

Does Human JC Virus Play a Role in the International Variations and Temporal Trends of Early-Onset Colorectal Cancer? A Meta-Analysis

Hytham K. S. Hamid¹, MRCSEd, Artur Marc-Hernández², MD, Jaime Ruiz-Tovar³, MD, PhD

1 Department of Surgery, Soba University Hospital, Khartoum, Sudan;

2 Department of Surgery, University Hospital Rey Juan Carlos, Madrid, Spain;

3 Department of Surgery, University Alfonso X, Madrid, Spain.

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Correspondence to: Hytham K. S. Hamid, Department of Surgery, Soba University Hospital, Khartoum, Sudan.

Email: kujali2@gmail.com

Telephone: +249902517760

ORCID No: 0000-0001-8068-0154

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ABSTRACT

The prevalence and incidence trend of early-onset colorectal cancer (EO-CRC) exhibit wide variations across the world, which could be attributed to early-life exposures such as diet and viral infections. We carried out a systematic review and meta-analysis to examine the association between John Cunningham virus (JCV) infection and EO-CRC. Literature was searched for studies comparing the presence of JCV DNA in colorectal cancerous and non-cancerous tissues. Countries where the studies were conducted were categorized into high and low prevalence settings for EO-CRC, and according to the time-weighted average annual per cent change in incidence rates of

EO-CRC into rising-trend, stable-trend, and decreasing-trend settings. A total of 19 studies with 3065 participants were included. The meta-analysis showed that JCV increased the odds for colorectal adenoma development (6-fold) more than colorectal cancer (4.1-fold), in comparison to controls. The odds for colorectal tumor development with JCV infection were significantly higher in the high-prevalence settings than in the low-prevalence settings when compared with controls and matched adjacent colorectal tissues. Similarly, the odds for colorectal tumor development trended to be higher in the rising-trend settings than in the stable-trend settings when compared with controls and matched adjacent colorectal tissues; no JCV DNA was detected in the decreasing-trend settings in both cancerous and non-cancerous colorectal tissues. These results suggest that JCV is likely involved in early stage of colorectal carcinogenesis, and may play a role in the international variations and temporal trends of EO-CRC in some parts of the world.

Key words: John Cunningham virus; Early-onset colorectal cancer; Colorectal adenoma; Prevalence; Incidence trend

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INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide, accounting for nearly 1.8 million new cases in 2018 and is projected to increase to more than 2.2 million new cases by 2030^[1,2]. The distribution of CRC burden varies widely, with more than two-thirds of cases occurring in countries with a high or very high human developmental index^[2]. For early-onset colorectal cancer (EO-CRC), defined as CRC diagnosed before the age of 50, the incidence rates were shown to be highest in developed countries like USA and Australia, while the lowest rates were recorded in

developing countries such as Uganda and India^[3]. Similarly, the prevalence of EO-CRC exhibits wide variations across the world with higher proportions of patients diagnosed under 50 years reported in Africa and Asia (16-52%) compared to Europe and North America (< 10 %)^[4]. This geographical disparity unlikely relates to hereditary factors, since familial tumors and hereditary syndromes are implicated in only a fraction (20%) of EO-CRC^[4], and would rather signal differences in early-life exposures such as diet and viral infections that influence colorectal carcinogenesis^[5,6].

John Cunningham virus (JCV) is a neurotropic human polyoma virus with cell transforming properties, which is highly prevalent in human upper and lower gastrointestinal tract^[7]. Since the first report by Laghi *et al*^[8], demonstrating presence of JCV DNA in malignant colorectal epithelium, several studies have pointed out an association between JCV infection and colorectal tumors^[9-17]. Considering that JCV infection occurs during childhood, we hypothesized that JCV may play a role in the international variation and temporal trends of EO-CRC. Therefore, we conducted this systematic review and meta-analysis to investigate the possible association between JCV infection and colorectal tumors with special reference to EO-CRC.

METHODS

Search strategy

A systematic review was performed following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement^[18]. The PubMed, Cochrane Collaboration, and Embase databases were explored to identify relevant studies published before November 1, 2019. Our search strategy included terms for: "JCV" OR "JC virus" OR "polyomavirus" AND "colorectal" OR "rectal" OR "rectum" OR "colonic" OR "colon" AND "cancer" OR "carcinoma" OR "adenoma" OR "adenocarcinoma" OR "malignancy" OR "tumor" OR "neoplasm", without any restrictions for language. The reference lists in each identified article were also searched.

Study selection

A single reviewer (H.H) screened the titles and abstracts. Full articles were assessed by 2 independent reviewers (H.H and A.H) and discrepancies were discussed and resolved by joint discussion. Inclusion criteria included (1) studies comparing the presence of JCV in colorectal cancer and/or adenoma with non-neoplastic colorectal tissues, and (2) studies employing polymerase chain reaction (PCR) to examine for presence of JCV nucleic acid material in tissues.

Exclusion criteria were as follows: non-comparative studies, studies not reporting on colorectal tumors separately, using non-colorectal tissue control, employing method other than PCR to detect JCV infection, and small-sampled studies (< 10 patients). Review articles, case reports, letters, and conference abstracts with no access to the entire report were also excluded.

Data extraction

Data extraction and methodologic quality assessment were carried out by two independent reviewers (H.H and A.M), and any disagreements were resolved through discussion and consensus. The methodological quality of each study was assessed using the Newcastle-Ottawa scale (NOS) for observational studies^[19], and any disagreements were resolved through discussion and consensus. Studies with a cumulative score ≥ 7 were considered to be of high quality^[20,21].

Data were collected on study design, year of publication, country,

method of JCV testing, type of tumor (cancer vs. adenoma), specimen, and control (adjacent vs. non-adjacent tissues), number of positive and negative cases in terms of the presence of JCV, and quality assessment. Adjacent tissues were defined as non-neoplastic colorectal tissues obtained from the same individual, whereas non-adjacent tissues were defined as non-neoplastic colorectal tissues obtained from non-cancer patients. The corresponding authors were contacted by email when required to request incomplete or unpublished data.

