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ASSOCIATION BETWEEN KHAT (*CATHA EDULIS*) CHEWING AND INFECTION WITH *HELICOBACTER PYLORI*: A CASE CONTROL STUDY IN NAIROBI COUNTY

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## ASSOCIATION BETWEEN KHAT (*CATHA EDULIS*) CHEWING AND INFECTION WITH *HELICOBACTER PYLORI*: A CASE CONTROL STUDY IN NAIROBI COUNTY

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### ABSTRACT

**Background:** Khat (*Catha edulis*) is a psycho-stimulant substance grown and widely chewed in East Africa. The use of Khat leads to a number of health complications however its adverse effects and prevalence are not well studied.

**Objective:** To compare the prevalence of Khat chewing among *H. Pylori* infected cases and controls.

**Design:** Individual matched case control study

**Setting:** KEMRI's Centre for Clinical Research (CCR) and St. Michael's Digestive Disease and Medical Care.

**Subjects:** Ninety three cases were selected using Rome III criteria for functional dyspepsia, and the controls (n=93) were matched on age and gender.

**Results:** Khat Chewing was associated with infection with *H. Pylori*. Of the 93 cases, 58.1% were *H. Pylori* positive with a majority being Khat chewers 67.2% (41/61) and 32.8% (20/61) non-Khat chewers; the two groups were significantly different (p-value=0.007). Functional dyspepsia was associated with *H. Pylori*. Therefore, participants with functional dyspepsia were twice more likely of being diagnosed with *H. Pylori* (OR 2.1, 95% CI: 1.2,3.9).

**Conclusion:** The prevalence of *H. Pylori* infection was found to be higher among khat chewers, indicating that Khat chewing could be a predisposing factor to *H. Pylori* infection and to gastrointestinal disorders. Community-based awareness creation about the adverse effect of Khat use is thus recommended.

### INTRODUCTION

*Helicobacter pylori* (*H. pylori*) was the first formally recognised bacterial carcinogen and is one of the most successful human pathogen. It has been etiologically associated with gastritis and gastritis associated diseases, peptic ulcer, gastric adenocarcinoma and primary gastric lymphoma (1,2).

*H. pylori* is a sheathed monoflagellated Gram-negative rod. It is microaerophilic, catalase negative and produces urease that enables it to survive in the hostile acidic environment of the stomach. Transmission in humans is usually oral-to-oral, or foecal-to-oral route (3).

Epidemiology of *H. pylori* infection demonstrated a high prevalence in developing countries of up to

90% than in developed countries (4). The prevalence of infection varies both between and within countries in relation with race, ethnicity, and geographical area of the population. The pattern of infection is an early childhood acquisition of *H. pylori* (30%-50%) that reaches over 90% during adult hood in developing countries. Unless treated colonisation persist lifelong. This has been attributed to the poor socio-economic status, hygienic practice and overcrowding conditions (5, 6).

The organism is non-invasive but stimulates chronic gastritis by provoking a local inflammatory response in the underlying stomach epithelium due to release of cytokines, e.g., VacA, cagA, BabA2, phospholipases and porins. However multiple factors contributed for the pathogenecity of the bacteria, alteration of gastric acid production and

tissue destruction which are characteristics of *H. pylori* disease. Initial colonisation is facilitated by bacterial acid inhibitory protein and naturalisation of gastric acid by ammonia, produced by bacterial urease activity (7, 8).

Diagnostic tests for *H. pylori* can be divided into non-invasive (non-endoscopic) and invasive test. Non-endoscopic methods include blood antibody detection tests, urea breath tests (UBT) and stool antigen tests. Stool antigen testing has emerged as a rapid non-endoscopic method of *H. pylori* detection. Studies have reported high sensitivities and specificities similar to those of UBT (>90%) in stool antigen testing (9).

