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# Intestinal Spirochaetosis as a Cause of Longstanding Diarrhoea

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## ABSTRACT

**This paper deals with the clinical history, the histopathological and scanning electron microscopy (SEM) pictures, treatment and follow up of four cases of intestinal spirochaetosis. Diarrhoea was a prominent symptom in all cases but distension and vague abdominal trouble were also present. It is suggested that heavy infestation of the gut surface epithelium by spirochaetes acts as a barrier for the normal absorptive processes and leads to diarrhoea.**

## INTRODUCTION

Harland & Lee (3) and Lee et al. (4) from the Western Infirmary in Glasgow described a condition in which the surface epithelium of the gut becomes colonised by a type of spirochaetes classified as Borrelia eurygyrata. This was accompanied by variable symptoms and was also seen in cases of bowel carcinoma. However, the pathogenicity of intestinal spirochaetosis remained obscure and only one more case was reported from North Carolina (2).

In this work we describe the first cases of intestinal spirochaetosis discovered so far in Sweden. The Scanning electron microscopy (SEM) findings suggest that the extent and heaviness of colonisation of the gut epithelium is the main cause of the clinical symptoms especially diarrhoea.

## MATERIALS AND METHODS

This study was based on four patients, three females and one male, with no history of other relevant diseases:

*Patient I*

A. B., a 54-year-old Swedish man, who since a visit to Kenya in 1972 suffered from watery diarrhoea up to 15 times per day. Barium enema revealed only dehaustration of the sigmoid colon. Colonoscopy showed slight oedema of the colon mucosa.

In March 1976 multiple biopsies were taken from the transition between the descending and sigmoid colon. These showed intestinal spirochaetosis. Further biopsies were taken from the duodenum and rectum to study the extent of the infestation. Material from the rectum was also fixed and processed for scanning electron microscopy (SEM). A rectal swab was taken in June for cytological examination. The patient received oral courses of penicillin-V (Kåvepenin) 1.2 g per day for ten days, doxycycline (Vibramycin) 0.1 g per day for ten days and neomycin 4.0 g per day for five days. Rectal biopsies were taken after every course to study the effect of the treatment.

*Patient II*

G. P., a 41-year-old Swedish woman with fibroadenosis of the right breast diagnosed in 1972 and uterine leiomyoma in 1974. In April 1976 hysterectomy and appendectomy were performed. Histological examination of the appendix showed heavy infestation with spirochaetes and subsequent thorough history-taking revealed periods of alternating diarrhoea and constipation for several years and that the patient has never been outside Sweden. Further biopsies were taken from the stomach, duodenum, sigmoid colon and rectum. The patient was treated with Neomycin and a subsequent rectal biopsy was examined by light microscopy.

*Patient III*

M. P., a 17-year-old Swedish girl (daughter of patient II), who suffered from abdominal distension for a few months before clinical examination in May 1976. She had never had any attacks of diarrhoea. Rectoscopy was performed in June 1976 and a biopsy at 15 cm was taken.

*Patient IV*

P. K., a 46-year-old Swedish woman who presented in August 1976 with a history of pain in the lower part of the abdomen for several months together with mucinous diarrhoea for several years but no bleeding per rectum. Rectoscopy showed oedematous rectal mucosa and rectal biopsy was taken.

All biopsies were fixed in 10% neutral buffered formalin prior to processing in the ordinary way for light microscopy. Sections were cut at 5$\mu$m and slides from all