Prevalence and incidence trends of EO-CRC

Data on the relative proportion of EO-CRC (ie. proportion of CRC cases diagnosed under age 50 years) in the countries where studies were conducted were retrieved from the most recent and comprehensive report published within the last 10 years^[22-33]. Moreover, the trends in the incidence rates of EO-CRC in these countries were obtained from population-based annual incidence data reported by the national cancer registries in each country^[3,34-38]. These trends spanned a minimum of 10-year period between 1990 and 2016. The epidemiologic knowledge was used to define high- and low-prevalence settings based on whether the relative proportion of EO-CRC was > 10% or < 10% in the respective countries. Similarly, countries were categorized into rising-trend, stable-trend, and decreasing-trend settings, according to the trends in the incidence rates of EO-CRC. The trend was described as "increasing", "stable", or "decreasing" based on the time-weighted average annual per cent change in incidence rates of EO-CRC^[3]. One study, conducted in Greece, was excluded from the final subgroup analysis as no data on the relative proportion and the incidence trend of EO-CRC was available^[39].

Statistical analysis

All analyses were performed using Review Manager (RevMan version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) and STATA software (version 16, StataCorp., College Station, TX). Dichotomous variables were expressed as odd ratios (ORs) and 95% confidence interval (CI).

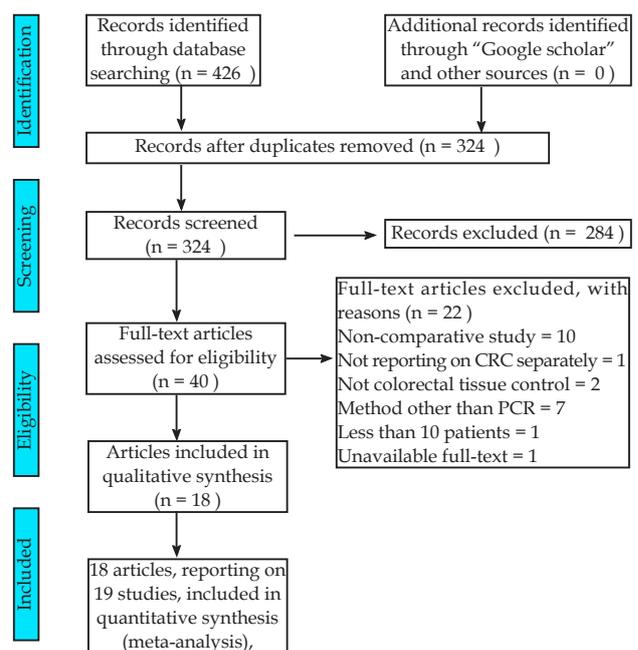


Figure 1 PRISMA flow chart.

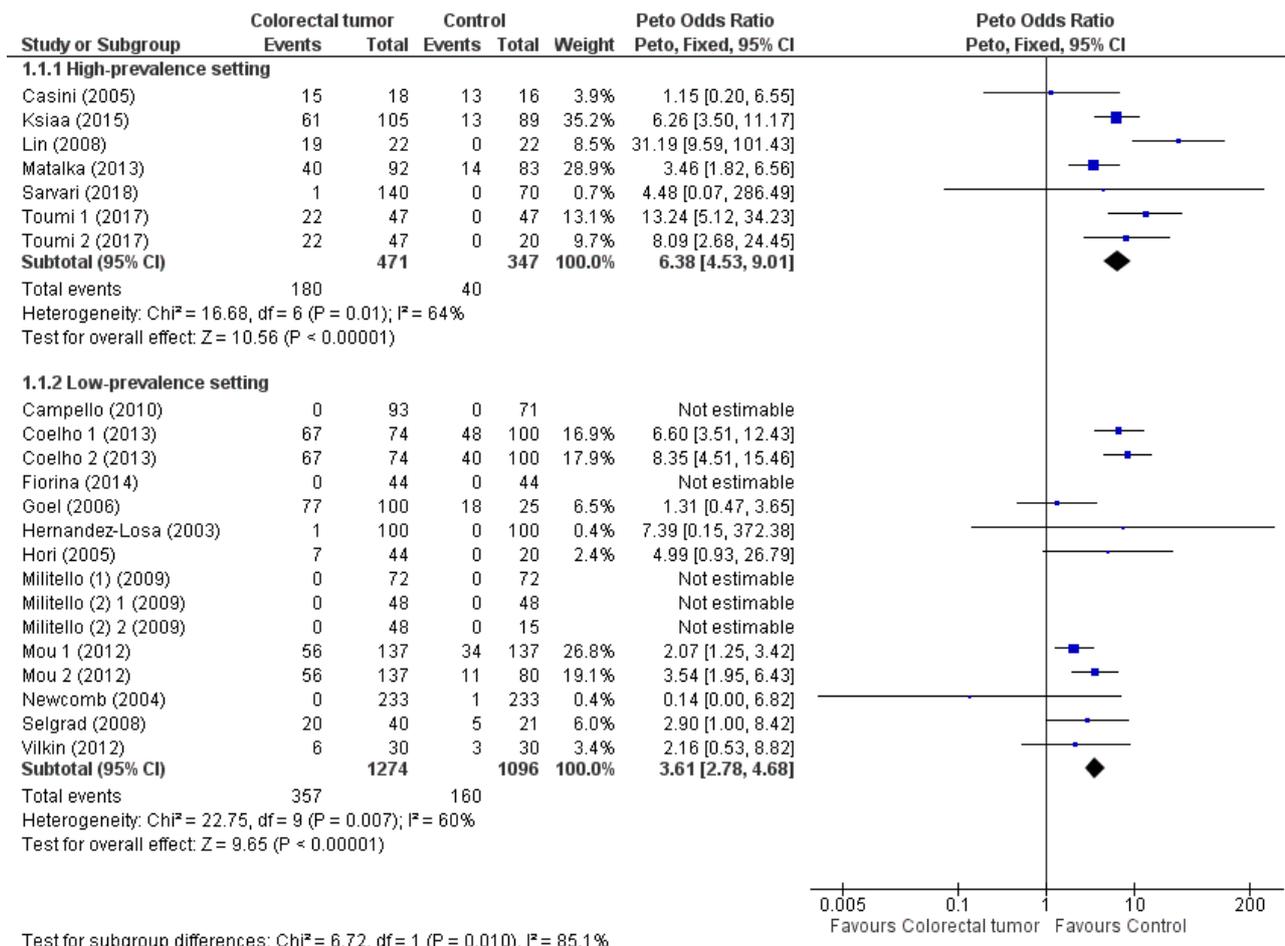


Figure 2 JCV infection incidence in colorectal tumors vs. controls according to the prevalence settings