Khat is the name generally used for *C. edulis*, a dicotyledonous evergreen shrub of the family Celastraceae. It is also known as 'Miraa' or 'Qat' in Kenya, while in Ethiopia the Amharas call it 'tchat' and the Gallas 'Jimma'. Khat is regularly cultivated in certain areas of East Africa and in the Arabian Peninsula (10-12). The custom of chewing Khat leaves continues to be practiced for its stimulant effects on the central nervous system (13). In Kenya the practice is widespread among Somali and Meru ethnic groups. Khat trees grow wild in the forests and are cultivated widely in various locations in Kenya, especially in the Nyambene Hills, a mountain range lying northeast of Mount Kenya. Khat leaves are crimson-brown and glossy but become yellow-green and leathery as they age. The leaves are up to five centimetres wide and up to ten centimetres long. The leaves emit a strong aromatic smell and have an astringent and slightly sweet taste. Khat is a cash crop profitable for a large number of people involved in its production and marketing including farmers, distributors and retail merchants. Taxes levied on the production and sale of Khat are an important source of revenue to the government (10, 14).

The chemical profile of Khat leaves varies depending on environmental and climate conditions (15). Fresh leaves of Khat may contain 60 different cathedulins (16). Compounds found in Khat include alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins and minerals (17). The pharmacologically active constituent of Khat is cathinone, which has amphetamine-like properties that affects the central nervous system (17, 18).

Khat use has a number of health-related complications that includes increased blood pressure, tachycardia, insomnia, anorexia, gastritis, stomatitis, oesophagitis, gastric ulcers, hemorrhoids, constipation, general malaise, irritability, migraine headaches, cardiovascular complications, loss of appetite, and impaired sexual potency in men (13, 15, 19, 20).

Clinical observations have shown that habitual Khat chewers often complain of symptoms suggestive of stomatitis, oesophagitis, gastritis and constipation.

In experiments on animals, Khat extract has been shown to cause gastritis and duodenitis (20). *H. pylori* infection is another major cause of chronic gastritis, peptic ulcer and also affects gastric acid secretion (21, 22).

Studies on the effect of Khat and infection with *H. pylori* are scarce despite the increase in the knowledge of Khat pharmacology and chemistry. Thus, the need for a study was determined to be useful to illuminate the association between Khat chewing and infection with *H. pylori*. This study, therefore, examines the association of Khat use and infection with *H. pylori* through a case-control design by enrolling cases with functional dyspepsia in Nairobi, Kenya.

## MATERIALS AND METHODS

**Study design:** The study design was a one-to-one case-control study stratified by overall age and gender distribution of the control group similar to that of the cases occurring in Nairobi County during the same period. The study was aimed at assessing the risks of Khat chewing among *H. pylori* infected cases and controls. The matching of the control group with the group of active cases was done to improve efficiency in the estimation of the effect of exposure by protecting against the situation in which the distributions of the confounder are substantially different between the two groups, for completeness of control for confounding variables, for control of unmeasured confounders, and for time comparability especially related to age (23).

**Study setting:** The study was conducted at the endoscopy centre at St. Michael's Digestive Disease and Medical Care, in partnership with the Centre for Clinical Research (CCR)-KEMRI, Nairobi, Kenya. Nairobi is the referral city for most patients who are sent for endoscopy: it also has a high population density.

Patients referred to the endoscopy centre, were seen by the gastroenterologist who took gastric biopsies – from the antrum and from the body of the stomach, for histology.

Patients were equally asked to give a stool sample for stool antigen testing using the ELISA method.

**Study procedure:** After initial participant interviews and data collection and after a full explanation of the study to the potential participant, the investigator booked the participant for an endoscopy procedure. Two biopsy specimens were taken during an upper gastrointestinal endoscopy, one from the corpus and one from the gastric antrum; this was done to undertake a histological assessment of gastritis and *H. pylori* infection. For *H. pylori* diagnosis, biopsy specimens were fixed in 10% formalin and stained with haematoxylin-eosin and Giemsa stain (24). The

version of the visual analogue scale in the updated Sydney system was used to grade the density of *H. pylori* (25). A histological examination of gastric mucosal biopsies was necessary in order to establish a diagnosis of gastritis.

*Stool antigen testing procedure:* Stool samples were collected in plastic polypot containers, carried in a cooler if necessary, transported to the lab and stored at -20°C to -80°C, until tested.

The monoclonal stool antigen test (Meridian Bioscience – Premier Platinum HpSA PLUS) uses the enzyme linked immunosorbent assay technique (ELISA) to detect *H. pylori* antigen. This test involved the following: - A mixture of murine monoclonal antibodies specific for *H. pylori* was already fixed to the microwells of a microtitration plate which came with the stool antigen kit.