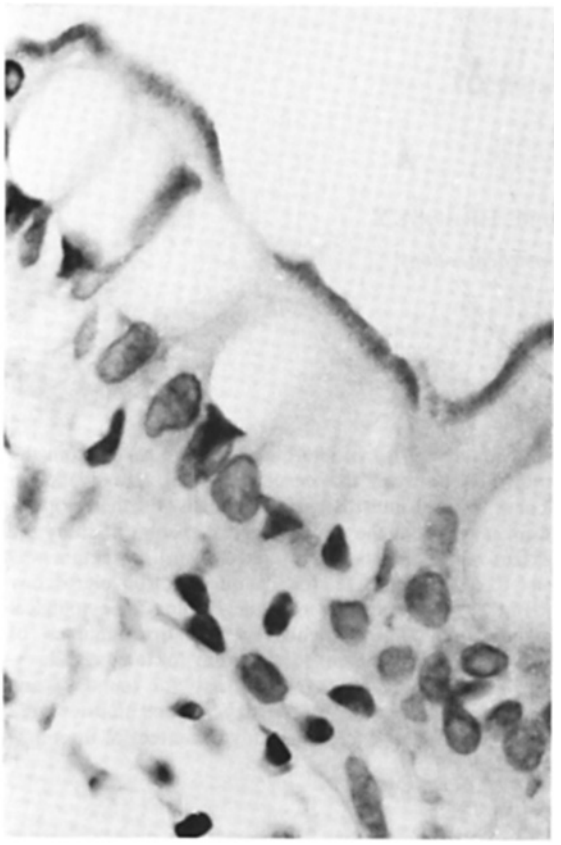

*Fig. 1.* The fuzzy haematoxyphilic band-like line of spirochaetal infestation of the surface epithelium of rectal mucosa. Haematoxylin-eosin. Original magnification ×400.

cases were stained with haematoxylin and eosin (H & E), Van Gieson (V. G.), periodic acid Schiff (PAS), Gram stain and Grocott method.

For scanning electron microscopy (SEM) tissues were cut into small cubes measuring about 3 $mm^3$. These tissue cubes were fixed for 12 hours at 0–4°C in 2% vacuum distilled glutaraldehyde in 0.1 M sodium cacodylate-buffer with 0.1 M sucrose (pH 7.2, 510 mOsm).

The tissues were rinsed briefly in 0.15 M cacodylate buffer pH 7.2 at 22°C then postfixed in 2% Os $O_4$ in coloidine buffer (pH 7.2) for 90 min at 22°C, dehydrated in graded ethanol (50, 70, 75 and 80%) then into acetone/water (80, 85, 90 and 95%) and absolute acetone. The tissues were subsequently dried in liquid carbon dioxide in a Polaron E 3000 apparatus (1). After mounting on stubbs with silver conductive paint, the specimens were vacuum coated with gold in a Polaron E 5000 diode sputtering system run at 30 mA, 1.2 kV, 90 s giving a gold layer of 270 Å in thickness. The specimens were studied in a Jeol JSM-S1 microscope run at 10 kV and using 100 sec exposure time for the micrographs.

## RESULTS

In all four cases the diagnosis of intestinal spirochaetosis was established by light microscopy using haematoxylin and eosin (H & E) stained slides. Colon and rectal biopsies as well as the appendix from the second case showed a fuzzy blue line, about 3 μm in thickness which covered the surface epithelium but did not extend deep into the mouths of the crypts or into the glandular epithelium. By higher magnifications (×40) this blue haematoxyphilic line was seen to consist of tiny rod-like structures perpendicular to the surface epithelium (Fig. 1). This line stained dark black by the Grocott method but both PAS and Van Gieson failed completely to demonstrate any trace of the spirochaetes. All other components of the large bowel mucosa were normal in all respects.

Biopsies from the descending and sigmoid colons and rectum of the first patient showed heavy colonisation in the form of a rather continuous line only interrupted by the mouths of the crypts and occasionally by a goblet cell. The same picture persisted after treatment with penicillin-V. The rectal swab revealed clusters of aggregated spirochaetes with individual organisms in the periphery. The presence of the spirochaetes in the swab was strongly suspected in haematoxylin and eosin stained preparations but was confirmed by the Grocott method which only stained the spirochaetes leaving the other bacteria and contaminants negative. Slides stained with haematoxylin and eosin, Grocott and Gram methods help in the identification and differentiation of the spirochaetes from the other components of the mixed bacterial flora of the gut (Fig. 2).

After the doxycycline course the degree of infestation was much reduced although patchy areas remained as prominent as in the first biopsy. In the biopsy taken after treatment with neomycin the surface of the gut was completely free of spirochaetes.