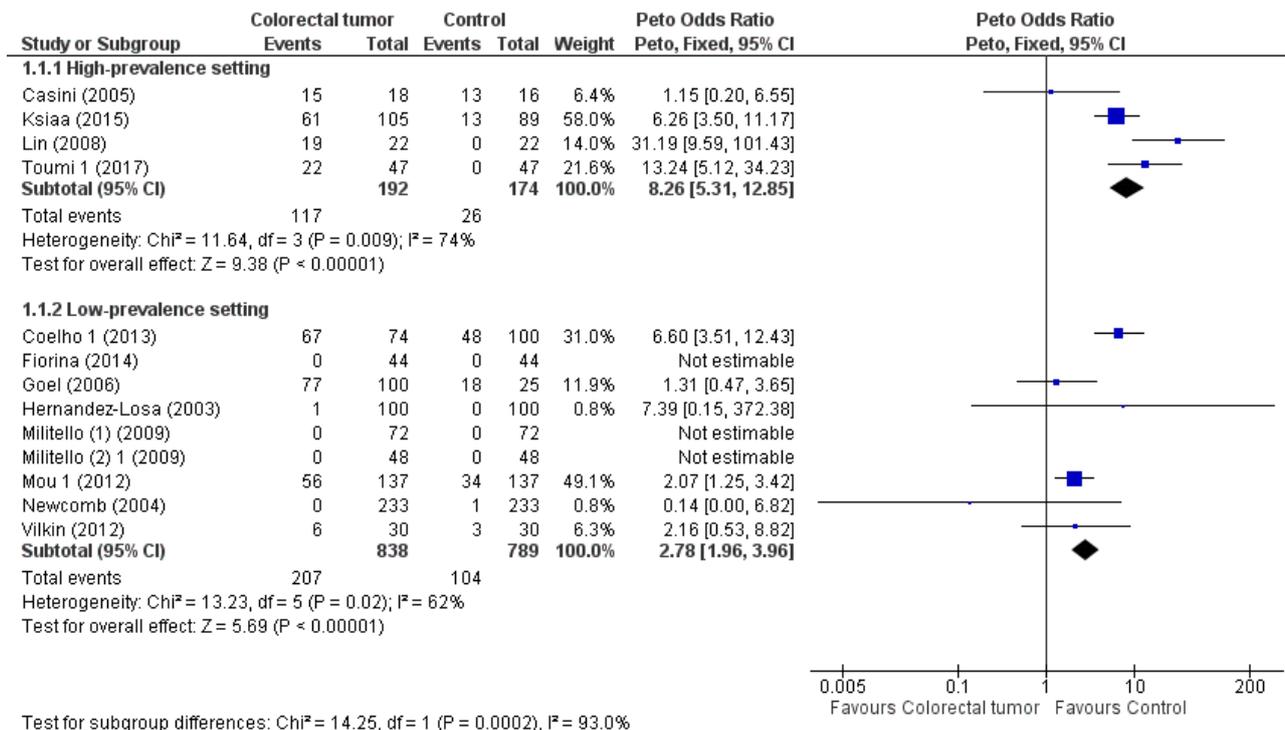


Figure 3 JCV infection incidence in colorectal tumors vs. adjacent tissues according to the prevalence settings.

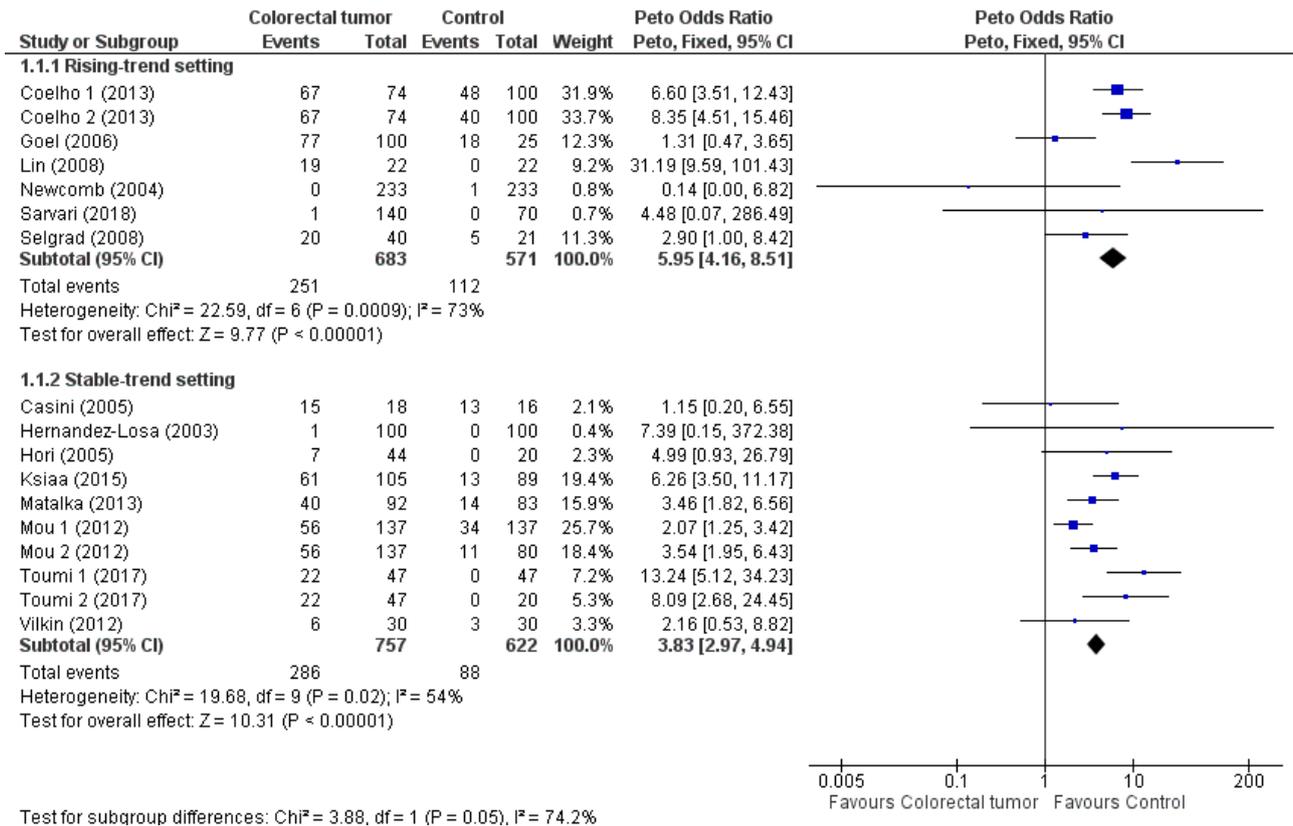


Figure 4 JCV infection incidence in colorectal tumors vs. controls according to the incidence trend settings.

