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Mycoplasma infection in the uterus of early postpartum dairy cows and its relation to dystocia and endometritis

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ABSTRACT

This study investigated the incidence of mycoplasma infection in the uterus of postpartum Holstein dairy cows and its relationship to the occurrence of endometritis. The genital tracts of 209 cows from three dairy farms in the Iwate Prefecture, Japan, were examined at Weeks 5 and 7 postpartum. The condition of the cervicovaginal mucus was assessed using a Metricheck device and assigned a score from 0 (clear mucus) to 4 (purulent material with fetid odor). Intrauterine samples (N = 418) were collected at Weeks 5 and 7 postpartum using a cytobrush. After its withdrawal, swab samples were placed in mycoplasma culture broth at 37 °C for 72 hours. A novel and rapid polymerase chain reaction was used to detect seven mycoplasma species (*Mycoplasma bovis*, *M. arginini*, *M. bovigenitalium*, *M. californicum*, *M. bovirhinis*, *M. alkalescens*, and *M. canadense*). The cytobrush was also rolled gently along the length of a glass slide for subsequent polymorphonuclear neutrophil count. The diagnostic criteria for cytological endometritis were 6% or more and 4% or more polymorphonuclear neutrophils at Weeks 5 and 7, respectively. From a subset of cows, additional swabs were rolled against the cytobrush and then placed in transport medium. These samples were then plated on specific agar plates and cultured under aerobic and anaerobic conditions to identify other bacteria present. The incidence of dystocia at the last calving was compared in mycoplasma positive and negative cows. Of the seven mycoplasma species, only *M. bovigenitalium* was detected; it was detected in 31 of the 418 uterine swabs (7.4%). Twenty-four cows were positive for *M. bovigenitalium* (eight cows at Week 5, nine cows at Week 7, and seven cows at both Weeks 5 and 7). The incidence of dystocia was higher ($P < 0.0001$) in mycoplasma positive (7/24; 29.2%) compared with mycoplasma negative (4/185; 2.2%) cows. However, there was no significant association between dystocia at last calving and subsequent uterine infection with other bacteria. In addition, the incidence of cytologic endometritis was higher ($P < 0.05$) in mycoplasma positive (8/16; 50%) than in mycoplasma negative (47/193; 24.4%) cows at Week 7. Therefore, we concluded that *M. bovigenitalium* infection in the uterus might be associated with recent dystocia and with cytologic endometritis in postpartum dairy cows.

1. Introduction

Heavy bacterial colonization subsequent to trauma, dystocia, or poor hygiene and poor uterine defense mechanisms can lead to establishment of puerperal uterine

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infection [1–4]. In cattle, nonpathogenic bacteria in the uterus disappear more quickly after a difficult calving than after normal parturition, and pathogenic isolates persist longer in dystocia-affected animals [5]. The presence of pathogenic bacteria in the uterus causes inflammation and histologic lesions in the endometrium, delays uterine involution, and hinders embryo survival [6].

After the first isolation of *Mycoplasma bovigenitalium* in 1947, numerous studies throughout the world have demonstrated the presence of this organism in the genital tracts of healthy and diseased cows and bulls [7,8]. Although the experimental pathogenicity of some isolates has been demonstrated, there is still doubt regarding the exact role of this organism in bovine reproductive disease [9]. *M. bovigenitalium* has been detected in the vaginal mucus of normal and repeat breeder cows, which has led to speculation concerning its role as a pathogen [10]. It has been found in cows with low fertility in which no other cause of infertility was identified [11]. *M. bovigenitalium* has been implicated in several outbreaks of granulopapular vulvovaginitis, which is characterized by lesions that resemble 'wolf bites' on the external vaginal labia and granulopustular lesions of the vagina [12]. In South Africa, *M. bovigenitalium* was recovered from the cervicovaginal mucus of 47% of gynecologically normal postpartum cows [13]. However, there is little information available regarding isolation of *M. bovigenitalium* from the uterus of live cows. Additionally, there has been little reported association between mycoplasma infection and cytological endometritis based on polymorphonuclear neutrophil (PMN) percentage. The percentage of PMN in the total number of endometrial cells is considered indicative of cytologic endometritis in cows, with 6% or more PMNs at the first reproductive exam (35 ± 3 days) and 4% or more PMNs at the second (56 ± 3 days) postpartum reproductive exam resulting in a diagnosis of endometritis [14].

