Original Article

Streptococcus Bovis Fecal Carriage is not Associated with an Increased Risk of Colorectal Neoplasm

Chi-Cheng Huang[1,2]
Shih-Chang Chang[3]
Ming-Hung Shen[3]
Jia-Uei Wong[3]
Hsueh-Yin Wu[4]
Henry Hsin-Chung Lee[2,3]
[1]*Division of General Surgery, Department of Surgery, Cathay General Hospital SiJhih, Taiwan*
[2]*School of Medicine, Fu-Jen Catholic University, Taipei, Taiwan*
[3]*Section of Colorectal Surgery, Department of Surgery*
[4]*Department of Laboratory Medicine, Cathay General Hospital, Taipei, Taiwan*

Key Words
Streptococcus bovis;
Colorectal neoplasms;
Carrier states;
Feces

Purpose. The aim of the study is to evaluate the association between fecal isolates of Streptococcus bovis and colorectal neoplasms.
Methods. From Dec 1993 to Dec 1995, 1,121 stool samples were obtained from 1,048 recruits including 62 with newly diagnosed colorectal neoplasms. Fecal isolates of S. bovis were identified with API 20 Strep System for both healthy (average risk) and colorectal cancer group. The demographic features, S. bovis fecal carriage status, colonoscopy and pathological findings of colonic lesions, if any, were collected. Medical records of the 986 average risk group subjects were retrieved to evaluate the incidence of colorectal neoplasm during the one-decade interval.
Results. The S. bovis fecal carriage rate of the colorectal cancer group was significant higher than that of the average risk group (25.8% vs. 14.5%, p = 0.015). The distribution of S. bovis biotypes did not show a statistical difference between the two groups (χ^2 test, p = 0.774). Full colonoscopy was conducted on 109 carriers and 217 non-carriers of the average risk group; the incidence of benign and neoplastic lesions was not increased among S. bovis carriers. 47 (32.9%) of healthy carriers remained enrolled during the one to nine-year follow-up interval (3.3 ± 0.8 years), and one rectal adenocarcinoma (2.1%) did occur two years after the prior positive S. bovis fecal culture.
Conclusion. The fecal carriages of S. bovis among average risk subjects with no symptomatic bacteremia are not associated with subsequent colorectal neoplasms. For each fecal isolate of S. bovis, the emphasis of intensive survey for occult colonic lesions is not justified.
[*J Soc Colon Rectal Surgeon* (*Taiwan*) *2008;19:71-77*]

Streptococcus bovis, part of the normal flora of human alimentary tract, is widely acknowledged as a causative agent of infective endocarditis and is associated with colorectal cancer once septicemia presents.[1-7] All patients with S. bovis bacteremia are advocated to have their colon and heart fully examined to exclude GI tract malignancies and valvular vegetations.[6] S. bovis overgrowth, breakdown of mucosal integration as an entry into circulation system and subsequent bacterial translocation were hypothesized mechanisms for S. bovis pathogenesis.[8] It remains inconclusive whether S. bovis plays a role of carcinogenesis or merely a subsequent phenomenon of colorectal neoplasms. The fecal carriage rate of S. bovis among patients with preneoplastic or neoplastic colonic lesions was reported higher than that of nor-

Received: March 5, 2008. Accepted: July 31, 2008.
Correspondence to: Dr. Henry Hsin-Chung Lee, MD, Section of Colorectal Surgery, Dept of Surgery, Cathay General Hospital, 280, Section 4, Jen-Ai Road, Taipei, Taiwan. Tel: +886-2-2708-2121; Fax: +886-2-2709-2063; E-maill: hhc.lee@gmail.com

mal controls from several studies.[5,9-10] From literature reviewed, the prevalence of S. bovis fecal carriage in non-neoplastic colons ranged from 2.3% to 13.0%.[4,9-13]

For general population without colon cancer and concurrent S. bovis septicemia, the association of fecal isolates of S. bovis with colorectal neoplasms and the clinical significance of S. bovis fecal carriages were rarely reported. The aim of the study is to compare the incidence of S. bovis fecal carriage between newly diagnosed colorectal cancer patients and average risk population. Colonoscopic findings of carriers were compared to those of non-carriers within non-neoplastic group. Furthermore, with medical records of the following one to nine years among average group reviewed, we are going to determine whether fecal isolation of S. bovis deserves extensive survey for occult colon and rectal neoplastic lesions.

