Comparison of Swabbing and Biopsy for Studying the Flora of the Bovine Uterus

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SUMMARY

The objectives of this study were to evaluate the efficacy of uterine biopsy as a sampling procedure for bacteriological examination, and to assess the importance of obligate anaerobes in the bovine uterus. The aerobic and anaerobic uterine flora of cows with postpartum metritis, cows in postpartum period without metritis and repeat-breeder cows was examined by using swab and biopsy sampling techniques. Obligate anaerobes were isolated in all the 11 cows with postpartum metritis and in three of the five normal cows. No obligate anaerobes were isolated from the six repeat-breeder cows. There was a significant difference (p < 0.01) in the number of bacterial isolates obtained from samples collected by biopsy and by swabbing. A total of 72 isolates was obtained with the biopsies compared to 48 by swabbing. Obligate anaerobes make up an important part of the postpartum uterine bacterial flora, and it seems that in some instances uterine biopsy would be more satisfactory than swabbing for bacteriological examination of the uterus.

Key words: Uterine flora, cattle, metritis, endometritis, diagnosis.

INTRODUCTION

The uterine flora has been studied extensively on different groups of cows: cows in postpartum period, with or without uterine infection (1, 2, 3, 4, 5) and cows with infertility problems (6, 7, 8). The purpose of these studies was to evaluate the role played by nonspecific pathogenic bacteria on the reproductive performances of cows. Nonspecific bacteria were associated in some occasions with infertility (5, 9, 10) and the economic losses brought about were quite considerable (9, 10, 11).

Swabbing of the uterine mucosa was the most widely used technique in collecting samples for bacteriology. In some instances the results obtained were negative or difficult to analyze, especially when bacteria are not isolated from cows with endometritis or infertility problems (7, 8, 12). Two studies of the uterine flora of cows were done using biopsies of the mucosa as sampling material. Sagartz and Hardenbrook (13) studied the bacterial flora of infertile cows by streaking a loopful of the collected endometrial material on different plates. Appleby and Gourlay (6) examined uterine biopsies from infertile cows for the presence of viruses and mycoplasmas and also checked for the presence of bacteria by plating the transport medium from each sample onto blood agar, before and after enrichment.

Only recently has the importance of obligate anaerobes been recognized in postpartum metritis of cows (14), although their significance in human postpartum uterine infection has been emphasized for almost ten years (15, 16).

The objectives of this work were to

Mots clés: flore utérine, bovins, métrite, endométrite, diagnostic.
evaluate the uterine biopsy as a specimen for bacteriological analysis and to determine the importance of obligate anaerobes as infectious agents in acute and chronic uterine infection.

MATERIALS AND METHODS

Animals

Twenty-two adult cows of the Holstein, Ayrshire or Jersey breeds were selected for this study. The cows were divided into three groups based upon the following criteria:

Group 1: Eleven cows between 3 to 21 days after parturition, hospitalized following abomasopexy at the Faculté de Médecine vétérinaire, Université de Montréal, and showing clinical signs of postpartum metritis (abnormal uterine discharge; uterine horns 5 cm or more in diameter).

Group 2: Five cows between 40 to 60 days after parturition, without any postpartum metritis and showing signs of estrus.

Group 3: Six cows that calved at least once showing regular estrus cycles but failing to conceive after three or more services.

Gross Genital Tract Examination

Before taking the samples, the genital tract of each cow was examined. The location and diameter of the uterine horns were determined by transrectal palpation. A visual inspection of any abnormal uterine discharge was done using a disposable vaginoscope (Alexander-Shaw Corp., Wellesley Hills, Massachusetts). The quantity of abnormal uterine discharge was evaluated roughly during the transrectal examination.

Sampling of Specimens

With the animal restrained, the perineal area was washed with water and disinfectant soap (Hibitane®, Rougier, Chambly, Québec). The vulva was then disinfected with a solution of povidone-iodine (Iodovet®, Rougier, Chambly, Québec). While the vulvar lips were parted by an assistant, a guarded culture instrument (Kalayjian Industries Inc., Long Beach, California) was inserted into the vagina. By manipulation of the cervix via the rectum, the instrument was advanced into the body of the uterus. The sterile swab was then pushed out of its protective sheath and pressed against the mucosa of one of the uterine horns. The swab was drawn back into its protective sheath and pulled out of the genital tract. The swab was streaked onto the different plates within 30 minutes.

