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Haemophilus vaginalis (Corynebacterium vaginale, Gardnerella vaginalis) in a family planning clinic population

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SUMMARY Vaginal specimens were obtained at 902 attendances from 522 women requiring vaginal examination at a family planning clinic. *Haemophilus vaginalis* was found in 8% of specimens either by culture or by at least two out of three microscopical tests; lactobacilli were seldom found in the presence of the organism. Increased signs and symptoms, especially an offensive odour, were found only when *H vaginalis* and staphylococci were isolated together. The organism was found less often in patients using contraceptive methods which protected the vagina, thus suggesting sexual transmission. Treatment is advisable when *H vaginalis* is in contact with a vascular bed.

Introduction

A *Haemophilus*-like organism was first described in 1953 by Leopold,[1] who isolated it from male urine and female cervical specimens. Gardner and Dukes[2] isolated a similar organism from a number of cases of bacterial vaginitis and proposed the name *Haemophilus vaginalis.* In 1963, Zinneman and Turner[3] renamed it *Corynebacterium vaginale,* but as it is a true member of neither species taxonomists are currently considering the newly proposed name of *Gardnerella vaginalis.* Dunkelberg's[4] review includes reports of prevalence ranging from 0 to 89% and a variety of clinical presentations. In this study of healthy women attending a family planning clinic we estimated the prevalence of *H vaginalis,* with and without other infections. We also report on the pathogenicity of the organism in the vagina and present evidence of sexual transmission, and compare various methods of diagnosis.

Patients and methods

STUDY POPULATION

Over a period of 12 months all 522 women attending a single-doctor family planning clinic who required a vaginal examination were included in the study, provided they were not menstruating at the time. Over half were aged 25-34 years and over half belonged to socioeconomic group III (husbands or fathers in skilled manual jobs or the equivalent); 2% were unmarried.

About a third of the women were taking oral contraceptives and another third used a intrauterine contraceptive device (IUCD). Details of recent coitus, contraceptive practice, and symptoms and treatment of any vaginal discharge during the past year were recorded. A clinical examination was made of the vulva, vaginal walls, and cervix. Any abnormality of these and the nature of any discharge were noted. A creamy white or clear mucoid discharge was regarded as normal while that of any other colour or consistency was regarded as abnormal.

SPECIMENS

The urethral orifice was cleaned and a urethral swab taken and placed in Amies's transport medium[5] for the culture of *Neisseria gonorrhoeae.* Vaginal secretion was collected with a plastic spoon for microscopical examination in the clinic. A Cusco's speculum was inserted and three high vaginal swabs taken; one was immersed in peptone starch dextrose (PSD) transport medium[6] for the culture of *H vaginalis,* one placed in reinforced clostridial medium (Oxoid Ltd) containing 0·2% paracresol for the culture of *Clostridium difficile,*[7] and the third used for microscopy in the laboratory. The cervix was then cleaned and a cervical swab taken and placed in Amies's transport medium for the culture of *N gonorrhoeae.*

MICROSCOPY

In the clinic, a saline preparation of the vaginal secre-

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tion was examined to determine the number of polymorphonuclear leucocytes and the presence of trichomonads, yeasts, and the "clue cells" associated with *H vaginalis* infection.[2] In the laboratory, a wet preparation was made in "vaginal identification of pathogens" stain[8] and examined as in the clinic; in addition a Gram-stained smear was prepared to verify the nature of the organisms present, in particular the presence of multitudes of Gram-variable cocco-bacilli found with "clue cells" and *H vaginalis* infection.

CULTURES

H vaginalis

Culture methods used for the isolation and identification of *H vaginalis* were similar to those devised by Dunkelberg[6] except that Columbia blood agar plates[9] were used in preference to PSD plates for primary isolation. Confirmatory tests performed on pure subcultures of the isolates included a Gram-stained film, hydrogen peroxide inhibition test, catalase test, and sugar utilisation reactions. Microscopically the organism is a slender Gram-variable bacillus easily over-decolorised and often showing a barred effect not unlike some members of the *Corynebacterium* species. It exhibits a wide zone of growth inhibition by hydrogen peroxide, is catalase-negative, and ferments glucose, maltose, and starch after 48 hours. Yeasts and other organisms present in the cultures were identified by their colonial morphology and Gram-stain reactions only.

Cl difficile

Cultures for *Cl difficile* were performed independently by Dr S Hafiz and this organism was identified primarily by its ability to grow in a medium containing 0·2% paracresol and then by confirmatory fermentation reactions.

N gonorrhoeae

Cultures for *N gonorrhoeae* were performed at the Public Health Laboratory, Sheffield. Swabs from the transport media were inoculated on to chocolate blood agar and a modified selective medium containing vancomycin, colistin, and trimethoprim. Confirmatory identification was by fluorescent antibody and sugar utilisation reactions.