Materials and Methods

From December 1993 to December 1995, 986 subjects were enrolled into study from a colorectal outpatient service. Exclusion criteria included ongoing symptomatic septicemia, receiving antibiotic treatment in recent one month, histories of colorectal neoplasms with or without prior treatment, inflammatory bowel diseases, functional bowel syndromes and acute gastroenteritis. Subjects receiving barium enema or colon preparation shortly before the visit were also excluded. Those who were younger than 20 years at the time of survey were discarded from analysis as well.

1,033 stool samples from the 986 subjects were sent to bacteriology laboratory for isolation of S. bovis. Stool specimens were inoculated onto 5% sheep blood agar plate and incubated at 37 °C for at least 24 hours. All colonies of the cultures were Gram stained first to confirm the presence of Gram positive cocci and tested for catalase. Streptococci species were recognized with the characteristics of Gram positive cocci in short chains and negative for catalase. Some unique features distinguishing group D streptococci, mainly S. bovis, from enterococci species included inability to grow in 6.5% NaCl and hydrolyzing starch.[14-16] We also adopted one commercial kit, the API 20 Strep system (Analytab Products, Inc., Plainview, N.Y.), to identify S. bovis to biotype level.[17-19] The test strips were inoculated, incubated and interpreted according to manufacturer's manual. These 986 subjects constituted the average risk group in current study.

In the mean time, 88 stool samples from 62 newly diagnosed colorectal cancer patients but had not undergone surgery yet were tested for S. bovis fecal carriages as the colorectal cancer group. The demographic features of the colon cancer and average risk group are summarized in Table 1.

Subjects with affirmed S. bovis fecal carriages were reported from laboratory and were advocated a full colonic study with the mention of possible underlying colorectal neoplasms. Full Colonoscopy was performed after adequate bowel preparation for some of the average risk and all colon cancer group patients. All suspicious lesions were well documented and snared for pathological examinations. In order to elucidate colorectal cancer incidence following the episode of S.bovis fecal carriage, medical records of the following one to nine years of the average risk group were retrieved and reviewed; the incident colorectal malignancy following S.bovis stool testing was evaluated accordingly.

Results

For the average risk group (n = 986), 149 stool

Table 1. Demographic features of subjects for *S. bovis* fecal carriage survey

		Average risk group	Colon cancer group	
Number		986	62	
Sex	Male	438 (44.4%)	36 (58.1%)	$p = 0.018$
	Female	548 (55.6%)	26 (41.9%)	
Age (year-old)		47.4 ± 1.0 (0-84)	58.0 ± 3.7 (30-81)	$p < 0.001$

samples of 143 subjects were positive for S. bovis. On the other hand, 20 samples of 16 subjects from 62 colorectal cancer patients had a positive stool culture. The S. bovis fecal carriage rate of the colorectal cancer group was significant higher than that of the average risk group (25.8% vs. 14.5%, p = 0.015). However, cancer and average risk group differed greatly in age and sex distribution (Table 1). In order to eliminate such bias, we used the fecal carriage rate from each age/sex stratum from the average group to estimate the number of carriers among the cancer group. The expected case number was 8.2, which was much lower than the observed number of 16 carriers in current study. The distribution of S. bovis biotypes were summarized in Table 2. For both the colorectal cancer and average risk groups, the proportion of biotype II/2 was greater than biotype II/1, and again greater than biotype I. The distribution among both groups, however, did not achieve a statistical significance (χ^2 test = 1.111, p = 0.774).

