Clinical Importance of *Streptococcus gallolyticus* Infection Among Colorectal Cancer Patients: Systematic Review and Meta-analysis

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Background. *Streptococcus bovis* has long been associated with colorectal cancer (CRC). However, not all genospecies are as closely related to CRC. With this systematic review, we aim to increase the awareness of the association between *S. bovis* biotype I (*Streptococcus gallolyticus*) and CRC and urge for uniform molecular microbiological classification.

Methods. In January 2011, the PubMed database was searched for all studies that investigated the association between *S. bovis*, infective endocarditis (IE), and CRC. A total of 191 studies were screened for eligibility and yielded 52 case reports and 31 case series, of which 11 were used for meta-analysis on the association between *S. bovis* biotype, IE, and adenomas/carcinomas (CRC).

Results. Among the *S. bovis*-infected patients who underwent colonic evaluation, the median percentage of patients who had concomitant adenomas/carcinomas was 60% (interquartile range, 22%), which largely exceeds the disease rate reported in the general asymptomatic population. Meta-analysis showed that patients with *S. bovis* biotype I infection had a strongly increased risk of having CRC (pooled odds ratio [OR], 7.26; 95% confidence interval [CI], 3.94–13.36) and IE (pooled OR, 16.61; 95% CI, 8.85–31.16), compared with *S. bovis* biotype II–infected patients. Notably, CRC occurred more often among patients with *S. bovis* IE than among patients with *S. bovis* infection at other sites (pooled OR, 3.72; 95% CI, 2.03–6.81).

Conclusions. Our meta-analysis clearly indicates that *S. bovis* should no longer be regarded as a single species in clinical practice, because *S. gallolyticus* (*S. bovis* biotype I) infection, in particular, has an unambiguous association with CRC.
these biotypes into *Streptococcus gallolyticus* subsp *gallolyticus*, *Streptococcus infantarius* subsp *infantarius* (II/1), *S. infantarius* subsp *coli* (II/1), and *S. gallolyticus* subsp *pasteurianus* (II/2), based on molecular characteristics (Table 1) [6–8]. The latter nomenclature has been embraced by some but not by the majority of studies that have appeared in literature since then. This lack of uniform microbiological classification in scientific literature has led to an underestimation of the relationship between *S. bovis* and CRC, because not all genospecies seem to be as closely related to colonic malignancies. With this systematic review, we aim to increase the awareness of the specific association between *S. gallolyticus* subsp *gallolyticus* (biotype I) and CRC. Furthermore, we urge for proper classification of *S. bovis*–related strains to increase the understanding of disease pathology, which could have major implications for the early detection of CRC and patients’ health.

**METHODS**

**Data Sources and Study Selection**

In January 2011, a total of 4 independent searches of PubMed (www.ncbi.nlm.nih.gov/pubmed) were performed using the following search terms: “*Streptococcus bovis* AND colorectal cancer (MeSH terms),” “*Streptococcus gallolyticus* AND colorectal cancer (MeSH terms),” “*Streptococcus infantarius* AND colorectal cancer (MeSH terms),” “*Streptococcus pasteurianus* AND colorectal cancer (MeSH terms),” “*Streptococcus bovis* and malignancy,” and “endocarditis AND colorectal cancer (MeSH terms).” After elimination of duplicates, this yielded a total of 191 records. Studies that reported infection with *S. bovis* or one of its subspecies were selected for individual evaluation. Case series from the period during 1970–2010 with a clear description of CRC rates among *S. bovis*–infected patients aged >20 years were selected for further evaluation. All narrative literature reviews, studies that were not published in the English language, and studies comprising in vitro research were excluded. Thus, a total of 52 case reports and 31 case series were included in this review. Of the 31 case series, 11 provided detailed information (see below) suitable for inclusion in the meta-analysis (Supplementary Figure 1; online only).

