HAEMOPHILUS VAGINALIS

Its Association with Puerperal Pyrexia and Leucorrhoea

BY

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FOR several years, this laboratory has reported in routine high vaginal swabs, mainly from cases of puerperal pyrexia, numerous small Gram negative bacilli, under the name of "*Haemophilus influenzae*-like bacilli". These have been identified, in the course of the work here reported, as *H. vaginalis* (Gardner and Dukes, 1955).

Until 1953, few authors had mentioned the occurrence of aerobic Gram negative bacilli in the vagina, with the exception of coliform bacilli and Ducrey's bacillus. The first recognizable description of *H. vaginalis* was by Leopold (1953), working in the U.S.A., who reported the isolation of small Gram negative aerobic bacilli from the urine of 53 of 965 males with mild prostatitis, and from 16 of 58 cervical swabs from cases of cervicitis. Growth occurred as pinpoint haemolytic colonies after 48 hours incubation on Casman's blood agar (Casman, 1947). Leopold suggested a close relationship of this organism with the genus "Haemophilus", but did not name its species.

The French workers Lutz and Wurch (1954) stated that numerous small Gram negative bacilli not yet identified were seen in many cases where mixed vaginal flora were present. Wurch and Lutz (1955) found in the vaginal flora in 500 cases of leucorrhoea that small Gram negative bacilli were present in 22 per cent. They noted that there were usually few leucocytes but very many bacilli, in spite of which cultures on various media remained sterile. In 1956, however, Lutz, Wurch and Grootten reported that the organism grew in 48 hours on 10 per cent blood agar plus 0·3 per cent glucose, giving a small haemolytic colony. Lutz, Grootten and Wurch (1956) revealed that they used sheep blood in their media, and named the organism "*Hemophilus hemolyticus vaginalis*". Lutz and Burger (1957) gave the incidence for this organism in normal pregnant women as 20 per cent, and 28 per cent in cases with trichomonas infection. They suggested that there was an association between the two organisms.

Gardner and Dukes (1954, 1955), in Texas, described a similar organism under the name of *H. vaginalis* and claimed it to be the cause of a newly defined, specific infection previously classified as "non-specific vaginitis". They investigated 602 obstetric and 579 gynaecological cases, and, of the total of 1,181, 12 per cent yielded cultures of *H. vaginalis* (13·3 per cent in gynaecological cases and 10·6 per cent in obstetric). The organism was not found in any of 78 normal control and 43 miscellaneous control cases, but of 138 cases diagnosed as "bacterial vaginitis", 92 per cent were attributed to *H. vaginalis*.

Ray and Maughan (1956) reported from Oregon, U.S.A., the results of an investigation into 447 clinic patients, two-thirds of which were gynaecological cases and one-third obstetric, together with 74 cases of vaginitis seen in private practice. Of the 447 clinic patients, 68 (15·2 per cent) showed *H. vaginalis*, and these constituted 94 per cent of the total of 72 cases diagnosed as "bacterial vaginitis", a group which apparently consisted of all cases with "infectious organisms" (not defined) where neither trichomonas nor

monilia were found microscopically, and regardless of whether any symptoms were present. These results were based on microscopic findings only, culture evidently having failed.

Amies and Jones (1957), in Canada, were able to isolate a similar organism from 5·1 per cent of 371 cervical swabs, using serum yeast agar plates, which gave dew-drop colonies after 24 hours aerobic incubation. They thought that V factor encouraged growth and suggested that X factor was not required. Their organism failed to grow under complete anaerobiosis, unlike descriptions by other authors. In common with Lutz, Grootten and Wurch (1956), Amies and Jones failed to produce definite disease in animals by inoculation of *H. vaginalis*.

Brewer, Halpern and Thomas (1957) reported an investigation into 211 gynaecological cases aged 20–62, all complaining of leucorrhoea. Of these, 89 showed small Gram negative bacilli, either by microscopic or cultural means. Casman's agar medium as described by Leopold (1953) was used, 5 per cent defibrinated rabbit blood being added. Smooth dew-drop pin-point colonies were regarded as typical of *H. vaginalis*. No mention was made of haemolysis occurring on the blood agar and pure cultures could not be maintained for more than 2–3 sub-cultures. Of the 89 cases with *H. vaginalis* present, 59 were without trichomonas or candida, 21 showed trichomonas, 6 candida, and 3 both.

