Isolation of *Bacteroides Ureolyticus* from the Equine Endometrium

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not be completely eliminated by hydrogen peroxide in methanol incubation. Occasional diffuse background staining was also detected. However, none of the background staining caused confusion in interpretation when appropriate negative control antiserum was compared to *Coxiella*-specific serum, and careful morphologic examination was performed.

We have adapted this technique for routine screening of ovine and caprine placental tissue from abortions. An immunoperoxidase technique applicable to paraffin sections for definitive detection of *Coxiella burnetii* in placental tissues can contribute to a rapid diagnosis while eliminating the source of laboratory infections from airborne dissemination of the rickettsiae present in fresh tissues.[9] In addition, sample submission is convenient, and retrospective studies of stored specimens are possible.[3] Definitive and rapid diagnosis of *Coxiella*-induced abortion will facilitate identification of infected dams in commercial and experimental flocks, thereby contributing significantly to prevention of Zoonotic transmission.

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Sources and manufacturers

a. Integrated Diagnostics, Baltimore, MD.
b. Abbott Laboratories, Abbott Park, IL.
c. Probe-On+, Microprobe, Fisher Scientific, Kent, WA.
d. Pro-par, Anatech, Battle Creek, MI.
e. Vector Labs, Burlingame, CA.
f. Human ExtrAvidin Peroxidase Staining Kit, Sigma Chemical, St, Louis, MO.
g Biogenex Labs, San Ramon, CA.
h. Geltol, Lemer Lab, Pittsburgh, PA.
i. Ortho Diagnostics, Raritan, NJ.

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Isolation of *Bacteroides ureolyticus* from the equine endometrium

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Infertility in the mare is of major concern to the equine breeding industry because of the economic losses attributable to mares that are unable to conceive or carry a foal to term. In a recent survey conducted among equine practitioners, endometritis was ranked as the third most common medical problem in adult horses.[23] Bacterial endometritis is thought to be a significant cause of decreased reproductive performance in mares.[2,7,16] Aerobic cultures from the genital organs of mares have been widely used to determine the possible etiology of bacterial endometritis. However, the information available on the identity and frequency of anaerobic bacteria in the equine endometrium is minimal. Anaerobic bacteria of the genus *Bacteroides,* particularly *B. fragilis,* have been isolated from equine endometrium;[8,19] these bacteria are generally penicillin and aminoglycoside resistant.[10,19] Because penicillin and aminoglycosides are commonly used to treat endometritis, they may not be effective if anaerobic bacteria are involved in the pathogenesis. Cytologic examination of the endometrium is useful to assess the significance of bacteria isolated from the endometrium.[7] Examining uterine smears for the presence of neutrophils is a practical and reliable way to diagnose acute endometritis.[10]

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The endometria of 71 mares from Prince Edward Island were cultured for anaerobic and aerobic bacteria and examined for the presence of inflammatory cells. Most of the mares were sampled during estrus, and the majority of the remainder were in anestrus. All the mares that were available for endometrial culture during the breeding season were included in this study. Special guarded swabs[a] that are designed for recovery of both aerobic and obligate anaerobic bacteria were used for endometrial culture. The swabs were cultured aerobically on Columbia blood agar and MacConkey agar plates and anaerobically on prereduced Columbia blood agar, Wilkins Chalgren anaerobic agar (WCA) with nonsporing anaerobe supplement, and WCA with gram-negative anaerobe supplement.[b] For anaerobes, swabs were also inoculated onto prereduced phenylethylalcohol blood agar and *Bacteroides* bile esculin agar. Finally, the swabs were inoculated into thioglycollate broth as a backup source of culture material. An anaerobic chamber[c] was used to facilitate recovery of anaerobes. The chamber was made anaerobic by following the manufacturer's instructions, using a gas mixture containing 5% H_2, 5% CO_2, and 90% N_2 in the presence of a catalyst. Initially, a solution of methylene blue (Smith modified methylene blue indicator)[3] contained in a test tube was checked for colorlessness to indicate anaerobiasis. Later, a disposable anaerobic indicator[d] which was changed every day, was used to check the anaerobic atmosphere in the chamber. A plate inoculated with *Fusobacterium necrophorum* (ATCC 25286)[e] was also included periodically, and the bacterial growth was checked as a control for the environment and medium.