100 uL of diluted stool was placed in microwells. Soluble microbial antigen (in stool) bound to this antibody. Washing was done to remove excess stool antigen. Antibody conjugated to an enzyme was added (a mixture of murine monoclonal antibodies specific for *H. pylori* conjugated to horseradish peroxidase). Conjugated antibody bound to the antibody-antigen complex. Washing was done to remove excess conjugated antigen. A chromogenic substrate joined to an indicator (Premier substrate solution - buffered solution containing urea peroxide and tetramethylbenzidine) was added. The enzyme in the conjugated antibody hydrolysed the substrate, producing a color reaction. Color was read visually and/or spectrophotometrically. Positive results on visual reading showed a definite yellow color, and spectrophotometrically was  $\geq 0.100$  at dual wavelength. Negative results on visual reading showed a colorless to faint yellow color, and spectrophotometrically was  $< 0.100$  at dual wavelength. This was done using a built-in control, which came with the kit, for quality control.

#### *Data Management and Analysis*

*Data management:* The quantitative data collected from the field was coded and double entered into a computer database designed using EpiData Version 3.1. Files were backed-up regularly to avoid loss or data tampering. All questionnaires were stored in a locked drawer for confidentiality purposes.

*Data analysis:* Data analysis was conducted using IBM SPSS Statistics Version 20 statistical software. Exploratory data techniques were used at the initial stage of analysis to uncover the structure of data and identify outliers or unusual entered values.

*Univariate analysis:* Descriptive statistics such as proportions were used to summarise categorical variables with measures of central tendency such as mean, SD, median and ranges used for continuous variables.

*Bivariate analysis:* Conditional Logistic Regression using age as the strata was used to test for the strength of association between categorical variables. When data were small (cell values less than 5) or the data were sparse, Fishers Exact Test was used to test the association. All Independent variables (including chewing of Khat) were associated with the presence or absence of *H. pylori* to determine which ones would have significant association. Odds Ratio (OR) and 95% Confidence Interval (CI) were used to estimate the strength of association between independent variables and *H. pylori* infection.

*Ethical considerations:* Ethical clearance and permission to carry out the study was obtained from the Ethical Review Board of Jomo Kenyatta University of Agriculture and Technology (JKUAT) and from the Kenya Medical Research Institute (KEMRI) Scientific Committee and Ethics Review Committee. Participants were enrolled into the study only after written voluntary informed consents were given. All patient data were handled with confidentiality and were used only for intended purposes. The participants were given the results of the tests they undertook.

## RESULTS

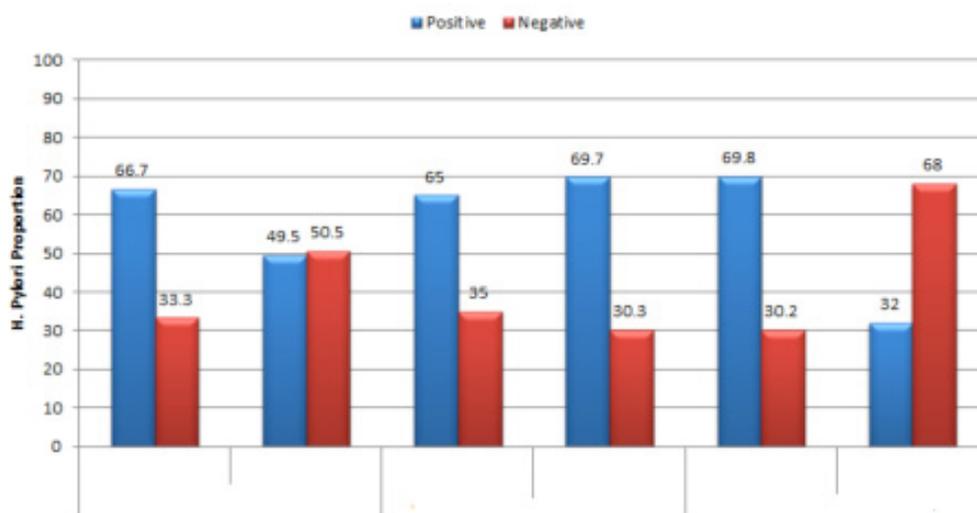
All 93 case-control pairs had results for stool antigen test. Of the 93 cases, 58.1% were *H. pylori* positive with a majority being Khat chewers 67.2% (41/61) and 32.8% (20/61) non-Khat chewers; the two groups were significantly different (p-value=0.007) (Table 1). Of the 93 controls, 49.1% were *H. pylori* positive with a majority being Khat chewers 63.60% (28/44) and 36.7% (16/44) non-Khat chewers; the two groups were borderline statistically significant (p-value=0.070) (Table 1). The prevalence of *H. pylori* among cases, control and case-controls is as shown in (Figure 1). Functional dyspepsia was associated with *H. pylori*. Therefore, participants with functional dyspepsia were twice more likely of being diagnosed with *H. pylori* (OR 2.1, 95% CI: 1.2,3.9). Among the controls Khat chewing was associated with *H. pylori* (OR 4.0, 95% CI: 1.7, 9.6). Therefore, Khat chewers were four times more likely to have *H. pylori* infection than non-chewers (Table 1).

