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Investigating the association between African spontaneously fermented dairy products, faecal carriage of *Streptococcus infantarius* subsp. *infantarius* and colorectal adenocarcinoma in Kenya

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ABSTRACT

Consumption of traditional fermented dairy products (tFDP) in Africa leads to the ingestion of up to 10^8 *Streptococcus infantarius* subspecies *infantarius (Sii)* per millilitre of spontaneously fermented milk. *Sii* is a member of the *Streptococcus bovis/Streptococcus equinus* complex (SBSEC) for which some members are associated particularly with colorectal cancer or endocarditis. The extent of health risks to tFDP consumers is largely unknown.

A hospital-based unmatched case-control study was conducted at Kenyatta National Hospital, Nairobi (Kenya) on 80 cases and 193 controls that were selected exhaustively from patients attending colonoscopy at the hospital. Logistic regression models adjusted for age, sex and residency were used in the statistical analysis. Consumption of tFDP was not associated with CRC (odds ratio (OR) 1.4; 95% Confidence interval (CI) 0.7–2.7; p = 0.34). Risk factors associated with CRC included age above 40 years, and consumption of processed meat and alcohol. Faecal carriage of *Sii* was significantly higher in persons with colon tumours and polyps compared to controls (8.4% vs 21.6%: OR: 4.6; CI 1.3–15.9). Patients with haemorrhoids represented an unexpected carrier group with significantly higher *Sii* faecal carriage (30.4%, CI: 17.7–45.8). Consumption of tFDP does not represent risk factors for CRC whereas *Sii* seems to be associated with CRC. However, there is urgent need to assess this finding also in the general population, investigate the causality of SBSEC, *Sii* and CRC as well as compare the phylogenetic, functional and genomic relationship between human and dairy *Sii* with regards to the ongoing application of *Sii* in FDP production.

1. Introduction

Globally, colorectal cancer (CRC) is the third most common cancer among men and second among women (International Agency for Research on Cancer, 2012) with nearly 1.4 million new cases diagnosed in 2012 (World Cancer Research Fund, 2016). In Kenya, CRC ranks 7th

Abbreviations: cFPD, commercial fermented dairy products; CRC, Colorectal cancer; tFDP, Traditional fermented dairy products; GIT, Gastrointestinal tract; KNH, Kenyatta National Hospital; OR^A, Odds ratio adjusted; SBSEC, *Streptococcus bovis/Streptococcus equinus* complex; *Sgg*, *Streptococcus gallolyticus* subsp. *gallolyticus*; *Sgm*, *Streptococcus gallolyticus* subsp. *macedonicus*; *Sgp*, *Streptococcus gallolyticus* subsp. *pasteurianus*; *Sii*, *Streptococcus infantarius* subsp. *infantarius*; yFPD, yoghurt fermented dairy product

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for cancers in both sexes with a cumulative incidence of 4.2 per 100,000 persons per year (International Agency for Research on Cancer, 2012; Korir et al., 2015). CRC is associated with a number of environmental factors including: age above 50 years, smoking, drinking alcohol, obesity, consumption of saturated fats and red meat (Joint WHO/FAO expert consultation group, 2003). Microorganisms such as members of the *Streptococcus bovis/Streptococcus equinus* complex (SBSEC) have also been associated with CRC (Ben-Chetrit et al., 2017; Boleij and Tjalsma, 2013; Corredoira et al., 2015). Despite this association, some SBSEC members are also given commensal status in the gastrointestinal tract of humans and animals, while recent studies have even shown that traditional fermented dairy products (tFDP) in Africa are predominated by *Streptococcus infantarius* subsp. *infantarius* (*Sii*) and to a lesser extent by *Streptococcus gallolyticus* subsp. *macedonicus* (*Sgm*) (Jans et al., 2013b) with up to 10^8 live *Sii* per millilitre of tFDP (Abdelgadir et al., 2008; Jans et al., 2012a). *Sii* and *Sgm* are members of the SBSEC (Jans et al., 2015; Schlegel et al., 2003). The SBSEC and CRC correlation was originally associated to *S. bovis* (Klein et al., 1977). Advancing taxonomic differentiation specified this association mainly to *Streptococcus gallolyticus* subsp. *gallolyticus* (*Sgg*) formerly classified as *S. bovis* biotype I and *Streptococcus gallolyticus* subsp. *pasteurianus* (*Sgp*), formerly biotype II.2 (Ben-Chetrit et al., 2017; Jans et al., 2015). However, these advances in taxonomic differentiation among the SBSEC opened questions on disease associations of the *S. infantarius* branch (biotype II.1) including *Sii* and *Streptococcus lutetiensis* (intermediately named *Streptococcus infantarius* subsp. *coli*). Disease association with infective endocarditis and other clinical syndromes was partially clarified for *S. lutetiensis* but not for *Sii* as accurate epidemiological data on *Sii* is scarce (Ben-Chetrit et al., 2017; Jans et al., 2015; Romero et al., 2011). In parallel to taxonomic advances, some *Sii* strains have been reclassified as *Streptococcus equinus* while others implicated as human or livestock pathogens were confirmed to be *Sii* suggesting the need to investigate the role of *Sii* with respect to public health (Jans et al., 2016). This is particularly important for the sub Saharan African setting where exposure of humans to live *Sii* through food such as tFDP is high in multiple countries (Abdelgadir et al., 2008; Jans et al., 2012a, 2016, 2013b; Wullschleger et al., 2013).