The age and sex were equally distributed among carriers and non-carriers of the average risk group (39.9% vs. 45.2% for male gender and 46.8 vs. 48.1 year-old for mean age).

Full colonoscopy was conducted on 109 of the 143 S. bovis fecal carriers and 217 of the 843 non-carriers of the 986 average risk subjects. The colonoscopic and pathological findings were listed in Table 3. Although attendance rate was discrepant between carriers and non-carriers, it seems that asymptomatic S. bovis fecal carriers were not associated with an increased chance of benign and neoplastic lesions compared with non-carriers.

Medical records were retrieved and reviewed for average risk group of the initial survey. Forty-seven (18 males, age: 47.2 ± 4.2 year-old) carriers were available for follow-up during one to nine years period. Table 4 showed that the only colon and rectum origin malignancy came from a 55 year-old female with an initial positive biotype II/2 culture and subsequently developed sigmoid cancer two years after the fecal isolate of S. bovis.

Discussion

The study evaluated the S. bovis fecal carriage of 986 average risk subjects without active bacteremia and prior colorectal malignancies with the carriage rate of 14.5% revealed. The fecal carriage rate of 25.8% from 62 newly diagnosed colorectal cancer patients during enrollment period at the same institute was apparently higher than that of the average risk group and achieved a statistical significance, even after age/sex distribution adjustment. The increased S. bovis fecal carriage among colon and rectal neoplasms was compatible with those described previously.[4]

Historically, McCoy and Mason reported the first

Table 2. The distribution of *S. bovis* biotypes

		Average risk group	Colon cancer group	
Biotype	I	9 (6.3%)	2 (11.8%)	$\chi^2 = 1.111$
	II/1	51 (35.7%)	7 (41.2%)	$p = 0.774$
	II/2	83 (58.0%)	8 (47.0%)*	
Total		143	17	

*including one with an initial biotype II/1 culture but shifted to biotype II/2 at a repeated culture four months later.

Table 3. Colonoscopic findings of some *S. bovis* fecal carriers and non-carriers

		Carriers	Non-carriers
Number		109	217
Findings	Benign polyp	32 (29.3%)	124 (57.1%)
	Colon lipoma	1 (0.9%)	1 (0.5%)
	Ileocecal lipoma		1 (0.5%)
	Benign cecal tumor		2 (0.9%)
	Colon adenocarcinoma		2 (0.9%)
	Anal adenocarcinoma		1 (0.5%)

Table 4. Follow-up events of initial healthy *S. bovis* fecal carriers during the one to nine-year period

	Number (incidence)	Interval to fecal isolate (year)
Colorectal cancer	1 (2.1%)	2
Breast cancer	3 (6.4%)	2, 3, 5
Gastric cancer with metastasis	1 (2.1%)	4 (liver), 5 (colon), 6 (bone)
Brain metastasis with unknown origin	1 (2.1%)	0.5

case of enterococcal endocarditis with colon cancer in 1951; several sporadic case reports followed.[1,20,21] Some of the causative agents were now believed to be group D streptococci, notably S. bovis, which were misidentified as enterococci species due to technical insufficiency. In 1977 Klein et al. reported the first series of 278 subjects: the fecal carriage rate of 63 colon cancer patients was apparently increased compared to that in 105 controls (55.6% vs. 10.5%).[4] Two years later the same author reported a prospective study on 15 S. bovis septicemia patients and found 8 colon cancers.[5] The association of S. bovis septicemia and colon cancer was well established since then; increased fecal carriage of S. bovis with concurrent colon malignancies was also documented on the side. In 1985 Burns et al. published the fecal carrier rate of 216 normal subjects and 18 colon carcinomas: 2.3% and 11.1% respectively.[9] The overall fecal carriage rate from this series, however, was much lower than that reported by Klein et al. Burns attributed the discrepancy to the false negative colon malignancies due to lack of direct colonoscopy with the resultant higher carriage rate in Klein's "normal controls", geographic and demographic variances, and differences in culture methods.[4,9] Series dealing with the fecal carriages of S. bovis among colon cancers and normal controls are summarized in Table 5: 2.3-13.0% for normal subjects and 11.1-55.6% for cancer group.[4,9-13] Potter et al. ever argued that no difference between controls and colorectal cancers in the fecal carriage rate in contrast to most reported.[13] Though our average risk group carriage rate was slightly higher than those from literatures, increased S. bovis fecal carriage of colorectal cancer group was confirmed again from current study.

