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Treponema pallidum Immunohistochemistry is positive in human intestinal Spirochetosis

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Abstract

Background: Human intestinal spirochetosis (IS) has been recognized for decades, but whether it represents commensalism or a pathogenic process remains controversial. IS is diagnosed on routine stains with confirmation by silver stains but these stains are labor intensive and slow to read. We evaluated the *Treponema pallidum* immunostain as a diagnostic adjunct for IS.

Methods: We retrieved biopsies from 33 patients with IS for this study. Each case was tested by Warthin-Starry (WS) and *T. pallidum* immunohistochemistry (IHC). Species specific genotyping was performed in 3 cases.

Results: Patients with IS ranged from 22 to 82 years without gender predilection. IS involved normal ($n = 15$), and inflamed ($n = 5$) mucosa and colonic polyps ($n = 13$). Warthin-Starry and *T. pallidum* IHC were positive in all cases including both species of *Brachyspira*. Six (18%) symptomatic patients were treated for IS, and experienced resolution. In patients diagnosed with incidental IS on cancer screening ($n = 5$), follow up biopsies, without therapy, were negative for IS. *T. pallidum* IHC required 75 min less hands-on time than WS for performance and was faster to interpret.

Conclusions: *T. pallidum* IHC can be used to confirm the diagnosis of IS and is easier to perform and faster to interpret than WS.

Keywords: Intestinal spirochetosis, *Treponema*, Diarrhea, *Brachyspira*, Warthin-starry, *B. aalborgi*, *B. pilosicoli*

Some of these data were presented at the 105th United States and Canadian Academy of Pathology Annual Meeting in Seattle, WA and have been published in abstract form [1].

Background

Intestinal spirochetosis (IS) is diagnosed on routine sections with identification of a thick band of basophilia at the apical aspect of the intestinal mucosa on low magnification. On high magnification, this basophilic band can be resolved as linearly arranged spiral organisms attached to the apical aspect of the colonocytes at the mucosal surface. The diagnosis is most often confirmed by a silver stain such as Warthin-Starry or the Steiner stain. Although most cases are identified initially by routine hematoxylin and eosin (H&E) stain, the presence of a microvillous

brush border along the surface of colonocytes can be a diagnostic pitfall, and confirmatory staining is sometimes necessary.

IS is caused by *Brachyspira* species, *B. aalborgi* and *B. pilosicoli* [2, 3], and has been recognized for decades, but whether it represents commensalism or a pathogenic process is still debated [4–6]. Authors have suggested that IS is often incidentally discovered and has no apparent clinical consequence but in some cases there is a diarrheal illness which resolves after metronidazole treatment and may be associated with an immunocompromised state [5, 7, 8]. It has been suggested that the presence of invasion into the cytoplasm of epithelial cells or lamina propria indicates symptomatic infection, but this has only been examined by ultrastructural study in a total of 5 cases [9, 10]. The *Treponema pallidum* immunostain has been shown to be positive in cases of intestinal spirochetosis in a prior study [8] but this was not the main focus of that study. We therefore evaluated the

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Treponema pallidum immunostain, which cross-reacts with *Brachyspira* species, as a diagnostic tool for IS.

The *T. pallidum* immunostain can be performed by standard automation methods unlike the manual Warthin-Starry method. In addition, the Warthin-Starry stain can be challenging to interpret with significant background staining. As such, we also designed this study to compare the technical hands on time to perform Warthin-Starry and *T. pallidum* immunohistochemistry and the interpretive time for the pathologist reviewing these stains.

Methods

Cases

In this institutional review board exempt study, we retrieved colonic biopsies diagnosed as IS from 1998 to 2015 where residual formalin fixed paraffin embedded tissue was available from the authors' institutional files. The diagnostic slides were reviewed to confirm the diagnosis, assess for concomitant pathology and to select a paraffin block for ancillary study (RPG, BVN, SSS, DLH). We also retrieved 10 control cases (normal colon ($n = 2$), lymphocytic colitis ($n = 3$), cryptosporidiosis ($n = 4$) and active chronic colitis ($n = 1$)). All cases and controls were reviewed and scored after performing special stains (RPG, BVN, DLH).