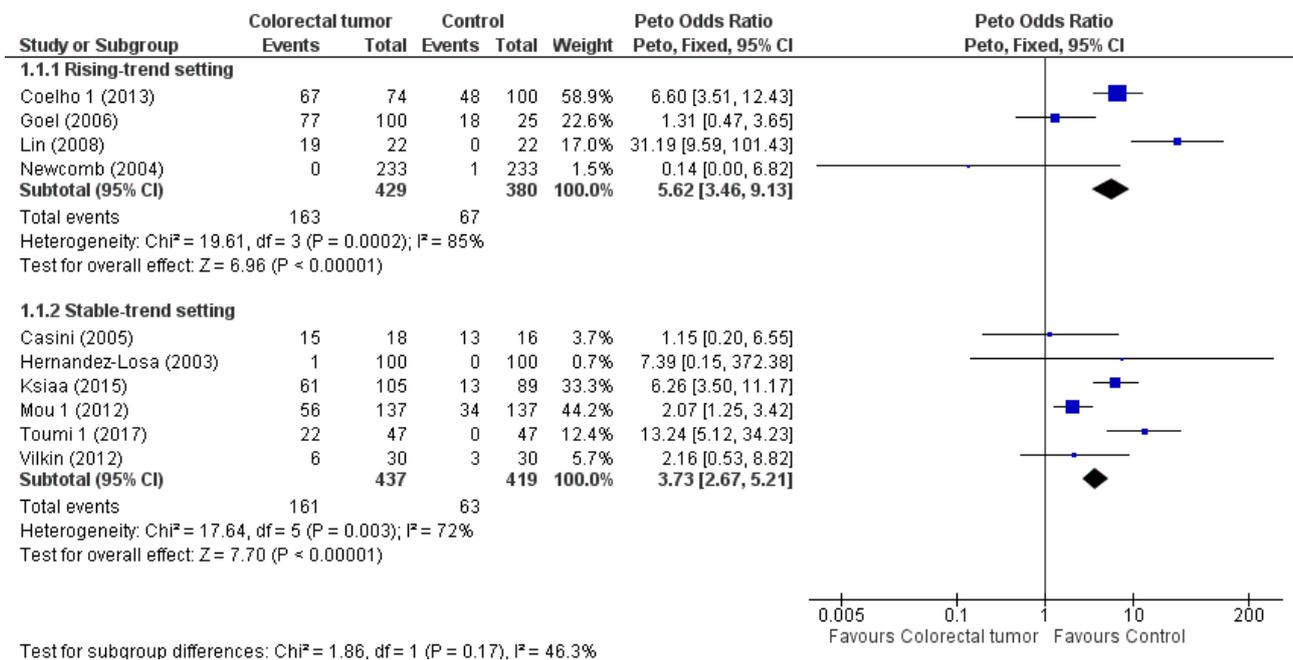


Figure 5 JCV infection incidence in colorectal tumors vs. adjacent tissues according to the incidence trend settings.

The Peto method along with an inverse-variance fixed-effect model was used to calculate the ORs, because this method performs well in the settings of low event rates and zero events, and does not require zero-cell corrections^[40]. Heterogeneity was assessed using the I^2 statistic. Pooled prevalence and Peto ORs were calculated for both colorectal cancer and adenoma. Subgroup analysis was performed to assess the difference in the Peto ORs according to the type of control used, and to evaluate a possible association between the prevalence

and incidence trend of EO-CRC and the effect size on evaluated outcomes. A leave-one-out sensitivity analysis was conducted to assess if our results were driven by any study. Publication bias was assessed through funnel plots and Egger’s regression test^[41].

RESULTS

Study Identification and Characteristics

The systematic search yielded a total of 426 unique articles. After title and abstract screening, 40 full-text articles were evaluated for eligibility. Finally, 18 articles reporting on 19 studies were included in the quantitative synthesis^[9-17,39,42-49]. Details of the study selection process are shown in Figure 1.

The characteristics and methodologic quality evaluation of 19 eligible studies are summarized in Table 1 and Supplementary Table 1. These studies were published between 2003 and 2018, and involved 3065 participants (1544 patients and 1521 controls). Of the 19 studies, 17 were case-control^[9-16,39,43-49], and 2 were cross-sectional^[17,42]. Eight studies were conducted in Europe, 6 in Asia, 3 in Africa, and 2 in North America. Testing for JCV infection was performed using PCR in 12 studies^[10,11,13,15,16,42-44,47-49], and nested PCR in 7 studies^[9,12,14,17,39,45,46]; all but one study^[39] employed primers targeting JCV large T antigen (T-Ag) sequence. Nine studies examined for JCV infection in colorectal cancer and adjacent tissues^[10,13,17,42,43,45,46,48,49]. Five studies compared JCV infection in colorectal cancer/adenoma with nonadjacent tissues^[12,15,16,44,47]. Another 5 studies included colorectal cancer/adenoma and controlled for both adjacent and non-adjacent tissues^[9,11,14,39,42]. The NOS scores of the included studies ranged from 5 to 9; 10 studies were considered high quality.