The secondary part of the study was to conduct colonoscopy for both healthy S. bovis fecal carriers and non-carriers. Our persuasion drove 109 (76.2%) of healthy carriers had their colon thoroughly studied. About one quarter of 843 non-carriers also underwent colonoscopic examinations. Due to the discrepancy of participation, we did not conduct statistical analysis for colonoscopic findings between carriers/non-carriers; our result still suggested that no increased risk of benign and neoplastic colonic diseases merely from fecal isolates of S. bovis from healthy individuals. The final part of the study tried to estimate the long-term risk of colorectal neoplasms once S. bovis fecal carriage had been identified. The crude incidence of colorectal cancer of the 47 initial healthy carriers during the one to nine years follow-up interval was 2.1% and an increased risk of colorectal cancer could not be concluded from this level of evidence. The long-term follow-up analysis, however, was hampered by a low follow-up rate.

Fecal isolates of S. bovis from healthy average

Table 5. *S. bovis* fecal carriage rate from literature reviews

Year	Author	Classifications	No. of subjects	Rate
1977	Klein et al.[4]	Controls	11/105	10.50%
		Colon cancer	35/63	55.60%
1978	Noble et al.[12]	Controls	2/39	5.10%
1985	Burns et al.[9]	Controls	5/216	2.30%
		Colon cancer	2/18	11.10%
1987	Klein et al.[10]	Controls	10/82	12.20%
1993	Norfleet et al.[11]	Controls	1/35	2.90%
1998	Potter et al.[13]	Controls	3/23	13.0%
		Colon cancer	2/19	10.5%

risk individuals still give rise to the concern about occult colorectal lesions. Our study suggested no increased association of S. bovis fecal carriages and colorectal cancers. The dynamic process has been hypothesized for the pathogenesis of S. bovis including microbe overgrowth, mucosal injuries and bacterial translocation. Any event that disturbs the equilibrium of normal flora may result in overgrowth of certain bacterial species.[8,22] The direct impact of mucosal injuries and bacterial translocation is catastrophic S. bovis septicemia. Fecal isolates of S. bovis without bacteremia represent the intestinal overgrowth of the species but no transmucosal invasion. Focal mucosal weakness, either from pre-cancerous metaplasia or other benign process such as ischemia, provides an entry for the pathogen to across the physical barrier and access blood stream and systemic circulation. S. bovis bacteremia, the translocation stage of the disease, may not necessarily take place synchronously with the overgrowth of intra-gut counterpart. We believe symptomatic bacteremia of the gut-origin species occurs imperatively following the completion of the transmucosal stage.

The bacterial virulence, partially defined by the propensity of mucosal adherence, invasion and endocardial vegetation, is now evaluated at the biotype level.[17-19] Jean et al. retrospectively analyzed 62 S. bovis septicemia patients and found two-thirds of infective endocarditis cases were biotype I while nearly 100% of primary bacteremia, all hepatobiliary infections and primary bacterial peritonitis cases came with biotype II.[7] Biotype I was more virulent than biotype II in regard to colon cancer and infective endocarditis[19] (71% vs. 17% for colon cancer and 94% vs. 18% for infective endocarditis, respectively). From our series, both colon cancer and average risk group had dominant biotype II/2 carriages but the cancer group seemed to have an evener distribution. Biotype of S. bovis fecal isolates, nevertheless, was considered neither a diagnostic nor a predictive marker from current study. Some subjects had repeated stool cultures (38 of the average group and 17 of the cancer group, data not shown). The pattern of appearance of S. bovis fecal isolates at different time slot was various and even one colorectal cancer patient demonstrated discordant biotypes as shown in footnotes of Table 2.