Histochemistry and Immunohistochemistry

Each case was uniformly tested by Warthin-Starry (WS) and *T. pallidum* immunohistochemistry (IHC). For WS, all glassware to be used in stain preparation was rinsed with acidulated water. Twenty and 30 ml solutions of 5% gelatin and 0.15% Hydroquinone were prepared in separate flasks and heated to 60 °C for an hour. Silver nitrate solution (2%) was prepared in a water rinsed flask. A coplin jar and 50 ml graduated cylinder were heated to 60 °C for an hour. The glass slides with formalin fixed paraffin embedded tissue were deparaffinized and then hydrated during that time. Next, the 2% silver nitrate, 5% gelatin and 0.15% hydroquinone were added sequentially to the warmed coplin jar with stirring. This was called the developer solution. The hydrated slides were placed in 1% silver nitrate solution and then into a 43 °C water bath for 30 min. The slides were then transferred to the developer solution and placed in a 43 °C water bath for between 30 s to 1 min with close observation. The slides were then washed quickly and thoroughly in running hot tap water. The slides were then rinsed with distilled water. Each slide was dehydrated in alcohol followed by xylene treatment and coverslipping. IHC for *T. pallidum* was performed as described by Arnold et al. [11] with the

exception that the Ventana platform was used instead of the Leica Bond platform.

The hands-on technical time for WS and *T. pallidum* IHC was determined by stopwatch for a group of 11 randomly selected cases simultaneously submitted for staining. Also, the microscope time for these 11 cases and 5 control cases was assessed for two blinded independent observers (MST and SSS) who reviewed each case and control with WS and *T. pallidum* IHC with a 10-day interval between WS and *T. pallidum* IHC review. During the 10-day interval, these observers (MST and SSS) were part of the daily routine clinical service providing a sufficient wash out between interpretation of WS and *T. pallidum*.

Genotyping

Species specific genotyping was performed in 3 cases using a previously published method [12]. In brief, genomic DNA was extracted from spirochetes that had been microdissected from formalin-fixed, paraffin-embedded histologic sections and was subjected to polymerase chain reaction (PCR) analysis. PCR primers were designed to amplify a non-conserved region of the bacterial 16S ribosomal RNA gene, permitting identification of *B. aalborgi* and *B. pilosicoli* based on sequence alignment.

Clinical information

Patient demographics, abdominal complaints (diarrhea, abdominal pain/cramping, bloating, rectal bleeding), indication for colonoscopy, colonoscopy reports, clinical follow up and follow up biopsies were examined blinded to the results of ancillary tests.

Results

We identified 33 patients with IS including 18 men and 15 women ranging from 22 to 82 years. The indications for colonoscopy were as follows: colorectal cancer screening ($n = 19$), unexplained diarrhea ($n = 8$), abdominal pain ($n = 2$), hematochezia ($n = 2$) and anemia or low vitamin B12 ($n = 2$). Four patients were human immunodeficiency virus (HIV) positive. The colonoscopic impressions were available for 32 (97%) patients and were normal ($n = 12$), polyps ($n = 13$), diverticular disease only ($n = 2$), diverticular disease with polyps ($n = 1$), aphthous ulceration ($n = 2$), changes of colitis ($n = 1$; in a patient with a history of Crohn's disease), ulcer in rectum but otherwise normal ($n = 1$).

IS involved normal mucosa ($n = 15$), inflamed mucosa ($n = 5$) and both the neoplastic and non-neoplastic mucosa adjacent to adenomatous ($n = 8$), serrated ($n = 3$) and hyperplastic polyps ($n = 2$). Representative photomicrographs are shown in Fig. 1.