Spirochaetosis was accidentally diagnosed in the second patient by examining the appendix which was removed during the hysterectomy operation done for leiomyoma of the uterus. Subsequent biopsies from the colon and rectum confirmed the presence of spirochaetes in both localities. However, gastric and duodenal biopsies from this patient and duodenal biopsy from the first patient were negative. In the rectal biopsy taken one week after

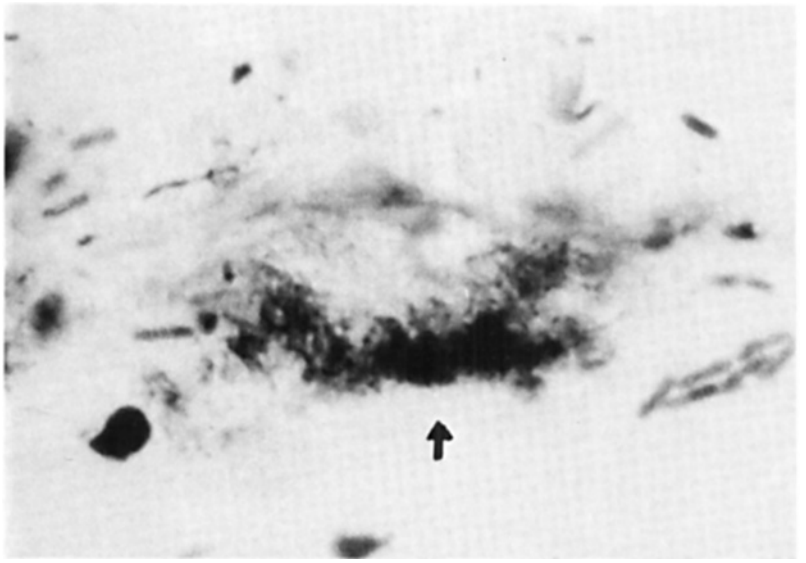

*Fig. 2.* Clusters of spirochaetes (arrow) from rectal swab. Grocott stain. Original magnification ×400.

treatment with neomycin the characteristic haematoxyphilic fuzzy line was absent from the surface epithelium. Clinically the patient was free from any symptom including the diarrhoea until the last visit in September 1976.

Rectal biopsy from the third patient showed only minimal, rather sporadic infestation of the surface epithelium. However, the patient was not fully investigated and subsequently not treated as she moved from Falun shortly after the diagnosis. The fourth patient is being investigated at the present time for studying the heaviness and extent of infestation of the intestinal tract by spirochaetes before starting the treatment.

Scanning electron microscopy (SEM) was done on material from the first patient. The surface of the gut was covered by a continuous thick layer of spirochaetes which almost completely masked the underlying structures. As in light microscopy the openings of the glands were less colonised. Only a few spirochaetes were seen hanging around the borders of the glandular openings on the surface epithelium. The individual spirochaetes were wavy, showing 3 to 4 incomplete spirals. Some spirochaetes were, however, seen in the non-contracted state as rather straight filaments with striated body showing alternating light and dark lines. In the contracted state the spirochaetes were almost equal in length to the diameter of the red blood corpuscle (Fig. 3), and the outline of the body of most organisms showed rather regular fine serrations.

No attempt was made to measure the exact length of the spirochaetes as the vast majority of them showed only parts of the body while the rest was either entangled in the forest of the surrounding spirochaetes or hidden deep in the underlying structures. Added to that is the inherent limitation of the Scanning method caused by the tilting angle error.

## DISCUSSION

The Genus Borrelia comprises several species of spirochaetes which are morphologically similar but exhibit widely different pathogenic tendencies and host range. The Borrelia group of spirochaetes are distinguished from treponema and leptospira by being longer, up to 30$\mu$m with the spirals deeper, more loosely wound and more flexible (5). These organisms are not readily cultivable on artificial media.

