Infectious agents and colorectal cancer: A review of *Helicobacter pylori, Streptococcus bovis*, JC virus, and human papillomavirus

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Abstract

Based on the high volume of bacteria and viruses that the intestine is exposed to and the importance of infectious agents in some gastrointestinal and anogenital cancers, it is not surprising the many studies have evaluated the association between colorectal cancer and infectious agents. This review highlights investigations of four agents in relation to colorectal cancer; *Helicobacter pylori*, *Streptococcus bovis*, JC virus (JCV), and human papillomavirus (HPV) have all been evaluated as possible etiologic agents for colorectal cancer. For each of these agents, a review of possible mechanisms for carcinogenesis and epidemiologic evidence is discussed, and future directions for research are proposed.

Introduction

Since the 1980s, there has been a dramatic increase in research on infections and cancer. In 2002, it was reported that infectious agents accounted for approximately 18% of all cancers worldwide (1). This estimate is based on the burden of disease associated with cancers that have known infectious etiologies, such as cervical, liver, and gastric cancers. However, as the technology to detect infectious agents improves and more studies are conducted, future research may reveal new associations between cancer and infection, and the proportion of cancers attributable to infection may rise. Furthermore, linking cancers to specific infectious agents may provide new avenues for effective cancer prevention, in particular vaccination.

In 2002, there were approximately 1 million new cases of colorectal cancer worldwide, accounting for 9.4% of all cancer (2). Colorectal cancer is the fourth most common cancer among men and third most common among women worldwide (2); nonetheless, much is still uncertain about its etiology. It is established that colorectal cancer has a strong association with certain hereditary gene mutations, but only 3-5% of colorectal cancers are due to these known mutations alone(3). Cigarette smoking, high alcohol consumption, low vegetable intake, obesity, and physical inactivity are associated with an increased risk of colorectal cancer; post-menopausal hormone use, non-steroidal inflammatory drug use, and high calcium intake are associated with a reduced risk(4).

Colorectal cancer originates in the epithelial cells lining the colon and rectum. The cells of the human colon replicate at a relatively high rate with 10^{10} epithelial cells being replaced every day (5). This high rate of replication is thought to contribute to the vulnerability of colon and rectal epithelium to mutation and consequent carcinogenesis, although this elevated risk does not seem to apply to the small intestine, despite comparably elevated cell turnover. If colonic epithelial cells accumulate mutations in oncogenes and tumor suppressor genes, the morphology of the cell changes, and there is a hyperproliferation of abnormal cells (6). This can result in a neoplastic growth, known as a polyp. Adenomatous polyps (adenomas) are benign lesions in the colon and rectum that have the potential to develop into cancer (7). Other pathways for colorectal cancer include those involving hyperplastic polyps (8-10) and ulcerative colitis (11).

The human intestine provides a habitat that is rich in nutrients, permitting for the growth of over 500 different species of bacteria, with the highest concentration of bacteria found in the colon (12). In addition to bacteria, the human colon is frequently exposed to both pathogenic and non-pathogenic viruses. Normally, the bacteria found in the colon have a symbiotic relationship with their host and can even provide some protection against pathogens (12). However, some microbes that are normally or incidentally found in the colon are pathogenic or potentially pathogenic if they breech the host mucosal barrier.

Due to the sheer numbers of microbes found in the colorectum and the recent interest in infectious agents as a cause of cancer, it is not surprising that researchers have begun, again, to consider infectious agents as a possible cause of colorectal cancer. In this article, we review the evidence on four infectious agents that have been most commonly studied in relation to colorectal cancer: *Helicobacter pylori*, *Streptococcus bovis*, JC virus (JCV), and human papillomavirus (HPV). For each infectious agent, we conducted a search of PubMed and reviewed all relevant studies with a manuscript or abstract written in English published prior to 2008.

Helicobacter pylori

Infection with *Helicobacter pylori* usually occurs at a young age and is extremely common, with one study showing that 49% of the people pooled from 17 populations across the world had antibodies to *H. pylori* (13). However, there is tremendous geographic variation in the prevalence of *H. pylori* infection with some of the highest infection rates nearing 90% in parts of Japan and the lowest infection rates around 33% in the United States (13). In addition, *H. pylori* infection is associated with low socioeconomic status, and person-to-person transmission is thought to be the dominant mode of transmission (14). Also, *H. pylori* infection prevalence varies markedly by age, either as a result of a cohort effect in which older generations were exposed to higher rates of *H. pylori* transmission or (less likely) accumulating risk of infection with age (13).

H. pylori is a gram-negative bacterium that has become well adapted to the human stomach via interaction with gastric epithelial cells (15). Chronic gastric infection with *H. pylori* causes inflammation and several gastric pathologies, including gastric ulcers

and gastric cancer (16-18). Carcinogenesis via *H. pylori* involves inflammation, as well as deregulation of the cell cycle via the *H. pylori* protein, CagA, which binds and activates SHP2 (a human phosphatase that can act as an oncoprotein) resulting in cell growth and motility (18). Due to the strong association between *H. pylori* and gastric cancer, *H. pylori* is classified as a class I carcinogen by the International Agency for Research on Cancer (19).

Despite the established relationship between *H. pylori* and gastric pathologies, the association between *H. pylori* and colorectal cancer is much less clear. Epidemiologic studies have used serology, polymerase chain reaction (PCR) methods, C-urea breath tests (C-UBT) and circulating gastrin levels to examine colorectal neoplasia in relation to *H. pylori* infection and have produced conflicting results.

We reviewed 16 epidemiologic studies examining associations between colorectal adenomas or adenocarcinomas and *H. pylori* seroprevalence. Six of these studies found statistically significantly associations between *H. pylori* antibodies and colorectal neoplasia with odds ratio (OR) estimates ranging from 1.4 to 4.0 (20-25). However, the other 10 studies did not find a statistically significant association between *H. pylori* seropositivity and colorectal neoplasia (26-35). Most of these null studies reported OR estimates between 1.0 and 1.5 (26, 28, 30-32, 34, 35). Only one of these studies reported an inverse association between *H. pylori* and colorectal neoplasia (OR=0.7; 95% CI=0.3-2.0) (27).

A recent meta-analysis examining studies published between 1991 and 2002 to determine the relationship between *H. pylori* and colorectal neoplasia found an overall statistically significant association between *H. pylori* and the risk of colorectal neoplasia (OR=1.4; 95% CI=1.1-1.8) (36). However, there is skepticism of this association, because the geographic distribution of colorectal cancer does not mirror that of gastric cancer, and in many areas, most notably in Japan, there are opposing trends over time for these two cancers (37, 38).

Discrepancies in the results between studies could be attributed to differences in the selection of controls, variation in adjustment for confounding variables, and limited power to detect associations due to small sample sizes in most of the studies. However, one study attributes these variable results to differences in the prevalence of cytotoxin-associated gene-A-positive (CagA+) strains of *H. pylori* between the populations studied (30). Studies of gastric cancer indicate that *H. pylori* CagA+ strains are more likely to cause inflammation and malignancy than CagA- strains (39, 40). Therefore, the Shmuely study compared CagA status between 41 colorectal adenocarcinoma patients seropositive for *H. pylori* and 24 hospital-based controls also seropositive for *H. pylori*. Infection with a CagA+ strain was associated with a statistically significant increased risk of colorectal adenocarcinoma (OR=10.6; 95% CI = 2.7-41.3) compared to infection with a CagA- strain. Although the design of this study is limited because it is hospital-based rather than population-based, it underscores the importance of examining pathogen characteristics as possible risk factors for disease. Not all *H. pylori* strains have the same

virulence, so combining mild strains together with more virulent strains may dilute possible associations.

