DETECTION OF LEPTOTRIX VAGINALIS IN OBSTETRIC PATHOLOGY

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SUMMARY

The Authors report on the presence and classification of Leptotrix vaginalis in the vaginal flora of 150 women and compare cytologic and cultural data.

This microorganism was detected in 7 cases through observation of stained smears (Papanicolaou's method). But the cultural examination in many elective media showed different microbic forms in 5 cases and mycetes in two cases only. The Authors conclude that the observation of the vaginal fluor, fresh or in preparations stained according to Papanicolaou's method, is insufficient to identify this germ. The smear should always be supplemented by the cultural examination. The vagina is an organ without glands which nevertheless has a potential of selfdefence thanks to its particular histological structure and the chemical processes occuring in the tissues. However, the frequence of vaginites is increasing; the infection of the vagina may be asymptomatic, or present a particular symptomatology suggestive of the pathologic agent present. On the other hand, variable symptomatology may be referred without being able to identify the etiologic agent.

The development of new laboratory methods has enabled us to detect an increasing number of germs that can cause vaginitis. Vaginites produced by gonococcus, or known as 'specific', have decreased while, at the same time, those caused by trychomonas, mycetes, Haemophilus vaginalis, opportunist bacteria or other agents of uncertain classification have increased.

Over the last few years vaginites have been caused by Mycoplasms, by agents belonging to the family Chlamydozoaceae and, finally, by a parasite microorganism, Leptotrix, that has been detected and studied by cytologists. While the available information has enabled us to classify mycoplasms and Chlamydozoaceae from the taxonomic and nosologic standpoints, views still diverge on what is known as to Leptotrix, because of the great difficulties to culture it and detect its presence.

The aim of this study is to contribute to its identification by comparing cytologic and cultural data.

MATERIAL AND METHODS

This study concerns 150 women aged between 20 and 71 years, who came to our colposcopy center. Some of them presented vaginal troubles like leukorrhea, itching, smarting whereas others were asymptomatic. All patients underwent colposcopy, colpocytology with subsequent staining according to Papanicolaou's method, and sampling of vaginal secretion by tampon from the posterior fornix.

The obtained samples were placed in various selective culture media like:

- Lactosate Bromotimol blue agar for the isolation of enterobacteria (Wartz agar);

Table 1.											
			Development in specific culture i								
Case	Symptomatology	Associated microorganisms	Size	Gram	Wurtz Agar	Mannitol salt agar	Broth Sabouraud	Columbia blood in anaerobis	Thayer Martin medium	Thioglycolate agar in anaerobiosis	Medium for the isolation of Leptotrix
Anaerobe	Absent	α-Haemolytic streptococci				_	_	_	_	+	
e actobacillus	Absent	Staphilococcus epidermidis		+	_	_	+	+	_	_	_

Case	Symptomatology	Associated microorganisms	Size	Gram	Wurtz Agar	Mannitol salt agar	Broth Sabouraud	Columbia blood in anaerobis	Thayer Marti medium	Thioglycolate agar in anaerobiosis	Medium for the isolation Leptotrix sphaerotilus
1 Anaerobe	Absent	α-Haemolytic streptococci				_	_	_	_	+	_
2 Lactobacillus	Absent	Staphilococcus epidermidis		+	_	_	+	+	-	_	-
3 Lactobacillus	Absent			+	_	_	+	+	_	_	-
4 Lactobacillus	Absent			+	_		+	+		_	_
5	Leukorrhea Smart Itching Dyspareunia	Trichomonas staphilococcus		+	_	_	+	_	-	_	_
6 Bacillus	Leukorrhea Itching Dyspareunia	Haemophilus vaginalis Klebsiella Proteus mirabilis		±*	+	+	+	+	_		_
7	Leukorrhea Itching	Haemophilus vaginalis		+	_	<i>—</i>	+	_	-	-	_

Τ

* Coexistence of Gram + and Gram - form.

- Mannitol salt agar for the isolation of staphylococci (MSA);

- Columbia agar plus Isovitalex and mutton blood (5%) for the isolation of streptococci, Haemophilus vaginalis, lactobacilli and microaerophile microorganisms;

- Thayer-Martin agar with VCN for the selective isolation of gonococci;

- Sabouraud-Broth agar for the isolation of mycetes;

- Thioglycolate agar for the isolation of anaerobe microorganisms;

- Basal medium containing glucose (0.5%) and peptone (0.5%) for the isolation of Leptotrix sphaerotilus.

These cultures were subsequently incubated at 37 °C; CO₂ was added to the Columbia blood agar and the agar for the isolation of gonococcus, accounting for 10% of the medium.

RESULTS

When the smears, stained with Papanicolaou's method, were microscopically examined, filamentous microbial forms were observed suggestive of the presence of Leptotrix vaginalis in 7 patients (4.6% of the cases). At the objective examination 4 of them showed leukorrhea, itching and dyspareunia. The three remaining patients had no symptom (see table). But the culture of the vaginal tampon in various media enabled us to see that the cytologic detection of Leptotrix may be associated to different microbic forms.