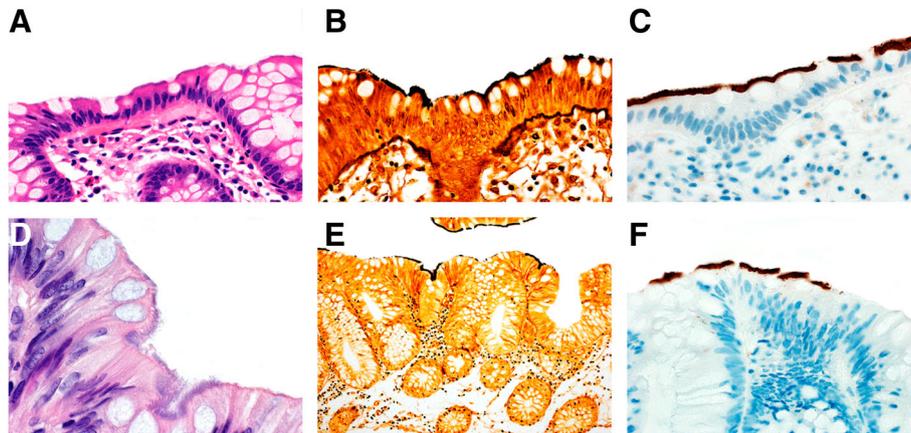


Fig. 1 **a** Hematoxylin and eosin stained section of colonic epithelium showing the characteristic histology of intestinal spirochetosis (Original magnification $\times 600$). **b** The Warthin–Starry stain highlights the *Brachyspira* species along the apical aspect of the colonic epithelium (Original magnification $\times 600$). **c** The *T. pallidum* immunostain robustly highlights the *Brachyspira* species without significant background staining (Original magnification $\times 600$). **d** A tubular adenoma removed as part of cancer surveillance screening is colonized by intestinal spirochetosis (Original magnification $\times 1000$). **e** The Warthin–Starry stain decorates the organisms (Original magnification $\times 400$). **f** The *T. pallidum* immunostain is similarly positive for intestinal spirochetosis (Original magnification $\times 1000$)

***T. pallidum* IHC is 100% sensitive for intestinal spirochetosis and highlights tissue invasion**

WS ($n = 33$ of 33) and *T. pallidum* IHC ($n = 31$ of 31) were positive in all cases of IS (Fig. 1) including both species of *Brachyspira*. *T. pallidum* IHC displayed no background staining. Spirochetes were highlighted within colonic epithelium, lamina propria and lamina propria macrophages by the *T. pallidum* immunostain in 30 (97%) of cases (Fig. 2). Only surface epithelial organisms were seen by WS. None of the 10 controls were positive by WS or *T. pallidum* IHC.

T. pallidum* IHC and Warthin-starry detect both *B. aalborgi* and *B. pilosicoli

Genotyping results from 3 cases revealed 2 cases of *B. aalborgi* and 1 case of *B. pilosicoli* affecting a male patient with HIV who presented with a history of diarrhea. No clinical follow up was available for these 3 cases but *T. pallidum* IHC and WS were positive in each of the three cases, confirming the sensitivity of these stains to both genotypes of *Brachyspira*.

Clinical follow up of intestinal Spirochetosis

In 8 patients, follow up biopsies/clinical information were available for review. Four of the patients with clinical follow up were treated with metronidazole because of diarrheal symptoms. Each of the four patients experienced symptom resolution. In one of these patients, symptoms recurred approximately 8 months later and a second course of metronidazole therapy was prescribed with sustained resolution thereafter. The other 3 patients remained symptom

free. In 2 of these patients, follow up biopsies were negative for IS. The other patients were not re-biopsied.

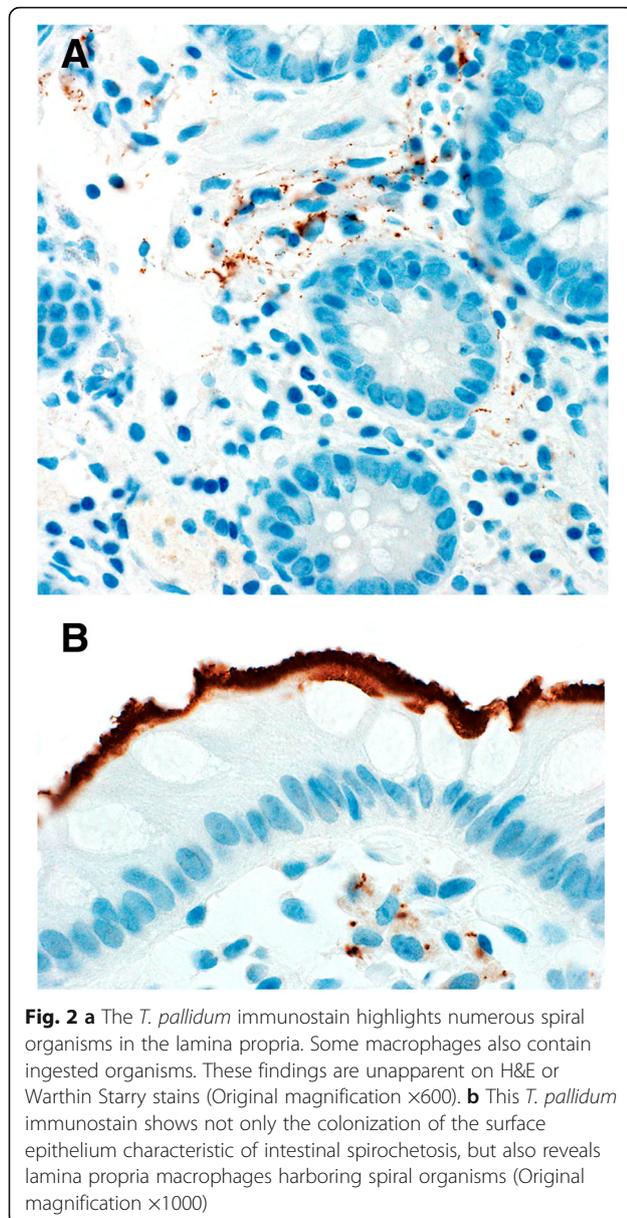
Four of the patients with follow up were not treated – each of these patients was undergoing endoscopy for colorectal cancer screening. Biopsies from subsequent screening colonoscopies were reviewed and did not show IS (median 5 year interval). A total of 6 (18%) patients were treated for IS including 2 without clinical follow up.