Although serology is the most common approach used to assess the relationship between *H. pylori* and colorectal cancer, several other techniques have been used. C-urea breath tests (C-UBT) can detect current gastric *H. pylori* infection with approximately 97% sensitivity and specificity (41). A Taiwanese study using this method found no association between current *H. pylori* infection and colorectal adenomas (42). However, another study in Japan using C-UBT, urease detection in biopsy specimens, or other histologic tests of biopsied gastric tissue to assess current infection with H. pylori found positive associations (OR=1.60; 95% CI=1.18-2.02 for adenomas and OR=1.80; 95% CI = 1.28-2.32 for adenocarcinomas). This association was modified by sex, with a stronger association among women (43).

Using PCR methods, one study found that 1.2% of malignant colorectal tissues (N=83) were positive for *H. pylori*, whereas 6.0% of normal tissues were positive (N=83) (44). Therefore, the authors concluded that *H. pylori* is not important in the pathogenesis of colorectal cancer. However, two other studies that used PCR to detect *H. pylori* in colorectal neoplasms indicated that a much greater proportion of these tissues were positive for *H. pylori*: one study detected *H. pylori* DNA in 27% of colorectal adenocarcinoma tissues (45); another found that detection of *H. pylori* DNA in colorectal tissue was associated with an increased risk of colorectal adenocarcinomas (OR= 8.13; 95% CI=1.4-47.0) (46).

It could be that it is not infection of colorectal tissue with *H. pylori* that may be responsible for an increased risk of colorectal cancer, but rather the byproducts of a gastric *H. pylori* infection (22). One theory stems from the fact that gastric *H. pylori* infection increases serum levels of gastrin leading to hypergastrinemia (47). Because hypergastrinemia is hypothesized to have proliferative effects on intestinal mucosa (48), some studies have assessed the relationship between serum gastrin levels and colorectal cancer risk. Several studies found a positive association between hypergastrinemia and colorectal neoplasia (22, 28, 33), including a prospective study assessing serum gastrin levels prior to the diagnosis of colorectal carcinoma (OR=3.9; 95% CI=1.5-9.8) (28).

If *H. pylori* is a cause of colorectal carcinoma, it is clear that the association is complex and perhaps mediated through pathogen-virulence factors. Future research addressing the relationship between *H. pylori* and colorectal neoplasia should be prospective and make attempts to increase the sensitivity of studies by focusing on the subsets of *H. pylori* that are most likely to cause malignancy and/or subsets of colorectal cancers that are most likely to be associated with infection or inflammation. In addition, studies should evaluate gastric H. pylori infection as a possible risk factor for colorectal neoplasia.

Streptococcus bovis

Streptococcus bovis, a nonenterococcal group D Streptococcus, is a bacterium that is found among the normal flora of the human gastrointestinal tract in 5-16% of adults (49).

In addition, *S. bovis* is commonly detected as a contaminant in packaged meat (50). If *S. bovis* enters the blood stream, it can cause bacteremia and endocarditis; approximately 11-12% of infective endocarditis is caused by *S. bovis* (51, 52). Endocarditis caused by *S. bovis* is more common in men and in the elderly (53). In two studies, patients with endocarditis caused by *S. bovis* type I, recently reclassified as *Streptococcus gallolyticus*, have an increased risk of prevalent colorectal neoplasia (54) (55).

Laboratory studies of *S. bovis* reveal that this bacterium releases proteins which stimulate inflammation (56). In addition, *S. bovis* proteins were associated with an *in vitro* over-expression of COX-2 (56), which is known to be frequently over-expressed in human colorectal cancers and which can inhibit apoptosis and increase angiogenesis (57).

The debate over the association between *S. bovis* and colonic neoplasia has a long history, going back as early as 1951 when the first case report of colon cancer associated with enterococcal endocarditis was published (58). Since then, numerous studies and case reports have linked *S. bovis* bacteremia and endocarditis with colon polyps and carcinomas (59-72). In a review of studies evaluating patients with *S. bovis* bacteremia who were examined for gastrointestinal disease, Gold et al noted that studies reported between 6% and 71% of those with *S. bovis* bacteremia had colonic neoplasia (71). Due to the high prevalence of colonic neoplasia in those with *S. bovis* bacteremia or endocarditis, colonoscopy to screen for occult colorectal cancer and pre-cancerous lesions has been recommended in this group (60, 62, 63, 65, 70, 71).

In addition, several cross-sectional studies have examined the association between *S. bovis* endocarditis and colonic neoplasia. A 1987 study found statistically significantly higher risks of colon polyps and colon cancer among 34 patients with *S. bovis* endocarditis compared to 43 patients with endocarditis caused by other bacteria (35% vs. 7% for polyps and 26% vs. 2% for cancer) (66). Since then, two additional studies have confirmed these results (68, 69). The Hoen et al study compared the prevalence of colon polyps and colon cancer in 32 colonoscopy screened cases of *S. bovis* endocarditis and 64 age and sex-matched controls without *S. bovis* endocarditis also screened via colonoscopy: 47% of *S. bovis* endocarditis patients had colon adenomas vs. 23% of those without *S. bovis* endocarditis, and 9% of *S. bovis* endocarditis cases had colorectal cancer compared to 3% of those without *S. bovis* endocarditis (68). The Pergola et al study examined colorectal neoplasia in 40 cases of *S. bovis* endocarditis and 166 patients with infective endocarditis caused by other bacterium: colorectal neoplasia was present in 55% of *S. bovis* endocarditis cases but in only 4% of other infective endocarditis cases (69). Although the above studies have been small, all suggest an association between *S. bovis* endocarditis and colorectal neoplasia.

Other studies examining the presence of *S. bovis* in stool and the risk of colorectal cancer have produced conflicting results. A study by Klein et al found that 35 of 63 colon cancer cases had *S. bovis* present in their stool compared to 11 of 105 hospital-based controls (OR=10.7; 95% CI = 4.8-23.7) (59). This finding was confirmed in a later study on a separate population (73). However, three studies found no association between the presence of *S. bovis* in stool and colorectal neoplasia (74-76).

Serologic studies assessing the association between *S. bovis* antibodies and colonic neoplasia have been performed. A 1993 study examining serum samples from 16 colon cancer cases and 16 age-matched controls whose sera was being tested for rheumatoid factor and anti-nuclear factor found that cases had a statistically significantly higher median IgG antibody titer to *S. bovis* than controls; however, IgM antibody titers were similar between the two groups (77). The authors concluded that immune stimulation caused by *S. bovis* occurred over a long period of time and was not a recent occurrence due to advanced clinical disease. Another study by Tjalsma et al found *S. bovis* antibodies in 11 out 12 colon-cancer patients, in 3 out of 4 colon-polyp patients, and in 0 out of 8 control subjects; antibodies to another bacterium commonly found in the human gut, *E. coli*, were not found more commonly in cases than controls (78).