Intestinal spirochaetosis was accidently discovered by Harland & Lee (3) when a rectal biopsy from a patient with persistent diarrhoea for three years, interpreted as being normal by light microscopy, was subjected to electron microscopy. This revealed the presence of the spirochaetes. A retrospective study of rectal biopsies and appendix

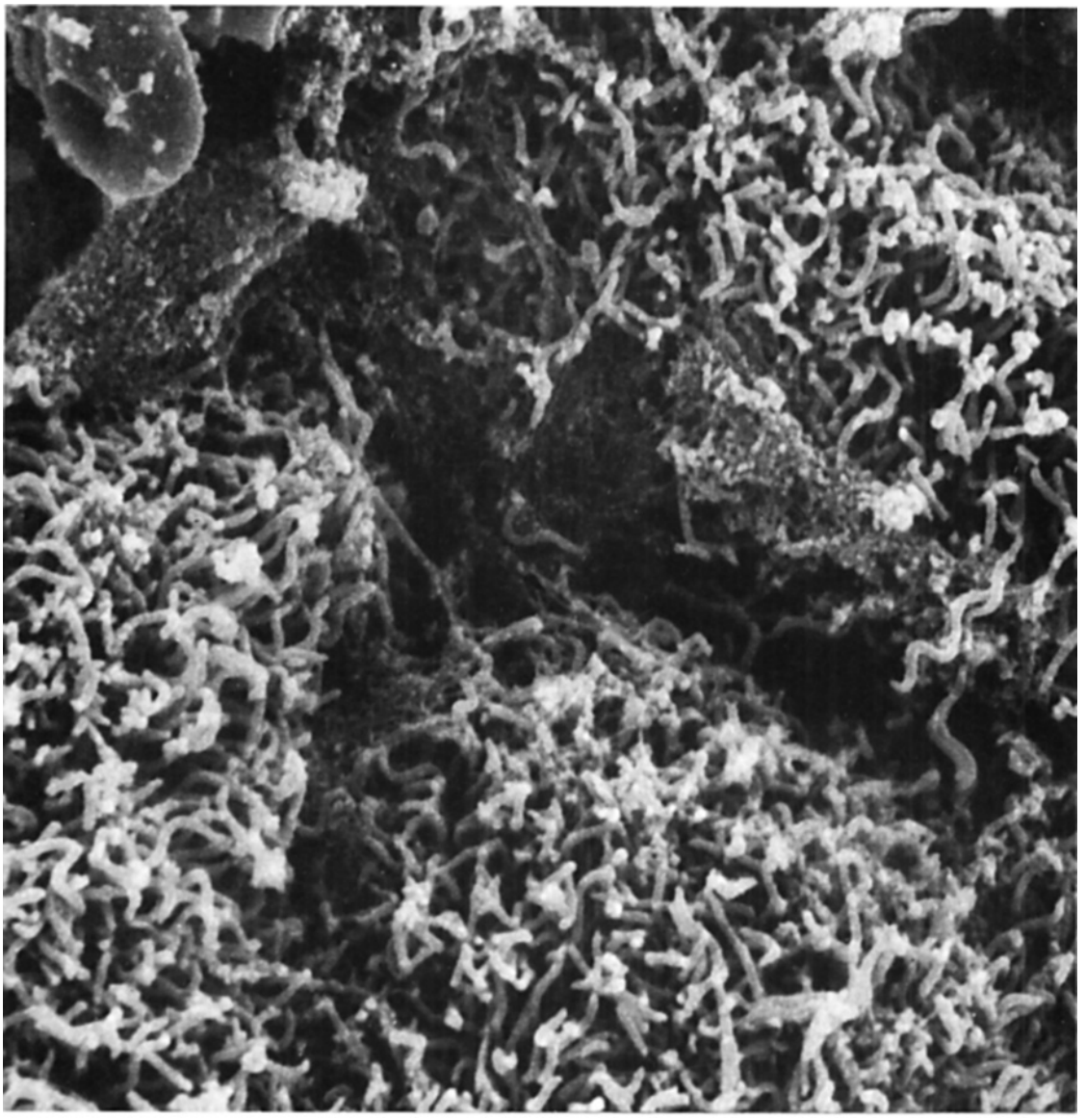

*Fig. 3.* Scanning electron microscopy picture of the surface of the bowel showing heavy infestation with spirochaetes. Compare the size of the spirochaetes with the red blod corpuscle in the upper left corner of the picture. Magnification is about ×6 000.

specimens revealed an incidence of spirochaetosis in 4.3% of rectal biopsies from non tumour cases, 3.7% in incidental appendectomy and 9.8% in cases simulating appendicitis. In this retrospective study the positive cases were diagnosed by ordinary light microscopy using haematoxylin and eosin stained slides and confirmed by electron microscopy.

