

## Prospective Study

**Association of *Streptococcus bovis* presence in colonic content with advanced colonic lesion**

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Received: October 17, 2014

Peer-review started: October 19, 2014

First decision: December 11, 2014

Revised: December 22, 2014

Accepted: February 5, 2015

Article in press: February 5, 2015

Published online: May 14, 2015

**Abstract****AIM:** To prospectively examine the association between presence of *Streptococcus bovis* (*S. bovis*) in colonic suction fluid and the endoscopic findings on colonoscopy.**METHODS:** From May 2012 to March 2013, 203 consecutivepatients who underwent colonoscopy for any reason were enrolled in the study. Exclusion criteria included: antibiotic use in the previous month, age younger than 18 years, and inadequate preparation for colonoscopy. The colonoscopy was performed for the total length of the colon or to the occluding tumor. The endoscopic findings were registered. Samples were obtained proximal to the colonoscopic part of the suction tube from each patient and sent to the clinical microbiology laboratory for isolation and identification of *S. bovis*. Samples were incubated in enrichment media with addition of antibiotic disks for inhibition of growth of Gram-negative rods. The samples were seeded on differential growth media; suspected positive colonies were isolated and identified with Gram staining, catalase, and pyrrolidonyl arylamidase tests, and further identified using a VITEK2 system. Statistical analyses were performed using the Student's *t* and  $\chi^2$  tests.**RESULTS:** Of the 203 patients recruited, 49 (24%) patients were found to be *S. bovis* carriers; of them, the endoscopic findings included: 17 (34.7%) cases with malignant tumors, 11 (22.4%) with large polyps, 5 (10.2%) with medium-sized polyps, 6 (12.2%) with small polyps, 4 (8.1%) with colitis, and 6 (12.2%) normal colonoscopies. Of 154 patients found negative for *S. bovis*, the endoscopic findings included: none with malignant tumors, 9 (5.8%) cases with large polyps, 11 (7.1%) with medium-sized polyps, 26 (16.9%) with small polyps, 7 (4.5%) with colitis, and 101 (65.6%) normal colonoscopies. *S. bovis* (Gram-positive coccus) is considered part of the normal intestinal flora. There is an association between *S. bovis* bacteremia and colonic neoplasia. It is not well understood whether the bacterium has a pathogenetic role in the development of neoplasia or constitutes an epiphenomenon of colorectal neoplasms. There was a clear relationship between positivity for *S. bovis* in colonic suction fluid and findings of malignant tumors and large polyps in the colon.

**CONCLUSION:** There is an association between *S. bovis* bacteremia and malignant colonic lesions; this should prompt for development of a reliable screening method for advanced colonic lesions.

**Key words:** Colon cancer; Colonoscopy; Culture; Screening; *Streptococcus bovis*

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**Core tip:** Currently, there is no available noninvasive, efficient, predictive tool for screening patients in high-risk populations for the purpose of colonoscopy examination. In this prospective study, we show a clear association between the presence of *Streptococcus bovis* in colonic suction fluid and findings of malignant tumors and large polyps during colonoscopy examination. Stool samples in different forms can be used as screening material for detection of the population at risk for advanced colorectal lesion. This may be preferable to repeated colonoscopy for surveillance in patients who underwent treatment of advanced colonic lesions by endoscopy or surgery.

Paritsky M, Pastukh N, Brodsky D, Isakovich N, Peretz A. Association of *Streptococcus bovis* presence in colonic content with advanced colonic lesion. *World J Gastroenterol* 2015; 21(18): 5663-5667 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i18/5663.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i18.5663>

## INTRODUCTION

*Streptococcus bovis* (*S. bovis*) is part of the normal flora of the human alimentary tract in 2.5%-15% of individuals<sup>[1]</sup>. The association between *S. bovis* bacteremia and colonic neoplasia is well described in the literature<sup>[1-4]</sup>, and all patients with *S. bovis* bacteremia are advised to undergo investigation to rule out gastrointestinal tract neoplasia<sup>[5,6]</sup>.

It has not yet been determined whether the relation between the bacterium and gastrointestinal neoplasia is etiologic or incidental. Data support the etiologic nature of this relation because of the proinflammatory potential and procarcinogenic properties of *S. bovis*, including the leukocyte recruitment driven by the bacterium, the tumor tissue-selective adhesion potential of *S. bovis*, selective colonization of *S. bovis* in tumor cells, tumor tissue microenvironment suitability for *S. bovis* proliferation, the local disruption of tumor tissues and capillaries that allow the entry of *S. bovis* into blood circulation, and the *S. bovis*-induced cytokines and transcriptional factors<sup>[6-9]</sup>. Some researchers have postulated that the increased load of *S. bovis* in the colon might be responsible for its association with colon cancer<sup>[1]</sup>.