FOLLOW-UP

Patients were asked to return for an anniversary visit, at which details of contraceptive practice during the previous year and any symptoms present were recorded. The clinical examination was repeated in the 380 women who punctually reattended, and specimens were taken for microscopy and culture as before so that a total of 902 specimens was available for study.

Results

H VAGINALIS PREVALENCE

H vaginalis was isolated from 27 (5%) of 522 women at their first attendance and from 26 (7%) of 380 at reattendance, making a mean prevalence of 6% for the 902 attendances. All the results are presented for initial and anniversary attendances combined. Our conclusions are unaltered when results at the two attendances are analysed separately.

Microscopical examination of vaginal secretion is thought to be a useful detector of *H vaginalis* infection,[2 6] and because the cultural viability of the organism is poor[10] we adopted a second definition of positivity. We regarded the organism to be present either when cultured or when at least two of the following conditions were met: "clue cells" were seen in the clinic wet film or in the laboratory wet film; or Gram-variable cocco-bacilli were present in the laboratory Gram-stained film. By this criterion a further 2% of specimens were regarded as positive, making a total prevalence of 8% either by culture or by microscopy.

OTHER ORGANISMS

Cl difficile was cultured from 17% of the 902 vaginal specimens, yeasts from 9%, *T vaginalis* from 1%, and *N gonorrhoeae* from only two (0·2%).

When "clue cells" were present, lactobacilli were reported in only 9% of 53 clinic wet films and were cultured from only 6% of the 53 specimens. This contrasts with the findings in women who were not infected with either *H vaginalis, N gonorrhoeae, T vaginalis,* or yeasts, in whom lactobacilli were seen in 90% of 736 wet films and cultured from 66% of 736 vaginal swabs. In the absence of yeasts, *T vaginalis,* and *N gonorrhoeae* there was no difference in the numbers of polymorphonuclear leucocytes seen in specimens from which *H vaginalis* was cultured and in those from which it was not.

H VAGINALIS INFECTION

Symptoms and signs

The signs and symptoms present when *H vaginalis* was cultured are compared with those when the organism was neither seen microscopically (by at least two tests) nor cultured (table I). In the presence of *H vaginalis* abnormalities of the vaginal discharge—in particular an offensive odour—were more common either as symptoms or signs. This increased frequency was found only when both *H vaginalis* and unclassified staphylococci were grown (table II).

Sexual transmission

Infection rates related to the degree of protection

TABLE I *Percentage frequency of symptoms and signs in women with and without H vaginalis (all attendances*)*

Symptom/sign	*H vaginalis infection (% of attendances)*	
	Present (by culture)	*Absent (by culture and microscopy)*
Vaginal symptoms		
Abnormal discharge	27	17
Irritation	8	6
Odour	16	6
Any of the above	29	18
Signs		
Abnormal vaginal discharge:		
Offensive	12	3
Purulent	29	23
Frothy	20	12
"Coloured"	39	30
Excessive	18	13
Any of the above	51	41
Vaginitis	2	2
Abnormal cervix†	78	73
Abnormal vulva		2
Total No of attendances (100%)	49	717

*Excluding attendances where yeasts, *T vaginalis*, or *N gonorrhoeae* were present.
†Excessive secretion, ectopic columnar epithelium, or cervicitis

TABLE III *Contraceptive usage and associated prevalence of H vaginalis (all attendances)*

Contraceptive method/degree of protection	*Total attendances (100%)*	*H vaginalis culture rate (%)**
Protective		
Sheath (with or without pessary)	116	3
Semi-protective		
Cap and cream	19	5
Foam or pessaries	3	
Coitus interruptus	34	3
Oral	333	5
All semi-protective	389	5
Non-protective		
IUD	339	8
Sterilisation	35	6
No method used	21	10
All non-protective	395	8
Method not known	2	
All attendances	902	6

*Mann-Whitney U tests for association between culture rate and degree of protection: first attendances—$P<0.05$; anniversary attendances—$P>0.05$

afforded to the vagina by various contraceptive methods are shown in table III. A culture rate of 3% was found when a sheath was used, of 8% with an IUCD or other non-protective method, and of 5% with partial protection, including oral contraceptives, since they are thought to lower the vaginal pH and so create conditions which are less favourable to *H vaginalis*.[11]

Diagnostic tests

At 879 of the 902 attendances, when all four tests for the presence of *H vaginalis* were performed, the apparent prevalence of the organism was 14·8% by clinic wet film, 7·2% by laboratory wet film, 7·5% by laboratory stained film, and 5·9% by culture. While "clue cells" were seen in 130 clinic wet films, *H vaginalis* was cultured from only 48 of the corresponding specimens. On the other hand, the clinic wet film gave a negative result in only four of 52 cases which were culture-positive.