It is well established now that the light microscopic picture with the 3 $\mu$m thick haematoxyphilic band like layer of spirochaetes covering the surface epithelium is diagnostic. However, one has to bear in mind the existence of this entity and to stain all large bowel biopsies with haematoxylin and eosin if this stain is not used routinely in the laboratory. In cases of doubt or in cases in which the infestation is sporadic or minimal the Grocott method should be helpful in establishing the diagnosis.

It seems that infestation of the intestinal tract by spirochaetes is more frequent than realised. From North Carolina Gear & Dobbins (2) demonstrated identical findings in a rectal biopsy obtained from a patient with systemic lupus erythematosus who had no gastrointestinal symptoms and a normal proctoscopy. It also seems that it is of a widespread geographical incidence. Although one of our four patients had been to Kenya before the diarrhoea started, the other patients have never been outside Sweden. Three of the four patients presented with periods of diarrhoea for several years which in the first case was almost five years. The main complaint in the third patient was only abdominal distension. Lee et al. (4) reported the association of intestinal spirochaetosis with carcinoma of the bowel as well as in cases of Crohn's disease, ulcerative colitis, ischaemic colitis, diverticulitis, haemorrhoids and polypi. They have also reported two out of 14 cases of intestinal spirochaetosis in rectal biopsies without any associated disease. In their retrospective study Lee and his collaborators used material most of which was from already diagnosed cases of different types of inflammatory disease or malignancy. This perhaps explains the low incidence of cases of intestinal spirochaetosis without other associated disease in their series. It also emphasizes the importance of suspecting spirochaetosis in conditions in which there is diarrhoea or other intestinal symptoms without positive radiologic, endoscopic or histologic positive findings.

Lee et al. (4) could not estimate the extent of spirochaetal colonisation and did not have enough evidence to substantiate the pathogenicity of infestation by this type of spirochaetes. Nevertheless they stated that they had indirect evidence that the condition might be quite extensive. Gear & Dobbins (2) thought that the spirochaetes in their case were harmless commensals in rectal mucin curiously adherent to surface columnar cells and orientated longitudinally between the microvilli. This motivated us to estimate the extent of infestation by studying multiple biopsies from different parts of the gastrointestinal tract. The Scanning Electron Microscope (SEM) was also of great help in revealing the remarkable degree of infestation of the gut as demonstrated in our first case.

This gives a clue to the way these organisms can cause symptoms. The presence of such a thick layer of organisms on the surface epithelium of the colon or rectum should at least act as a mechanical barrier for the reabsorptive processes which take place in the large bowel lumen and hence the watery diarrhoea. Furthermore the organism might lead to mechanical irritation of mucin-secreting cells on the surface of the gut leading to more production of mucin. The pathogenecity of the organism thus seems to depend on the extent and degree of infestation. In cases in which the whole surface of the large bowel is heavily infested one expects that the main symptom could be watery diarrhoea. Cases with only limited, sporadic or light infestation could be either silent or manifest themselves with other minor troubles such as distension. On the other hand as Lee and his group suggested the spirochaetes seem to be common survivors in the gut of many persons. It could be advanced that the extent and degree of infestation of the bowel are the two main factors which determine whether the condition would be symptomatic or not. The overgrowth of the spirochaetes in the symptomatic cases could be due to some factor or factors which upset the balance of the bacterial flora in the gut.

The most effective treatment in cases of intestinal spirochaetosis from our own limited experience is a short course of Neomycin. Penicillin-V has failed completely to affect the infestation whereas doxycycline did not cause complete disappearance of the spirochaetes. Recurrence of the symptoms and reappearance of spirochaetes in biopsies should be expected if the cause or causes which upset the balance of the intestinal flora and overgrowth of the spirochaetes persist or recur. A long period of follow-up of our patients is planned to try to answer some of the obscure aspects of this entity. However, we feel that on the evidence provided by the scanning microscopy, intestinal spirochaetosis should be considered as an entity. It should be suspected and looked for in any condition of long-standing diarrhoea or other intestinal symptoms of obscure nature.

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