***T. pallidum* IHC required less hands-on technical time and was faster to interpret than Warthin-starry**

T. pallidum IHC required 15 min hands-on technical time compared to 90 min for WS. Two independent pathologists blindly reviewed the hematoxylin and eosin stained sections, *T. pallidum* IHC and WS for the same 16 cases including 11 examples of IS and 5 controls while being timed. In the first sitting, the pathologists were timed reviewing the routine stains and WS and then they were timed 2 weeks later reviewing the routine stains and *T. pallidum* IHC. The 2 pathologists took 10 and 18 min to review the cases with WS and 6 (40% less time) and 12 (33% less time) minutes respectively to review the cases with *T. pallidum* IHC. The average time saved was 5 min over the set of 16 cases or 0.3 min per case.

Discussion

Human IS is identified infrequently in approximately 1% of colonic biopsies [8, 13]. The diagnosis is made on routine sections and confirmed most often by silver stains such as Warthin–Starry. The prevalence has been reported to be increased in resource limited countries and in immunocompromised hosts [5, 13, 14]. Despite being



recognized histologically since the nineteenth century [15], there remain several controversial points regarding IS. First, there continues to be debate as to whether IS is a pathogenic condition or whether it represents commensalism [2, 4–7, 9, 10, 12–14]. Another point of contention is focused on whether IS can involve adenomatous epithelium or not. Some authors have proposed that adenomatous epithelium bears a disrupted brush border which is inhospitable to *Brachyspira* organisms and as such IS does not colonize adenomatous epithelium [16]. Others have shown individual examples of IS involving adenomatous polyps [17]. Yet another controversial issue is the detection of invasion by *Brachyspira* organisms. Existing literature describes detection of *Brachyspira* organisms from the

serum of immunocompromised patients with *B. pilosicoli* [18, 19] but not from patients with *B. aalborgi*. Ultrastructural studies have described invasion into the colonic epithelium [9, 10] but no correlation with symptoms has been examined in a series with both symptomatic and asymptomatic cases.

From our study, *T. pallidum* IHC is positive in 100% of IS, a finding also noted by recent observers [20]. Furthermore, *T. pallidum* IHC is easier to perform and faster to interpret than the commonly used WS stain. This is useful information for laboratories trying to optimize the use of hands-on time for technical staff and for pathologists who are trying to optimize sign out time in time-constrained settings. Other ancillary immunohistochemical, molecular and in situ hybridization assays have been used to detect intestinal spirochetosis. *Brachyspira* PCR is reportedly used in the Netherlands [21]. Tanahashi et al. have described that *T. pallidum* IHC and also *Mycobacterium* IHC highlight the organisms of IS [22]. Jensen et al. described a fluorescent in situ hybridization assay which is specific for *Brachyspira* species [23] and a Swedish group described an immunostain for *Brachyspira* species [24]. Unfortunately, these antibodies are not available in the United States and fluorescent microscopy is not readily available or practical for routine gastrointestinal biopsy examination. These limitations in availability impede the implementation of these assays. In contrast, *T. pallidum* IHC is widely available in the United States and so others can readily employ it for confirmation of the diagnosis of IS. In their description, Willen et al. [24] posit that IHC is preferable to silver stains and our study provides objective evidence to support their hypothesis.

We also were able to highlight IS involving multiple adenomatous polyps and serrated polyps. This is similar to findings from prior case reports¹⁶ and confirms that IS can involve neoplastic epithelium. In the authors' experience, *T. pallidum* IHC can cross-react with luminal bacteria giving rise to faint staining of individual bacilli and cocci separate from the tissue. This should not be interpreted as evidence of IS. Also, as expected, *T. pallidum* IHC shows no reactivity with the microvillous brush border of the colonocytes, and therefore can be used to exclude IS when a prominent brush border is present. Notably, IS is reported to share similar risk factors as syphilis but the morphologies of the 2 diseases are quite distinct. Syphilis is characterized by active colitis with prominent plasma cell infiltration and occasional ulceration in contrast to the above described features of IS.