Based on this body of evidence, there is a strong association between *S. bovis* bacteremia and colorectal neoplasia. However, many debate the temporality of this association. One view is that ulcerating colorectal carcinomas allow increased growth of S. bovis, invasion of the blood stream, and establishment of infection (59). Others argue that *S. bovis* is a direct cause of colon carcinogenesis. Supporters of the latter argument point to the fact that pre-cancerous polyps, and not just ulcerative carcinomas, are associated with *S. bovis* (73, 78). Furthermore, a 1982 study found an increased risk of subsequent colonic neoplasia among those with previous *S. bovis* endocarditis (63). Finally, molecular evidence points towards *S. bovis* as a possible carcinogen in a rat model (56, 79).

Despite this evidence, large gaps exist in the literature assessing the relationship between *S. bovis* and colon cancer. There are currently no published case-control studies using PCR to detect *S. bovis* in colorectal tissue from cases and controls. In addition, *S. bovis* type I is the subtype of *S. bovis* that is most commonly associated with colorectal neoplasia in patients with *S. bovis* bacteremia (54, 55). However, most colorectal cancer epidemiology studies have not classified *S. bovis* according to subtype. Therefore, studies may dilute a possible association by combining the more pathogenic subtype, type I, with less pathogenic subtypes. Finally, large prospective studies are absent from the literature. Because colorectal cancer screening has become relatively common, prospective studies of *S. bovis* colorectal infection and later development of colorectal neoplasia are feasible and should be pursued.

JC Virus

Human infection with the polyomavirus, JC virus (JCV), is extremely common, affecting up to 80% of the population (80). Although the route of transmission for JCV is unknown, primary infection generally occurs in early childhood. The vast majority of those infected with JCV have no symptoms, and the virus travels to the kidneys, where it remains latent (81). However, severe immunsupression, as seen in transplant patients and those with advanced HIV disease, can trigger reactivation of the virus causing a serious demyelinating disease known as progressive multifocal leukoencephalopathy (PML) (82, 83).

The oncogenic properties of JCV are well described in the literature and attributed to the viral protein, large T-antigen. Laboratory studies of this viral protein have demonstrated that the large T-antigen has the ability to immortalize cells in culture (84, 85). The mechanism for this cellular transformation has been studied: the large T-antigen binds p53 and members of the pRb family of proteins, thereby blocking tumor suppression and inducing unchecked cellular replication (86, 87). This is hypothesized to result in chromosomal instability, which is common in colon carcinogenesis (88).

Despite molecular evidence showing the potential for JCV to induce carcinogenesis, there is not a strong consensus linking JCV to human cancers. Most of the studies involving JCV and cancer have been conducted in tumors of the CNS and, although many detect the presence of JCV in CNS tumors, most of these studies have examined only case tissue and did not compare their results to separate disease-free controls (89-91).

Recently, attention has turned to examining JCV in relation to colorectal neoplasia. Several studies have demonstrated the presence of JCV in both normal and neoplastic tissues from the colon and rectum (92-95). One of the earliest studies to detect JCV DNA via PCR in colorectal epithelial tissues was published in 1999. This study found that, although JCV DNA was in both cancerous and normal colon tissue, the viral copy number was statistically significantly higher in the cancerous cells. In addition, these researchers recommended the use of topoisomerase I treatment to improve PCR sensitivity for detecting JCV. Because JCV contains a supercoiled DNA genome, topoisomerase I is thought to relax the supercoiling and allow better detection and amplification of JCV DNA sequences by PCR (92).

Since then, several studies have examined the presence of JCV DNA in colonic tissue using PCR. A study by Ricciardiello et al in 2000 confirmed the presence of JCV in the upper and lower gastrointestinal (GI) tract using normal GI tissue samples from 33 patients. This study detected JCV DNA sequences in the upper GI for 70.6% of patients and in the lower GI for 81.2% of patients and concluded that infection of the GI track with JCV is common in those without immune suppression (93).

In addition, we reviewed five studies examining colorectal neoplastic tissue detecting JCV DNA in colorectal neoplasias at varying frequencies, finding from 26% to 89% of carcinomas positive for JCV (94-98). Two of these studies also tested colorectal adenomatous tissue from separate patients with adenomas and normal colorectal tissues from controls. One study found JCV infection in 61% of cancerous tissue (N=80), 60% of adenomatous tissue (N=25) and 30% of normal tissue samples from controls (N=20), resulting in an OR = 6.2 (95% CI = 2.4-16.6) comparing neoplastic tissue to normal tissue. This same study found JCV viral copy numbers were statistically significantly higher in neoplastic colorectal tissue compared to normal colorectal tissue (94). The other study had lower rates of detection for JCV, finding 26% of cancerous colorectal tissue (N=23), 5% of adenomas (N=21) and 0% of normal tissue (N=20) positive for JCV (95).

Two studies found no association between JCV and colorectal cancer using PCR (99, 100). One of these null studies tested 233 cancerous colorectal tissue samples and 233 normal surrounding colorectal tissue samples from the same patients using a lab that had never previously been used for JCV or other viral studies; only 1 normal colorectal specimen was positive for JCV (100). The authors concluded that there is no association between colorectal cancer and JCV, and that previous studies detecting JCV in colonic tissue may have had problems with contamination due to the ubiquity of JCV.

Recently, a nested case-control study tested blood samples collected at least 3 months prior to cancer diagnosis in 386 male cases of colorectal cancer and 386 matched controls and found no association between JCV seropositivity and colorectal cancer (OR = 0.9; 95% CI = 0.7-1.3) (101). Although this test was sensitive for detecting exposure to JC virus, it was not specific to the detection of colonic JCV infection. Because JCV infection is so common, it is important to identify infection site.

Despite the inconsistencies in the epidemiologic evidence, there is molecular evidence that the JC virus large T-antigen causes chromosomal mutations resulting in chromosomal instability in colonic epithelial cell lines *in vitro* (102). In addition, Ricciardello et al demonstrated that a specific subset of JCV, the Mad-1 strain, is the only type of JC virus found in the colon and that a specific variant of this strain (a variant lacking a 98-bp repeat) is associated with colorectal cancer (103). If this finding is confirmed, differentiating this subset of JCV from other potentially non-pathogenic types will be important in establishing an etiologic association between JCV and colorectal cancer.

Future studies need to establish a standard, reliable, and reproducible test for the detection of JC virus DNA. This test should be evaluated in masked specimens, multiple populations, and different laboratories to insure the validity of the results. In addition, prospective studies of fecal carriage of JC virus in relation to colorectal cancer are absent from the literature. Such studies could help determine specificity, and possibly causality, in the association between JC virus and colorectal cancer if fecal carriage of virulent JCV subtypes occurs prior to the development of colorectal neoplasia and at higher rates in cases than controls.

Human papillomavirus

HPV is a double-stranded DNA virus that infects basal-layer epithelial cells through microscopic abrasions or tears (104). There are more than 100 types of HPV, and about 40 of these types are known to infect genital epithelial cells (105). Genital HPV is transmitted via sexual contact and is the most common sexually transmitted infection among Americans ages 15-49 years of age (106, 107). For most people, anogenital HPV infections resolve on their own and have few to no clinically apparent symptoms (108). However, women who are unable to clear cervical HPV infection and are persistently infected with certain types of HPV are at increased risk for the development of cancer (108).