When the results of tests were combined, the effect on the estimated prevalence of *H vaginalis* was: 5·9% of specimens were culture-positive (group A), an additional 2·6% were culture-negative but had positive results by at least two microscopical tests (group B), a further 6·7% showed "clue cells" in the clinic wet film when all other tests, including culture, were negative (group C), and 84·6% gave negative results by all tests (group D). For each of the three groups, A, B, and C, the symptoms and signs of vaginal abnormality were more frequent than when

TABLE II *Unclassified staphylococci and H vaginalis: percentage frequency of selected symptoms and signs (all attendances*)*

Symptom/sign	*Unclassified staphylococci (% of attendances)*			
	Cultured		*Not cultured*	
	H vaginalis +	*H vaginalis − †*	*H vaginalis +*	*H vaginalis − †*
Symptoms				
Abnormal discharge	37	18	11	15
Odour	23	7	5	5
Signs				
Abnormal discharge (any sign)	63	42	32	38
Odour	20	4		2
Total attendances (100%)	30	391	19	326

+ Positive − negative
*Excluding those where yeasts, *T vaginalis*, or *N gonorrhoeae* were identified.
†By culture and microscopy (see text)

all four tests gave negative results (group D) (table IV), suggesting that each of the three combinations (A, B, and C) of tests may have contributed to the diagnosis of a pathogen.

TABLE IV *Percentage frequency of symptoms and signs by various diagnostic criteria for H vaginalis (all attendances*)*

Symptom/sign	*Test result* (% of attendances)*			
	A	*B*	*C*	*D*
Symptoms				
Abnormal discharge	31	26	36	17
Irritation	10	17	8	8
Odour	21	13	14	6
Any of the above	33	35	36	19
Signs				
Any abnormal vaginal discharge	54	48	54	42
Offensive odour	15	26	19	2
Total (100%)	52	23	59	744

*Where all four tests were performed.
A = culture positive; B = culture negative, two or more others positive; C = clinic wet-film positive, rest negative; D = all four negative

Discussion

The study population consisted mainly of healthy, married women from social class III, so the low prevalence of infection with *N gonorrhoeae* and *T vaginalis* was not unexpected. On the other hand, the high prevalence of *Cl difficile* was surprising because of the much lower prevalence in the normal female bowel.[12] The low culture rate of *H vaginalis* (6%) is at variance with the results of McCormack, who isolated the organism from the vagina of 32% of 466 undergraduate women.[13]

Differences in the reported prevalence of *H vaginalis* could be due to variations in (a) its real incidence in the different kinds of population studied, (b) the duration of an infection which could terminate naturally or as a result of coincidental treatment, or (c) diagnostic methods. The poor viability of the organism could result in underdiagnosis owing to death or overgrowth by contaminants in transit to the laboratory or before completion of confirmatory tests. Conversely, microscopical tests could give rise to considerable overdiagnosis.

H vaginalis has been suggested as the principal cause of bacterial vaginitis,[14] although McCormack found no relationship between the signs and symptoms of vaginal discharge and the presence of *H vaginalis*. We unexpectedly found that the higher percentage of abnormal vaginal signs and symptoms was associated only with the concurrent presence of *H vaginalis* and unclassified staphylococci.

True inflammation of the vaginal mucosa was found only once with *H vaginalis* and this is consistent with our microscopical findings when similar numbers of polymorphonuclear leucocytes were found in both infected and uninfected specimens. The organism may colonise a normal discharge and, if present in sufficient numbers, produce the distinctive odour. Lactobacilli were seldom found in the presence of *H vaginalis,* and this could be a useful indicator in diagnosis.

The 6% prevalence of *H vaginalis,* when compared with the 60% found by Rogers in a sexually transmitted disease clinic,[15] suggests that the infection could be sexually transmitted. In the present study, infection rates were higher when contraceptive methods affording little protection to the vagina were used, thus supporting the theory of sexual transmission.[2]

None of our patients was treated for *H vaginalis* infection, as antibiotic treatment of vaginal infections can cause monilial vaginitis and sulphonamide pessaries can produce local irritation. Perhaps treatment should therefore be avoided unless there is a persistent complaint. When in contact with a vascular bed, *H vaginalis* may cause a bacteraemia.[16] Infected women should therefore be treated in the third trimester of pregnancy, before any gynaecological surgery, and perhaps before the insertion of an IUCD.

"Clue cells" were reported more often in the clinic than in the laboratory. If culture is the criterion of diagnosis, clinic microscopy produced few false-negative and many false-positive results. Whether *H vaginalis* was diagnosed by clinic microscopy alone or by culture alone, the pattern of signs and symptoms was similar and more frequent than in uninfected women. Positive laboratory microscopical findings in wet films and Gram-stained films were also commoner than positive culture results, probably because of the poor viability of the organism.

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