacterial infection may impair reproductive performance but the presence of *E coli* was not associated with isolation of *T pyogenes* 3 weeks later. Increased endometrial flux of PMNs in cows early postpartum may be a physiological process and improve reproductive performance.

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1. Introduction

Postpartum, most cows have bacteria present in the uterus. However, most cows undergo self-resolution with time [1]. A wide diversity of bacterial species is present in the uterus of cows, but differences in isolated bacterial species occur between healthy cows and those with reproductive tract disease [2]. Bacteria that are considered potentially pathogenic to uterine tissue include *Escherichia coli* and *Trueperella pyogenes* [3]. Risk factors for uterine disease include low body condition score (BCS) at calving, hyperketonemia, and peripartum disease [1,4].

Endometritis may be defined as cytologic evidence of inflammation of endometrial tissue, which is associated with reduced reproductive performance [5]. Recent studies have reported that purulent vaginal discharge (PVD) may be associated with, but is not identical to endometritis [6]. Therefore, a diagnosis of PVD is applied to cows with grossly evident purulent material in the vagina, whether there is evidence of endometritis defined by cytology [5]. Purulent vaginal discharge can be diagnosed by the retrieval of purulent material from the vagina by placement of a gloved hand [7] or Metricheck device [1] into the vagina or visualization by vaginoscopy [8]. The Metricheck device, a soft rubber hemisphere connected to a stainless steel rod, is inserted into the vagina. Vaginal discharge is evaluated after retracting the device caudally [1]. Endometritis may also be diagnosed by endometrial biopsy [9]. Uterine cytology after sample collection by cytobrush or uterine lavage is now frequently used, at least in the research environment [5]. The cytobrush technique collects cells for cytology by rolling a small brush against the endometrium [10].

The same sample collected to define the degree of uterine inflammation may also be used for bacteriology [2,9,11]. Isolation of some species of bacteria from the uterus early postpartum has been associated with reduced dominant follicle growth rate, reduced estradiol concentrations, and a smaller CL after ovulation [12]. However, not all studies have found associations between isolation of bacteria and reproductive outcomes [13]. Specific strains or virulence factors of *E coli* seem to be more pathogenic than others [14,15], and intrauterine presence of a specific virulence factor for *E coli* (fimH) was associated with reduced pregnancy rate [15]. Cows infected with *T pyogenes* took longer to conceive compared with those without *T pyogenes* [16]. The presence of *T pyogenes* has also been associated with an increased influx of inflammatory cells in uterine tissue [9] and a higher risk of PVD [3].

The prevalence of *E coli* decreases and that of *T pyogenes* increases with time postpartum [11,12]. Additionally, a small study in which approximately half of the cows had retained fetal membranes (RFMs) found an association between *E coli* within 2 days postpartum and *T pyogenes* at 14 days in milk (DIM) [17].

The primary objective of this study was to test the hypothesis that the presence of *E coli* within the first week postpartum increases the risk of subsequent isolation of *T pyogenes*. Secondary objectives were to assess associations between early isolation of *E coli*, *T pyogenes*, or any bacteria (i.e., isolation of any bacterial species irrespective of type) and subsequent isolation of these bacteria, reproductive tract inflammation measured by PVD or polymorphonuclear cells (PMNs) in endometrial cytology, and reproductive performance. The final objectives were to assess associations between early reproductive tract inflammation and subsequent inflammation and reproductive performance.

2. Materials and methods

Animal ethics approval was obtained before study commencement (AgResearch Animal Ethics Committee; Ruakura; Hamilton; New Zealand; application number, 12395).

2.1. Herds and cows

Dairy cows (n = 272) from six commercial herds in the Waikato region of New Zealand were enrolled between 18 July and 12 August 2011 (Table 1, Fig. 1). Data collection ended March 7, 2012, the date of the last pregnancy diagnosis. The enrollment of herds was on a convenience basis, with herds being recruited based on willingness to participate, agreeing to the study protocol and allowing access to herd- and cow-level records. Cows were managed in spring-calving, predominantly pasture-fed herds. At each enrollment day (Day 0), herd owners were asked to present multiparous cows calved between 1 and 4 days for veterinary examination. Cows with peripartum disease (e.g., dystocia, RFMs) were included, but cows were excluded if an antibiotic or anti-inflammatory treatment had been administered for 30 days or less before enrollment or if rectal temperature greater than 39.5 °C was recorded at the initial examination. When 25 cows or less were available on a given enrollment day (maximum 2 days for each herd), all cows that met the enrollment criteria were enrolled. When greater than 25 were available, the first or second cow in the row was selected for exclusion by flip of a coin. Thereafter, every second cow in the row was excluded from the study until total 25 cows remained.

2.2. Sampling methods

Cows were sampled on Days 0, 21 (range, ±0 days), and 42 (±2 days). On Day 21, cytobrush samples were taken by one of two veterinarians (Melvin de Boer and Stephen J. LeBlanc), whereas all other samples and measurements including those taken at Days 0 and 42 were taken by the one veterinarian (Melvin de Boer). On Day 0, the following measurements and samples were collected from each study cow: rectal temperature, uterine tone score (firm vs. large and flaccid without striations), BCS on a 1 to 10 scale [18], vaginal discharge score (VDS) evaluated using a Metricheck device (Simcro Tech, Hamilton, New Zealand), and two intrauterine samples using cytobrushes (Ebos Group Ltd., Auckland, New Zealand). A VDS of 0 was given when no mucus was visible; a score of 1, 2, 3, 4, or 5 was given when clear mucus, flecks of purulent discharge, more than flecks but less than 50% purulent discharge, more than 50% purulent discharge, more than 50% purulent discharge plus odor, respectively, was visualized [1]. Two intrauterine