Not all types of HPV are associated with cancer. Currently, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are classified as "high-risk" oncogenic infections (104). Of these, types 16 and 18 are the most common types found in cervical cancer tumors, with one review finding that approximately 70% of cases were positive for HPV-16, 18 or both (109). HPV infection is a necessary cause of cervical cancer and is associated with other epithelial malignancies, such as oropharyngeal, penile, vaginal, vulvar, and anal cancer (110-112). Although 100% of cervical cancer is attributable to HPV, other anogenital cancers vary in the proportion of site-specific cancer positive for "high-risk" HPV (111).

Oncogenic HPV allows for the growth of cancerous cells through the expression of viral proteins E6 and E7. These interfere with tumor suppressor proteins, p53 and pRb, and induce telomerase, thereby immortalizing cells (111). Thus, HPV-related tumors infrequently contain p53 mutations (113). Infection with "high-risk" HPV, however, is not sufficient by itself to cause cancer. Additional cellular alterations are necessary for tumorigenesis, and an accumulation of mutations appear to occur over time (111).

Early case-only studies of colorectal neoplastic tissue failed to detect HPV DNA (114-117). However, the sample size in each of these studies was small, ranging from 10 to 50 cases. In addition, HPV detection techniques have improved, and more recent studies of the association between HPV and colorectal neoplasia suggest a positive association.

We reviewed 9 case-control studies of the association between HPV and colorectal neoplasia. Studies varied by the type of tissue analyzed, with some including carcinomas, others adenomas, and still others both. In addition, control tissues varied among studies and included: adjacent normal tissue from cases, benign colon polyps from separate individuals, and normal colon tissue from disease-free controls. Eight of these studies used PCR techniques to detect HPV DNA in colorectal neoplastic tissues and control tissues (118-125). One study used immunohistochemistry to detect HPV antigen in case and control tissues (126). Despite limited sample sizes in these studies (ranging from 19 to 72 cases), all case-control studies indicated a positive association between HPV infection and colorectal neoplasia. Estimates of the ORs associated with these studies ranged from 2.7 (95% CI = 1.1 to 6.2) to 9.1 (95% CI = 3.7 to 22.3) (119, 124).

In addition to these case-control studies, a recent study found that, among 56 HPV positive colorectal tumors, only 3.6% contained p53 mutations (127). This is in contrast to the fact that approximately 50% of all colorectal cancers contain p53 mutations (128). The authors concluded that this is evidence that HPV may contribute to colorectal cancer through HPV-mediated p53 inactivation, thereby simulating a p53 mutation. Based on this, HPV may play a role in the subset of colorectal cancers that lack p53 mutations. This is consistent with studies of oral carcinomas, which find the association with HPV to be strongest in the subset without p53 mutations (129).

Large cohort studies, with sample sizes ranging from 21,222 to 104,760 cases of cervical cancer, have compared colorectal cancer risk in women with a history of cervical cancer to women in the general population (130-132). Two of these studies reported no

association between cervical cancer and subsequent colorectal cancer (130, 131). The other study found an increased risk of cancer of the anus/rectum among cervical cancer survivors, and an increased risk of colon cancer in women treated with radiotherapy but no increased risk of colon cancer in women who were not treated with radiotherapy (132). This suggests that any increase in the risk of colon cancer among those with previous cervical cancer is related to radiation treatment and not because of a common etiology, HPV, for the two cancers.

Due to the conflicting evidence concerning the association between colorectal cancer and HPV, further investigation is needed. First, there are no studies of colorectal cancer and sexual risk factors, such as number of sexual partners, age at first intercourse, and history of anal intercourse. Because HPV is a sexually transmitted virus, one would expect colorectal cancer to be positively associated with some or all of these sexual risk factors if HPV plays a role in colorectal carcinogenesis. In addition, current case-control studies are small and do not adjust for potential confounding variables, such as age, sex, and smoking status. Finally, no prospective studies have been done to establish the temporal association between HPV and colorectal neoplasia. Therefore, despite some suggestive evidence of an association between HPV and colorectal neoplasia, large, well-designed studies are needed to test this hypothesis rigorously.

Summary and recommendations

It is notable that studies of colorectal cancer and infection have resulted in at least four possible candidates that may be involved in colorectal carcinogenesis. This lack of specificity between one etiologic agent and colorectal cancer may be a clue that the relationship between colorectal cancer and infection is not due to any of these agents, but instead is tied to a general disruption in the microflora of the gut, resulting in an increased susceptibility to pathogenic infection. Several review articles have discussed colorectal health in relation to normal flora in the gut (133-135). This is an area that deserves further investigation, and any studies of this topic should be especially vigilant in assessing the temporal association between disruptions in the normal flora of the gut and colorectal cancer.

In addition, the HPV and JC virus literature is dominated by studies that use PCR to detect viral DNA in colorectal neoplatic tissue. Because PCR is susceptible to false positive results due to contamination, further tests, such as serologic assays, should be done for exposure assessment. Another criticism of studies comparing tumor tissue in cases to normal tissue in controls is that the two types of tissues are not comparable, and the sensitivity and specificity of PCR-based testing techniques on normal control tissue is unknown. Again, serologic tests would allow for more comparable exposure assessment between cases and controls. However, serologic assays do not identify the site of infection, so site-specific testing is still an important component to evaluating the etiologic relationship between infectious agents and colorectal cancer.

Also, three of the infectious agents reviewed, *H. pylori*, HPV, and JC virus, were first evaluated as causes of cancer in other parts of the body, with two of them (*H. pylori* and

HPV) having clear positive associations with other cancers. This resulted in a whole host of studies assessing these agents' possible role in multiple cancers. However, neither HPV nor *H. pylori* is particularly suited to infect the colon, but hundreds of bacterial species that are adapted to colon have not been evaluated as possible causes of colorectal cancer.

Several sets of guidelines for establishing causality between an exposure and disease have been proposed, with one of the most famous of these being the Bradford Hill criteria (136). The Bradford Hill criteria are applied in epidemiologic studies and include the following: strength of the association (often measured by the magnitude of the odds ratio or relative risk estimate), temporality (exposure proceeds disease), consistency of studies, specificity (a one-to-one relationship in which the exposure leads to a single specific outcome), biological plausibility, coherence with prior knowledge, biological gradient (sometimes considered a dose-effect), analogy, and experimentation. A compilation of these criteria can be used to assess the likelihood that an exposure causes an outcome as opposed to being incidentally associated with the outcome. Table 1 summarizes an evaluation of each infectious agent in relation to colorectal cancer using Hill's criteria. Based in this table, it is clear that none of these agents show unequivocal, strong evidence for a causal association with colorectal cancer. None have been evaluated to determine if the infectious agent precedes the development of colorectal cancer. In addition, studies which assess viral or bacterial copy number in relation to disease severity, a way to evaluate the biological gradient criteria, are either limited or absent for these infectious agents.

Colorectal cancer clearly does not have one single necessary and sufficient cause. It is almost certain, on the basis of existing data that multiple pathways involving host genetics and environmental factors play a role in colorectal cancer carcinogenesis, and that subsets of colorectal cancer may be related to particular risk factors. For example, much of colorectal cancer (close to 85% of cases) is characterized by chromosomal instability in which the tumor cells display aneuploidy, an unusual chromosome number (137). However, approximately 15-17% of all colorectal carcinomas are characterized by microsatellite instability with mutations in, or methylation of, mismatch repair genes (138, 139). This subset appears to be associated with smoking (140), and it is hypothesized that colorectal tumors with chromosomal versus microsatellite instability result from different carcinogenic pathways and may have different etiologies (137).

