J Vet Diagn Invest 2:167-170 (1990)

# Isolation of *Gardnerella vaginalis* from the reproductive tract of four mares

Sarah **A.** Salmon, Robert D. Walker, Carla L. Carleton, Barbara E. Robinson

**Abstract.** A gram-variable pleomorphic bacillus was isolated from the reproductive tracts of 4 mares during routine prebreeding soundness examinations. Using a commercial bacterial identification system, these organisms were identified as *Streptococcus acidominimus.* However, colonial and Gram-staining characteristics did not support this identification. Subsequent testing indicated the organism was similar to *Gardnerella vaginalis.* Additional growth and biochemical analysis performed in our laboratory and at the Michigan Department of Public Health and by the Center for Disease Control, Atlanta, Georgia, confirmed the identification of G. *vaginalis.*

In 1955, a catalase-negative, oxidase-negative, gram-negative pleomorphic bacillus was isolated from the vaginae of women with nonspecific vaginitis.[3] When first isolated, the organism was identified as *Haemophilus vaginalis,* based on growth and Gram-staining characteristics.[3] Subsequently, this bacterium was renamed *Corynebacterium vaginale.* [25] Based on more recent taxonomic studies of the organism utilizing biochemical analysis of the cell wall constituents, DNA hybridizations, and electron microscopy, the organism was renamed *Gardnerella vaginalis.* [5] Although it is frequently associated with nonspecific (bacterial) vaginitis, the pathogenic role of this organism is unclear.[11,15,18] In addition to the vagina, this organism has been isolated from other sites in humans and has been implicated in infections associated with these sites.[11,13,15,18] This organism has also been isolated from the genitourinary tract of asymptomatic women. [11,13,15,18] Recently, we isolated an organism from the reproductive tracts of 4 mares, which was identified as *G. vaginalis.* We believe that this is the first report of *G. vaginalis* isolated from a nonhuman animal species. This report describes the characteristics of the equine isolates and discusses the clinical conditions associated with the mares from which this organism was isolated.

## Materials and methods

*Sample collection.* Uterine cultures were obtained, using standard guarded culture[a] techniques, from 4 barren Standard-bred brood mares, ages 5-22 yr, that were resident at 2 central Michigan stud farms. To avoid desiccation, the swab from the guarded culture instrument was placed into a transport medium[b] for transport to the laboratory.

*Primary isolation media.* Primary isolation media consisted of enriched blood agar (EBA), phenyl ethanol agar[c] (PEA) supplemented with 5% defibrinated sheep's blood,[d] and MacConkey agar[e] (MAC). Enriched blood agar was made using a tryptic soy agar base[e] supplemented with 5% defibrinated sheep's blood, 1% yeast extract," and 1% horse serum.[f] Thioglycollate broth[d] supplemented with 1.0% hemin[g] and 1.0% vitamin K[g] was used as an enrichment broth. All media were prepared within 7 days of use. Inoculated EBA and PEA plates were incubated at 35-37 C in a 5% CO, environment. The MAC plates and thioglycolate broths were incubated at 35-37 C aerobically.

*Isolation of Gardnerella vaginalis.* All primary plates were examined after 24 and 48 hr of incubation. Single colonies of gram-negative to gram-variable pleomorphic bacilli that were catalase and oxidase negative were subcultured to EBA and incubated at 35-37 C in 5% CO, an additional 24 hr. These subcultures were used as inocula for further biochemical characterization.

*Identification of Gardnerella vaginalis.* Subcultures of G. *vaginalis-like* colonies were suspended in 2 ml of triple distilled water to a turbidity equal to that of a number 4 McFarland standard. These bacterial suspensions were then used to inoculate a commercial bacterial identification system.[h] The strips were incubated at 37 C in an aerobic atmosphere for 4 and 24 hr. Interpretation of the biochemical reactions on the bacterial identification system were in accordance with written instructions for that system. Oxidase tests were performed by touching single colonies of the organism with a sterile swab and placing a drop of Oxichrome reagent' on the colony. Colorless to blue reactions were recorded as positive reactions. Human blood Tween (HBT)[c] agar was used to detect diffuse ,&hemolysis on human blood bilayer agar. Vaginalis agar (V-agar)[c] was used to detect diffuse ,&hemolysis on human blood single-layer agar. Tryptic soy agar base supplemented with 5% defibrinated rabbit's

From the Animal Health Diagnostic Laboratory (Salmon, Walker) and the Large Animal Clinical Sciences (Carleton), Michigan State University, East Lansing, MI 48824, and the Michigan Department of Public Health, Lansing, MI 48909 (Robinson).