Therefore, the objectives of the present study were to investigate the incidence of mycoplasma infection in the uterus of postpartum cows and to examine the association of mycoplasma infection with calving conditions and cytologic endometritis.

2. Materials and methods

2.1. Animals

Holstein cows (N = 209) from three herds in the Iwate Prefecture, Japan, were used in this study. All cows were housed in free stalls, fed an identical diet formulated according to standard guidelines, and milked twice daily. The average parity and age of the cows were 1.4 ± 0.1 and 3.6 ± 0.1 years, respectively. The ease of the previous calving (eutocia or dystocia) was recorded for all cows. Dystocia was defined as any calving that required an intervention to assist the cow. To determine whether dystocia was a predisposing factor for bacterial infections of the postpartum uterus, the incidence of dystocia in a subset of cows (N = 88) with or without postpartum uterine bacterial infection was calculated. The study period was from June 2011 to February 2012.

2.2. Evaluation of cervicovaginal mucus using a Metricheck device

Before examination, the vulva was cleaned with paper towels. Cows were examined using a Metricheck device (Simcro Tech Ltd., Hamilton, New Zealand) at Week 5 (34.9 ± 0.2 days) and Week 7 (48.6 ± 0.2 days) postpartum. Cervicovaginal mucus was collected as described [15]. The material within the concave surface of the device and/or adherent to the convex surface was visually examined and scored on a 0 to 4 scale as follows: 0 = clear mucus; 1 =mucus with flecks of pus; 2 = mucopurulent discharge; 3 = purulent discharge; and 4 = foul smelling discharge [16,17].

2.3. Endometrial cytology

A cytological sample of the endometrium from the uterine body was collected at Weeks 5 and 7 using a cytobrush (Puritan Medical Products Company L.L.C., Guilford, ME, USA) adapted for use in cattle [17,18]. The brush was retracted into a stainless steel tube before removal from the uterus. Slides for cytologic examination were prepared on farm by rolling the cytobrush on a clean glass microscope slide and fixing with cytofixative (Cytokeep II, Alfresa Pharma Corporation, Osaka, Japan). Fixed slides were stained with Diff-Quik (Sysmex, Kobe, Japan) for 20 seconds, washed in distilled water, and dried. A quantitative cytologic assessment of endometrial inflammation was carried out by counting a minimum of 200 endometrial cells at magnification ×400 and determining the percentage of PMNs within those cells. The diagnostic criteria for cytologic endometritis were 6% or more and 4% or more PMNs, at postpartum Weeks 5 and 7, respectively [14].

2.4. Mycoplasma culture techniques

After the withdrawal of the cytobrush from the uterus and before rolling it on the glass slide, swab samples were taken, placed in mycoplasma culture broth (Kanto Kagaku, Japan), and incubated at 37 °C for 72 hours. Then, 100 μL of the broth culture was plated on a mycoplasma agar plate (Kanto Kagaku, Japan) and incubated in 5% CO_2 at 37 °C for 3 to 14 days to produce typical mycoplasma colonies. If typical mycoplasma colonies appeared within 3 days, species identification was performed using polymerase chain reaction (PCR). However, if no typical mycoplasma colonies grew during the first 3 days of incubation, the culture was continued for a total of 14 days to ensure that the sample was mycoplasma negative (this was subsequently confirmed using PCR). Each broth culture was analyzed using the simplified PCR procedure described below.

2.5. Detection and identification of mycoplasma species by PCR

A novel and rapid PCR was used to detect seven mycoplasma species (*Mycoplasma bovis*, *M. arginini*, *M. bovigenitalium*, *M. californicum*, *M. bovirhinis*, *M. alkalescens*, and *M. canadense*) [19]. A simplified PCR was performed in