Studies that have examined the issue of *S. bovis* presence in stool and the risk of colorectal cancer have produced conflicting results. A correlation between *S. bovis* presence in stool and colorectal neoplasia was found in some of the studies<sup>[2,4,5]</sup>, whereas that association was not found in others<sup>[6-13]</sup>.

Our primary goal is a further examination of the nature of that association. We prospectively investigated the association of *S. bovis* presence in colonic content and the nature of endoscopic findings in colonoscopy of 203 patients.

## MATERIALS AND METHODS

### Patient characteristics

From May 2012 to March 2013, a total of 203 consecutive patients (101 men and 102 women) who underwent colonoscopy for any reason were enrolled in the study. Mean age of the recruited patients was 62.5 years (range: 20-95 years). Exclusion criteria included an age younger than 18 years and inadequate preparation for colonoscopy. None of the patients included in the study received antibiotic treatment of any kind in the month preceding the colonoscopy procedure.

The colonoscopy procedure examined the entire length of the colon or until an occluding tumor was detected. The study protocol was reviewed and approved by the Poria-Baruch Padeh Medical Center Institutional Review Board. All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

### Bacterial culture

Samples were obtained proximal to the colonoscopic part of the suction tube that was used in the colonoscopy procedure. The end of the tubule, 3 cm in length, was placed in a sterile container and transferred to Laboratory of Clinical Microbiology following the colonoscopy procedure. Each tubule was transferred sterilely into a test tube with 2 mL of Brain-Heart Infusion Broth (Hylabs, Rehovot, Israel); an antibiotic disk impregnated with ertapenem (BD Diagnostics, Sparks, MD, United States) was added in order to delay growth of Gram-negative rods. Samples were incubated for 18 h at 37 °C. At the end of the incubation period, 5 µL of the test tube contents were seeded on Bile esculin agar (Hylabs), in accordance with the relevant protocol. Culture samples were incubated for a period of 18 h at 37 °C under CO<sub>2</sub> atmosphere.

### Identification of microorganisms

At the end of the incubation period, plates were examined for presence of *S. bovis* suspicious colonies. Presence of black-colored colonies on the plates indicated suspicion of *S. bovis* (black color attests to esculin hydrolysis). These colonies were sampled for Gram-staining and determination of catalase

**Table 1** Endoscopic findings in accordance with *Streptococcus bovis* culture results *n* (%)

Variable	Negative ( <i>n</i> = 154)	Positive ( <i>n</i> = 49)	<i>P</i> value
Age, yr	60.1	70.1	< 0.0001
Sex, female:male	1:1 (77:77)	1:1 (25:24)	0.9010
Normal colonoscopy	102 (66.2)	6 (12.2)	< 0.0001
Colitis/ileitis	7 (4.5)	4 (8.1)	0.4428
Small polyp	26 (16.9)	6 (12.2)	0.4990
Medium-sized polyp	11 (7.1)	5 (10.2)	0.4885
Large polyp	8 (5.2)	11 (22.4)	0.0003
Malignant tumor	0	17 (34.7)	< 0.0001

and pyrrolidonyl arylamidase presence. Colonies that were found to be Gram-positive cocci and were catalase and pyrrolidonyl arylamidase negative were further identified using a VITEK2 system (bioMérieux, Durham, NC, United States).

### Statistical analysis

For statistical analysis, differences between continuous variables, summarized as mean  $\pm$  SD, were analyzed with a Student's *t* test. Differences between frequencies were analyzed using a  $\chi^2$  test. A *P* < 0.05 was set to indicate statistical significance.

## RESULTS

Two hundred three consecutive patients with adequate preparation for the colonoscopy procedure were recruited and underwent whole colonoscopy procedures in the Gastroenterology Department (except in cases of occluding tumor). Forty-nine patients (24%) were found to be *S. bovis* carriers.

Among the study population, 17 malignant tumors were diagnosed. In all these cases *S. bovis* was identified in suction fluid. Among 49 patients who were found positive for *S. bovis*, the endoscopic findings included: 17 (34.7%) cases with malignant tumors, 11 (22.5%) with large polyps, 5 (10.2%) with medium-sized polyps, 6 (12.2%) with small polyps, 4 (8.2%) with colitis, and 6 (12.2%) with normal colonoscopies.

Among 154 patients who were found negative for *S. bovis*, the endoscopic findings included: none with malignant tumors, 8 (5.2%) cases with large polyps, 11 (7.1%) with medium-sized polyps, 26 (16.9%) with small polyps, 7 (4.6%) with colitis, and 102 (66.2%) normal colonoscopies (Table 1). In all cases, histopathologic examination was performed with confirmation of malignant or benign nature of every lesion.