All the plates were incubated at 35 C for 24-72 hours. The anaerobic plates were checked for growth up to 7 days. The aerobic cultures were identified as per standard procedures.[9] The anaerobic bacteria isolated were tested initially for their obligate anaerobic property by incubating inoculated secondary plates aerobically with 5% CO_2. They were then examined for morphology by Gram stain and were tested for catalase, oxidase, urease, indole, and nitrate reactions by previously described methods.[22] Bacteria were also tested for their pattern of susceptibility to special identification discs containing antimicrobial agents.[b] The potencies of these discs were colistin, 10 μg, kanamycin, 1,000 μg, and vancomycin, 5 μg. Discs of penicillin (2 units), rifampicin (15 μg), and erythromycin (60 μg) were also used for some isolates. Gram-positive anaerobic cocci were tested for sensitivity to sodium polyanethol sulfonate.[b] Most of the anaerobic isolates could be identified by conventional bacterial identification procedures[22] based on colony morphology, appearance in Gram's smear, selected biochemical reactions, and sensitivity to An-ident discs. Isolates not identified by the above tests were further tested by 2 commercial anaerobe-identification systems.[f,g]

For cytology, guarded aerobic culture swabs[h] were introduced immediately after collecting the bacteriology samples. The swabs were rolled onto glass slides and the smears were stained with Wright-Giemsa stain and examined under high-power microscopy (400 x). The presence of 1 or more neutrophils per high-power field (HPF) was considered positive evidence for inflammation. Any association between the occurrence of specific bacteria and inflammation was statistically analysed by a chi-square test and Fisher's exact test.[21]

Of 71 mares sampled, 63 were culture positive, 83% of these yielding more than 1 species of bacteria. Forty-five of 71 mares yielded 1 or more types of anaerobic bacteria (Table 1). The most common anaerobes were of the genus *Bacteroides;* 25 mares were positive for the predominant species, *B. ureolyticus*. *Bacteroides ureolyticus* isolates were characteristically nonpigmented and produced pitting colonies on the agar surface. Typical colonies were evident in many instances only after 72 hours of incubation. All of these were urease positive and showed biochemical properties typical for this species. Of 25 swabs positive for *B. ureolyticus, 2* yielded heavy growth with colonies appearing in all the quadrants of primary plates, 10 yielded colonies in 2 or 3 quadrants of the plates (moderate growth), and 12 swabs yielded growth with 2-20 colonies appearing only in the first quadrant. One swab yielded only 1 colony. Most of the recoveries of *B. ureolyticus* were made from WCA with gram-negative anaerobic supplement, although a minority were recovered from WCA with nonsporing anaerobic supplement or solely from Columbia blood agar. Only 2 mares yielded *B. ureolyticus* in pure culture, and in both cases, growth on primary plates was light. From the other mares, these organisms were associated with several other species of bacteria, the most common of which was *Peptostreptococcus* spp. *Peptostreptococcus assaccharolyticus* was isolated from 11 mares, and the other anaerobes in order of frequency were *B. fragilis* and other *Bacteroides* spp., *P. magnus,* and *Veillonella* spp.

Aerobic bacteria were isolated from 57 endometrial swabs of the 71 mares (80%) examined. A total of 108 aerobic bacterial isolates were recovered. The most commonly occurring aerobic bacteria were *Bacillus* spp.; 40 (37%) swabs were positive for this organism. Seventy-five percent of the 40 *Bacillus* spp. isolates yielded only light growth, with colonies evident only in the first quadrant of aerobic blood agar plates. Typically, these organisms were catalase-positive, spore-forming large gram-positive rods. The next most commonly isolated aerobe was *Streptococcus zooepidemicus,* which comprised 17% of the total isolates. This was followed by a-hemolytic streptococci, coagulase-negative staphylococci, *Escherichia coli,* P-hemolytic streptococci, *Staphylococcus aureus, Klebsiella pneumoniae,* and *Pseudomonas* spp.