With regard to the issue of tissue invasion, the *T. pallidum* IHC highlighted organisms within colonic epithelial cells and the lamina propria. Interestingly, rare spiral organisms were visualized engulfed within lamina propria macrophages (Fig. 2). This along with observations from prior ultrastructural studies [9, 10] suggests

that *Brachyspira* species are capable of tissue invasion. However, it is not possible to definitively confirm the species of these organisms within the lamina propria in view of the fact that the *T. pallidum* antibody cross reacts with other bacterial organisms. Notably, the frequency of detection of organisms within the lamina propria by IHC is in excess of the frequency of symptoms and there were no symptomatic differences between cases where there was invasion and those without. Taken together, these data indicate need for further study into the capacity of *Brachyspira* organisms to invade and the physiology surrounding symptomatic infection.

Less than half (42%) of patients diagnosed with IS during the study were symptomatic indicating that if IS is a pathogenic infection it is of low virulence as has been suggested by others. There were 4 patients with HIV with IS but 3 of these patients were lost to follow up and in 1 patient, clinical follow up is pending at the time of writing. The lack of follow up precludes assessment of the impact of immunodeficiency on clinical characteristics. Indeed, a limitation of this study is the limited clinical follow up overall. Nonetheless, where follow up information as available, symptomatic patients (i.e. those with diarrheal illnesses) who were treated for IS experienced resolution of symptoms. Interestingly, asymptomatic patients who were followed up experienced resolution without any antimicrobial treatment. Spontaneous resolution in asymptomatic individuals has been described by several others [4, 7, 8, 12, 13]. Taken together, it would appear that antimicrobial treatment is not indicated in incidentally diagnosed IS, but treatment to prevent forward transmission may be prudent.

Conclusion

In summary, *T. pallidum* IHC is 100% sensitive for the confirmation of the diagnosis of IS and is faster to perform and interpret than WS. As such, *T. pallidum* IHC is a useful adjunct in the diagnosis of IS.

Abbreviations

H&E: Hematoxylin and eosin; HIV: Human immunodeficiency virus; IHC: Immunohistochemistry; IS: Intestinal spirochetosis; PCR: Polymerase chain reaction; WS: Warthin-Starry

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Some of these data were presented at the 105th United States and Canadian Academy of Pathology Annual Meeting in Seattle, WA and have been published in abstract form [24].

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RPG conceived and designed the study, was involved in acquisition of data, performed molecular analysis, interpreted data, and was critical in drafting and revising the manuscript. BVN was involved in the study design and acquisition of data, analysis/interpretation of data, and was involved in revising the manuscript for important intellectual content. SSS was involved in the study design, acquisition of data, analysis/interpretation of data, and was involved in revising the manuscript for important intellectual content. CAA was involved in the study design, interpretation of data, and revision of manuscript for important intellectual content. RK was involved in the study design, interpretation of data, and revision of manuscript for important intellectual content. MST conceived and designed the study, was involved in acquisition of data, interpretation data, and revision of manuscript for important intellectual content. DLH was involved in the study design, acquisition of data, analysis/interpretation of data, and was involved in revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study is exempt by the institutional review board at Mayo Clinic.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- Graham R, Naini BV, Shah SS, Arnold CA, Kannangai R, Torbenson M, Lam-Himlin D. Treponema pallidum immunohistochemistry is a sensitive diagnostic adjunct for intestinal spirochetosis. *Laboratory Investigation*. 2016; 96:173A–4A.
- Hovind-Hougen K, Birch-Andersen A, Henrik-Nielsen R, Orholm M, Pedersen JO, Teglbjaerg PS, Thaysen EH. Intestinal spirochetosis: morphological characterization and cultivation of the spirochete *Brachyspira aalborgi* gen. nov., sp. nov. *J Clin Microbiol*. 1982;16:1127–36.
- Lee JI, Hampson DJ. Genetic characterisation of intestinal spirochaetes and their association with disease. *J Med Microbiol*. 1994;40:365–71.
- Smith JL. Colonic spirochetosis in animals and humans. *J Food Prot*. 2005;68:1525–34.
- Tsinganou E, Gebbers JO: Human intestinal spirochetosis—a review. *German medical science: GMS e-journal* 2010, 8:Doc01.
- Weisheit B, Bethke B, Stolte M. Human intestinal spirochetosis: analysis of the symptoms of 209 patients. *Scand J Gastroenterol*. 2007;42:1422–7.
- Esteve M, Salas A, Fernandez-Banares F, Lloreta J, Marine M, Gonzalez CI, Forne M, Casalots J, Santaolalla R, Espinos JC, et al. Intestinal spirochetosis and chronic watery diarrhea: clinical and histological response to treatment and long-term follow up. *J Gastroenterol Hepatol*. 2006;21:1326–33.
- Tanahashi J, Daa T, Gamachi A, Kashima K, Kondoh Y, Yada N, Yokoyama S. Human intestinal spirochetosis in Japan; its incidence, clinicopathologic features, and genotypic identification. *Mod. Pathol*. 2008;21:76–84.