Immediately after swabbing, a first biopsy of the uterine mucosa was taken using a biopsy instrument set (Instrumentarium, Terrebonne, Québec) that had previously been inserted in a protective plastic sheath and sterilized with ethylene oxide. Using the same technique as described above, the biopsy instrument set was advanced into the body of the uterus. The biopsy instrument was pushed out of its protective cover and moved into the same uterine horn where the swabbing was done. A piece of endometrium was clipped off. The biopsy instrument was withdrawn into its protective sheath and removed from the genital tract. The piece of tissue was immediately dropped into a tube of brain heart infusion pred-reduced anaerobically sterilized (BHI-PRAS, Carr-Scarborough Microbiologicals Inc., Decatur, Georgia). This sample was processed for bacteriological evaluation within 30 minutes. A second biopsy was taken in the manner already described except that the biopsy instrument was not inserted inside a protective sheath. This specimen was placed into buffered 10% formalin and used to evaluate histological lesions.

In all but five cows of group 1, the three samples could be collected. In these five cows, only a swabbing of the uterine mucosa could be done due to the fact that the uterus was distended by a large quantity of fluid and could not be retracted.

Bacteriological and Fungal Examination

The different plates used and the purpose for which they were used are listed in Table I. The TSA plates were incubated at 37°C in 5-10% CO₂ up to seven days. The BHIA and LKV plates were incubated at 37°C inside an anaerobic jar (Oxoid Limited, Montréal, Québec) containing 10% CO₂, 10% H₂ and 80% N₂ up to ten days. The plates for fungi isolation were incubated in surrounding atmosphere for up to 14 days.

When using a swab, the TSA, BHIA and LKV plates were streaked in a manner described by Hirsh and Wiger (17). This procedure allowed a semi-quantitative evaluation of the number of bacteria of each isolate present by giving a score ranging from < 1 to up to 4.

After determining the weight of the biopsy sample used for bacteriological examination on a precision balance (Sartorius, Postfach, R.F.A.) it was ground with a manual tissue grinder (Radnoti Glass Technology Inc., Arcadia, California). The suspension obtained was then diluted and 0.1 mL aliquots from the 10⁻², 10⁻⁴ and 10⁻⁶ dilutions were spread on different TSA, BHIA and LKV plates. This procedure allowed a quantitative evaluation of the number of living bacteria per gram of uterine tissue (cfu/g) as determined by the number of colonies on the plate.

Three drops of the 10⁻²-dilution were deposited on the mycobiotic and Sabouraud plus thiamine plates.

Identification of the aerobic and facultative anaerobes was performed by the API system (API 20E, Société de Biologie, Paris, France) and also by using specific media (Brain Heart Infusion Agar, Trypticase Soy Agar, Laked Blood Kanamycin Vancomycin). The identity of fungi was done by using Sabouraud dextrose agar and Ciment's medium (Ciment et al., 1970).

TABLE I

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<tr>
<th>Media Used and Purpose of Their Utilization in the Isolation of Microorganisms From the Uterus of Cows</th>
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<td>Purpose</td>
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⁺ Difco Laboratories, Detroit, Michigan.
ᵇ I.A.F. Production Inc., Laval-des-Rapides, Québec.
facultative anaerobic bacteria was based upon colonial morphology and pigmentation, classification of the bacteria after a Gram stain and by adequate biochemical testing (18, 19, 20, 21, 22, 23, 24, 25). On some occasions the API 20E (Analytab Products Inc., Montréal, Québec) was used.

Strict anaerobic bacteria identification was based on colonial morphology and pigmentation, classification of the bacteria after a Gram stain and the results of the API 20A identification system. Four isolates had to be sent to the Direction des Laboratoires, Ministère des Affaires sociales du Québec Ste-Anne-de-Bellevue, Québec for identification.

Fungi were identified by their macroscopic and microscopic characteristics (26).

Histopathological Examination

Biopsy specimens for histopathological examination were embedded in paraffin. Sections 6 µm thick were stained with hematoxylin and eosin. The stained sections were examined for the presence of histological lesions by an observer ignoring the origin of the specimen.

Statistical Analysis

A t-test pair was done to compare the total number of bacterial and fungal species that were isolated from cows where both swabbing and biopsy samples could be obtained.

RESULTS

The clinical examination of the cows in group 1 revealed that the uterine horns were generally 10 cm or more in diameter and were filled with more than 100 mL of abnormal uterine fluid. In group 2, the diameter of the uterine horns was between 2.0 and 4.5 cm. No abnormal uterine discharge was noticed. In group 3, the uterine horn diameter ranged from 2.5 to 4.5 cm. In one of the cows 20 to 25 mL of white purulent exudate was present in the uterus while all the others had no abnormal uterine content.

Table I shows the relative frequency of isolation of the different organisms in the three groups of cows. In some instances more than one species of the same genus was isolated from the same cow.

