Defining postpartum uterine disease in cattle

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Abstract

Uterine function is often compromised in cattle by bacterial contamination of the uterine lumen after parturition, and pathogenic bacteria often persist, causing uterine disease, a key cause of infertility in cattle. However, the definition or characterization of uterine disease frequently lacks precision or varies among research groups. The aim of the present paper was to provide clear clinical definitions of uterine disease that researchers could adopt. Puerperal metritis should be defined as an animal with an abnormally enlarged uterus and a fetid watery red-brown uterine discharge, associated with signs of systemic illness (decreased milk yield, dullness or other signs of toxemia) and fever >39.5 °C, within 21 days after parturition. Animals that are not systemically ill, but have an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina, within 21 days post partum, may be classified as having clinical metritis. Clinical endometritis is characterised by the presence of purulent (>50% pus) uterine discharge detectable in the vagina 21 days or more after parturition, or mucuopurulent (approximately 50% pus, 50% mucus) discharge detectable in the vagina after 26 days post partum. In the absence of clinical endometritis, a cow with subclinical endometritis is defined by >18% neutrophils in uterine cytology samples collected 21–33 days post partum, or >10% neutrophils at 34–47 days. Pyometra is defined as the accumulation of purulent material within the uterine lumen in the presence of a persistent corpus luteum and a closed cervix.

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conclusion, we have suggested definitions for common postpartum uterine diseases, which can be readily adopted by researchers and veterinarians.

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1. Introduction

Uterine function is often compromised in cattle by bacterial contamination of the uterine lumen after parturition; pathogenic bacteria frequently persist, causing uterine disease, a key cause of infertility [1]. Bacteria can be cultured from samples collected from the uterine lumen of most dairy cattle in the first 2 weeks after parturition in many situations, although in some countries the frequency is lower [2–6]. Although many cows eliminate these bacteria during the first 5 weeks after parturition, in 10–17% of animals, persistence of bacterial infection causes uterine disease detectable by physical examination [7,8]. The presence of pathogenic bacteria in the uterus causes inflammation, histological lesions of the endometrium, delays uterine involution and perturbs embryo survival [9–11]. In addition, uterine bacterial infection, bacterial products or the associated inflammation, suppress pituitary LH secretion, and perturbs postpartum ovarian follicular growth and function, which disrupts ovulation in cattle [5,12–14]. Thus, uterine disease is associated with lower conception rates, increased intervals from calving to first service or conception, and more cattle culled for failure to conceive [7,8,15,16].

The goal of reproduction management is to have cows become pregnant at a biologically optimal time and at an economically profitable interval after calving. The timing of examination of animals after parturition should allow for the normal process of involution, yet also provide sufficient time for treatment and response prior to the start of the breeding period. The aims of uterine disease treatments are to reverse inflammatory changes that impair fertility, whilst enhancing uterine defence and repair.

As bovine uterine disease is so important, it is not surprising that more than 500 papers have been published in the last 40 years on the most common uterine diseases, metritis, endometritis and pyometra [17]. Indeed, there are several review articles with extensive citation lists [1,18–23]. However, frequently the definition or characterization of the various manifestations of uterine disease either lack precision or definitions vary among research groups and/or were not validated as to their effect on reproductive performance, making assessment of the effects of treatment difficult [24]. Often the term endometritis incorrectly includes metritis, endometritis and pyometra, and/or is determined solely on the basis of transrectal palpation of an enlarged uterus [25,26]. During the 15th International Congress on Animal Reproduction [19], it was suggested that the research field would be aided by clear definitions of uterine disease that researchers could adopt. Whilst it may not be possible to categorise every animal, it is important to have practical definitions, particularly for research. Thus, in the present paper, we outline the key clinical features of uterine disease in cattle and suggest working definitions to reflect the character of these diseases.
2. Uterine bacterial contamination and infection

The majority of postpartum inflammatory conditions of the uterus begin with bacterial contamination of the uterine lumen. One should differentiate between uterine contamination and uterine infection. The uterus of postpartum cows is usually contaminated with a range of bacteria, but this is not consistently associated with clinical disease. Infection implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium, and/or release of bacterial toxins that lead to establishment of uterine disease [27]. The development of uterine disease depends on the immune response of the cow, as well as the species and number (load or challenge) of bacteria. The number of pathogenic bacteria in the uterus of postpartum cows may be great enough to overwhelm uterine defence mechanisms and cause life-threatening infections, although these are relatively uncommon [1]. Indeed, it is non-life-threatening uterine infections that are most common and associated with impaired reproductive performance [8]. Furthermore, inflammation, even in the absence of active bacterial infection, may perturb embryo survival [28,29].