Presented at the 32nd Annual Meeting of the AAVLD, Las Vegas, NV, October 28-31, 1989.

Received for publication October 30, 1989.

**Table 1.** Summary of microorganisms isolated from uterine swabs obtained from mares during routine prebreeding soundness examination.

| Mare no. | No. of microorganisms isolated* | | | | | |
|---|---|---|---|---|---|---|
| | *Gardnerella vaginalis* | *Staph.* sp. | *Strep.* spp. | *Coryne.* sp. | Coliform | *Cryptococcus laurentii* |
| 1 | <5 | <50 | ... | ... | ... | ... |
| 2 | <10 | ... | FBO† | <5 | FBO | <50 |
| 3 | <5 | ... | <10 | ... | ... | ... |
| 4 | <10 | ... | ... | ... | ... | ... |

* Quantities are expressed in colony-forming units.
† From broth only.

blood,[d] 1% yeast extract, and 1% horse serum was used to detect P-hemolysis on rabbit blood agar. Isolated colonies of the organism were suspended in 2 ml brain-heart infusion (BHI) broth," and incubated for 2-4 hr. These suspensions were diluted with additional BHI broth to the equivalent of a number 0.5 McFarland turbidity standard and used to inoculate Dunkelberg's proteose peptone-starch-dextrose (PSD) agar[2] plates for susceptibility testing to 80 µg/ml metronidazole[c] and 0.25 mg/ml sulfisoxazole.[c] Susceptibility to sodium polyanetholesulfonate[e] (SPS) was performed following the previously described procedure. [19] Susceptibility to a-hemolytic *Streptococcus* was performed by inoculating EBA with a confluent layer of the previously prepared number 0.5 McFarland turbidity standard BHI broth and streaking *Streptococcus sanguis* across the center of the plate. Zones of inhibition around the streak were reported as positive. Fermentation media for dextrose and maltose were prepared using Proteose Peptone No. 3[e] as previously described.*

## Results

*Isolation of Gardnerella vaginalis.* Growth of G. *vaginalis* was not observed on any of the primary cultures until after 48 hours of incubation. At that time, colonies of *G. vaginalis* appeared as pinpoint to small in size (05-1.0 mm), opaque, domed, entire, and gray-ish-white. Growth of *G. vaginalis* was not observed on PEA or MAC nor in the enriched thioglycolate. The quantities of *G. vaginalis* colonies, as well as other organisms isolated, are listed in Table 1.

*Identification of Gardnerella vaginalis.* The *G. vaginalis* isolates were gram-negative to gram-variable pleomorphic bacilli and were approximately 0.5 µm in diameter and 1.0-2.5 µm in length. Because there was very little biochemical activity after 4 hours of incubation using the commercial bacterial identification system, a full 24 hours of incubation was required before the strips could be read. Interpretation of the bacterial identification system yielded a 7-digit profile number. Three of the 4 profiles yielded an identification of *S. acidominimus.* The fourth profile indicated an identification of 47.9% *G. vaginalis* and 45.9% *S. acidominimus.* Gram-stain reaction, cellular morphology and colonial morphology did not support the identification of *S. acidominimus.* The profiles of the isolates, the positive reactions from those test strips, and their resulting identifications are summarized in Table 2. Using the bacterial identification system, all isolates were positive for hippurate hydrolysis, leucine arylamidase, and ß-glucuronidase, and negative for mannitol acidification, maltose acidification, and ß-galactosidase production. Three of the isolates were positive for lactose acidification and negative for starch acidification. Biochemical and hemolytic reactions for the equine isolates are shown in Tables 3 and 4.