**Data Extraction**

The 52 case reports were screened for the following information: type of infection, *S. bovis* biotypes involved, stage of premalignant and malignant lesions, and underlying diseases other than CRC. The 31 case series were screened to extract the following information: number of patients, mean age of patients, number of gastrointestinal evaluations, number of infective endocarditis (IE) cases, biotypes reported, number of adenomas and carcinomas, and presence of other infections or gastrointestinal disorders. Adenomas include neoplastic polyps (adenomatous polyps, villous adenomas, tubular adenomas, and adenomas) but exclude nonneoplastic polyps (Supplementary Table 1; online only). Because most articles did not use the genotypic designation of the *S. bovis/Streptococcus equinus* complex (Table 1) [5], we used the former phenotypic designation and assumed that species that were characterized as *S. bovis* biotype I belonged to the genospecies *S. gallolyticus* subsp *gallolyticus*. The synonyms for the different genotypes (Table 1) were used throughout this review, and the *S. bovis* biotype II strains were grouped together, because 6 studies did not specify the number of species belonging to biotype II/1 and II/2. When no further classification was reported, the general term “*S. bovis*” was used. From these data, the CRC occurrence among all patients with *S. bovis* infection and among colonic evaluated subjects was calculated. Unless stated otherwise, the term “CRC” relates both to carcinomas and to adenomas. CRC occurrence was subcategorized by *S. bovis* biotype when possible. The median prevalence with corresponding interquartile range (IQR; defined as Q3–Q1) was calculated for a combined overview of the case series. The methods used to determine the rate of CRC, the *S. bovis* biotype involved, and the presence of IE are presented in Figure 1.

**Meta-analysis**

For quantitative meta-analysis, 11 of the 31 case series were selected that discriminated between *S. bovis* biotypes or between IE and other infection sites. The aim of this meta-analysis was to assess the risk of CRC or IE among *S. bovis* biotypes. Furthermore, the risk of CRC among *S. bovis* IE or infections at other sites was assessed. Data were extracted independently by 2 reviewers, and discrepancies in data extraction were resolved by repeated manuscript review to reach consensus. We reported only patients who underwent gastrointestinal evaluation when possible. We calculated odds ratios (ORs) with 95% confidence intervals (CIs) for all studies with sufficient data on the previously specified associations. Pooled ORs were calculated as the weighted mean of the ORs for the associations of interest. Weights were assigned according to the inverse of the variance.
Figure 1. Flow diagram of methods used to assess biotype, infective endocarditis (IE), colorectal cancer (CRC). This flow-diagram shows the methods used for the determination of S. bovis biotypes, diagnosis of IE, and assessment of colonic disease in 31 case series. The number of studies that used the indicated method is shown in brackets. In 21 of these case series, colonic evaluations were performed. Abbreviations: TEE, transesophageal echocardiography; TTE, transthoracic echocardiography.

Heterogeneity was tested using I² tests and was quantified using the I² statistic [9]. Because all values of the I² statistic were <50%, fixed-effects models were used. Statistical analyses were performed using RevMan software version 5.0.25 for Windows (The Nordic Cochrane Centre, The Cochrane Collaboration).

RESULTS

Case Reports and Case Series
A total of 12 case series provided detailed information on S. bovis biotypes. Of these studies, 3 provided the number of S. bovis biotype I and II infections but did not report the number of CRC cases stratified by biotype [28, 36, 38]. Another 3 studies comprised almost exclusively S. bovis biotype I–infected patients, with reported prevalence of CRC of 33%, 52%, and 59% (Table 2) [26, 29, 34]. The remaining 6 studies were included in the meta-analyses [8, 18, 31, 32, 35, 37] and showed that patients with a S. bovis biotype I infection had a statistically significantly increased risk of CRC, compared with S. bovis biotype II–infected individuals (pooled OR, 7.26; 95% CI, 3.94–13.36) (Figure 3). However, it should be noted that the inconsistent naming of S. bovis because of characterization through phenotypic and genotypic methods could have biased the results of this analysis. Nonetheless, from these studies, it is evident that infection with S. bovis biotype I, currently known as S. gallolyticus, is strongly associated with CRC (prevalence range, 33%–71%) that markedly exceeds the normal prevalence of CRC (10%–25%) in the general population.