Cytologic examination revealed that 12 of the 71 endometrial smears were positive for 1 or more neutrophils/HPF, providing evidence of inflammation. Fifty percent or more of the endometrial swabs yielded mixed cultures of aerobic and anaerobic bacteria, regardless of whether or not neutrophils were present. Anaerobes alone were isolated from neutrophil-positive and -negative groups with almost the same frequency. There was no significant difference in the occurrence of *Bacteroides ureolyticus* between neutrophil-negative and neutrophil-positive groups. The predominant aerobes were *Bacillus* spp., and these were isolated from 62% of the neutrophil-negative group, whereas only 38% of the neutrophil-positive group yielded these bacteria. The difference was significant only at $P = 0.08$ with both chi-square and Fisher's exact tests.

Anaerobic culturing has been a neglected area, and the presence and significance of obligate anaerobes in the equine uterus has not been well addressed. The results of the current study tend to agree in general with those of other workers.[8,19] in that *Bacteroides* was the most common anaerobe cultured

from the equine endometrium. Although *B. fragilis* was the most common species isolated by other workers,[8,19] *B. ureolyticus* was the most common species in the present study; 25/71 mares were culture positive for this bacterium. However, *B. ureolyticus,* which was called *B. corrodens,* has been isolated from the uterus of a single mare in an earlier study.[8] Although *B. ureolyticus* is a part of the normal flora of the human female genital tract[5] and has been found in a variety of clinical conditions, including a case of uterine infection in humans,[11] its role in animal disease is unknown.

In the present study, *B. ureolyticus* was rarely isolated in pure culture. Several bacteria were associated with it, but the most common was *Peptostreptococcus* spp. A similar association between *B. ureolyticus* and gram-positive anaerobic cocci was found in human clinical specimens.[11] Although it has been suggested that *B. ureolyticus* is a microaerophilic organism,[13] all our isolates were recovered under strict anaerobic conditions. Microaerophilic cultivation was not attempted. The *B. ureolyticus* isolates were tested for ß-lactamase enzymes, using cefinase paper disks.[d] None of the isolates gave a positive reaction for ß-lactamase, whereas a control *B. fragilis* strain (ATCC 25285)[e] showed production of the enzyme, as evidenced by a reddish color on the area of the disc where the culture was applied. A study of human isolates of *B. ureolyticus*[12] showed that the majority of strains were extremely susceptible to several antibiotics, including benzylpenicillin. However, it is known that resistance to penicillins and cephalosporins is common among *Bacteroides* species in general isolated from horses.[15]

Fusobacterium necrophorum, an important anaerobic pathogen of animals, was isolated from only 1 mare (1.4%). Other workers[8] isolated *Fusobacterium* spp. from just 1.4% of 570 cervicoendometrial swabs. Our results confirm the results of other surveys,[8,19] in that anaerobes belonging to the genera *Veillonella, Fusobacterium,* and *Clostridium* can occasionally be isolated from the uterine swabs of mares. Their significance, as in the case of several other anaerobes, is yet to be established.