## Discussion

The biochemical and morphological characteristics of the gram-variable pleomorphic bacilli isolated from these 4 mares closely resembled the organism isolated from human vaginae and identified as *G. vaginalis.* Critical tests used to identify *G. vaginalis* from human sources include colonial and cellular morphology, negative catalase, negative oxidase, hydrolysis of hippu-

**Table 2.** Biochemical reactions of equine and human *G. vaginalis* isolates, using a commercial bacterial identification system.[h]

| Samples | Positive reactions* | Profile no. | Identification | % identified |
|---|---|---|---|---|
| Mare no. | | | | |
| 1 | HIP, βGUR, LAP, LAC, AMD | 2440401 | *S. acidominimus* | 99.3 |
| 2 | HIP, βGUR, LAP, LAC, AMD | 2440401 | *S. acidominimus* | 99.3 |
| 3 | HIP, βGUR, LAP, | 2440000 | *G. vaginalis* | 47.9 |
| | | | *S. acidominimus* | 45.9 |
| 4 | HIP, βGUR, LAP, LAC | 2440400 | *G. vaginalis* | 99.3 |
| Human isolate | | | | |
| 1 | HIP, βGAL, LAP | 2050000 | *G. vaginalis* | 98.1 |
| 2 | HIP, LAP | 2040000 | *G. vaginalis* | 88.6 |
| 3 | HIP, LAP | 2040000 | *G. vaginalis* | 88.6 |
| 4 | HIP, LAP | 2040000 | *G. vaginalis* | 88.6 |
| 5 | HIP, βGAL, LAP, RIB, AMD | 2052001 | *G. vaginalis* | 99.9 |

* HIP—hippurate hydrolysis; βGUR—β-glucuronidase; βGAL—β-galactosidase; LAP—leucine arylamidase; RIB—ribose acification; LAC—lactose acidification; AMD—starch acidification.

**Table 3.** Critical tests for the identification of *G. vaginalis.*

| Tests | Isolate from mare no. 1 | 2 | 3 | 4 | Human isolates* |
|---|---|---|---|---|---|
| Gram-variable, pleomorphic | + | + | + | + | 100 |
| PP† growth on BA at 48 hr | + | + | + | + | 100 |
| Catalase | – | – | – | – | 0 |
| Oxidase | – | – | – | – | 0 |
| Hippurate hydrolysis‡ | + | + | + | + | 100 |
| β-hemolysis on HBT§ agar | + | + | + | + | 100 |
| δ-hemolysis on sheep blood agar | + | + | + | + | 100 |

* Percent of human isolates with positive reactions.
† Pinpoint colonies.
‡ Test performed using commercial bacterial identification system.[h]
§ Human blood Tween agar (a bilayer medium).

**Table 4.** Additional tests recommended for the identification of *G. vaginalis.*

| Tests | Isolate from mare no. 1 | 2 | 3 | 4 | Human isolates* |
|---|---|---|---|---|---|
| Susceptible to: | | | | | |
| α-hemolytic strep | + | + | + | + | 100 |
| Metronidazole | + | + | – | + | 96 |
| SPS† | – | – | – | + | 96 |
| Sulfisoxazole | – | – | + | – | 0 |
| Starch acidification‡ | – | – | + | – | 70 |
| Starch hydrolysis | – | – | – | + | 100 |
| Dextrose acidification | + | + | + | + | 97 |
| Lactose acidification‡ | + | + | + | – | 17 |
| Mannitol acidification‡ | – | – | – | – | 0 |
| Maltose acidification | – | – | – | – | 97 |
| β-galactosidase‡ | – | – | – | – | 53 |
| Leucine arylamidase‡ | + | + | + | + | 100 |
| β-hemolysis on RB§ | + | + | – | + | 100 |
| β-hemolysis on V-agar‖ | + | + | + | + | 88 |

* Percent of human isolates with positive reactions.
† Sodium polyanetholesulfonate disk.
‡ Test performed on commercial bacterial identification system.[h]
§ EBA with 5% rabbit blood.
‖ Vaginalis agar, single-layer human blood.

rate, presence of diffuse ,&hemolysis on human blood bilayer agar, and absence of hemolysis on agar containing sheep blood.[2,4,6,12,16,23,24] The *G. vaginalis* isolates from the mares were identical to those reported from human sources in all of these critical biochemical tests. Like the isolates described from humans, pinpoint growth of *G. vaginalis* from the mares in this study was not observed on primary culture media until after approximately 48 hours of incubation at 35-37 C in 5% CO, and then was only observed on EBA.[8,14,20,22] Subsequently, the transferring of single pinpoint colonies from the primary plate to subculture yielded sufficient growth at 24 hours for inoculation of bacterial identification system media (i.e., a number 4 McFarland turbidity).[8,14,20,22]