**Table 1**  
Stool antigen results

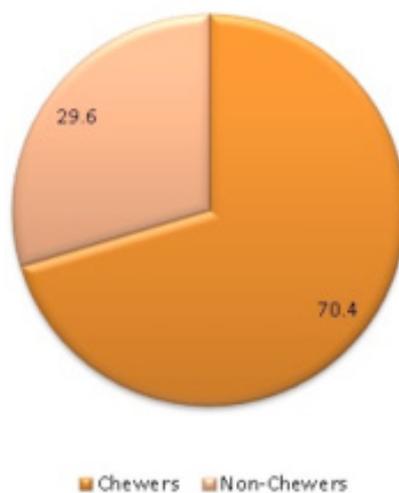
Condition	Cases				Controls			
	Chewers (60)	Non-Chewers (33)	OR (95% CI)	P-value	Chewers (43)	Non-Chewers (50)	OR(95% CI)	P-value
StoolAntigenTest- <i>H. pylori</i> (positive)	41(67.2)	20(32.8)		0.0074	28(63.64)	16(36.36)		0.0704
Positive	41(68.3)	20(60.6)	1.4 (0.6, 3.4)	0.4543	28(65.1)	16(32.0)	4.0(1.7,9.6)	0.0023
Negative	19(31.7)	13(39.4)	1		15(34.9)	34(68.0)	1	