Establishment of uterine bacterial infection may depend in part on the endocrine environment; in particular, progesterone seems to suppress uterine immune defences. Formation of the first corpus luteum after parturition and secretion of progesterone often precedes the onset of uterine disease [21,30]. Indeed, intrauterine infusions of Arcanobacterium pyogenes and Escherichia coli into postpartum beef cows under experimental conditions did not establish infections, unless peripheral plasma progesterone concentrations had started to increase [31]. However, many spontaneous uterine infections are established within 3 weeks after parturition, before ovulation of the first dominant follicle [5,32]. Furthermore, chronic uterine infection and increased plasma concentrations of lipopolysaccharide (LPS) were associated with disruption of the LH surge and failure of ovulation [1,14,33]. The relationships among uterine bacteria, the immune or inflammatory response, and ovarian function are complex and require more investigation, although it appears that uterine disease is associated with anovulatory anestrus and cystic ovarian disease [12,34]. On the other hand, the immunosuppressive effects of progesterone from the corpus luteum, or possibly adrenal steroids, may contribute to the progression of uterine contamination into uterine infections. The mechanisms of action of progesterone are complex, not completely understood, and not within the scope of this article. However, the knowledge that progesterone is immunosuppressive at a number of levels and is involved in regulating the synthesis of prostaglandin F2α (PGF2α), plus a variety of immuno-modulatory cytokines, can be useful for managing uterine infections. In cows with a functional corpus luteum, administration of exogenous PGF2α is used to stimulate luteolysis, reduce progesterone and increase estrogen concentrations, induce estrus and resolve uterine infections [21,35,36]. Estrus appears to be particularly beneficial to the resolution of uterine infection [37]. Exogenous PGF2α may enhance immune functions or increase uterine motility to help the uterus resolve infections in animals that do not have active corpora lutea [38,39]. However, the results of clinical trials of PGF2α for treatment of clinical endometritis in the absence of an active corpus luteum are inconsistent [40–42]. Indeed, investigation by meta-analysis of many studies reporting the effect of PGF2α given post partum, indicated little benefit to the
reproductive performance of dairy cattle [43]. An effective alternative treatment for endometritis is intrauterine infusion of antimicrobials [35,40,42].

Involution of the genital tract after parturition also aids the resolution of uterine infection, and conversely may be delayed by uterine disease. In addition, evaluating uterine and cervical involution may help to differentiate between physiological and pathological observations. In normal cattle, the cervix reopens after 1-week post partum [44]. Lochia is passed until 15–20 days post partum; over the course of involution, lochia changes from a red-brown fluid to a more viscous yellow-white material. Healthy cows achieve a uterine horn diameter of 3–4 cm by 25–30 days post partum, and cervical diameter <5 cm by 40 days post partum, but involution of the uterus and cervix is not complete until approximately 40–50 days post partum [18,45]. However, uterine involution can also be affected by age, breed, nutrition and other factors so that delayed uterine involution is not a specific indicator of uterine disease [46].

In summary, bacteria from the surface of the animal and the environment contaminate the uterine lumen of most postpartum cows. Elimination of this contamination is dependent on uterine involution, regeneration of the endometrium, and uterine defence mechanisms. The influx of polymorphonuclear neutrophils (PMN), attracted by chemokines such as interleukin-8 (IL-8), plays a key role in the uterine immune response [47]. However, ovarian activity and luteal progesterone modulate many of the processes that make a cow resistant or susceptible to uterine infections, and exogenous PGF$_2\alpha$ or intrauterine antimicrobials are effective treatments.

3. Pathology

To the pathologist, the general definitions of inflammation of the genital tract are simple. Inflammation limited to the endometrium is termed endometritis; involvement of the entire thickness of the uterine wall is metritis; of the serosa, perimetritis; and of the suspensory ligaments, parametritis [20,48]. Metritis can be distinguished from endometritis; in the former, all layers of the uterine wall show evidence of inflammation such as edema, infiltration by leukocytes, and myometrial degeneration. In both conditions, the mucosa is congested, and there is a prominent leukocyte infiltration in response to the common pathogens A. pyogenes, Fusobacterium necrophorum, Prevotella species and E. coli. In particular, infection with A. pyogenes is associated with increased time to pregnancy [16,49]. Endometritis is a superficial inflammation of the endometrium, extending no deeper than the stratum spongiosum [20]; with histological evidence of inflammation (Figs. 1a and 2a). During recovery from acute endometritis, there is fibrosis and leukocytosis, with depletion of endometrial glands and atrophy of the remainder.