Up to the time of writing this, no reports have apparently been published on *H. vaginalis* infections in Britain.

The present work describes the diagnosis and incidence of *H. vaginalis* infection in various clinical groups, and its relation to other vaginal flora.

Materials and Methods

A. Collection of Specimens

Through the co-operation of the clinicians concerned, two well-soaked high vaginal swabs and two smears were obtained from 276 out- and in-patients in two Edinburgh hospitals. In addition, there were 3 cases of vaginal discharge in children (aged 2½–3½) seen at the Out-Patients' Department of a children's hospital. Thirty-nine cases had to be dropped from the series, due to treatment having been given before the specimen was taken, gross contamination of cultures having occurred, or later investigation having showed that they did not fit into the groups being studied. (Seven of these were, however, available for analysis of results as regards the relation of *H. vaginalis* to other pathogens, to pleuropneumonia-like organisms (PPLO), to pH and to type of vaginal flora.)

The 240 cases thus remaining were divided up as follows:

Antenatal group (*AN*): 44 normal pregnant women attending an ante-natal clinic at one of the hospitals. These were taken at random and consisted mainly of 2½–5½ month pregnancies. None of the women had more than a slight vaginal discharge, 2 patients with a definite discharge having been excluded from the group.

Puerperal pyrexia group (*PP*): 45 in-patients whose temperature rose to 99° F. (oral) or over, during the first week after delivery, without any obvious explanation such as breast abscess, urinary infection or venous thrombosis.

Puerperal control group (*PC*): 26 normal puerperal women in the same hospital as the PP group, whose temperature remained below 99° F. during the first week after delivery.

Gynaecological group (*G*): 42 women of reproductive age, who attended out-patient clinics at one of the hospitals, suffering from leucorrhoea, vaginitis and/or cervical erosion or cervicitis.

Gynaecological control group (*GC*): 42 cases in the same category as the above, except that they suffered from some condition other than those indicated, usually pelvic floor weakness.

Post-menopausal group (*PM*): it was considered advisable to separate these cases from G group, in view of the change in vaginal secretion and flora occurring at the menopause. The 18 women in this group suffered from the same conditions as those in G group.

Post-menopausal control group (*PMC*): 14 cases, the same as the PM group except that they suffered from conditions other than those just mentioned, mostly pelvic floor weakness.

Miscellaneous cases: 6 postnatal women with leucorrhoea, seen 6 weeks after delivery, and 3 cases of vaginal discharge in very young girls, already mentioned.

B. Laboratory Methods used in Examining Specimens

Measurement of pH

Preliminary tests, carried out with 5 per cent plasma in water, showed that the material of the swabs used had no effect on the pH of the fluid in which they were soaked, over the pH range likely to be encountered in vaginal secretions. It was considered therefore that the purposes of this study would be sufficiently served by measuring the pH of the vaginal secretion with a B.D.H. capillator, after expressing it from the specimen swab into 0·5 ml. of sterile tap-water (which was neutral in reaction).

Microscopic Examination

One smear was fixed by heat and stained Gram, alcohol being used for de-colorizing, the other was stained by Leishman's method. Diagnosis of flora type, cell contents, yeast infection and trichomoniasis (*v.* Liston and Liston, 1939) was made by examination of these slides. For diagnosing *H. vaginalis*, reliance was placed on culture rather than on film appearance, although it was usually possible to tell in advance from seeing numerous small Gram negative bacilli that this organism would be isolated.

Culture Technique

For routine culture, the digest blood agar medium developed by Professor Levinthal (when in this unit) was used. This consists of 2 per cent Evans peptone agar with the addition of 5 per cent horse digest and 6 per cent human citrated blood (rejected from the Blood Transfusion Service on account of age, etc.). The digest was prepared by the action of pancreatin on lean horse-flesh, as described by Levinthal (1931). The media used for isolation of pleuropneumonia-like organisms (PPLO) included thallium acetate soft blood agar, plasma thallium acetate agar and plasma thallium acetate sloppy agar, based on Edward (1947). *H. vaginalis* was recognized, after 48–72 hours incubation, by its minute haemolytic colony and confirmed by filming (Figs. 3, 4 and 5). Only those organisms which were definitely Gram negative were included although in the original smear and later in fluid cultures, a tendency to be Gram positive was noted in some strains. PPLO colonies were diagnosed from their microscopic appearance on soft agar plates, using oblique transmitted light as recommended by Edward (1954). The presence or absence of other organisms was also noted.