Association between JCV and colorectal tumors

Overall, the pooled prevalence of JCV infection in colorectal cancer and adenoma was 0.34 (95% CI 0.29-0.40) and 0.37 (95% CI 0.08-0.66), respectively. Compared to controls, JCV infection was significantly associated with increased risk of colorectal cancer (Peto OR 4.07 [95% CI 3.29-5.04], *p* < 0.001; *I*² = 58%) and colorectal adenoma (Peto OR 5.95 [95% CI 3.99-8.85], *p* < 0.001; *I*² = 0%) (Supplementary Fig. 1 and 2). On subgroup analysis, JCV infection was significantly associated with increased risk of colorectal cancer when compared with adjacent tissues (Peto OR 4.01 [95% CI 3.06-5.25], *P* < 0.001; *I*² = 73%) and with non-adjacent tissues (Peto OR 4.19 [95% CI 2.96-5.92], *p* < 0.001; *I*² = 0%) (Supplementary Fig. 3). Similarly, significant association was also found between JCV

infection and increased risk of colorectal adenoma compared with adjacent (Peto OR 6.63 [95% CI 3.41-12.92], *p* < 0.001) and non-adjacent tissues (Peto OR 5.59 [95% CI 3.40-9.19], *p* < 0.001; *I*² = 21%) (Supplementary Figure 4).

Association between JCV and EO-CRC

Of the 18 studies included in this analysis, 6 were conducted in a high-prevalence setting^[11,13,15,17,47,49], and 12 in a low-prevalence setting for EO-CRC^[9,10,12,14,16,42-46,48]. Stratifying studies by the incidence trend of EO-CRC, 6^[9,10,16,17,46,47], 8^[11-15,45,48,49], and 4 studies^[42-44] were conducted in a rising-trend, stable-trend, and decreasing-trend setting, respectively (Table 1). The pooled OR for JCV infection in colorectal tumors (ie. cancer and/or adenoma) compared to controls was significantly higher in the high-prevalence settings compared to the low-prevalence settings (Peto OR 6.38 [95% CI 4.53-9.01] vs. 3.61 [95% CI 2.78-4.68], *p* = 0.01; Fig. 2). This difference was also noted when analysis was restricted to studies testing paired samples of colorectal tumors and adjacent colorectal tissues (Peto OR 8.26 [95% CI 5.31-12.85] vs. 2.78 [95% CI 1.96-3.96], *p* = 0.0002; Fig. 3). Likewise, the pooled OR for JCV infection in colorectal tumors compared to controls and adjacent tissues trended to be higher in the rising-trend settings compared to the stable -trend settings (Peto OR 5.95 [95% CI 4.16-8.51] vs. 3.83 [95% CI 2.97-4.94], *p* = 0.05) and (Peto OR 5.62 [95% CI 3.46-9.13] vs. 3.73 [95% CI 2.67-5.21], *p* = 0.17), respectively (Figure 4 and 5). No JCV infection was detected in both colorectal tumors and controls in the decreasing-trend settings^[42-44].

Sensitivity analysis and Publication bias

On sensitivity analysis of association between JCV infection and colorectal cancer and adenoma, leaving out one study at a time did not significantly alter the effect size, indicating that the results were stable and reliable. For the comparisons (colorectal cancer vs. controls and colorectal adenoma vs. controls), the funnel plot appeared symmetric (Supplementary Fig. 5 and 6), and egger’s test did not detect publication bias (*p* = 0.27 and *p* = 0.70, respectively).

Table 1 Characteristics and quality assessment of the included studies.

Author	Year	Study design	Country	Temporal trend of EO-CRC	Prevalence of EO-CRC	NOS quality score
Hernandez- Losa et al.	2003	Case-control	Spain	Stable	Low	6
Newcomb et al.	2004	Case-control	USA	Rising	Low	9
Theodoropoulos et al.	2005	Case-control	Greece	NA	NA	7
Hori et al.	2005	Case-control	Japan	Stable	Low	6
Casini et al.	2005	Case-control	Tunisia	Stable	High	6
Goel et al.	2006	Case-control	USA	Rising	Low	8
Lin et al.	2008	Cross-sectional	Taiwan	Rising	High	8
Selgrad et al.	2008	Case-control	Netherlands	Rising	Low	5
Militello et al ^[1]	2009	Case-control	Italy	Decreasing	Low	7
Militello et al ^[2]	2009	Cross-sectional	Italy	Decreasing	Low	6
Campello et al.	2010	Case-control	Italy	Decreasing	Low	5
Mou et al.	2012	Case-control	China	Stable	Low	8
Vilkin et al.	2012	Case-control	Israel	Stable	Low	9
Coelho et al.	2013	Case-control	Portugal	Rising	Low	7
Matalka et al.	2013	Case-control	Jordan	Stable	High	6
Fiorina et al.	2014	Case-control	Italy	Decreasing	Low	6
Ksiaa et al.	2015	Case-control	Tunisia	Stable	High	8
Toumi et al.	2017	Case-control	Tunisia	Stable	High	7
Sarvari et al.	2018	Case-control	Iran	Rising	High	5

EO-CRC early-onset colorectal cancer, NOS Newcastle-Ottawa scale, NA not available.

DISCUSSION

This meta-analysis demonstrated that JCV infection was associated with increased risk of colorectal cancer and adenoma, regardless of the type of control, suggesting a possible causal role for JCV in colorectal carcinogenesis. Additionally, JCV infection increased the odds for colorectal adenoma development (6-fold) more than colorectal cancer (4.1-fold). Our findings corroborate the results of recent meta-analyses indicating that the presence of JCV in colorectal tissues increased the odds of colorectal tumors by 4.4 to 4.7 times compared to the absence of the virus^[50,51]. In these studies, however, colorectal cancer and adenoma were not looked at individually, and the association of JCV infection with EO-CRC was not assessed.

According to our results, it is clearly evident that the presence of JCV increased the odds of CRC in countries with high prevalence of EO-CRC significantly more than in their low-prevalence counterparts. This difference was further substantiated when tumor-adjacent colorectal tissues were used as the sole control. Tumor-adjacent colorectal tissues are more valid controls as they share the same genetic conditions and environmental exposures with the tumors. These results indicate that JCV may play a key role in the higher prevalence of EO-CRC observed in certain parts of the world, notably developing countries. Indeed, JCV-associated CRC has a strong predilection for the distal colorectum^[52], a pattern analogous to that described in the context of CRC in developing countries^[5,24,26,31]. Further evidence supporting this hypothesis was a recent study employing the Next Generation Sequencing technology from Sudan, where EO-CRC represents almost 40-50% of CRC cases. Whole-exome sequencing of CRC disclosed significant centrality of *NFKB1* and *ELAVL1* pointing to a possible underlying viral etiology in these tumors^[53].