In the group of cows with metritis, 48 bacterial isolates were identified when a swab was used. Of this number 28 were aerobic or facultative anaerobic bacteria and 20 were strict anaerobes. Escherichia coli represented 32.1% of the aerobic or facultative anaerobic isolates; Corynebacterium pyogenes 28.6% and Streptococcus faecalis 17.9%. Fifty-five percent of the strict anaerobes isolates were bacterial species belonging to the Bacteroides genus. The other 45% included ten bacterial species distributed into six different genera. The Gram negative bacteria prevailed over the Gram positive bacteria with 57.1% of the isolates versus 42.9%.

When a biopsy was used as a bacteriological specimen, 34 bacterial isolates were identified, 17 aerobic or facultative anaerobic and 17 strict anaerobes. Escherichia coli and C. pyogenes each accounted for 23.5% of the aerobic or facultative anaerobic isolates and S. faecalis for 17.6%. In the strict anaerobes group, species belonging to the Bacteroides genus counted for 35% of the isolates and species of the Clostridium genus for 23.5%. The Gram negative bacteria again prevailed over the Gram positive bacteria (55.9% vs 44.1%). For the different isolates the number of bacteria ranged from 8.3 x 10^3 to 4.7 x 10^10 cfu/g.

Bacteria were isolated from every cow of group 2 when a swab was used. A total of 16 bacterial isolates and one fungus were identified. Fifteen of these isolates were aerobic or facultative anaerobic bacteria. The fungus isolated was identified as being Aspergillus fumigatus. The Gram positive bacteria prevailed largely over the Gram negative bacteria (81.3% vs 18.7%).

Sixteen bacterial isolates and one fungus were also isolated when an uterine biopsy was used as a bacteriological specimen. Twenty-five percent of the bacterial isolates were strict anaerobes. As with the swab, 81.3% of the isolates were Gram positive bacteria and 18.7% Gram negative. The number of bacteria of the different isolates ranged between 2.1 x 10^3 and 7.2 x 10^1. The A. fumigatus could not be quantified due to its confluent growth.

Two of the infertile cows gave negative results when a swab was used to take a sample for bacteriological analysis. Seven bacterial isolates were cul-
tured from the four remaining cows and all of them were Gram positive. No strict anaerobes and no fungus were isolated using this method of sampling.

Bacteria could be isolated from the uterus of every cow of group 3 when an uterine biopsy was used as a bacteriological specimen. Twenty-one bacterial isolates representing eight species were found. Gram positive bacteria accounted for 89.5% of the isolates. No strict anaerobes and no fungus were isolated. The number of cfu/g ranged between $1.3 \times 10^3$ and $1.2 \times 10^6$. However not taking into consideration the result obtained for the *C. pyogenes* isolate the number of cfu/g varied between $1.3 \times 10^3$ and $1.0 \times 10^4$.

Figure 1 shows the number of isolates in the different groups of cows having a same score or the same number of cfu/g of tissue.

In animals where both type of samples could be obtained the number of bacterial and fungal species isolated by swabbing was compared to the one isolated by biopsy (Table III). In groups 1 and 3, the number of aerobes and facultative anaerobes as well as strict anaerobes isolates was greater when a biopsy was used. In group 2 this observation was noticed only for the strict anaerobic bacteria. There was a significant difference ($p < 0.01$) between the total number of isolates obtained by swabbing and by biopsy in animals from which these two samples could be collected.

Histopathological Examination

In the group of cows with metritis (group 1), similar type of lesions were seen in all of the animals. The lesions were characterized by an infiltration, variable in intensity, of the superficial uterine epithelium by neutrophils and macrophages. Numerous uterine glands were missing and periglandular fibrosis was seen around those still present.

Four of the five cows in group 2 did not have any significant lesions of the endometrium. The fifth cow of this group had moderate fibrosis of the *lamina propria*, atrophy and disappearance of many uterine glands, and infiltration of the *stratum compactum* by some mononuclear cells. Further investigation into this cow's reproductive performance revealed that four services were required before it conceived.

Important variations in the extent of histological lesions present in the group of infertile cows (group 3) were seen. Two cows had only a minimal inflammatory reaction of the endometrium characterized by diffuse infiltration of mast cells along with slight periglandular fibrosis. In two other cows lesions were a little more extensive. The endometrium was infiltrated by a large number of mast cells and moderate periglandular fibrosis was present. The two remaining cows showed severe lesions of the endometrium. Ulcerative lesions of the superficial epithelium were present. The chorion was infiltrated with eosinophils and periglandular fibrosis causing compression of the uterine glands was also noticed.

**Discussion**

The isolation of obligate anaerobes from all the cows with metritis (group 1) and the fact that they account for nearly half of the total number of isolates confirms observations made previously (14) that, as in women (15, 16, 27), anaerobic bacteria are a major part of the bacterial flora found in postpartum metritis. Bacteria of the *Bacteroides* and *Clostridium* genera were isolated the most frequently in our study while Ruder et al (14) isolated mainly bacteria belonging to the *Fusobacterium* and *Bifidobacterium* genera.
Only a few strict anaerobic bacteria isolates could be recovered from cows without postpartum infection (group 2) and no such type of bacteria could be isolated from repeat-breeder cows (group 3). This might suggest that obligate anaerobes are progressively eliminated from the uterine mucosa as necrotic tissue is removed and normal vascularization of the mucosa is restored.