Results

The total numbers of isolations of different organisms in culture, or their detection in films (excluding Döderlein's bacillus) are given below, in descending order of frequency. (Percentages are shown in brackets.)

Name of Organism	*No. of Times Isolated*
Staph. albus	157 (63·6)
H. vaginalis	95 (38·1)
Diphtheroids	64 (25·9)
PPLO	45 (18·4)
Coliform bacilli	40 (16·2)
Anaerobic streptococci, etc.*	35 (14·2)
Non-haemolytic streptococci	32 (12·9)
Strep. faecalis	32 (12·9)
Monilia or yeasts	18 (7·3)
Strep. viridans	15 (6·1)
Trichomonas	13 (5·3)
Anaerobic Gram —ve bacilli	8 (3·2)
Staph. aureus	7 (2·8)
Bacilli of subtilis group	4 (1·6)
H. influenzae	3 (1·2)
Cl. welchii	2 (0·8)

Total number of specimens examined=247. No *Strep. pyogenes* or *N. gonorrhoeae* were isolated during the investigation. There were usually only a few colonies of *Staph. albus*, when present.

It will be seen that, apart from *Staph. albus*, *H. vaginalis* was the commonest organism, followed by diphtheroid bacilli, PPLO and other organisms. True *H. influenzae* was confirmed to be rare in high vaginal specimens, only 1 isolation occurring in a woman of reproductive age (a case of puerperal pyrexia). The two other isolations were from the children already mentioned.

Comparative Incidence of H. vaginalis *in the Various Clinical Groups*

The incidence of *H. vaginalis* in the various groups is shown in Table I and Figure 1. The

* Including micro-aerophilic streptococci and Veillonella.

TABLE I

Incidence of H. vaginalis *in the Various Groups*

	No. of Cases in Group	No. *H. vaginalis* +ve	Per cent *H. vaginalis* +ve
Puerperal pyrexia group	45	32	71·0
Puerperal control group	26	8	30·8
Antenatal group	44	12	27·3
Gynaecological group	42	18	42·8
Gynaecological control group	42	9	21·4
Post-menopausal group	18	7	38·9
Post-menopausal control group	14	4	28·6
Totals	231	90	38·9

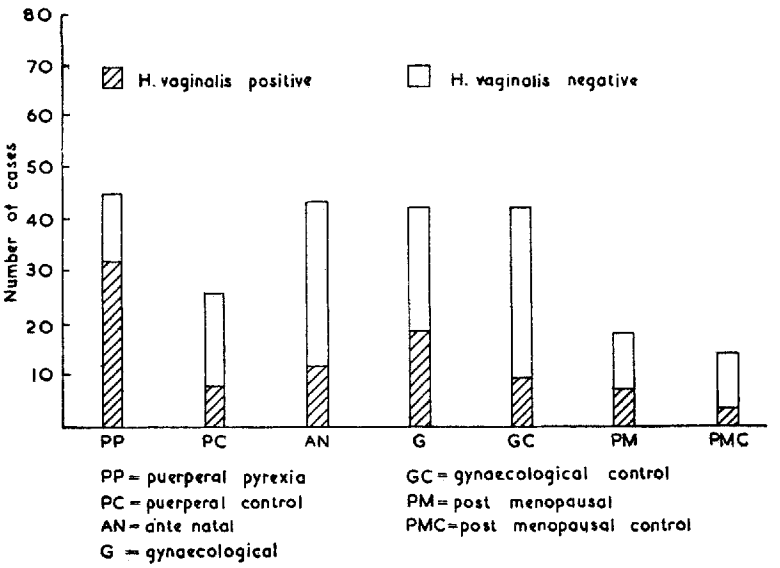

FIG. 1

Incidence of *H. vaginalis* in the different clinical groups.

highest incidence was recorded in the puerperal pyrexia (PP) group at 71 per cent. Next came the gynaecological group, 43 per cent, followed by the post-menopausal group, 39 per cent, and the 3 control groups, 31–21 per cent.