The role of S. bovis as merely a marker of colon cancer has been challenged. Recently Ellmerich et al. extracted antigens from S. bovis cell wall to mimic neoplastic transformation with increased IL-8 secrections in animal models.[23,24] S. bovis acting as an etiological factor during the induction or promotion phase of colon cancer carcinogenesis is gaining popularity and under intense investigations.

The study showed no increased risk of colorectal neoplasms from fecal isolates of S. bovis. On the contrary, S. bovis bacteremia is highly associative with colon cancer and infective endocarditis regardless of its fecal carriage status and deserves extensive survey. At mean time, there is no benefit of intensive colon and rectum survey for merely a fecal isolation of S. bovis without systemic involvement.

References

1. McCoy WC, Mason JM. Enterococcal endocarditis associated with carcinoma of the sigmoid; report of a case. *J Med Assoc State Ala* 1951;21:162-6.
2. Ballet M, Gevigney G, Gare JP, Delahaye F, Etienne J, Delahaye JP. Infective endocarditis due to Streptococcus bovis. A report of 53 cases. *Eur Heart J* 1995;16:1975-80.
3. Leport J, Leport C, Vilde JL, Cerf M. Endocardites a Streptococcus bovis et pathologie colique: a propos de 42 observations. *Gastroenterol Clin Biol* 1987;11:25A.
4. Klein RS, Recco RA, Catalano MT, Edberg SC, Casey JI, Steigbigel NH. Association of Streptococcus bovis with carcinoma of the colon. *N Engl J Med* 1977;297:800-2.
5. Klein RS, Catalano MT, Edberg SC, Casey JI, Steigbigel NH. Streptococcus bovis septicemia and carcinoma of the colon. *Ann Intern Med* 1979;91:560-2.
6. Beeching NJ, Christmas TI, Ellis-Pegler RB, Nicholson GI. Streptococcus bovis bacteraemia requires rigorous exclusion of colonic neoplasia and endocarditis. *Q J Med* 1985;56: 439-50.
7. Jean SS, Teng LJ, Hsueh PR, Ho SW, Luh KT. Bacteremic Streptococcus bovis infections at a university hospital, 1992-2001. *J Formos Med Assoc* 2004;103:118-23.
8. Zarkin BA, Lillemoe KD, Cameron JL, Effron PN, Magnuson TH, Pitt HA. The triad of Streptococcus bovis bacteremia, colonic pathology, and liver disease. *Ann Surg* 1990;211: 786-91.
9. Burns CA, McCaughey R, Lauter CB. The association of Streptococcus bovis fecal carriage and colon neoplasia: possible relationship with polyps and their premalignant potential. *Am J Gastroenterol* 1985;80:42-6.
10. Klein RS, Warman SW, Knackmuhs GG, Edberg SC,