The different species of aerobic and facultative anaerobic bacteria isolated in this study are similar to those reported in the literature for cows in the postpartum period with metritis (1, 2, 5, 14) or without metritis (3, 12) and with infertility problems (6, 7, 8, 13).

Important variations in the Gram positive and Gram negative composition of the uterine bacterial flora were noticed among the three groups of cows. Gram negative bacteria predominated in the early postpartum period, but as cows further away from their calving date were sampled (groups 2 and 3) the Gram positive bacteria prevailed. In a different type of study (28), the cervix bacterial flora of women showed similar variations in its composition in regard to the Gram type of bacteria isolated. Gram negative bacteria were the major type of bacteria isolated early after delivery until the sixth week postpartum. Following this period their frequency of occurrence was down to levels found during the pregnancy, and Gram positive bacteria were the major constituents of the cervical flora.

When an uterine biopsy was used as a sampling method, bacteria could be isolated from all the cows, even the two in group 3 where swabbing gave negative result. Overall 33% more isolates were obtained using a biopsy compared to using a swab. Results obtained by Palmer et al (29) showed that in 32% of the cases they studied, *Salmonella* could be cultured from a rectal mucosal biopsy while a simple fecal culture gave negative result.

Except for the one of the repeat-breeder cow no correlation seems to exist between the presence or severity of histological lesions and isolation of one or more specific microbial species. The only cow where a correlation between lesions and microbial isolation could be found was one in which *C. pyogenes* was isolated in pure culture from the uterus. Previous studies (1,30) had shown that the isolation of *C. pyogenes* correlated with the presence of more severe lesions of the endometrium than when other species of bacteria were isolated. No correlation could be established between the number of bacteria per gram of uterine tissue and the severity of the lesions.

Human medical studies (31,32) have indicated that a better understanding of the microbial status of the female genital tract, and a more accurate interpretation of the results of bacteriological analysis in uterine infection are possible only with the use of quantitative technique of analysis. By using this type of technique in our study it was possible to show that obligate anaerobic bacteria can be numerous in bovine postpartum metritis and should be considered of major importance in this type of infection.

It appears also that the use of an uterine biopsy might in some instances be a better sampling method than a swab to perform a thorough bacteriological analysis of the uterus.

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References
BOOK REVIEW


The Foreword of the book begins as follows:

“Our era has been characterized by an exponential growth in the science of surgery. The burgeoning of science in itself generates excitement on the part of all students of surgery. The excitement is temporally satisfying and pleasurable; it stimulates the ongoing quest for new knowledge, additional facts, and statistically substantiated conclusions.

It cannot be disregarded that the science of surgery is irrevocably wedded to the art of surgery — to judgment calls related to individual patients, to conscious and subconscious responses based on personal reactions. The surgical equation is predicated on the interplay between two unique human beings — surgeon and patient.

Appreciation of a historical perspective, with its sense of heritage and, at times, romance, adds a desired dimension to the surgical personality. Unfortunately, modern students of the discipline have little concern for the past, shun the romantic constituents, and discount the evolution of modern concepts. A sense of history is now almost an anachronism and represents an encroachment on an overly taxed memory process constantly bombarded with scientific facts.”

The author describes many “firsts” in surgical history. The first laparatomy was carried out at Danville, Kentucky in 1809, by Ephraim McDowell on Mrs. J. T. Crawford, without anaesthesia. Descriptions of the first ligation of the aorta and gastrectomy followed. Ambroise Pare, born in 1510, demonstrated that wounds need not be cauterized with boiling oil and he replaced the hot iron with a ligature in controlling bleeding from large vessels, particularly for amputations.

Ether was first used for anaesthesia in 1846 in Massachusetts General Hospital by William Morton, while John Warren operated. The use of antiseptics for prevention of postsurgical infections is well described. Wounds then begin to heal by first intention.

The author describes surgical procedures on several famous people — the amputation of Nelson’s arm in 1797, including a photograph of the tourniquet which was used, removal of Napoleon III’s bladder stones, Edward the VII’s appendiceal abscess, a rib resection for empyema in George V and the pneumonectomy of George VI. Photographs accompany many of the surgical descriptions. In addition, the careers of the famous surgeons involved are briefly described, usually with a photograph of each.

This book will be of interest to surgeons and those with an interest in medical history. The photographs and drawings are also well presented and interesting. R.G. Thomson.