The χ^2 test was carried out and the probability of the observed differences in incidence being due to chance was shown to be negligible ($\chi^2=29{\cdot}23$, $P=<0{\cdot}01$). When this test was applied to the control groups only, however, (antenatal, puerperal control, gynaecological control and post-menopausal control) χ^2 was found to be only $0{\cdot}84$ ($P=>0{\cdot}8$), i.e., there was no significant difference in incidence of *H. vaginalis* in the various control groups.

Association of H. vaginalis *with Puerperal Pyrexia*

It will be seen that a very much higher incidence of *H. vaginalis* occurred in the PP group (71 per cent) than in the PC group (30·8 per cent). The Standard Error of the Difference

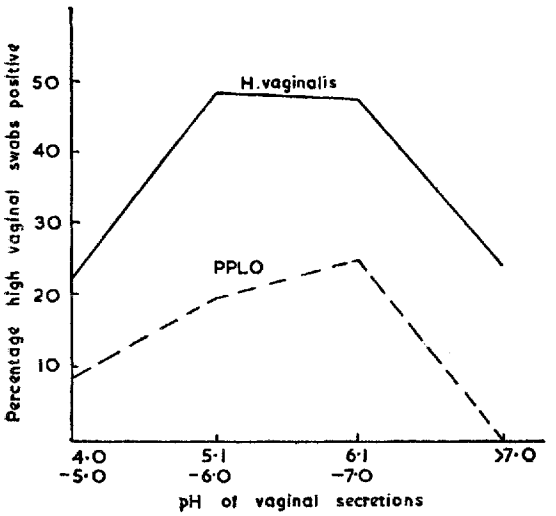

FIG. 2

pH and frequency of isolation of *H. vaginalis* and PPLO.

between these 2 proportions was found to be ±12·2 per cent. The observed difference was 40·2 per cent, i.e., 3¼ times S.E. The difference is therefore highly significant, indicating that an association exists between the occurrence of puerperal pyrexia of 99° F. or over in the first week after delivery and the isolation of *H. vaginalis* from high vaginal swabs.

Further analysis of the PP group findings seems to show a tendency for the incidence of *H. vaginalis* to vary within the group according to the severity of the pyrexia. This is seen in Table II.

A progressive increase seems to occur in the frequency of isolations of this organism from 59 per cent in the 99·0–99·4° F. (once only) group to 69 per cent in the intermediate pyrexia range and to 92 per cent in the 100° F. or over section. These figures are too small to be statistically significant. Similarly, the proportion of *H. vaginalis* +ve cases where this was the only likely causative organism was apparently higher in the 100° F. or over section than in the lower temperature groups. Again the figures are not statistically significant.

Association of H. vaginalis *with Leucorrhoea*

From Table I it can be seen that the incidence of *H. vaginalis* in the gynaecological group (42·8 per cent) is exactly double that in the control group (21·4 per cent). Statistical analysis shows that the S.E. of the difference between these proportions is ±10·2 per cent, while the observed difference is 21·4 per cent, i.e., just over twice the S.E. and therefore statistically significant. When the incidence of *H. vaginalis* in the groups G+PM (25 out of 60=42 per cent) is compared with that in groups GC+PMC (13 out of 56=23 per cent), the observed difference is 19 per cent, the S.E. being ±8·7 per cent, so that the observed difference is more than 2×S.E. and therefore again statistically significant.

TABLE II

Incidence of H. vaginalis *and Other Recognized Pathogens According to the Degree of Puerperal Pyrexia*

	Temperature		
	99–99·4° F. Once Only	99–99·4° F. More Than Once; or 99·5–99·9° F.	100° F. or More
No. of cases	17	16	12
No. *H. vaginalis* +ve	10 (59%)	11 (69%)	11 (92%)
No. *H. vaginalis* +ve showing other recognized pathogens	2	1	0

"Other recognized pathogens" defined as follows:
Strep. pyogenes isolated.
Anaerobic streptococci isolated.
Cl. welchii isolated.
H. influenzae isolated.
Staph. pyogenes isolated in moderate or heavy growth.
E. coli isolated in heavy growth.
Strep. faecalis isolated in heavy growth.