Virus association with cancer may be influenced by geographic and ethnic factors, as clearly illustrated by the Epstein-Barr virus.^[54] Infection with JCV is endemic in almost all populations, often acquired during late childhood, and is caused by different genotypes in different geographical regions^[55]. Furthermore, viral DNA sequence variability was detected in normal colorectal mucosa between and within subjects in the same population, suggesting that the same individuals are infected by different JCV strains^[7]. Recently, the International Agency for Research on Cancer has classified JCV as a probable, class 2B, biological carcinogen in humans^[56]. It has been proposed that JCV induces malignant transformation through “hit and run” mechanism, during early stage of carcinogenesis, which is often followed by genome silencing or loss of the T-Ag sequence as a consequence of viral integration. Our analysis showed that the association with JCV was stronger for colorectal adenoma compared to CRC, which supports this concept. The JCV T-Ag, a multi-potent virally-encoded oncoprotein, promotes carcinogenesis by inactivating tumor suppressor proteins p53 and pRb, and inducing chromosomal instability^[52]. The latter is frequently implicated in the early stages of carcinogenesis in EO-CRC^[4].

In another aspect, there has been a rising trend in the incidence of EO-CRC, particularly rectal cancer, in several countries over the last two decades, often against a backdrop of rapidly declining rates in older individuals^[3,38]. In some countries like the USA and Korea, the cohort effect, lifestyle changes, antibiotic use, and dietary transitions, have been pointed as likely and plausible culprits^[3]. In Taiwan, a recent large-scale population-based study, using serological markers to diagnose infection, has evidenced that chronic hepatitis B virus was significantly associated with the risk of CRC, particularly in young patients^[57], nevertheless, isolation of the viral DNA from

the tumor cell nuclei is necessary to indicate causality. In other regions, such as Europe, the causes of this rising trend are yet to be determined. Considering that the inclines in the incidence of EO-CRC pertains predominantly to distal cancers, and the association between CRC and JCV infection in our analysis was stronger in the rising-trend settings, we postulate that JCV may contribute to the development of a subset of EO-CRC in this rising trend.

An important issue related to this meta-analysis is that two of the studies conducted in high-prevalence and rising-trend settings failed to detect JCV DNA in cancerous or noncancerous colorectal tissues^[46,47]. This could be ascribed in large part to the method employed for JCV testing. In these studies, formalin-fixed specimens were used. Formalin fixation breaks the DNA and makes it more difficult to recover sufficient quantities for amplification^[58]. In addition, one of these studies used PCR assay for JCV DNA testing rather than nested PCR, which is known to have lower sensitivity. In this setting, the use of topoisomerase I treatment before PCR amplification may enhance the sensitivity of JCV DNA detection^[8].

In conclusion, JCV may play a role in the international variations and the rising trend of EO-CRC in some parts of the world. The current meta-analysis is limited by the relatively small number of studies. Further studies, comparing the prevalence of JCV in EO-CRC and average-onset CRC, and including larger numbers of patients with colorectal adenomas with special reference to the different JCV rearranged genotypes, are warranted to elucidate the role of JCV in colorectal tumorigenesis.

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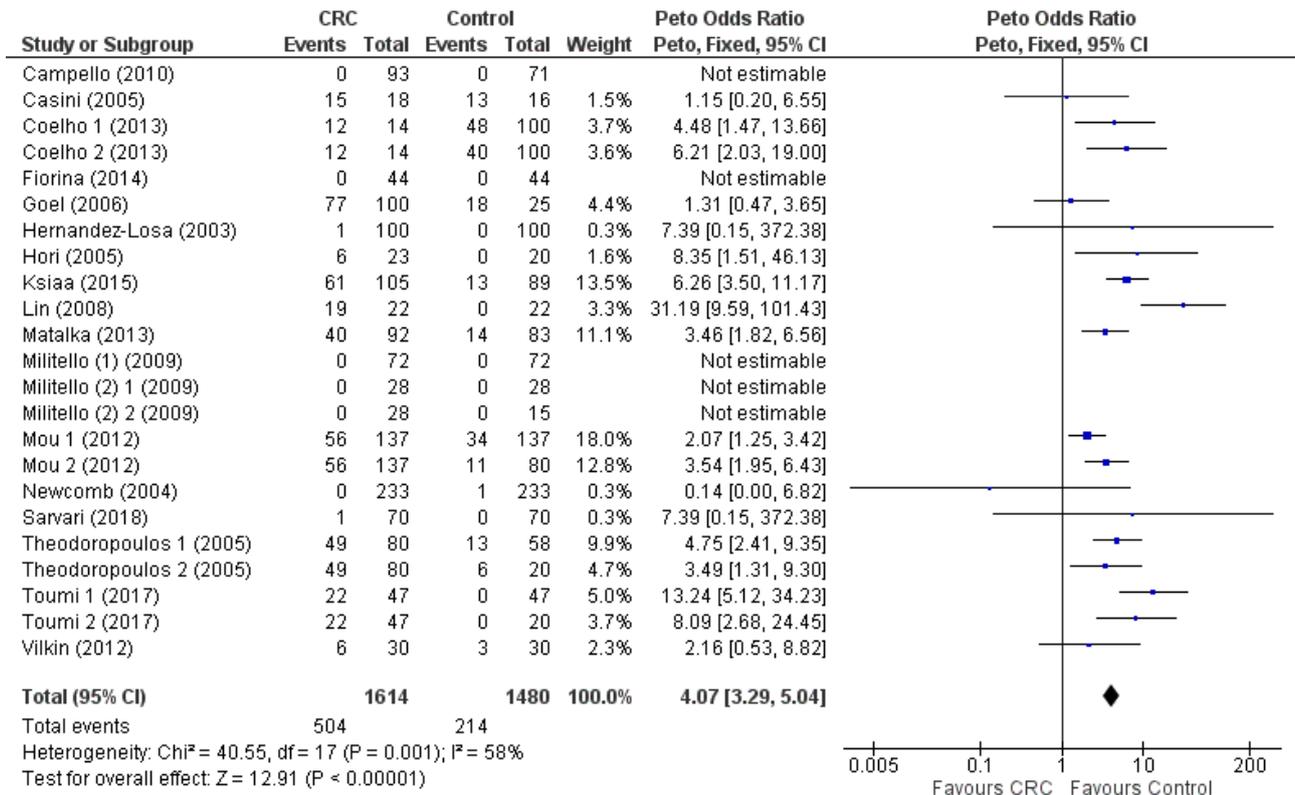
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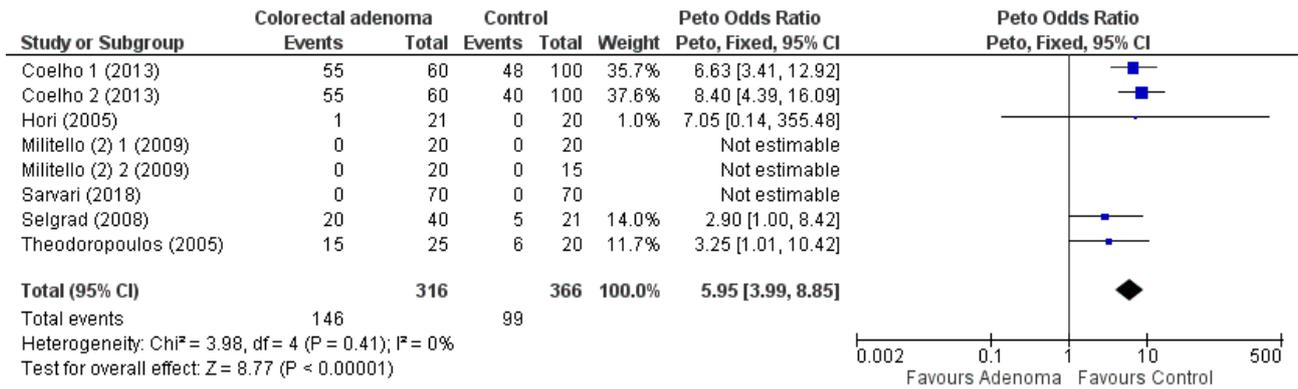
Supplementary Table 1 Methods and outcomes of JCV testing in the included studies.