A significant difference was observed between *S. bovis* carriers (patients positive for *S. bovis*) and patients without *S. bovis* (negative) among those who were diagnosed by colonoscopy with malignant tumors, large polyps, or normal-nature colonoscopy. There was no difference between *S. bovis*-positive and

-negative patients among those who were diagnosed with small- and medium-sized polyps and with colitis/ileitis.

The mean age of *S. bovis* carriers was significantly higher (10 years older) than the non-carriers group (*P* < 0.05), which could thereby predict potential appearance of advanced lesions in later stages of life.

## DISCUSSION

Colorectal cancer is one of the most common cancers (fourth among men and third among women)<sup>[14-17]</sup>. There is an association between *S. bovis* bacteremia and colonic neoplasia. It is not well understood whether the bacterium has a pathogenic role in the development of neoplasia or constitutes an epiphenomenon of colorectal neoplasms. Possible mechanisms that may explain this association include *S. bovis* overgrowth, breakdown of mucosal integrity, and subsequent bacterial translocation<sup>[18-21]</sup>.

Performance of a routine colonoscopy examination in high-risk populations could result in detection, early treatment of premalignant lesions, and subsequent prevention of colorectal cancer. Early detection of a malignant lesion can be crucial in the success of treatment.

It is important to state that none of the available noninvasive tests are efficient enough tools for screening patients in high-risk populations, or to eventually predict the patients that must undergo colonoscopy examination. In this prospective study, we found a clear association between the presence of *S. bovis* in colonic suction fluid and findings of malignant tumor and large polyps in the colon. These findings confirm the previous data that correlates the presence of *S. bovis* with colorectal cancer<sup>[22-24]</sup>. Findings shown in these studies demonstrate an association of stool positivity with colorectal cancer, indicating that bowel suction fluid is representative of stool positivity for *S. bovis*. Stool samples in different forms can be used as screening material for detection of the population at risk for advanced colorectal lesion. This can become a preferred surveillance tool instead of repeated colonoscopy performance in patients who have undergone treatment of advanced colonic lesions by endoscopy or surgery<sup>[25,26]</sup>.

Moreover, patients who had *S. bovis*-positive colonic content and whose colonoscopy examination showed a normal nature should be alerted and carefully observed in the future because of the possible pathogenic role of *S. bovis* in colorectal cancer. For this reason, culture of the suction fluid could shed light and predict the length of time required until the next colonoscopy. At present, there is no good noninvasive screening method for advanced colonic lesion detection.

In conclusion, we have found an association of *S. bovis* presence in colonic content and formation of malignant colonic tumors or large polyps, which could

be detected in colonoscopy. We suggest considering our finding of this association in order to develop a reliable screening method for detection of advanced colonic lesions. In light of significant developments in molecular biology and greater sensitivity of these methods, it is a good idea to utilize this platform for identification of *S. bovis*<sup>[27-30]</sup>.

## COMMENTS

### Background

*Streptococcus bovis* (*S. bovis*) is part of the normal flora of the human alimentary tract. *S. bovis* bacteremia is often associated with colonic gastrointestinal neoplasia, because of the pathogen's proinflammatory potential and procarcinogenic characteristic. Patients with *S. bovis* bacteremia are advised to undergo colonoscopic investigation to rule out gastrointestinal tract neoplasia. Currently, there is no available noninvasive, efficient, predictive tool for screening patients in high-risk populations for the purpose of colonoscopy examination. The results of this study indicate that there is an association of *S. bovis* presence in colonic content and the formation of malignant colonic tumors or large polyps; this knowledge may suggest development of a reliable screening method for detection of advanced colonic lesions.

### Research frontiers

In this study, the authors demonstrate the association between positive presence of *S. bovis* bacteria in the colon with precancerous changes and malignancies of colon. In recent years, there has been growing interest in research of the human microbiome; this is an example of a human disease that is associated with bacteria that are natural inhabitants of the human body.

### Innovations and breakthroughs

Most studies examine the association between the presence of bacteria in a clinical culture, such as a blood culture, and malignancies of the digestive tract. In this study the authors examined the association between bacterial carriage in the intestine without knowledge about active infection and malignancies in the colon. In addition, in this study, differential enrichment of growth media were performed in order to increase *S. bovis* detection and identification.

### Applications

The findings of the association of *S. bovis* presence in colonic content and formation of malignant colonic tumors or large polyps could be widely applied for the development of diagnostic and screening methods for detection of advanced colonic lesions. For example, *S. bovis* presence in colonic content may be applied in molecular biology methods for rapid identification of colonic malignancy.

### Terminology

The VITEK 2 is an automated microbiology system utilizing growth-based technology for identification and susceptibility testing of the most clinically important bacteria.

### Peer-review

The authors established an association between *S. bovis* bacteremia and malignant colonic lesions, it's interesting and applicable.

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**P- Reviewer:** Al-Jashamy K **S- Editor:** Yu J **L- Editor:** AmEditor  
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ISSN 1007-9327