TABLE III

Relation of H. vaginalis *to Other Pathogens*

	No. of Cases	No. *H. vaginalis* +ve	Per cent +ve
Trichomonas present	14	3	21
Yeasts present	18	2	11
Other recognized pathogens present*	41	10	24
Yeasts absent	213	88	41

* Defined as in Table II.

TABLE IV

Relation of H. vaginalis *and PPLO Isolations to pH of Vaginal Secretion*

	pH Group			
	4·0–5·0	5·1–6·0	6·1–7·0	>7·0
No. of cases	74	46	100	8
No. *H. vaginalis* +ve	16 (21·6%)	22 (48%)	47 (47%)	2 (25%)
No. PPLO +ve	7 (9·4%)	9 (19·6%)	25 (25%)	0
No. *H. vaginalis* +ve (+growth only)	5	1	7	0

+growth=scanty growth, with few widely separated colonies in well only.

However, the difference between the PM and PMC groups is obviously not significant.

Relation of H. vaginalis *to Trichomonas, Yeast and Other Infections*

Table III shows the relation, for the combined groups, of *H. vaginalis* to trichomonas, yeasts and other recognized pathogens. The incidence of *H. vaginalis* in trichomonas positive cases is seen to be about 20 per cent and in cases with other recognized pathogens (not yeasts) about 25 per cent. These compare with the total *H. vaginalis* incidence of 38·9 per cent for the 7 main groups, the difference not being significant in either case. On the other hand, comparatively few instances were found of isolation of *H. vaginalis* from yeast infections (2 out of 18). This proportion of 11 per cent is significantly lower than the corresponding proportion of 41 per cent for the yeast-negative specimens (observed difference, 30 per cent, S.E. of difference ±12·0 per cent).

Table IV shows the incidence of *H. vaginalis* isolations in 4 different pH groups of vaginal secretions. The majority occurred in groups pH 5·1–7·0. It is surprising how many were isolated from secretions with quite low pH levels (21·6 per cent of 74 in the pH 4·0–5·0 group). As would be expected from the type of flora associated with this group, a higher proportion of these specimens gave a scanty (+) growth (about one-third) than the pH 5·0–7 group (about one-eighth). Table IV shows that PPLO isolations also occur more frequently from secretions in the upper pH range (6·1–7·0) than in the lower ranges.

Table V shows that the great majority of all isolations of *H. vaginalis* occur in +++ growth. This applies equally in disease and control groups (although this fact is not shown in the Table). The incidence of isolations was highest (53·2 per cent) from specimens whose microscopic appearance was that of type III (so-called "mixed bacterial") flora (see Cruickshank and

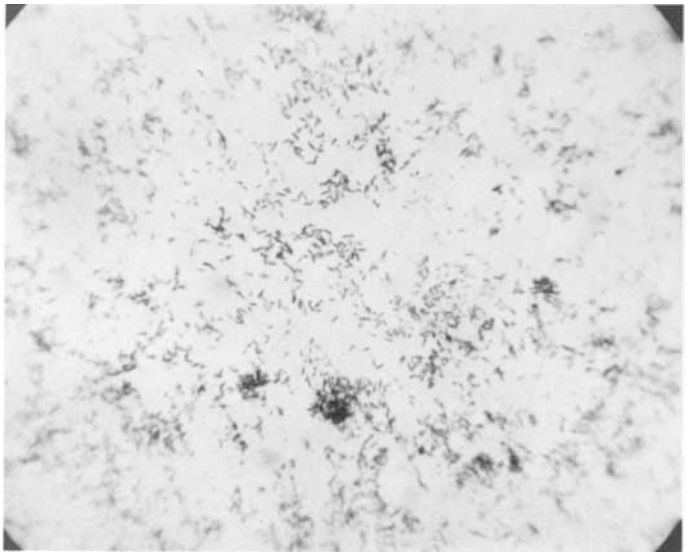

FIG. 3

3-day aerobic culture of *H. vaginalis* on blood agar, showing minute haemolytic colonies. ×2.