Author	Type of tissue	JCV DNA detection method	Tumour (n)		Adjacent tissues (n)		Non-adjacent tissues (n) †	
			JCV positive	Total	JCV positive	Total	JCV positive	Total
Hernandez- Losa et al.	FF	Nested PCR	1	100	0	100	---	---
Newcomb et al.	FFPE	Nested PCR	0	233	1	233	---	---
Theodoropoulos et al.	FFPE	Nested PCR	64 (15)	105 (25)	13	58	6	20
Hori et al.	FFPE	Nested PCR	7 (1)	44 (21)	---	---	0	20
Casini et al.	FFPE	PCR	15	18	13	16	---	---
Goel et al.	FFPE	PCR	77	100	18	25	---	---
Lin et al.	FFPE	Nested PCR	19	22	0	22	---	---
Selgrad et al.	FFPE	PCR	20 (20)	40 (40)	---	---	5	21
Militello et al. (1)	FFPE	PCR	0	72	0	72	---	---
Militello et al. (2)	FF	PCR	0	48 (20)	0	48	0	15
Campello et al.	FF	PCR	0	93	---	---	0	71
Mou et al.	FF	Nested PCR	56	137	34	137	11	80
Vilkin et al.	FFPE	PCR	6	30	3	30	---	---
Coelho et al.	FF	Nested PCR	67 (55)	74 (60)	48	100	40	100
Matalka et al.	FFPE	PCR	40	92	---	---	14	83
Fiorina et al.	NA	PCR	0	44	0	44	---	---
Ksiaa et al.	FFPE	PCR	61	105	13	89	---	---
Toumi et al.	FFPE	PCR	22	47	0	47	0	20
Sarvari et al.	FFPE	PCR	1 (0)	140 (70)	---	---	0	70

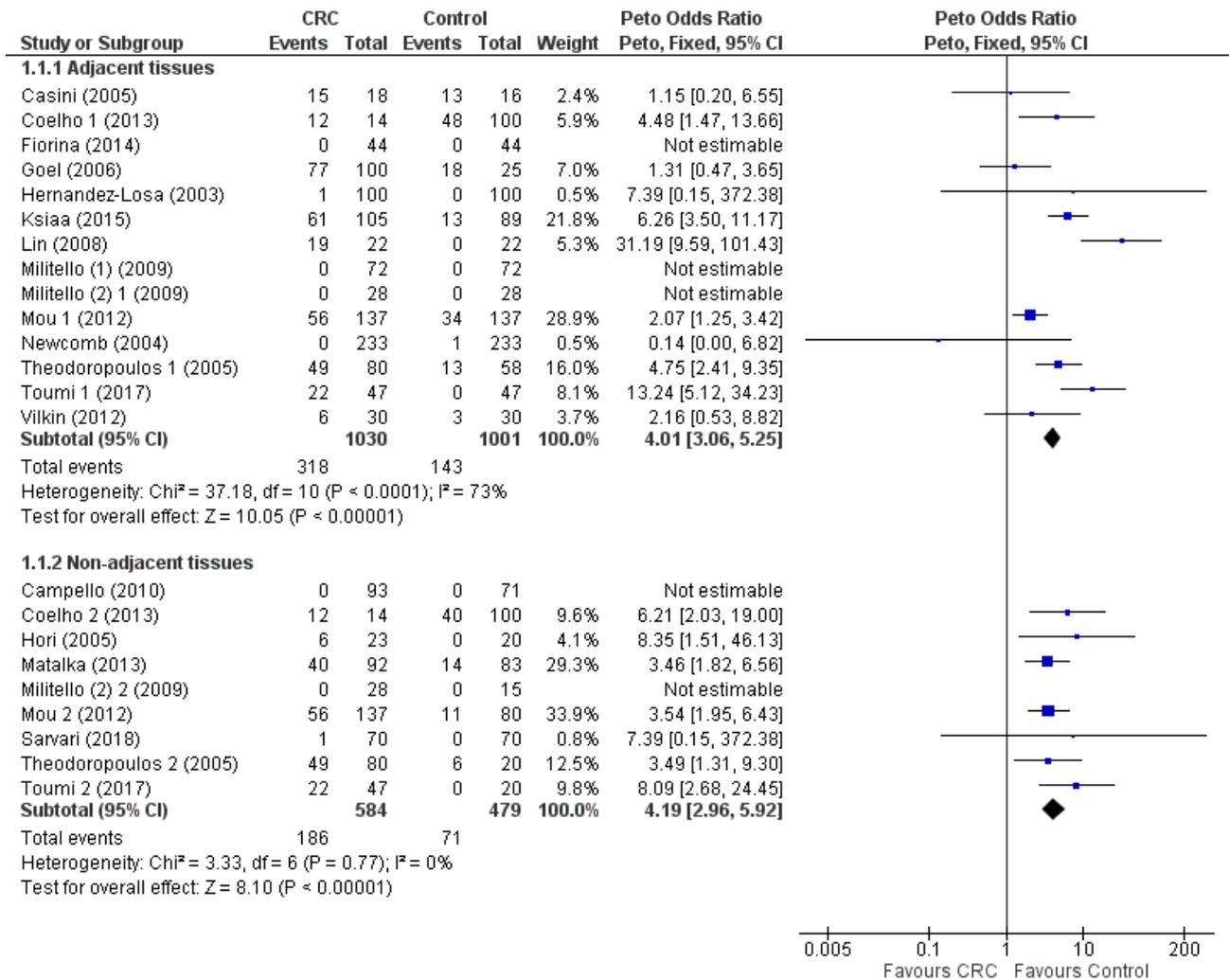
JCV John Cunningham virus, FFPE formalin-fixed paraffin-embedded, FF fresh-frozen, PCR polymerase chain reaction, NA not available. Figures in parentheses are the numbers of patients with colorectal adenoma. † Non-adjacent colorectal tissue samples were obtained from non-cancer patients.