Pyometra is associated with corpus luteum activity in the ovary; it often persists longer than the expected duration of the luteal phase. It has been suggested that it is the presence of this structure, with its secretion of progesterone that results in endometritis developing into pyometra [48]. Early ovulation after parturition and formation of an active corpus luteum may predispose to pyometra [50,51]. On the other hand, the retention of the corpus luteum may be associated with failure of luteolysis. The role of progesterone may be to maintain
Fig. 1. Photomicrographs from the same animal of: (a) histological section of an endometrial biopsy of normal tissue; and (b) cytology of normal fluid collected by flushing the uterine lumen. Note endometrial gland (arrow a) and blood vessel (arrow b) in histology. Cytology consists predominantly of endometrial epithelial cells (bar = 5 μm).
Fig. 2. Photomicrographs from the same animal of: (a) histological section of an endometrial biopsy specimen of inflamed tissue, with an influx of polymorphonuclear neutrophils (arrow a), some slight periglandular fibrosis (arrow b) and overall increased cell density due to cellular infiltration (arrow c); and (b) cytology of inflamed uterine fluid collected by flushing the uterine lumen, with prominent polymorphonuclear neutrophils (arrows) amongst the epithelial cells (bar = 15 μm).
functional closure of the cervix, as well as increasing the susceptibility to persistent infection, especially with *A. pyogenes* and anaerobic bacteria [51].

4. Clinical definitions

*Puerperal metritis* is an acute systemic illness due to infection of the uterus with bacteria, usually within 10 days after parturition. Puerperal metritis is characterized by the following clinical signs: a fetid red-brown watery uterine discharge and, usually, pyrexia [52]; in severe cases, reduced milk yield, dullness, inappetance or anorexia, elevated heart rate, and apparent dehydration may also be present. The term metritis should be used for cows that have delayed involution and a fetid discharge, in the absence of detected fever. Indeed, in some cases of severe bacterial infection pyrexia was not detected, even with daily monitoring of rectal temperature and did not depend on the load or species of bacteria [53]. Puerperal metritis is often associated with retained placenta, dystocia, stillbirth or twins, and usually occurs toward the end of the first week post partum, being rare after the second week post partum [52,54]. We propose that a case of puerperal metritis should be defined as an animal with an abnormally enlarged uterus and a fetid watery red-brown uterine discharge, associated with signs of systemic illness (decreased milk yield, dullness or other signs of toxemia) and fever >39.5 °C, within 21 days post partum. Animals that are not ill, but have an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina, within 21 days after parturition, may be classified as having clinical metritis.

*Clinical endometritis* is characterised by the presence of purulent (>50% pus) or mucopurulent (approximately 50% pus, 50% mucus) uterine exudate in the vagina, 21 days or more post partum, and is not accompanied by systemic signs [7,42]. Diagnostic criteria for clinical endometritis in postpartum dairy cows have been validated by examining factors associated with an increased interval from parturition to conception [7]. The significant factors were the presence of purulent vaginal mucus or a cervical diameter >7.5 cm, 21 days or more post partum; or after 26 days post partum, the presence of a mucopurulent material in the vagina. Using this classification for endometritis, the incidence was 16.9% for the 1865 cows examined. The temporal differences in the significant factors probably reflect the progress of uterine involution and immune defence. Classifying animals as having clinical endometritis if they are <21 days post partum may include a greater proportion of animals that are spontaneously resolving bacterial contamination, and so reflect the presence of disease less accurately. Furthermore, variations in appearance of normal lochia confound diagnosis of endometritis at this stage. Similarly, using delayed involution alone to diagnose endometritis is unreliable, as the enlarged uterus may reflect physical damage or variations associated with breed, age, or nutrition rather than bacterial infection, whilst the small increase in diameter of the uterine horns may be difficult to detect in mild cases of endometritis. Thus, although the pathology of interest involves the uterus, palpation of the uterus to assess its size lacks diagnostic accuracy [7,55]. However, estimation of cervical diameter by palpation was associated with clinical endometritis and appears to be a more reliable marker of delayed involution [7,56,57]. In summary, we propose that the definition of clinical endometritis in a cow is the
presence of purulent uterine discharge detectable in the vagina 21 days or more post partum, or mucuopurulent discharge detectable in the vagina after 26 days post partum.