Future studies of colorectal cancer and infectious agents should attempt to determine the subset of colorectal cancer that is most likely to be associated with the agent of interest, such as by studying the association between the subset colorectal cancer lacking p53 mutations and HPV infection. In addition, future studies should make the assessment of exposure more specific by focusing on the subtype of the agent of interest that is most pathogenic. For example, studies should focus on CagA positive strains of *H. pylori* or *S. bovis* type I instead of collapsing across all subtypes of these organisms. By focusing on more homogenous subsets of disease and subtypes of infectious agents, investigators may be able to increase the sensitivity of their studies to detect associations that may otherwise be masked by competing risk factors and misclassification of exposure status.

Also, prospective studies that establish temporality in the relationship between colorectal cancer and infection are necessary for evaluating causality.

Linking cancer to infectious agents has created a whole new direction for cancer prevention. Over the past 100 years, we have witnessed the eradication of infectious disease, such as smallpox, through vaccination, and we have seen the dramatic reduction in other vaccine-preventable diseases, such as measles and polio. With the advent of the Hepatitis B vaccine and the more recent HPV vaccine, we are likely to see dramatic decreases in morbidity and mortality due to liver cancer and cervical cancer. Through continued research in infectious agents and cancer, we may observe new associations as well as develop new effective means of prevention.

References

1. Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer 2006;118(12):3030-44.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55(2):74-108.
3. Kaz AM, Brentnall TA. Genetic testing for colon cancer. Nat Clin Pract Gastroenterol Hepatol 2006;3(12):670-9.
4. Potter JD. Colorectal cancer: molecules and populations. J Natl Cancer Inst 1999;91(11):916-32.
5. Komarova NL. Cancer, aging and the optimal tissue design. Semin Cancer Biol 2005;15(6):494-505.
6. Mak T, Lalloo F, Evans DG, Hill J. Molecular stool screening for colorectal cancer. Br J Surg 2004;91(7):790-800.
7. Muto T, Bussey HJ, Morson BC. The evolution of cancer of the colon and rectum. Cancer 1975;36(6):2251-70.
8. O'Brien MJ. Hyperplastic and serrated polyps of the colorectum. Gastroenterol Clin North Am 2007;36(4):947-68, viii.
9. Jass JR. Hyperplastic-like polyps as precursors of microsatellite-unstable colorectal cancer. Am J Clin Pathol 2003;119(6):773-5.
10. Hawkins NJ, Ward RL. Sporadic colorectal cancers with microsatellite instability and their possible origin in hyperplastic polyps and serrated adenomas. J Natl Cancer Inst 2001;93(17):1307-13.
11. Wong NA, Harrison DJ. Colorectal neoplasia in ulcerative colitis-recent advances. Histopathology 2001;39(3):221-34.
12. Guarner F. Enteric flora in health and disease. Digestion 2006;73 Suppl 1:5-12.
13. An international association between Helicobacter pylori infection and gastric cancer. The EUROGAST Study Group. Lancet 1993;341(8857):1359-62.
14. Malaty HM. Epidemiology of Helicobacter pylori infection. Best Pract Res Clin Gastroenterol 2007;21(2):205-14.
15. De Luca A, Iaquinto G. Helicobacter pylori and gastric diseases: a dangerous association. Cancer Lett 2004;213(1):1-10.
16. Logan RP. Helicobacter pylori and gastric cancer. Lancet 1994;344(8929):1078-9.

17. Parsonnet J, Friedman GD, Vandersteen DP, et al. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991;325(16):1127-31.
18. Lochhead P, El-Omar EM. Helicobacter pylori infection and gastric cancer. Best Pract Res Clin Gastroenterol 2007;21(2):281-97.
19. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum 1994;61:1-241.
20. Meucci G, Tatarella M, Vecchi M, et al. High prevalence of Helicobacter pylori infection in patients with colonic adenomas and carcinomas. J Clin Gastroenterol 1997;25(4):605-7.
21. Breuer-Katschinski B, Nemes K, Marr A, et al. Helicobacter pylori and the risk of colonic adenomas. Colorectal Adenoma Study Group. Digestion 1999;60(3):210-5.
22. Hartwich A, Konturek SJ, Pierzchalski P, et al. Helicobacter pylori infection, gastrin, cyclooxygenase-2, and apoptosis in colorectal cancer. Int J Colorectal Dis 2001;16(4):202-10.
23. Zumkeller N, Brenner H, Chang-Claude J, et al. Helicobacter pylori infection, interleukin-1 gene polymorphisms and the risk of colorectal cancer: evidence from a case-control study in Germany. Eur J Cancer 2007;43(8):1283-9.
24. Mizuno S, Morita Y, Inui T, et al. Helicobacter pylori infection is associated with colon adenomatous polyps detected by high-resolution colonoscopy. Int J Cancer 2005;117(6):1058-9.
25. Aydin A, Karasu Z, Zeytinoglu A, Kumanlioglu K, Ozacar T. Colorectal adenomateous polyps and Helicobacter pylori infection. Am J Gastroenterol 1999;94(4):1121-2.
26. Penman ID, el-Omar E, Ardill JE, et al. Plasma gastrin concentrations are normal in patients with colorectal neoplasia and unaltered following tumor resection. Gastroenterology 1994;106(5):1263-70.
27. Moss SF, Neugut AI, Garbowski GC, et al. Helicobacter pylori seroprevalence and colorectal neoplasia: evidence against an association. J Natl Cancer Inst 1995;87(10):762-3.
28. Thorburn CM, Friedman GD, Dickinson CJ, et al. Gastrin and colorectal cancer: a prospective study. Gastroenterology 1998;115(2):275-80.
29. Fireman Z, Trost L, Kopelman Y, Segal A, Sternberg A. Helicobacter pylori: seroprevalence and colorectal cancer. Isr Med Assoc J 2000;2(1):6-9.
30. Shmuely H, Passaro D, Figer A, et al. Relationship between Helicobacter pylori CagA status and colorectal cancer. Am J Gastroenterol 2001;96(12):3406-10.
31. Siddheshwar RK, Muhammad KB, Gray JC, Kelly SB. Seroprevalence of Helicobacter pylori in patients with colorectal polyps and colorectal carcinoma. Am J Gastroenterol 2001;96(1):84-8.
32. Limburg PJ, Stolzenberg-Solomon RZ, Colbert LH, et al. Helicobacter pylori seropositivity and colorectal cancer risk: a prospective study of male smokers. Cancer Epidemiol Biomarkers Prev 2002;11(10 Pt 1):1095-9.
33. Georgopoulos SD, Polymeros D, Triantafyllou K, et al. Hypergastrinemia is associated with increased risk of distal colon adenomas. Digestion 2006;74(1):42-6.
34. Machida-Montani A, Sasazuki S, Inoue M, et al. Atrophic gastritis, Helicobacter pylori, and colorectal cancer risk: a case-control study. Helicobacter 2007;12(4):328-32.