Indeed, the cultural examination revealed (see table) in one case a leptotri-

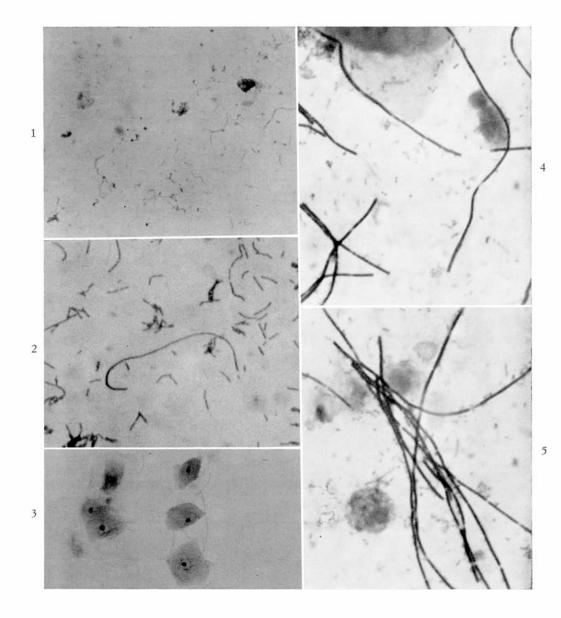


Fig. 1. — Leptotrichia vaginalis. From a thioglycolate culture in anaerobiosis $(40 \times)$. Fig. 2. Leptotrichia or leptotrichiform lactobacilli in a smear stained according to Papanicolaou's method $(70 \times)$. Fig. 3. Bacteria belonging to the Bacillus type in a smear stained according to Papanicolaou's method. Fig. 4. Leptotrix vaginalis, picture in "immersion" $(100 \times)$. Fig. 5. Enlargement of a particular of the previous preparation.

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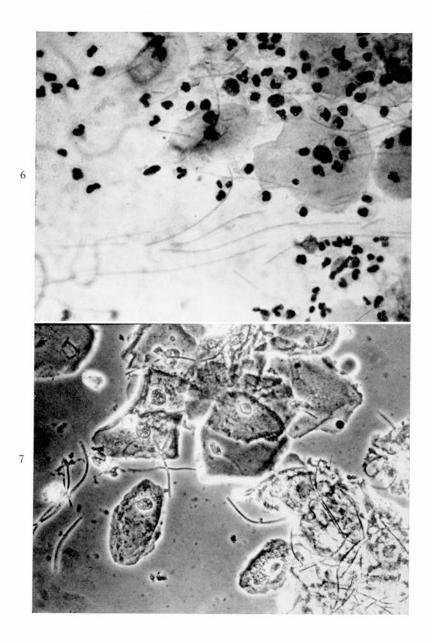


Fig. 6. — Leptotrix vaginalis in a phase-contrast fresh preparation (600 \times). Fig. 7. — Leptotrix vaginalis in a smear stained according to Papanicolaou's method.

chia vaginalis (Leptotrix vaginalis according to some Authors), a gram-negative anaerobic microorganism taxonomically classified as Fusobacterium (fig. 1); in three cases bacilli belonging to the kind 'Lactobacillus', known in the past as Döderlein's bacilli (Lactobacillus acidophilus, lactobacillus casei, etc.) which, at low pH levels, may assume the morphology of a leptotrichia (fig. 2) though being grampositive; in one case a microorganism belonging to the Bacillus kind (fig. 3) which, in an unfavourable medium (low pH) may assume its normal shape and in a lengthened shape that can easily be mistaken for Leptotrix.

Only in two cases did the cultural examination reveal microorganisms finding favourable conditions for their development exclusively in the Sabouraud agar which is specific for mycetes. The size of these microorganisms was similar to that of mycetes and their septate structure was typical of mycelial hyphae, unlike all the other cases (figs. 4, 5, 6, 7).

DISCUSSION AND CONCLUSIONS

Leptotrix vaginalis is known as a microorganism which entails good many problems with regard to both its taxonomic classification and etiologic significance.

In the smears, stained according to Papanicolaou's method it usually appears as a long, filiform, dark, non-branched, often isolated or gathered in hair-lock-like masses of microorganisms (⁷). It can sometimes be S- or U-shaped and its length can exceed the diameter of the vaginal surface cells. It is often associated to Trichomonas vaginalis or Candida.

Most Authors (1, 3) consider it as a bacterium, whereas others (4, 10) refer to it as a filamentous, unclassified fungus.

However, both views rely on the mere cvtologic examination not supplemented by the cultural examination of vaginal secretion. Thanks to the cultural examination performed on patients presenting Leptotrix forms, we have been able to identify microorganisms which can present the same morphology in particular conditions of vaginal pH, though with different characteristics of size and colour.

The form cytologists regard as 'Leptotrix' can be caused by:

1) some lactobacilli (Lactobacillus cereus, Lactobacillus acidophilus, L. fermentosus, L. cellobiosus);

2) bacteria belonging to the Bacillus type, which, in unfavourable pH conditions (acid pH) can become either a long shape (leptotrix-like) or the typically stumpy one;

3) the anaerobic bacterium classified as Fusobacterium, known as Leptotrichia vaginalis, gram-negative unlike the two previous forms;

4) a microorganism which, in view of its cultural characteristics (it grows in antibiotized Sabouraud agar, which is the elective medium for mycetes), its size and septate structure, should be classified as a mycete.

Only the last form should be regarded as Leptotrix vaginalis. At the bacteriologic examination (fresh) it presents the characteristics of mycelial hyphae.

No specific pathogenesis can be identified for this microorganism since it was detected in association with Trichomonas in one case and Haemophilus in the other. The symptomatology observed by these patients cannot therefore be exclusively ascribed to Leptotrix.

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