**Subclinical endometritis** can be defined as endometrial inflammation of the uterus usually determined by cytology, in the absence of purulent material in the vagina [58]. In animals without signs of clinical endometritis, subclinical disease was diagnosed by measuring the proportion of neutrophils present in a sample collected by flushing the uterine lumen (Figs. 1b and 2b), or using a cytobrush [19,59,60]. Subclinical endometritis was determined in one study by the presence of >18% neutrophils in uterine cytology samples collected 20–33 days post partum or >10% neutrophils at 34–47 days post partum [60]. The incidence of clinical and subclinical endometritis was 53% at 40–60 days post partum, and was associated with delayed conception and increased culling [61]. The assessment of inflammation at 40–60 days post partum corresponded approximately to >5% neutrophils [61]. Although investigation of subclinical endometritis is at an early stage, we have used available data to suggest a working definition. We propose that a cow with subclinical endometritis is defined by >18% neutrophils in uterine cytology samples collected 21–33 days post partum, or >10% neutrophils at 34–47 days, in the absence of clinical endometritis.

**Pyometra** is characterized by the accumulation of purulent or mucopurulent material within the uterine lumen and distension of the uterus, in the presence of an active corpus luteum. There is often an increased number of pathogenic bacteria within the uterine lumen when the corpus luteum forms and pyometra occurs [51]. Although there is functional closure of the cervix, the lumen is not always completely occluded and some pus may discharge through the cervix into the vaginal lumen. Pyometra is sonographically characterised by a corpus luteum in an ovary, accumulation of mixed echodensity fluid in the uterine lumen and distension of the uterus. In summary, we propose that pyometra is defined by the accumulation of purulent material within the uterine lumen, in the presence of a persistent corpus luteum and a closed cervix.

**5. Diagnosis**

It is important to be able to diagnose the presence of uterine infection to facilitate timely and appropriate treatment and to quantify the severity of disease, which allows a prognosis to be given for subsequent fertility. Unfortunately, there is no “gold standard” for diagnosis of uterine disease, making it difficult to measure the sensitivity and specificity of clinical definitions. In addition, there is little information on the correlation between clinical and histopathological observations, although the presence of pus in the vagina was correlated with the presence of pathogenic bacteria in the uterus [62,63]. The definitions we propose should allow classification of disease in most animals as long as a full reproductive examination is performed, whilst accepting that clinical parameters may not reflect the impact on economic or reproduction outcomes in all countries. Furthermore, there is a continuum of peri-partum disease, so the consideration of what is abnormal and the selection of cost-effective therapy will depend on the production system. Therapeutic cut-points and decision trees would be useful for the different production systems.
The diagnosis of puerperal metritis is made readily on the basis of clinical signs of illness and fetid uterine discharge detectable on clinical examination. The diagnosis of pyometra depends on transrectal palpation of a distended uterus and/or transrectal ultrasonography of mixed echodensity fluid, and the presence of a persistent corpus luteum, with a history of anestrus. The uterine luminal fluid of a pyometra can be displaced from one horn to the other, which cannot be done with fluids associated with a pregnancy, and often the uterine wall is doughy and thicker than that of a gravid uterus. The sensitivity, specificity and positive predictive value of palpation for identifying mid-cyclic corpora lutea were 85, 95.7 and 89.5%, respectively, whilst for ultrasonography the values were 95, 100 and 100%, respectively [64]. If necessary, progesterone concentration in milk or plasma can be measured to avoid a small proportion of animals being misclassified by errors in the physical detection of a corpus luteum.

The definitive diagnosis of endometritis is made on the basis of histological examination of endometrial biopsies (Figs. 1a and 2a), and these are predictive for subsequent fertility [49]. However, the technique is costly and time consuming, not clinically accessible in most situations, and may depress fertility. Cytology is more practical (Figs. 1b and 2b) and is necessary to diagnose subclinical endometritis [58,60]. However, neither of these procedures produces a rapid clinical diagnosis. Thus, in the field, diagnosis of uterine disease usually relies on clinical examination.

Transrectal palpation for delayed involution is not a good technique for evaluating uterine infection because it is subjective, uterine involution varies among cows, and there is little association with reproductive performance [7,21]. In addition, although a marked delay of uterine or cervical involution is associated with lower conception rates, it is the evaluation of animals where the effects on uterine involution are less obvious that is more difficult. Similarly, observation for abnormal vulval discharge is unreliable for diagnosis of endometritis [65]. The use of clinical records of stillbirth, twins, retained fetal membranes, dystocia and hypocalcemia helps identify animals at risk of uterine disease, but does not provide a specific diagnosis [54,65,66]. Further examination of data from a large clinical trial confirms that disease history alone lacks sensitivity for identifying cattle with clinical endometritis (Table 1). However, the use of historical information may decrease the cost of diagnosis and hence increase the cost:benefit of treatment for uterine disease for some producers.