35. D'Onghia V, Leoncini R, Carli R, et al. Circulating gastrin and ghrelin levels in patients with colorectal cancer: correlation with tumour stage, Helicobacter pylori infection and BMI. Biomed Pharmacother 2007;61(2-3):137-41.
36. Zumkeller N, Brenner H, Zwahlen M, Rothenbacher D. Helicobacter pylori infection and colorectal cancer risk: a meta-analysis. Helicobacter 2006;11(2):75-80.
37. Inoue M, Tsugane S. Epidemiology of gastric cancer in Japan. Postgrad Med J 2005;81(957):419-24.
38. Sung JJ, Lau JY, Goh KL, Leung WK. Increasing incidence of colorectal cancer in Asia: implications for screening. Lancet Oncol 2005;6(11):871-6.
39. Beales IL, Crabtree JE, Scunes D, Covacci A, Calam J. Antibodies to CagA protein are associated with gastric atrophy in Helicobacter pylori infection. Eur J Gastroenterol Hepatol 1996;8(7):645-9.
40. Maeda S, Mentis AF. Pathogenesis of Helicobacter pylori infection. Helicobacter 2007;12 Suppl 1:10-4.
41. Chen TS, Chang FY, Chen PC, et al. Simplified 13C-urea breath test with a new infrared spectrometer for diagnosis of Helicobacter pylori infection. J Gastroenterol Hepatol 2003;18(11):1237-43.
42. Liou JM, Lin JW, Huang SP, Lin JT, Wu MS. Helicobacter pylori infection is not associated with increased risk of colorectal polyps in Taiwanese. Int J Cancer 2006;119(8):1999-2000.
43. Fujimori S, Kishida T, Kobayashi T, et al. Helicobacter pylori infection increases the risk of colorectal adenoma and adenocarcinoma, especially in women. J Gastroenterol 2005;40(9):887-93.
44. Bulajic M, Stimec B, Jesenofsky R, et al. Helicobacter pylori in colorectal carcinoma tissue. Cancer Epidemiol Biomarkers Prev 2007;16(3):631-3.
45. Grahn N, Hmani-Aifa M, Fransen K, Soderkvist P, Monstein HJ. Molecular identification of Helicobacter DNA present in human colorectal adenocarcinomas by 16S rDNA PCR amplification and pyrosequencing analysis. J Med Microbiol 2005;54(Pt 11):1031-5.
46. Jones M, Helliwell P, Pritchard C, Tharakan J, Mathew J. Helicobacter pylori in colorectal neoplasms: is there an aetiological relationship? World J Surg Oncol 2007;5:51.
47. Mulholland G, Ardill JE, Fillmore D, et al. Helicobacter pylori related hypergastrinaemia is the result of a selective increase in gastrin 17. Gut 1993;34(6):757-61.
48. Sobhani I, Lehy T, Laurent-Puig P, et al. Chronic endogenous hypergastrinemia in humans: evidence for a mitogenic effect on the colonic mucosa. Gastroenterology 1993;105(1):22-30.
49. Noble CJ. Carriage of group D streptococci in the human bowel. J Clin Pathol 1978;31(12):1182-6.
50. Knudtson LM, Hartman PA. Comparison of fluorescent gentamicin-thallous-carbonate and KF streptococcal agars to enumerate enterococci and fecal streptococci in meats. Appl Environ Microbiol 1993;59(3):936-8.
51. Ballet M, Gevigney G, Gare JP, et al. Infective endocarditis due to Streptococcus bovis. A report of 53 cases. Eur Heart J 1995;16(12):1975-80.

52. Kupferwasser I, Darius H, Muller AM, et al. Clinical and morphological characteristics in Streptococcus bovis endocarditis: a comparison with other causative microorganisms in 177 cases. Heart 1998;80(3):276-80.
53. Hoen B, Chirouze C, Cabell CH, et al. Emergence of endocarditis due to group D streptococci: findings derived from the merged database of the International Collaboration on Endocarditis. Eur J Clin Microbiol Infect Dis 2005;24(1):12-6.
54. 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(2):305-8.
55. Corredoira JC, Alonso MP, Garcia JF, et al. Clinical characteristics and significance of Streptococcus salivarius bacteremia and Streptococcus bovis bacteremia: a prospective 16-year study. Eur J Clin Microbiol Infect Dis 2005;24(4):250-5.
56. Biarc J, Nguyen IS, Pini A, et al. Carcinogenic properties of proteins with pro-inflammatory activity from Streptococcus infantarius (formerly S.bovis). Carcinogenesis 2004;25(8):1477-84.
57. Harris RE. Cyclooxygenase-2 (cox-2) and the inflammogenesis of cancer. Subcell Biochem 2007;42:93-126.
58. Mc CW, Mason JM, 3rd. Enterococcal endocarditis associated with carcinoma of the sigmoid; report of a case. J Med Assoc State Ala 1951;21(6):162-6.
59. Klein RS, Recco RA, Catalano MT, et al. Association of Streptococcus bovis with carcinoma of the colon. N Engl J Med 1977;297(15):800-2.
60. Klein RS, Catalano MT, Edberg SC, Casey JI, Steigbigel NH. Streptococcus bovis septicemia and carcinoma of the colon. Ann Intern Med 1979;91(4):560-2.
61. Murray HW, Roberts RB. Streptococcus bovis bacteremia and underlying gastrointestinal disease. Arch Intern Med 1978;138(7):1097-9.
62. Marshall JB, Gerhardt DC. Polyposis coli presenting with Streptococcus bovis endocarditis. Am J Gastroenterol 1981;75(4):314-6.
63. Friedrich IA, Wormser GP, Gottfried EB. The association of recent Streptococcus bovis bacteremia with colonic neoplasia. Mil Med 1982;147(7):584-5.
64. Reynolds JG, Silva E, McCormack WM. Association of Streptococcus bovis bacteremia with bowel disease. J Clin Microbiol 1983;17(4):696-7.
65. 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(220):439-50.
66. Leport C, Bure A, Leport J, Vilde JL. Incidence of colonic lesions in Streptococcus bovis and enterococcal endocarditis. Lancet 1987;1(8535):748.
67. Zarkin BA, Lillemoe KD, Cameron JL, et al. The triad of Streptococcus bovis bacteremia, colonic pathology, and liver disease. Ann Surg 1990;211(6):786-91; discussion 791-2.
68. Hoen B, Briancon S, Delahaye F, et al. Tumors of the colon increase the risk of developing Streptococcus bovis endocarditis: case-control study. Clin Infect Dis 1994;19(2):361-2.
69. Pergola V, Di Salvo G, Habib G, et al. Comparison of clinical and echocardiographic characteristics of Streptococcus bovis endocarditis with that caused by other pathogens. Am J Cardiol 2001;88(8):871-5.