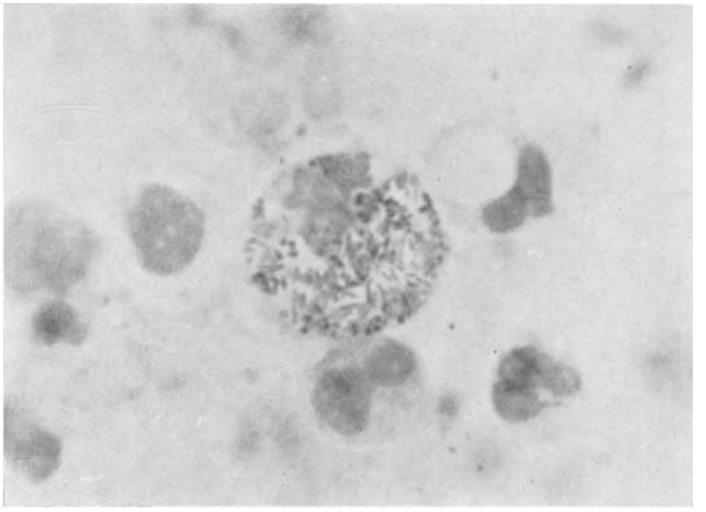

FIG. 4

3-day aerobic culture of *Strep. pyogenes* on blood agar. Note the very much larger size of the colonies as compared with *H. vaginalis* in Fig. 3. ×2.

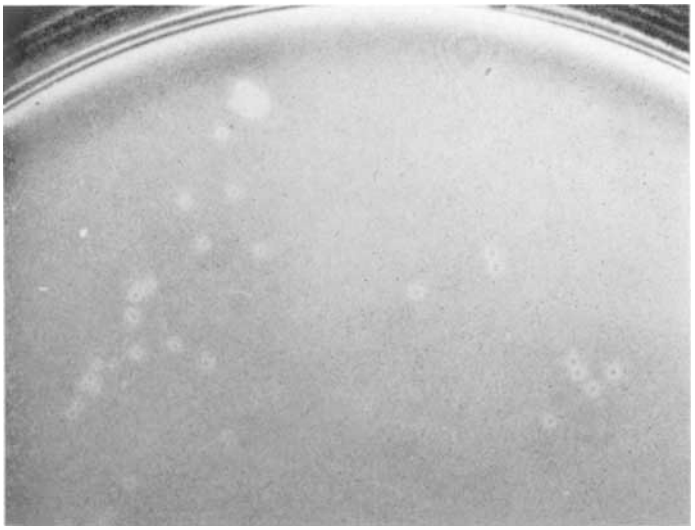

FIG. 5

48 hours maltose blood agar culture of *H. vaginalis* stained Gram. ×750.

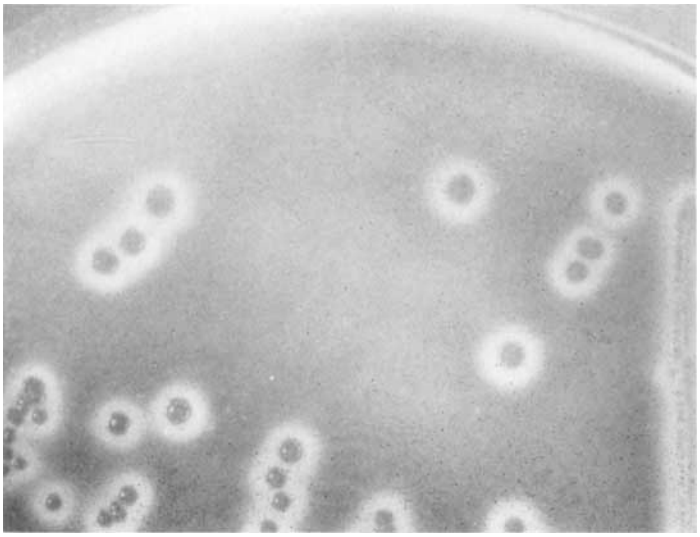

FIG. 6

High vaginal smear from case of puerperal pyrexia showing intracellular *H. vaginalis*. ×1,000.