The long tradition and wide distribution of tFDPs as food products in sub-Saharan Africa reflects the important role of tFDP in nutrition, food safety and food security for communities in this region (Franz et al., 2014; Jans et al., 2017). Considering the large population consuming and relying on tFDP as part of their diet, the elucidation of the role of *Sii* is pivotal. Comparative genomics and phylogenetic analyses of *Sii* strains indicated two main African *Sii* lineages with a rather recent and currently ongoing process of dairy adaptation (Jans et al., 2016, 2013a, 2012b). The dairy adaptations of *Sii* lineages seem to parallel the evolution of *Streptococcus thermophilus* as starter culture in Western fermented dairy products and suggest an important technological role during tFDP manufacturing (Jans et al., 2013a). In this context, African variants of *Sii* have been suggested for evaluation as indigenous starter culture for African tFDP (Jans et al., 2016, 2013a). Therefore, a thorough safety assessment of *Sii* for any future product development and estimation of its public health risk is necessary. However, basic appropriate epidemiological data on *Sii* in Africa and even worldwide is currently lacking.

To provide the first epidemiologic data on CRC, tFDP and *Sii,* we implemented a hospital-based study and enrolled patients at the endoscopy unit of Kenyatta National Hospital (KNH) in Nairobi, Kenya, a country with well-characterized *Sii*-containing tFDP. The main objectives of this study were: i) assessing the relationship between the consumption of tFDP and colon-related health conditions, ii) determining the prevalence of *Sii* in patients with different colon-related health conditions, and iii) identifying additional environmental risk factors such as consumption of other food products, smoking, drinking alcohol, obesity and level of physical activity.

2. Materials and methods

2.1. Study design

2.1.1. Sample size determination of participants and dairy diet

The anticipated sample size of individuals involved as study subjects was determined assuming a proportion exposed to the consumption of tFDP of 60% (Jans et al., 2013b) among controls and 75% among cases and a case/control ratio of 1:3. Applying Fleiss' formula for unmatched case control studies, 104 cases and 310 controls are required to detect a statistically significant difference with a power of 80% at the 95% level of confidence. Concerning the dairy diet, it was distinguished between (1) traditional FDP (tFDP) from spontaneously fermented raw milk including informal sour milk (tFDP1) and home-made sour milk (tFDP2); (2) sour milk from heat-treated milk and subsequently fermented with commercial non-*Sii*-starter cultures (cFDP), often named mala/lala; (3) Western-type yoghurt with light/medium to firm consistency made from heat-treated milk by commercial starter cultures containing *S. thermophilus* (yFDP); and (4) cereal-based porridge with either type cFDP or tFDP before cooking the porridge.

2.1.2. Case definition, inclusion and exclusion criteria

Participants were stratified by the outcome of the colonoscopy to either patients with normal colon (including minor complications such as constipation, abdominal pains or helminths), CRC, adenocarcinoma (polyps), colitis, haemorrhoids or other. A case was defined as an individual whose diagnosis showed colon tumours or polyps while controls were defined as individuals with normal colon. To increase statistical power and to prove that the results are not sensitive to the case definition, two alternative definitions were considered by including patients with colitis, haemorrhoids or other conditions once in the case and once in the control group (Fig. 1). The inclusion criteria were persons of 18 years and above referred for colonoscopy examination. Participants matching the following exclusion criteria were not considered for the study: undertaking chemotherapy, radiotherapy or surgery and pregnancy. Age was self-reported by participants or derived from their date of birth where available.

2.1.3. Participant enrolment and interview

All adult individuals attending KNH for colon examination by colonoscopy at the Endoscopy unit were invited to participate. They were briefed on the study goals and risks, according to the ethical guidelines including the signing of a consent form for voluntary study participation. This was then followed by interviewer-guided filling of the questionnaire, a process that took approximately 30 min. Questionnaires were administered in English, Swahili or local dialect with the help of a translator if needed.

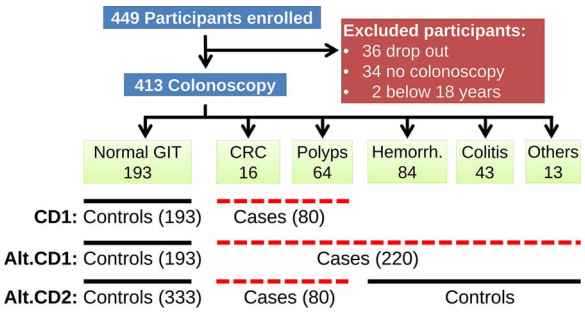

Fig. 1. Data flow diagram of participant recruitment among endoscopy patients at Kenyatta National Hospital, stratification by endoscopy examination outcome and case definition (CD) to determine colorectal cancer (CRC), *Sii*/SBSEC and FDP association. Alternative case definitions (Alt.CD) were formulated for sensitivity analysis.