42-proportion Z-test, 3Chi-Square Test,

**Figure 1**  
*H. Pylori* prevalence among cases and controls (Biopsies)

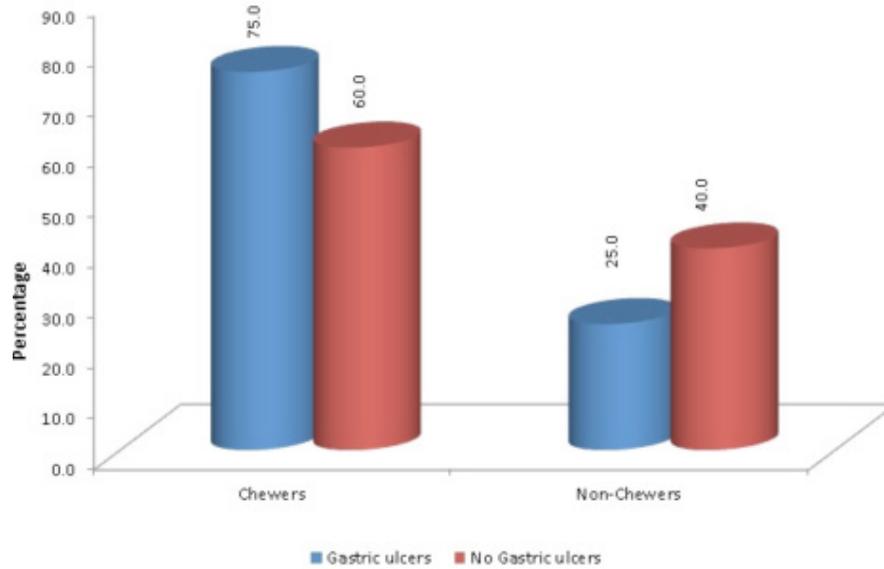


**Figure 2**  
Upper GIT endoscopy: Cases with Duodenal ulcers among the chewers and the non-chewers



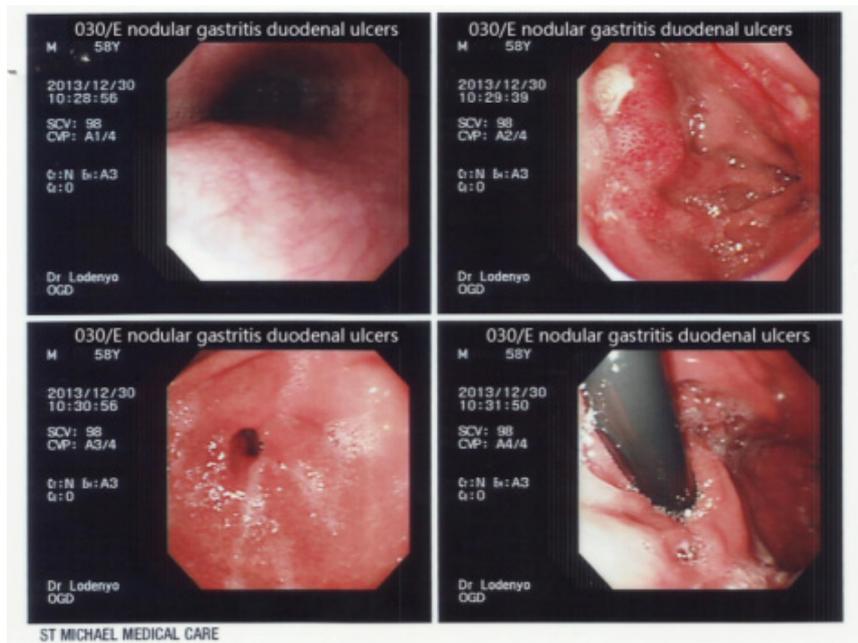
**Figure 3**

*Upper GIT endoscopy: Cases with Gastric ulcers among the chewers and the non chewers*

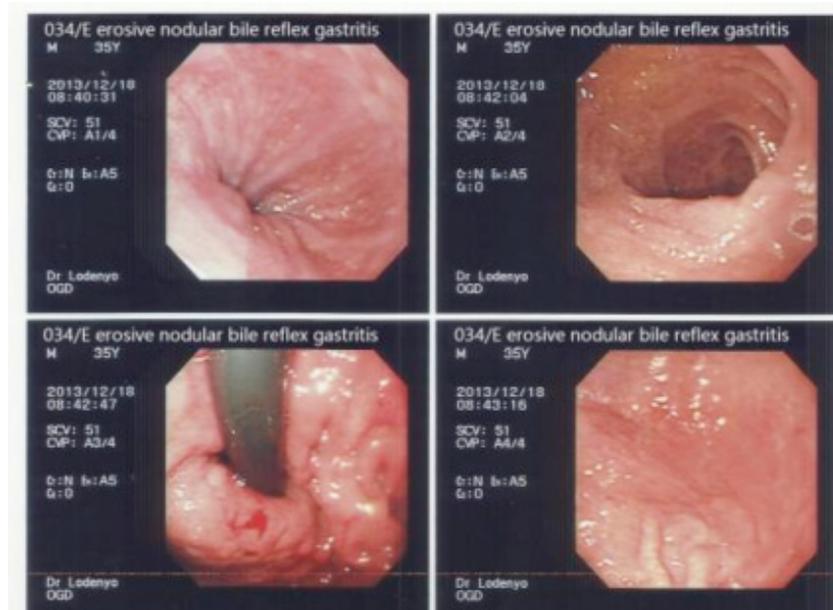


**Figure 4**

*Endoscopic evidence of Gastritis with duodenal ulcer of Khat chewer case*



**Figure 5**  
Endoscopic evidence of Erosion among Khat chewer cases



This concurs with the histology results, where of all the cases, 67.7% were diagnosed as having *H. pylori* with the majority being Khat chewers 62.9%; and the two groups (Khat chewers (62.9%) and non-chewers (37.1%) were significantly different ( $p$ -value=0.042) and the prevalence distribution among cases, controls and case-controls is shown in (Figure 1).