TABLE V

Incidence and Volume of Growth of H. vaginalis, *and Incidence of PPLO, According to Vaginal Flora Type*

	Amount of Growth	Flora type			Total
		I	II	III	
No. of cases		78	17	115	210
No. *H. vaginalis* +ve	+	7	1	3	11 (5·2%)
	++	3	0	7	10 (4·8%)
	+++	2	4	57	63 (30·0%)
	Total	12 (15·4%)	5 (29·4%)	67 (53·2%)	84 (40·0%)
No. PPLO +ve		4 (5·1%)	3 (17·7%)	35 (30·5%)	42 (20·0%)

\+ as in Table IV.
++ moderate growth, with numerous closely set but separate colonies in well, few or none in periphery of plate.
+++ heavy growth, confluent in well and numerous colonies, after successive stroking, in periphery.

Baird, 1929–30, for definition of flora types). There was, however, a surprisingly large number isolated from type I flora (15·4 per cent). Most of these were + or ++ growth, and in only 2 cases was a heavy (+++) growth obtained from this flora type (both of these, and the majority of other isolations from type I flora, occurred in pregnant women).

A similar tendency can be seen for PPLO isolations, 30·5 per cent of type III flora specimens yielding this organism as against 17·7 per cent of type II and 5·1 per cent of type I.

Table VI shows the relation between isolations of *H. vaginalis* and PPLO from all specimens in the main groups. The incidence of PPLO isolations among *H. vaginalis* positive specimens (27·8 per cent) was more than twice as high as among *H. vaginalis* negative specimens (11·4 per cent). The observed difference between these percentages (16·4 per cent) is highly significant, being 3 times the S.E. of the difference (±5·15 per cent). There is therefore a definite association between the presence of *H. vaginalis* and PPLO in the specimens as a whole.

TABLE VI

Relation of H. vaginalis *to PPLO in All Main Groups and in 2 pH Groups*

	No. of Cases	No. *H. vaginalis* +ve	No. *H. vaginalis* −ve	No. PPLO +ve in *H. vaginalis* +ve Group	No. PPLO +ve in *H. vaginalis* −ve Group
All cases	231	90	141	25 (27·8%)	16 (11·4%)
pH of specimen 5·4 or less* ..	93	22	71	4 (18·2%)	3 (4·2%)
pH of specimen >5·4	134	64	70	21 (32·8%)	11 (15·7%)

* pH readings were not obtained from all specimens.

This association could be due to a common relation between the two organisms on the one hand and the vaginal pH and flora type on the other, as it has already been pointed out that both *H. vaginalis* and PPLO are associated with the higher pH ranges and with type III flora rather than with type I or II. However, the relationship still seems to hold good when the pH range is split into two separate parts, pH 5·4 or under, and over 5·4 (*v.* Table VI). The percentage of PPLO positives among *H. vaginalis* positive specimens of pH 5·4 or less is 18·2 per cent, and among *H. vaginalis* negative specimens only 4·2 per cent. In the higher pH range the corresponding percentages are 32·8 per cent and 15·7 per cent, the two proportions even in this case being significantly different (observed difference 17·2 per cent, S.E. ±7·45 per cent). There seems no doubt therefore that these two organisms are genuinely associated in high vaginal specimens.

The growth requirements of *H. vaginalis* have been investigated and details of this work will be published later. It grows well on autoclaved blood agar and so does not require either V factor or catalase. No evidence has yet been found for a requirement of X factor, the addition of which in varying concentrations fails to permit growth on serum digest agar aerobically, although anaerobic incubation allows growth to occur without X factor on this medium. Good growth is given by digest agar to which washed red cell stromata have been added, and red cell stroma constituents such as cholesterol and cephalin will allow some growth when anaerobic incubation is used. Good growth from heavy inocula is also obtained in thioglycollate broth, in the absence of eH indicator, but no growth has been obtained aerobically on any medium not containing major blood constituents.

The viability of the organism is poor, death being rapidly brought about by drying and storage at room temperature for 24 hours, even when kept moist. Rapid transport of specimens to the laboratory must therefore be arranged.

Sensitivity to Antibiotics and Local Applications

H. vaginalis is uniformly sensitive to the main antibiotics, particularly to penicillin. The majority, however, are resistant to sulphonamide and all are resistant to polymyxin. Of four local preparations tested, namely nystatin, acetarsol, "pruvagol" and gentian violet, only the last inhibited growth in the concentrations tested.

Animal Pathogenicity

Repeated attempts were made to produce disease by inoculation of rabbits, guinea-pigs and mice by a variety of routes with cultures of *H. vaginalis*. All failed, except that mild fever, lasting several days, followed the intra-peritoneal or intravenous administration to rabbits of large doses of the organisms.