9. Guccion JG, Benator DA, Zeller J, Termanini B, Saini N. Intestinal spirochetosis and acquired immunodeficiency syndrome: ultrastructural studies of two cases. *Ultrastruct Pathol.* 1995;19:15–22.
10. Padmanabhan V, Dahlstrom J, Maxwell L, Kaye G, Clarke A, Barratt PJ. Invasive intestinal spirochetosis: a report of three cases. *Pathology.* 1996;28:283–6.
11. Arnold CA, Limketkai BN, Illei PB, Montgomery E, Voltaggio L. Syphilitic and lymphogranuloma venereum (LGV) proctocolitis: clues to a frequently missed diagnosis. *Am J Surg Pathol.* 2013;37:38–46.
12. Koteish A, Kannangai R, Abraham SC, Torbenson M. Colonic spirochetosis in children and adults. *Am J Clin Pathol.* 2003;120:828–32.
13. Korner M, Gebbers JO. Clinical significance of human intestinal spirochetosis—a morphologic approach. *Infection.* 2003;31:341–9.
14. van Mook WN, Koek GH, van der Ven AJ, Ceelen TL, Bos RP. Human intestinal spirochaetosis: any clinical significance? *Eur J Gastroenterol Hepatol.* 2004;16:83–7.
15. Escherich T. Beitrage zur Kenntniss der Darmbakterien. *Munch Med Wschr.* 1886;33:815 and 833.
16. Coyne JD, Curry A, Purnell P, Haboubi NY. Colonic tubular adenomas and intestinal spirochaetosis: an incompatible association. *Histopathology.* 1995;27:377–9.
17. Palejwala AA, Evans R, Campbell F. Spirochaetes can colonize colorectal adenomatous epithelium. *Histopathology.* 2000;37:284–5.
18. Bait-Merabet L, Thille A, Legrand P, Brun-Buisson C, Cattoir V. *Brachyspira pilosicoli* bloodstream infections: case report and review of the literature. *Ann Clin Microbiol Antimicrob.* 2008;7:19.
19. Prim N, Pericas R, Espanol M, Rivera A, Mirelis B, Coll P. Bloodstream infection due to *Brachyspira pilosicoli* in a patient with multiorgan failure. *J Clin Microbiol.* 2011;49:3697–9.
20. Ruiz SJ, Procop GW. Cross-reactivity of anti-Treponema Immunohistochemistry with non-Treponema spirochetes: a simple call for caution. *Arch Pathol Lab Med.* 2016;140:1021–2.
21. Westerman LJ, Stel HV, Schipper ME, Bakker LJ, Neefjes-Borst EA, van den Brande JH, Boel EC, Seldenrijk KA, Siersema PD, Bonten MJ, Kusters JG. Development of a real-time PCR for identification of *brachyspira* species in human colonic biopsies. *PLoS One.* 2012;7:e52281.
22. Tanahashi J, Daa T, Gamachi A, Kashima K, Kondoh Y, Yada N, Yokoyama S. Human intestinal spirochetosis in Japan; its incidence, clinicopathologic features, and genotypic identification. *Mod Pathol.* 2008;21:76–84.
23. Jensen TK, Boye M, Ahrens P, Korsager B, Teglbjaerg PS, Lindboe CF, Moller K. Diagnostic examination of human intestinal spirochetosis by fluorescent in situ hybridization for *Brachyspira aalborgi*, *Brachyspira pilosicoli*, and other species of the genus *Brachyspira* (Serpulina). *J Clin Microbiol.* 2001;39:4111–8.
24. Willen R, Wanders A, Engstrand L. Intestinal spirochaetosis. *Histopathology.* 2006;49:658–9.

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