A variety of aerobic bacteria, including pathogens, may be found in the uteri of mares whether or not they have endometritis. In a study of 4,024 mares,[24] 65% of the swabs yielded aerobic bacteria, whereas in the present study 80% were positive for aerobes. Although several authors[17,20,24] reported gram-positive cocci, particularly streptococci, as the most common aerobes found in equine endometrium, only a few listed *Bacillus* spp. In one study,[17] the endometria of 2/29 mares were positive for *Bacillus,* whereas in another,[24] 15% of the endometria of 4,024 mares harbored these organisms. In a third study,[14] only 1 uterine swab of 48 mares sampled was positive for *Bacillus,* and the overall isolation rate of aerobes was only 31%. The reasons for a higher rate of isolation (37%) of *Bacillus* spp. in the present study are not clear. The possibility of contamination from the environment or the lower genital tract was unlikely because guarded swabs were used with careful precautions. *Bacillus cereus* has been suspected as a cause of endometritis in mares;[1] however, the *Bacillus* isolates in the present study were not identified to species. In a recent study of dairy cows,[6] the presence of *Bacillus* spp. was correlated negatively with endometrial inflammation. Although some evidence of a negative association between the presence of *Bacillus* spp. and endometrial neutrophils was observed in the present study, the difference was not statistically significant, possibly because only a small number of mares had evidence of endometrial inflammation. Some authors[18] believe strongly that bacterial interference plays a major role in preventing disease and have cited examples of interference by *Bacillus* spp. and *Lactobacillus* spp. with the adhesion of pathogens to intestinal and urogenital mucosa.

Table 1. Anaerobic bacteria isolated from 45 of 71 endometrial swabs of mares.

Isolate	No. positive swabs	% total isolates
Bacteroides ureolyticus	25	37.3
Peptostreptococcus asaccharolyticus	11	16.4
Bacteroides fragilis	6	9.0
B. thetaiotaomicron	3	4.5
Peptostreptococcus magnus	3	4.5
Veillonella sp.	3	4.5
Bacteroides ovatus	2	3.0
B. uniformis	2	3.0
B. vulgatus	2	3.0
Peptostreptococcus anaerobius	2	3.0
P. tetradius	2	3.0
P. prevotii	1	1.5
P. indolicus	1	1.5
Prevotella disiens	1	1.5
Clostridium butyricum	1	1.5
Eubacterium lentum	1	1.5
Fusobacterium necrophorum	1	1.5
Total	67	

In general, the rates of isolation of *Bacillus* spp. and *Bacteroides ureolyticus* are higher in this study than previously reported. The higher isolation of *B. ureolyticus* is probably due to the simultaneous use of selective and nonselective media, and examination of plates for up to 1 week.

A variety of potentially pathogenic aerobic and anaerobic bacteria can be cultured from the endometria of apparently normal mares, and colonization of the equine endometrium with *Bacteroides ureolyticus* may not be uncommon.

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Sources and manufacturers

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b. Anaerobe supplements and An-ident discs, Unipath/Oxoid Canada, Nepean, ON.
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d. Becton Dickinson Microbiology Systems, Cockeysville, MD.
e. American Type Culture Collection, Rockville, MD.
f. RapID ANA II System, Innovative Diagnostic Systems, Atlanta, GA.
g. MicroScan Rapid Anaerobe Identification Panel, Baxter Healthcare Corp., West Sacramento, CA.
h. Continental Culture Swab, CDMV, Montreal, PQ.

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Multiple sarcomas of dental follicular mononuclear cells in a five-month-old dog

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Destructive tumors of the jaw in young dogs are most commonly of dental epithelial origin.[2] Papillary squamous cell carcinoma[5] and papillomatosis also frequently occur in the oral cavity. No cases of systemic metastasis originating from jaw tumors in young dogs have been reported.

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A 5-month-old mixed breed dog was presented for veterinary care because of a 6- x 5- x 3-cm ulcerative mass of the left maxilla caudal to the canine tooth (Fig. 1). Thoracic radiographs demonstrated multiple round radiopaque masses in all lung lobes. Because of the poor prognosis, the dog was euthanized. Postmortem examination revealed 7 tumors in the jaws. Three of these tumors were detectable grossly; 4 more were detected histologically. In addition to the original tumor, 3-cm masses were found associated with the left mandibular incisors and the left mandibular first molar. All of those masses were characterized by tan soft tissue masses invading into the bone (Fig. 2). The 4 tumors found on mi-