Discussion and Conclusions

Clinically, the outstanding feature of this organism is its apparent ability to cause puerperal pyrexia. It was unfortunately not possible to inoculate volunteers with cultures of *H. vaginalis*, owing to the natural reluctance of clinicians to put their patients at risk during the puerperium, so that the evidence for its pathogenic role in this condition is limited to the association with pyrexia revealed by comparison with control cases. Thus, the incidence was 71 per cent in cases of puerperal pyrexia of 99° F. or over, as compared with 31 per cent in a control group collected at the same time. The high incidence of positive cultures throughout the series, even in control groups, naturally throws some doubt on the pathogenicity of the organism and it is clear that it does not rank with, for example, *Strep. pyogenes* as a pathogen of the female genital tract. A truer comparison would be with *H. influenzae*, a commonly occurring organism in the respiratory tract which is capable of both commensal and pathogenic roles.

The effects of puerperal infection with *H. vaginalis* are not confined entirely to mild degrees of pyrexia, as is seen by the occurrence of 12 cases with a temperature of 100° F. or more, from 11 of which *H. vaginalis* was the only likely causative organism isolated (*v.* Table II). This power to produce quite severe reactions in a minority of cases has been confirmed by experience gained since this series. In one case, a temperature of 103° F. has been recorded, without there being any cause found other than the

presence of *H. vaginalis* in large numbers in the genital tract.

The association found of *H. vaginalis* infection with the presence of leucorrhoea was not so marked as with puerperal pyrexia, but it does seem probable that this organism plays a part in vaginal and cervical pathology, particularly where no other recognized pathogens can be found.

Previous authors, e.g., Gardner and Dukes (1955), Wurch and Lutz (1955) have stressed the predominance of epithelial cells, and the absence or rarity of polymorphs in smears taken from vaginas infected with organisms similar to *H. vaginalis*. It has been found in the present work, however, that polymorphs are usually present, both in leucorrhoea and puerperal pyrexia cases, and often in large numbers. Moreover, some of these polymorphs exhibit marked phagocytosis of *H. vaginalis* (Fig. 6).

Bacteriological Diagnosis of H. vaginalis *Infections*

The tendency of the bacteriologist, when confronted with the confusing microscopic picture of a high vaginal smear, is to eliminate trichomonas, yeasts, gonococci and Döderlein's bacillus, and to lump together any other organisms under the term "mixed flora". It is suggested that, in many cases, these specimens contain in fact an almost pure flora of *H. vaginalis*. This is supported by the author's experience of cultures from such specimens, where even aerobic and anaerobic cultures incubated for 3 days have frequently revealed little other than a copious growth of *H. vaginalis*.

If anaerobic or aerobic 37° C. culture of a vaginal or cervical swab is carried out on the blood agar medium previously described, the appearance in 48 hours of minute haemolytic, transparent, apparently smooth colonies, consisting of small, slender or pleomorphic Gram negative bacilli, which fail to grow on ordinary medium, but grow well on autoclaved blood agar, indicates the presence of *H. vaginalis*.

Summary

A small haemophilic Gram negative bacillus, now identified as *Haemophilus vaginalis*, has been isolated from high vaginal swabs of 38·9 per cent of 231 women.

An association has been found between the isolation of this organism, usually in large numbers, and the presence of puerperal pyrexia and leucorrhoea. The incidence was 71 per cent in 45 cases of mild to moderate puerperal pyrexia, but only 31 per cent in a control group of 26 cases without pyrexia. The incidence in 42 gynaecological cases suffering from leucorrhoea, vaginitis and/or cervicitis was 43 per cent, but only 21 per cent in a control group of 42 women without these conditions.

The organism has been found to occur in association with pleuropneumonia-like organisms, but is rarely found along with yeast infections. Although present more frequently in the pH range of vaginal secretions of 5·1–7·0 many were also isolated from secretions of pH 4·0–5·0.

It is suggested that *H. vaginalis* is the main organism concerned in cases of so-called "mixed bacterial" infection of the vagina.

Although haemophilic, the organism does not require V factor or catalase, and probably does not require X factor. The main source of growth factor in blood is the red cell stromata.

Acknowledgments

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