Like the human isolates of *G. vaginalis,* those from the mares were inhibited by a-hemolytic *Streptococcus* [1,19] Three of the 4 isolates were also resistant to sulfisoxazole and sensitive to metronidazole, as described for human isolates.[1,4] Unlike the human isolates, 3 of the isolates from the mares were resistant to SPS.[19] Isolates from both animal species are also similar in that all of the isolates from the mares showed diffuse ,&hemolysis on single-layer human blood agar and 3 of the 4 showed ,&hemolysis on enriched blood agar with rabbit blood.[16,21] Using the bacterial identification system,[h] those reactions from the equine isolates that were the same as those reported from humans included negative mannitol acidification and positive leucine arylamidase. Those which were not similar included starch and lactose acidification, ß-galactosidase, and ß-glucuronidase. Seventy percent of the human isolates are reported as positive for starch acidification on the commercial bacterial identification system used in this study.[7] In our laboratory, 2 of the 4 equine isolates cultured and only 1 of the 5 human isolates tested were positive for this reaction. We found that 3 of the 4 isolates from the mares acidified lactose, compared with none of the 5 human isolates tested and 17% of those from humans reported in the literature.[12] The *G. vaginalis* isolates cultured from the mares were ß-galactosidase negative and ß-glucuronidase positive. Isolates from humans are reported as being positive for ß-galactosidase 53% of the time[4] and negative for ß-glucuronidase 100% of the time.[12]

Maltose acidification and starch hydrolysis reactions, which were not run on the commercial bacterial identification system used in this study, did not show agreement between human and equine isolates. None of the equine isolates acidified maltose, and only one hydrolyzed starch. Human isolates reported 97% and 100% positive for these two reactions, respectively.[4,12,17,22] However, because of the small number of isolates in this report, complete characterization of G. *vaginalis* from the mares cannot be made at this time. Analysis of additional isolates of *G. vaginalis* from the horse will be necessary to provide conclusive data regarding the biochemical characteristics of equine G. *vaginalis* isolates.

The clinical significance of the isolation of *G. vaginalis* from the equine reproductive tract is yet to be determined. In humans with bacterial vaginitis, the detection of epithelial cells with sheets of *G. vaginalis*-like bacilli noted on microscopic examination and the isolation of *G. vaginalis* from copious vaginal secretions are used for the diagnosis of nonspecific vaginitis associated with *G. vaginalis.* [3,10] Because there was no evidence of abnormal discharge at the time of culturing, microscopic examination was not performed on the specimens obtained from these 4 mares. However,

as a routine procedure, endometrial biopsies were performed on 2 of the 4 mares. Cytology reports for these 2 samples indicated that both mares had a mild endometritis, based on Kenney's classification.[9] After the initial isolation of *G. vaginalis,* one of the biopsied mares was negative on repeat culture. Since the isolation of *G. vaginalis,* all 4 of the mares have conceived and carried their foals to term in the absence of antimicrobial chemotherapy. Such results may indicate that the presence of *G. vaginalis* in the reproductive tracts of horses is of similar significance as isolates obtained from the genital tracts of asymptomatic women. Further studies concerning the relationship of *G. vaginalis* to equine breeding efficiency are in progress.

## Sources and manufacturers

a. VETKEM, Zoecon Corp., Dallas, TX.
b. Marion Scientific, Kansas City, MO.
c. BBL, Becton-Dickinson, Cockeysville, MD.
d. Cleveland Scientific, Bath, OH.
e. Difco, Detroit, MI.
f. GIBCO Laboratories, Lawrence, MA.
g. Sigma Chemical Co., St. Louis, MO.
h. Analytab Products, Inc., Rapid STREP@ bacterial identification system, Plainview, NY.
i. Innovative Diagnostics, VITEK Systems, Inc., Hazelwood, CT.