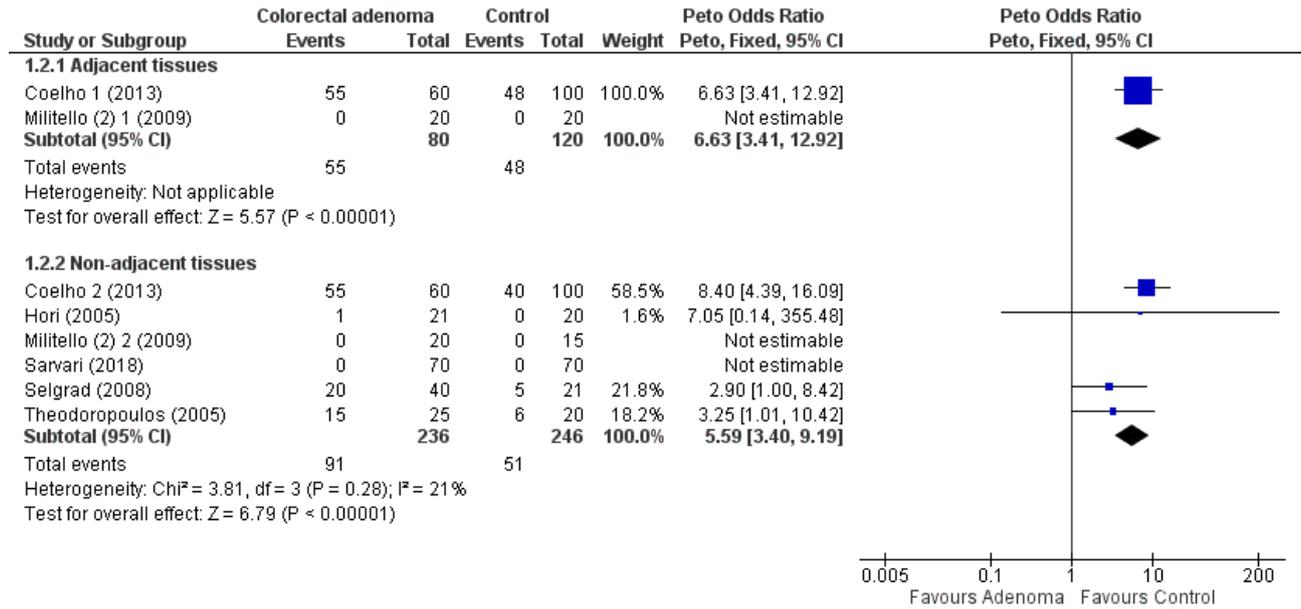
Supplementary Figure 1 Meta-analysis of JCV infection incidence in colorectal cancer compared with controls.



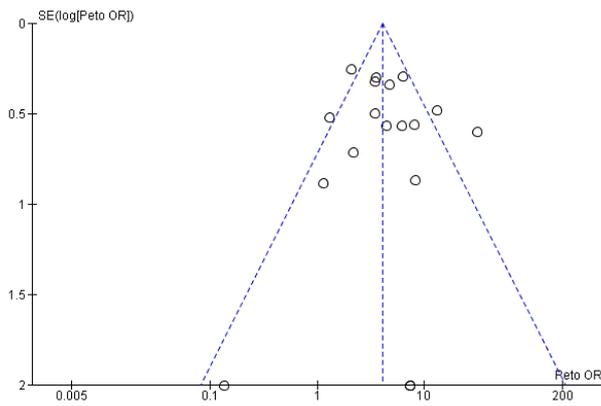
Supplementary Figure 2 Meta-analysis of JCV infection incidence in colorectal adenoma compared with controls.



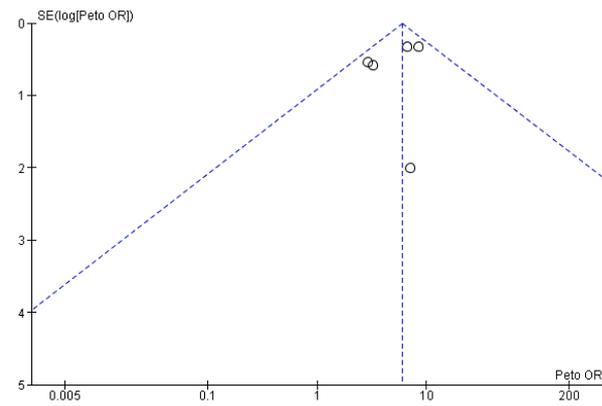
Supplementary Figure 3 Subgroup analysis of JCV infection incidence in colorectal cancer compared with adjacent and non-adjacent tissue control.



Supplementary Figure 4 Subgroup analysis of JCV infection incidence in colorectal adenoma compared with adjacent and non-adjacent tissue control.



Supplementary Figure 5 Funnel plot for studies detecting JCV infection in colorectal cancer vs. controls.



Supplementary Figure 6 Funnel plot for studies detecting JCV infection in colorectal adenoma vs. controls.