70. Waisberg J, Matheus Cde O, Pimenta J. Infectious endocarditis from Streptococcus bovis associated with colonic carcinoma: case report and literature review. Arq Gastroenterol 2002;39(3):177-80.
71. Gold JS, Bayar S, Salem RR. Association of Streptococcus bovis bacteremia with colonic neoplasia and extracolonic malignancy. Arch Surg 2004;139(7):760-5.
72. Alazmi W, Bustamante M, O'Loughlin C, Gonzalez J, Raskin JB. The association of Streptococcus bovis bacteremia and gastrointestinal diseases: a retrospective analysis. Dig Dis Sci 2006;51(4):732-6.
73. 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(1):42-6.
74. Dubrow R, Edberg S, Wikfors E, et al. Fecal carriage of Streptococcus bovis and colorectal adenomas. Gastroenterology 1991;101(3):721-5.
75. Norfleet RG, Mitchell PD. Streptococcus bovis does not selectively colonize colorectal cancer and polyps. J Clin Gastroenterol 1993;17(1):25-8.
76. Potter MA, Cunliffe NA, Smith M, et al. A prospective controlled study of the association of Streptococcus bovis with colorectal carcinoma. J Clin Pathol 1998;51(6):473-4.
77. Darjee R, Gibb AP. Serological investigation into the association between Streptococcus bovis and colonic cancer. J Clin Pathol 1993;46(12):1116-9.
78. Tjalsma H, Scholler-Guinard M, Lasonder E, et al. Profiling the humoral immune response in colon cancer patients: diagnostic antigens from Streptococcus bovis. Int J Cancer 2006;119(9):2127-35.
79. Ellmerich S, Scholler M, Duranton B, et al. Promotion of intestinal carcinogenesis by Streptococcus bovis. Carcinogenesis 2000;21(4):753-6.
80. Walker DL, Padgett BL. The epidemiology of human polyomaviruses. Prog Clin Biol Res 1983;105:99-106.
81. Khalili K, Del Valle L, Otte J, Weaver M, Gordon J. Human neurotropic polyomavirus, JCV, and its role in carcinogenesis. Oncogene 2003;22(33):5181-91.
82. Hou J, Major EO. Progressive multifocal leukoencephalopathy: JC virus induced demyelination in the immune compromised host. J Neurovirol 2000;6 Suppl 2:S98-S100.
83. Shitrit D, Lev N, Bar-Gil-Shitrit A, Kramer MR. Progressive multifocal leukoencephalopathy in transplant recipients. Transpl Int 2005;17(11):658-65.
84. Haggerty S, Walker DL, Frisque RJ. JC virus-simian virus 40 genomes containing heterologous regulatory signals and chimeric early regions: identification of regions restricting transformation by JC virus. J Virol 1989;63(5):2180-90.
85. Bollag B, Chuke WF, Frisque RJ. Hybrid genomes of the polyomaviruses JC virus, BK virus, and simian virus 40: identification of sequences important for efficient transformation. J Virol 1989;63(2):863-72.
86. Khalili K, Del Valle L, Wang JY, et al. T-antigen of human polyomavirus JC cooperates withIGF-IR signaling system in cerebellar tumors of the childhood-medulloblastomas. Anticancer Res 2003;23(3A):2035-41.
87. Staib C, Pesch J, Gerwig R, et al. p53 inhibits JC virus DNA replication in vivo and interacts with JC virus large T-antigen. Virology 1996;219(1):237-46.
88. Niv Y, Goel A, Boland CR. JC virus and colorectal cancer: a possible trigger in the chromosomal instability pathways. Curr Opin Gastroenterol 2005;21(1):85-9.

89. Krynska B, Del Valle L, Croul S, et al. Detection of human neurotropic JC virus DNA sequence and expression of the viral oncogenic protein in pediatric medulloblastomas. Proc Natl Acad Sci U S A 1999;96(20):11519-24.
90. Caldarelli-Stefano R, Boldorini R, Monga G, et al. JC virus in human glial-derived tumors. Hum Pathol 2000;31(3):394-5.
91. Del Valle L, Gordon J, Assimakopoulou M, et al. Detection of JC virus DNA sequences and expression of the viral regulatory protein T-antigen in tumors of the central nervous system. Cancer Res 2001;61(10):4287-93.
92. Laghi L, Randolph AE, Chauhan DP, et al. JC virus DNA is present in the mucosa of the human colon and in colorectal cancers. Proc Natl Acad Sci U S A 1999;96(13):7484-9.
93. Ricciardiello L, Laghi L, Ramamirtham P, et al. JC virus DNA sequences are frequently present in the human upper and lower gastrointestinal tract. Gastroenterology 2000;119(5):1228-35.
94. Theodoropoulos G, Panoussopoulos D, Papaconstantinou I, et al. Assessment of JC polyoma virus in colon neoplasms. Dis Colon Rectum 2005;48(1):86-91.
95. Hori R, Murai Y, Tsuneyama K, et al. Detection of JC virus DNA sequences in colorectal cancers in Japan. Virchows Arch 2005;447(4):723-30.
96. Enam S, Del Valle L, Lara C, et al. Association of human polyomavirus JCV with colon cancer: evidence for interaction of viral T-antigen and beta-catenin. Cancer Res 2002;62(23):7093-101.
97. Casini B, Borgese L, Del Nonno F, et al. Presence and incidence of DNA sequences of human polyomaviruses BKV and JCV in colorectal tumor tissues. Anticancer Res 2005;25(2A):1079-85.
98. Goel A, Li MS, Nagasaka T, et al. Association of JC virus T-antigen expression with the methylator phenotype in sporadic colorectal cancers. Gastroenterology 2006;130(7):1950-61.
99. Hernandez Losa J, Fernandez-Soria V, Parada C, et al. JC virus and human colon carcinoma: an intriguing and inconclusive association. Gastroenterology 2003;124(1):268-9; author reply 269-70.
100. Newcomb PA, Bush AC, Stoner GL, et al. No evidence of an association of JC virus and colon neoplasia. Cancer Epidemiol Biomarkers Prev 2004;13(4):662-6.
101. Lundstig A, Stattin P, Persson K, et al. No excess risk for colorectal cancer among subjects seropositive for the JC polyomavirus. Int J Cancer 2007;121(5):1098-102.
102. Ricciardiello L, Baglioni M, Giovannini C, et al. Induction of chromosomal instability in colonic cells by the human polyomavirus JC virus. Cancer Res 2003;63(21):7256-62.
103. Ricciardiello L, Chang DK, Laghi L, et al. Mad-1 is the exclusive JC virus strain present in the human colon, and its transcriptional control region has a deleted 98-base-pair sequence in colon cancer tissues. J Virol 2001;75(4):1996-2001.
104. Wiley D, Masongsong E. Human papillomavirus: the burden of infection. Obstet Gynecol Surv 2006;61(6 Suppl 1):S3-14.
105. Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. J Natl Cancer Inst Monogr 2003(31):14-9.
106. Koutsky L. Epidemiology of genital human papillomavirus infection. Am J Med 1997;102(5A):3-8.

107. Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. Jama 2007;297(8):813-9.
108. Giuliano AR, Harris R, Sedjo RL, et al. Incidence, prevalence, and clearance of type-specific human papillomavirus infections: The Young Women's Health Study. J Infect Dis 2002;186(4):462-9.
109. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348(6):518-27.
110. Munoz N. Human papillomavirus and cancer: the epidemiological evidence. J Clin Virol 2000;19(1-2):1-5.
111. Steenbergen RD, de Wilde J, Wilting SM, et al. HPV-mediated transformation of the anogenital tract. J Clin Virol 2005;32 Suppl 1:S25-33.
112. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007;356(19):1944-56.
113. Tommasino M, Accardi R, Caldeira S, et al. The role of TP53 in Cervical carcinogenesis. Hum Mutat 2003;21(3):307-12.
114. Boguszakova L, Hirsch I, Brichacek B, et al. Absence of cytomegalovirus, Epstein-Barr virus, and papillomavirus DNA from adenoma and adenocarcinoma of the colon. Acta Virol 1988;32(4):303-8.
115. Koulos J, Symmans F, Chumas J, Nuovo G. Human papillomavirus detection in adenocarcinoma of the anus. Mod Pathol 1991;4(1):58-61.
116. Shroyer KR, Kim JG, Manos MM, et al. Papillomavirus found in anorectal squamous carcinoma, not in colon adenocarcinoma. Arch Surg 1992;127(6):741-4.
117. Shah KV, Daniel RW, Simons JW, Vogelstein B. Investigation of colon cancers for human papillomavirus genomic sequences by polymerase chain reaction. J Surg Oncol 1992;51(1):5-7.
118. McGregor B, Byrne P, Kirgan D, et al. Confirmation of the association of human papillomavirus with human colon cancer. Am J Surg 1993;166(6):738-40; discussion 741-2.
119. Cheng JY, Sheu LF, Lin JC, Meng CL. Detection of human papillomavirus DNA in colorectal adenomas. Arch Surg 1995;130(1):73-6.
120. Zhu Q, Cao J, Li S. [Detection of human papillomavirus gene in biopsies from colon carcinoma by PCR]. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 1999;13(4):352-4.
121. Lee YM, Leu SY, Chiang H, Fung CP, Liu WT. Human papillomavirus type 18 in colorectal cancer. J Microbiol Immunol Infect 2001;34(2):87-91.
122. Perez LO, Abba MC, Laguens RM, Golijow CD. Analysis of adenocarcinoma of the colon and rectum: detection of human papillomavirus (HPV) DNA by polymerase chain reaction. Colorectal Dis 2005;7(5):492-5.
123. Bodaghi S, Yamanegi K, Xiao SY, et al. Colorectal papillomavirus infection in patients with colorectal cancer. Clin Cancer Res 2005;11(8):2862-7.
124. Buyru N, Tezol A, Dalay N. Coexistence of K-ras mutations and HPV infection in colon cancer. BMC Cancer 2006;6:115.
125. Damin DC, Caetano MB, Rosito MA, et al. Evidence for an association of human papillomavirus infection and colorectal cancer. Eur J Surg Oncol 2007;33(5):569-74.
126. Kirgan D, Manalo P, Hall M, McGregor B. Association of human papillomavirus and colon neoplasms. Arch Surg 1990;125(7):862-5.

127. Buyru N, Budak M, Yazici H, Dalay N. p53 gene mutations are rare in human papillomavirus-associated colon cancer. Oncol Rep 2003;10(6):2089-92.
128. Slattery ML, Curtin K, Schaffer D, Anderson K, Samowitz W. Associations between family history of colorectal cancer and genetic alterations in tumors. Int J Cancer 2002;97(6):823-7.
129. Braakhuis BJ, Snijders PJ, Keune WJ, et al. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. J Natl Cancer Inst 2004;96(13):998-1006.
130. Weinberg DS, Newschaffer CJ, Topham A. Risk for colorectal cancer after gynecologic cancer. Ann Intern Med 1999;131(3):189-93.
131. Rex D. Should we colonoscope women with gynecologic cancer? Am J Gastroenterol 2000;95(3):812-3.
132. Chaturvedi AK, Engels EA, Gilbert ES, et al. Second cancers among 104,760 survivors of cervical cancer: evaluation of long-term risk. J Natl Cancer Inst 2007;99(21):1634-43.
133. Gorbach SL, Goldin BR. The intestinal microflora and the colon cancer connection. Rev Infect Dis 1990;12 Suppl 2:S252-61.
134. McGarr SE, Ridlon JM, Hylemon PB. Diet, anaerobic bacterial metabolism, and colon cancer: a review of the literature. J Clin Gastroenterol 2005;39(2):98-109.
135. O'Keefe SJ. Nutrition and colonic health: the critical role of the microbiota. Curr Opin Gastroenterol 2008;24(1):51-8.
136. Hill AB. The Environment And Disease: Association Or Causation? Proc R Soc Med 1965;58:295-300.
137. Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. Nature 1997;386(6625):623-7.
138. Samowitz WS, Slattery ML, Kerber RA. Microsatellite instability in human colonic cancer is not a useful clinical indicator of familial colorectal cancer. Gastroenterology 1995;109(6):1765-71.
139. Niv Y. Microsatellite instability and MLH1 promoter hypermethylation in colorectal cancer. World J Gastroenterol 2007;13(12):1767-9.
140. Chia VM, Newcomb PA, Bigler J, et al. Risk of microsatellite-unstable colorectal cancer is associated jointly with smoking and nonsteroidal anti-inflammatory drug use. Cancer Res 2006;66(13):6877-83.

Table 1. Evaluation of the association between colorectal cancer and *H. pylori*, *S. bovis*, JCV and HPV using the Bradford Hill criteria

	H. pylori	***S. bovis***	**JCV**	**HPV**
Strength	Meta-analysis OR=1.4 +	ORs for detection of *S. bovis* in stool range from 1.0-10.7 +	ORs for JCV DNA in cases vs. controls range from 1.0-6.2 +	ORs for HPV DNA in cases vs. controls range from 2.7-9.1 ++
Temporality	Not evaluated 0	Not evaluated 0	Not evaluated 0	Not evaluated 0
Consistency	Most studies OR≥1 ++	*S. bovis* endocarditis studies consistently show elevated risks for colorectal neoplasia. However, stool studies of colorectal cancer cases and controls are not consistent. ++	Inconsistent study results +	Early studies do not detect HPV DNA in colorectal neoplastic tissue. Nine recent case-control studies consistently report an increased risk. ++
Specificity	*H. pylori* is a known cause of gastric cancer. +	*S. bovis* is a cause of septicemia and endocarditis. +	JCV is known to cause PML +	HPV is a known cause of several anogenital cancers. +
Biological Plausibility	Association between gastrin, gastric *H. pylori* infection, and colorectal cancer is plausible. Direct infection of the colon is unlikely. ++	*S. bovis* is known to inhabit the colon, and molecular studies indicate that *S. bovis* proteins have carcinogenic properties. +++	JCV large T-antigen has carcinogenic properties, but its ability to infect the colon is under debate. +	HPV infects epithelial cells, and its oncogenic properties are well-described, but the ability of HPV to enter the colon and rectum is debatable. ++
Coherence	Geographic distribution of colorectal cancer differs significantly from gastric cancer. +	Many colorectal cancers exhibit over-expression of COX-2. *S. bovis* proteins upregulate COX-2 *in vitro*. +++	JCV is a neurotrophic virus and is very common in the population. +	Colorectal cancer occurs in epithelial cells, but the risk factors for HPV infection are not known for colorectal cancer. +
Biological gradient	No evidence for gastrin. Not evaluated for *H. pylori*. 0	Not evaluated 0	One study finds viral copy number higher in cases than in controls. +	Not evaluated 0
Analogy	*H. pylori* causes gastric cancer. ++	*H. pylori* induces inflammation in the stomach, resulting in cellular proliferation and increased gastric cancer risk. *S. bovis* could have a similar mechanism in the colon. ++	SV40, another polyomavirus, is hypothesized to cause certain human cancers, including brain cancer. However, this has not been proven. +	HPV causes adenocarcinoma in the cervix. ++
Experiment	Not evaluated, but it is possible to treat *H. pylori* infection. 0	Not evaluated, but it is possible to treat *S. bovis* infection. 0	Not evaluated, and currently it is not possible to prevent JCV through vaccination. 0	Not evaluated, but it is possible through HPV vaccination studies. 0

0 = Not evaluated, + = Weak evidence, ++ = Moderate evidence, +++ = Strong evidence