## References

1. Bailey RK, Voss RL, Smith RF: 1979, Factors affecting isolation and identification of *Haemophilus vaginalis (Corynebacterium vaginale).* J Clin Microbiol 9:65-71.
2. Dunkelberg WE Jr, Skaggs R, Kellogg DS Jr: 1970, Method for isolation and identification of *Corynebacterium vaginale (Haemophilus vaginalis).* Appl Microbiol 19:47-52.
3. Gardner HL, Dukes CD: 1955, *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified "nonspecific" vaginitis. Am J Obstet Gynecol 69:962-976.
4. Greenwood JR, Pickett MJ: 1979, Salient features of *Haemophilus vaginalis.* J Clin Microbiol 9:200-204.
5. Greenwood JR, Pickett MJ: 1980, Transfer of *Haemophilus vaginalis* Gardner and Dukes to a new genus, *Gardnerella: G. vaginalis* (Gardner and Dukes) comb. nov. Int J Syst Bacteriol 30:170-178.
6. Greenwood JR, Pickett MJ, Martin WJ, et al.: 1976, *Haemophilus vaginalis (Corynebacterium vaginale):* method for isolation and rapid biochemical identification. Health Lab Sci 14:102-106.
7. Human RP, Tillotson GS: 1985, Identification of *Gardnerella vaginalis* with the API 20 Strep System. J Clin Microbiol 6: *985-986.*
8. Jolly JLS: 1983, Minimal criteria for the identification of *Gardnerella vaginalis* isolated from the vagina. J Clin Patho1 36:476-478.
9. Kenney RM: 1978, Cyclic and pathologic changes of the mare endometrium as detected by biopsy, with a note on early embryonic death. J Am Vet Med Assoc 172:241-262.
10. Krohn MA, Hilier SL, Escenbach DA: 1989, Comparison of methods for diagnosing bacterial vaginosis among pregnant women. J Clin Microbiol 27:1266-1271.
11. Lam MH, Birch DF, Fairley KF: 1988, Prevalence of *Gardnerella vaginalis* in the urinary tract. J Clin Microbiol 26:1130-1133.
12. Leighton PM: 1983, *Gardnerella vaginalis:* laboratory isolation and clinical significance. Can J Public Health 73:335-340.
13. McCormack WM, Hayes CH, Rosner B, et al.: 1977, Vaginal colonization with *Corynebacterium vaginale (Haemophilus vaginalis).* J Infect Dis 136:740-745.
14. O'Brien SM, Lewis JF: 1969, Identification of *Haemophilus vagina/is.* Am J Med Tech 35:158-161.
15. Phiefer TA, Forsyth PS, Durfee MA, et al.: 1978, Nonspecific vaginitis, role of *Haemophilus vaginalis* and treatment with metronidazole. N Engl J Med 298: 1429-1434.
16. Piot P, Van Dyck E: 1983, Isolation and identification of *Gardnerella vaginalis.* Scand J Infect Dis Suppl 40:15-18.
17. Piot P, Van Dyck E, Totten PA, Holmes KK: 1982, Identification of *Gardnerella (Haemophilus) vaginalis.* J Clin Microbiol 15:19–24.
18. Ratnam S, Fitzgerald BL: 1983, Semiquantitative culture of *Gardnerella vaginalis* in laboratory determination of nonspecific vaginitis. J Clin Microbiol 18:344-347.
19. Reimer LG, Barth Reller L: 1985, Use of sodium polyanetholsulfonate disk for the identification of *Gardnerella vagina/is.* J Clin Microbiol 21:146-149.
20. Shaw CE, Forsyth ME, Bowie WR, et al.: 1981, Rapid presumptive identification of *Gardnerella vaginalis (Haemophilus vaginalis)* from human blood agar media. J Clin Microbiol 14: 108-110.
21. Smith RF: 1979, Comparison of two media for isolation of *Haemophilus vaginalis.* J Clin Microbiol 9:729-730.
22. Taylor E, Phillips I: 1983, The identification of *Gardnerella vaginalis.* J Med Microbiol 16:83-92.
23. Totten PA, Amsel R, Hale J, et al.: 1982, Selective differential human blood bilayer media for isolation of *Gardnerella (Haemophilus) vaginalis.* J Clin Microbiol 15:141-147.
24. Yong DCT, Thompson JS: 1982, Rapid microbiochemical method for identification of *Gardnerella (Haemophilus) vaginalis.* J Clin Microbiol 16:30-33.
25. Zinneman K, Turner GC: 1963, The taxonomic position of *"Haemophilus vaginalis" (Corynebacterium vaginale).* J Pathol Bacteriol 85:213-219.