Steigbigel NH. Lack of association of Streptococcus bovis with noncolonic gastrointestinal carcinoma. *Am J Gastroenterol* 1987;82:540-3.
11. Norfleet RG, Mitchell PD. Streptococcus bovis does not selectively colonize colorectal cancer and polyps. *J Clin Gastroenterol* 1993;17:25-8.
12. Noble CJ. Carriage of group D streptococci in the human bowel. *J Clin Pathol* 1978;31:1182-6.
13. Potter MA, Cunliffe NA, Smith M, Miles RS, Flapan AD, Dunlop MG. A prospective controlled study of the association of Streptococcus bovis with colorectal carcinoma. *J Clin Pathol* 1998;51:473-4.
14. Facklam RR. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. *Appl Microbiol* 1972;23:1131-9.
15. Gross KC, Houghton MP, Senterfit LB. Presumptive speciation of Streptococcus bovis and other group D streptococci from human sources by using arginine and pyruvate tests. *J Clin Microbiol* 1975;1:54-60.
16. Knudtson LM, Hartman PA. Routine procedures for isolation and identification of enterococci and fecal streptococci. *Appl Environ Microbiol* 1992;58:3027-31.
17. Clarridge JE, Attorri SM, Zhang Q, Bartell J. 16S ribosomal DNA sequence analysis distinguishes biotypes of Streptococcus bovis: Streptococcus bovis Biotype II/2 is a separate genospecies and the predominant clinical isolate in adult males. *J Clin Microbiol* 2001;39:1549-52.
18. Songy WB, Ruoff KL, Facklam RR, Ferraro MJ, Falkow S. Identification of Streptococcus bovis biotype I strains among S. bovis clinical isolates by PCR. *J Clin Microbiol* 2002; 40:2913-8.
19. Ruoff KL, Miller SI, Garner CV, Ferraro MJ, Calderwood SB. Bacteremia with Streptococcus bovis and Streptococcus salivarius: clinical correlates of more accurate identification of isolates. *J Clin Microbiol* 1989;27:305-8.
20. Roses DF, Richman H, Localio SA. Bacterial endocarditis associated with colorectal carcinoma. *Ann Surg* 1974;179: 190-1.
21. Hoppes WL, Lerner PI. Nonenterococcal group-D streptococcal endocarditis caused by Streptococcus bovis. *Ann Intern Med* 1974;81:588-93.
22. Sabbaj J, Sutter VL, Finegold SM. Comparison of selective media for isolation of presumptive group D streptococci from human feces. *Appl Microbiol* 1971;22:1008-11.
23. Ellmerich S, Scholler M, Duranton B, Gosse F, Galluser M, Klein JP, Raul F. Promotion of intestinal carcinogenesis by Streptococcus bovis. *Carcinogenesis* 2000;21:753-6.
24. Ellmerich S, Djouder N, Scholler M, Klein JP. Production of cytokines by monocytes, epithelial and endothelial cells activated by Streptococcus bovis. *Cytokine* 2000;12:26-31.

原　著

糞便中的牛鏈球菌帶原並不會增加大腸直腸腫瘤的發生

黃其晟[1,2]　張世昌[3]　沈明宏[3]　黃家偉[3]　吳雪穎[4]　李興中[2,3]

[1]國泰綜合醫院汐止分院　一般外科

[2]輔仁大學　醫學系

[3]國泰綜合醫院　大腸直腸外科

[4]國泰綜合醫院　檢驗科

目的　本研究的目的是評估糞便培養出牛鏈球菌與大腸直腸腫瘤的關係。

方法　從 1993 年 12 月到 1995 年 12 月，從 1048 受檢者中取得 1121 個糞便標本，其中有 62 個新診斷出大腸直腸惡性腫瘤病患。牛鏈球菌是用 API-20 Strep system 檢測分離出來。受試者糞便牛鏈球菌的帶原情況、大腸鏡檢及鏡檢過程中病理報告皆收集並加以分析。此外並回顧 986 名一般族群受試者十年內之病歷，以了解此期間內大腸直腸腫瘤發生率。

結果　大腸直腸癌患者糞便牛鏈球菌的帶原比率確實比一般族群高 (25.8% 相對於 14.5%，p 值 = 0.015)，然而兩者間牛鏈球菌的亞型分佈並無統計學上的意義。在一般族群受試者中，各有 109 位糞便帶原者及 217 位非帶原者接受大腸鏡檢查，在帶原者這一組的腫瘤發生率並未增加。其中 47 位健康帶原者在後續一至九年 (3.3 ± 0.8 年) 的追蹤中，只有一位在糞便培養出牛鏈球菌二年後發現直腸癌。

結論　糞便牛鏈球菌帶原者若無菌血症並不會增加得大腸直腸腫瘤的機會，對於糞便中培養出牛鏈球菌者，並不須密集進行大腸直腸腫瘤的檢查。

關鍵詞　糞便牛鏈球菌帶原、大腸直腸腫瘤、帶原狀態、糞便。