Biotypes and Infective Endocarditis
It has previously been reported that S. bovis biotype I infection is more often associated with IE than is S. bovis biotype II infection [44]. To investigate this further, 7 case series that stratified the number of IE cases by biotype were selected for statistical analysis [8, 18, 31, 32, 36–38]. This analysis showed that 43%–100% of the S. bovis biotype I–infected patients presented with IE, whereas IE was markedly less common (8%–29%) among S. bovis biotype II–infected patients (pooled...
Table 2. Case Series on *Streptococcus bovis* and Colorectal Cancer

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OR, 16.61; 95% CI, 8.85–31.16) (Figure 3). This finding strongly suggests that S. bovis biotype I is a more potent causative agent for IE than is S. bovis biotype II.

Infective Endocarditis in Relation to Colorectal Cancer
Because S. bovis biotype I, in particular, is strongly associated with both CRC and IE, it can be envisaged that CRC occurs more often among S. bovis endocarditis cases than among cases of S. bovis infection at other locations. Therefore, 6 case series that stratified the number of CRC cases by infection site were included in this analysis [10, 19, 23, 36–38]. As indicated in Figure 3, the percentage of CRC among patients with IE ranged from 12% to 93%, whereas this was lower among patients with S. bovis infection at other locations (10%–50%), yielding a pooled OR of 3.72 (95% CI, 1.4–9.4) for tumors; 3.4 (95% CI, 1.2–9.2) for adenomas, and 5.7 (95% CI, 9–48.5) for adenocarcinomas.

DISCUSSION
Summary of Evidence
To our knowledge, this is the first meta-analysis on S. bovis infection that evaluated the relationship between different subtypes of this bacterium, infection sites, and adenomatous and carcinomatous colonic lesions. These analyses clearly showed that adenomas or carcinomas (CRC) were significantly more prevalent among patients with S. bovis biotype I infection than among patients who were diagnosed with a S. bovis
biotype II infection. In fact, the incidence of CRC among S. bovis biotype II–infected patients may not even exceed that in the general asymptomatic population [39–43]. These different associations may have accounted for the wide prevalence range of CRC that has been reported over the years (Figure 2). Of importance, because most studies did not discriminate S. bovis biotypes, the association of S. bovis biotype I and CRC has systematically been underestimated. With our current meta-analysis, we emphasize that S. bovis biotype I is a sign of occult CRC. Conspicuously, both S. bovis IE and CRC are more frequently diagnosed in the older population [44–47]. In this respect, it is important to know that S. bovis biotype I has been recently renamed S. gallolyticus. We and others have shown that collagen I/IV binding is one of the distinguished features of S. gallolyticus strains [48–50]. Superior binding to collagen I on heart valves could be responsible for the increased occurrence of S. gallolyticus IE, compared with that caused by other S. bovis biotypes. Intriguingly, polyps and early colorectal tumors are surrounded by a continuous and increased expression of collagen IV, which is distinct from healthy tissues [51–53]. Thus, S. gallolyticus strains may also have a competitive advantage to colonize collagen-rich premalignant or malignant sites in the intestine. Together, this could explain why S. gallolyticus (S. bovis biotype I) is strongly associated with both IE and CRC. Recently, S. gallolyticus DNA was detected in 49% of CRC tissue samples, whereas only 8% of healthy colonic samples were found to be positive [54], which correlates with increased fecal carriage of S. bovis in patients with CRC [3, 55]. Previous research showed that the antibody response against S. gallolyticus antigens was significantly increased in patients with early stages of CRC, compared with asymptomatic control subjects [56, 57]. Moreover, S. bovis has been cultured from blood samples from asymptomatic individuals with occult CRC [58, 59]. Together, these findings show that these infection can occur at the subclinical level, which accentuates the potency of S. gallolyticus as a diagnostic tool for CRC.