Of the 29% (27/93) duodenal ulcer case, 70.4% (19/27) were Khat chewers (Figure 2). Equally of the 30.1% (28/93) gastritis ulcer cases, Khat chewers (75.0%) and non-chewers (25.0%) were significantly different ( $p$ -value=0.008) (Figure 3) above.

Majority of the *H. pylori* infected cases that were chewers were either having ulcers and or erosions (Figure 4 and 5).

## DISCUSSION

The findings of this study reveal that there is a significant relationship between Khat chewing and infection with *H. pylori* among adults in Nairobi County. Both the histological and stool antigen test had demonstrated high prevalence of *H. pylori* infection among Khat chewers in cases and controls. Of all the cases, 67.7% were diagnosed as having *H. pylori* with the majority being Khat chewers 62.9%; and the two groups (Khat chewers (62.9%) and non-chewers (37.1%) were significantly different ( $p$ -value=0.042). In this study the stool antigen findings concur with that of the biopsies, where of the 93 cases, 58.1% were *H. pylori* positive with a majority being Khat chewers 67.2% (41/61) and 32.8% (20/61) non-Khat chewers; the two groups were significantly different ( $p$ -value=0.007).

Functional dyspepsia was associated with *H. pylori* infection. Therefore, participants with functional dyspepsia were twice more likely to be diagnosed with *H. pylori* (OR 2.1, 95% CI: 1.2,3.9). Among the controls Khat chewing was associated with *H. pylori* (OR 4.0, 95% CI: 1.7, 9.6). Therefore, Khat chewers were four times more likely to have *H. pylori* infection than non-chewers.

As has been shown by this and previous studies that Khat chewers complain of GIT related disorders, the high prevalence of *H. pylori* noted among Khat chewers may contribute to the gastritis, stomatitis, ulcers constipation and other GI disorders. *H. pylori* is a known etiological factor in gastrointestinal disease. It causes gastric inflammation, ulceration and cancer through different mechanisms, including the production of virulence factors by the bacteria itself, host inflammatory responses and associated environmental factors (26).

There were a number GIT related complaints such as bloating, constipation, epigastric pain, blood in the stool. This may be due to the high prevalence of *H. pylori*. Previous studies had shown that patients with *H. pylori* infection present with recurrent epigastric pain related to food and episodic in occurrence. Other symptoms may include burping, bloating, nausea and vomiting. Complications of *H. pylori* include progression to gastric cancer (27).

The study has demonstrated that Majority of chewer cases had; gastric ulcers, duodenal ulcers and or erosions in the upper GIT. Of the gastric ulcer cases 75.0% (21/28) were Khat chewers. Khat chewers and non-chewers were significantly different ( $p$ -value=0.008). This is supported by an observational

study that documented a significant association of stomach and duodenal ulcers with Khat chewing (28).

In this study we also found that Khat chewers share common rooms, food and utensil during chewing. Such unhygienic practices increase the rate of *H. pylori* infection. Most of Khat chewers don't wash the plant before chewing. Reports to the Advisory Council on the Misuse of Drugs (ACMD) Khat Working Group suggested that some Khat users were reluctant to wash Khat in a belief that it would cause the plant to lose its potency.

Poor sanitation, such as the lack of sanitary services at home, is believed to be an important risk factor for *H. pylori* infection (29).

Although an important pathogen of medical significance, *H. pylori* transmission pathways are still vague (30, 31) and currently more than 50% of the world's population is infected. The risks of transmission include precarious hygiene standards, crowding and contaminated environment and water sources (32-34)

Eastleigh in Nairobi that hosts many Somali refugees, we noted most of the chewers were living in crowded houses with poor sanitation facilities and hence high prevalence of *H. pylori*.

In some countries political unrest and economic quagmire have led to migration of people resulting in resettlement elsewhere hampered with poor hygiene standards. This also has resulted in increased number of people living sleeping in the same house. Such significant crowded living conditions may heighten the potential for a person to- person transmission pathway of the organism (35, 36).

In conclusion, the results of this study on association between Khat chewing and infection with *H. Pylori* are ideal as a preliminary investigation of a suspected risk factor for Gastrointestinal disorders; these findings indicate that Khat chewing is associated with *H. Pylori*, infection. It therefore calls for further research and gives justification for a more costly and time-consuming longitudinal study.

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