<table>
<thead>
<tr>
<th>Clinical endometritis</th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Had ≥1 of twins, RFM, metritis</td>
<td>Yes</td>
<td>73</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>122</td>
<td>976</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
<td>1086</td>
<td>1281</td>
</tr>
</tbody>
</table>

Although prior diseases were strongly associated with the risk of clinical endometritis, over half of the cows with endometritis did not have these risk factors. RFM, retained fetal membranes; sensitivity = 37%; specificity = 90%; OR = 5.3; \( P < 0.0001 \). Data from LeBlanc et al. [7] and unpublished.
Fig. 3. Typical samples of vaginal mucus character: score 0 = clear or translucent mucus; score 1 = mucus containing flecks of white or off-white pus; score 2 = discharge containing ≤ 50% white or off-white mucopurulent material; and score 3 = discharge containing ≥ 50% purulent material, usually white or yellow, but occasionally sanguineous. Previously published in [63].
The use of transrectal ultrasonography permits more objective measurement of the diameter of the uterine horns and cervix, and visualization of mucus and pus within the uterine lumen [11,60,67]. At present, there is little evidence that ultrasonography provides more information about clinical endometritis than examination of the contents of the vagina. Although, there is limited information on the relationship between ultrasonographic findings and clinical or subclinical endometritis, this is likely to be a fruitful area for research.

To diagnose clinical endometritis we advocate the examination of the contents of the vagina for the presence of pus [7,22,42]. The simplest method is to perform a manual examination of the vagina and withdraw the mucus for inspection [68]. The advantage of this technique is that it is inexpensive, quick, provides additional sensory information such as detection of vaginal lacerations and detection of the odor of the mucous in the vagina. One procedure is to clean the vulva using a dry paper towel and insert a clean, lubricated gloved hand through the vulva into the vagina. The lateral, dorsal and ventral walls of the vagina and the external cervical os are palpated and the mucus contents of the vagina withdrawn for examination. The hand usually remains in the vagina for <30 s. Manual vaginal examination has been validated and does not cause uterine bacterial contamination, provoke an acute phase protein response, or delay uterine involution [68]. However, the operator has to be aware that vaginitis, cervicitis, cystitis or purulent nephritis may give false results. Vaginoscopy can be performed using autoclavable plastic, metal or disposable foil-lined cardboard vaginoscopes, which allow inspection of the contents of the vagina. However, there may be some resistance to the use of vaginoscopes because of the perceived inconvenience, potential for disease transmission and cost [7]. A new device for examination of vaginal mucus (Metricheck™, Simcro, New Zealand) consists of a stainless steel rod with a rubber hemisphere that is used to retrieve vaginal contents.

Several scoring systems have been described to estimate the severity of clinical endometritis [35,42,63]. The character of the vaginal mucus can be scored (Fig. 3) as well as odor (Score 0 for no odor and Score 3 for a fetid odor) [63]. The character score reflects the presence and semiquantitative load of certain bacteria in the uterus. In one study [63], muco-purulent discharge was associated with *F. necrophorum* and purulent discharge was associated with *A. pyogenes* and *Proteus* species, whilst a fetid odor was associated with a greater load of *A. pyogenes*, *E. coli*, *Streptococci*, and *Mannheimia haemolytica*. Clear mucus with flecks of pus was not associated with the presence of higher numbers of pathogenic bacteria in the uterine lumen, but the effects on fertility were not consistent [7,63]. These mild cases of endometritis may be resolving the uterine infection, and the discriminators may not be sensitive enough to detect perturbation of reproduction. However, subclinical endometritis is associated with lower conception rates to first service and overall [19,60]. Thus, the absence of pus in the vagina does not always reflect the absence of inflammation in the uterus.

6. Conclusions

In conclusion, we have suggested clinical definitions for the common postpartum uterine diseases that can be readily adopted by researchers and veterinarians. Given the
diagnostic criteria that we present, it is possible to identify individual cows that are likely to have a meaningful impairment of reproductive performance. A practical question is whether it is economically beneficial to invest the time and resources necessary to find and treat cows with endometritis. The optimal answer likely varies among farms, depending on the prevalence of endometritis, the cost and accuracy of diagnosis, the efficacy and cost of treatment, the pressure for early postpartum breeding, and the use of systematic breeding programs including PGF$_{2\alpha}$ for first insemination. Further research is needed to refine the inputs into economic decision-making tools to answer these questions under a variety of management conditions.

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