Limitations
During the course of this meta-analysis, some difficulties emerged during data extraction and evaluation that could have introduced bias. The first and most important limitation that could have led to information bias was the inconsistent naming of S. bovis subspecies among studies. Evolving insights and microbiological techniques have extended the knowledge on its genotypic and phenotypic characteristics [5]. However, this new nomenclature has only slowly been adopted by clinicians and is still not consistently used throughout studies and case reports. Second, for various reasons, not all included patients were screened by colonoscopy, leading to underestimation of the association between S. bovis and CRC. Conversely, some studies did not specify whether polyps were adenomas or belonged to nonneoplastic polyps, which could have overestimated the association. Furthermore, most studies included cases based on retrospective chart evaluation, which may have introduced selection bias. For example, if only patients who already were suspected of having gastrointestinal disease were screened, the observed association would have been overestimated. Finally, the diagnosis of IE according to (modified) Duke’s criteria is a subjective procedure that is based on the opinion of the individual physician and could therefore differ substantially among hospitals. This could have introduced detection bias across studies [60, 61].

CONCLUSIONS AND RECOMMENDATIONS
The most important conclusion of this meta-analysis is that S. bovis biotypes should no longer be regarded as a single species, because S. gallolyticus (S. bovis biotype I), in particular, has an unambiguous association with CRC. In this respect, we would like to increase the awareness of the new nomenclature of different S. bovis subspecies among physicians [62, 63] and recommend that clinical microbiologists, researchers, and physicians use the classification as proposed by Schlegel et al [5], both in clinical practice and in scientific publications (Table 1). To implement such a classification regime implies that, in all cases, molecular genetic techniques should be used for the determination of S. bovis group bacteria. Second, we stimulate researchers to conduct properly designed case-control studies.
including colonoscopic evaluation of all included participants, to further unravel the association between S. gallolyticus infection and different stages of CRC. Finally, we encourage research that aims at the elucidation of those virulence features (e.g., collagen-binding properties) that are responsible for the specific association between S. gallolyticus IE and CRC. We believe that microbiological classification tools based on such features will allow further improvement of the guidelines to screen for underlying occult malignancy in case of bacterial infection. Furthermore, this may provide tools for the early detection of subclinical infections that are associated with CRC in a larger part of the population. The annual incidence of this disease is approximately 1 million cases in Western societies. Unfortunately, approximately 40% of the cases are detected during an advanced stage, resulting in a sharp decline in prognosis. Therefore, the early detection of CRC is one of the great challenges in the battle against this disease. Ultimately, S. gallolyticus–related diagnostic tools may aid CRC screening programs and, thereby, contribute to a decrease in the morbidity and mortality associated with this disease.

Figure 3. Forest plots on the relationship between biotype, infective endocarditis (IE), and colorectal cancer (CRC). Forest plots were generated to provide a more detailed view on the relationship between Streptococcus bovis biotype, IE, and CRC. Pooled odds ratios (ORs) were calculated as the weighted mean of the ORs for the associations of interest. The occurrence of (A) CRC and (B) IE among S. bovis biotype I–infected patients, compared with that among S. bovis biotype II–infected patients, and (C) CRC among patients with S. bovis IE, compared with patients with S. bovis infection at other sites, is shown.
References


Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/).

Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Wilbert Peters, Rian Roelofs, Tanja Schulin, Albert Bolhuis, Philippe Glaser, Shaynoor Dramsi, and Ikuko Kato for valuable discussions on this subject; Juan Corredoira and Vikram Vaska for giving us additional information on their studies; and the anonymous reviewers for their valuable comments.

Financial support. This work was supported by the Dutch Cancer Society (KWF; project KUN 2006-3591 to A. B.). The funding source had no involvement in the study design.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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