J Appl Biomed
DOI: 10.32725/jab.2024.018
journal homepage: http://jab.zsf.jcu.cz

Journal of Applied Biomedicine

Original research article

Salivary glands – a new site of *Helicobacter pylori* occurrence

Jan Rotnágl [1, 2] *, Jiří Hložek [1, 2], Richard Holý [1, 2], Emil Pavlík [3, 4], David Kalfeřt [4, 5], Jaromír Astl [1, 2]

[1] *Military University Hospital Prague, Department of Otorhinolaryngology and Maxillofacial Surgery, Prague, Czech Republic*
[2] *Charles University, Third Faculty of Medicine, Prague, Czech Republic*
[3] *General University Hospital In Prague , Institute of Immunology and Microbiology, Czech Republic*
[4] *Charles University, First Faculty of Medicine, Prague, Czech Republic*
[5] *University Hospital in Motol, Department of Otorhinolaryngology and Head and Neck Surgery, Prague, Czech Republic*

Abstract
Objective: The role of *Helicobacter pylori* (Hp) in the pathological processes of the gastric mucosa is well understood. Decreasing trend in successful eradication of HP from the stomach was observed in last years. This lack of succes is mainly caused by increasing ATB resistance. Nevertheless other possible causes of this phenomenon are being explored.
Thus, many studies have focused on the search for extragastric reservoirs as potential sources of persistence or reinfection after successful Hp eradication. The pathological potential of Hp at these localities has also been studied.
Methods: Our study aimed to determine the presence of Hp inside the salivary glands ductal system through its detection from sialolites. Subsequently, we tried to prove the possible ability of Hp to penetrate the salivary gland parenchyma by detecting Hp from the tissue of salivary tumors. Concrements and salivary tumor tissue samples were collected using sialendoscopy or standard surgery, and Hp detection and genotyping were performed through PCR.
Results: Hp was detected in 68.3% of the sialopathy samples. *VacA* S1AM1 was the most common genotype. *CagA*-positive genotype represented only 34% of the total number of positive samples.
Conclusion: Our findings of Hp positivity in concrements provide compelling evidence of Hp presence in the ductal system of salivary glands. Confirmation of Hp presence in tumor tissue suggests its potential ability to infiltrate the gland's parenchyma. Further research is needed to confirm Hp's ability to cause local infection, as well as the possible causal association between Hp presence in the studied region, sialolithiasis, and salivary gland tumors.

Keywords: Extragastric reservoirs; Genotyping; *Helicobacter pylori*; Salivary tumors; Sialolithiasis

Highlights:
- The results of our study clearly confirm the presence of Hp inside the salivary gland.
- The pathological potencial of Hp in this location has not been studied yet.
- Further research is needed to confirm Hp's ability to participate in local pathologies.

Introduction

Helicobacter pylori (Hp) is one of the most widespread pathogens in our population, with an approximate prevalence of 50%, depending on age, geographic, ethnic, and socioeconomic factors (Adler et al., 2014; Lukeš et al., 2012). Its role in inflammatory pathological processes in the stomach and, in particular, its carcinogenic potential has been extensively researched. Since 1994, Hp has been recognized as a Group 1 carcinogen. Approximately 10% of patients with Hp develop peptic ulcers, 1–3% develop gastric adenocarcinoma, and less than 0.1% develop mucosa-associated lymphoid tissue lymphoma (Adler et al., 2014).

Hp genomic analysis identified genes responsible for the production of virulence factors. These genes are found in the pathogenicity island (PAI). The most important Hp virulence factors are cytotoxin-associated gene A (*CagA*) and vacuolating cytotoxin A (*VacA*). *CagA* is associated with a higher risk of gastric pathologies, including carcinogenic potential. *VacA* is an important pluripotent cytotoxin. Its structure differs among individual Hp strains according to the various coding regions. Thus, *VacA* S1A, S1B, S2, M1, and M2 can be identified. Individual Hp strains with different *VacA* virulence factors are associated with various pathologies (Flores-Treviño et al., 2019; Lukeš et al., 2012; Mendoza-Cantú et al., 2017; Nártová et al., 2014; Román-Román et al., 2013; Wang et al., 2002).

* **Corresponding author:** Jan Rotnágl, Military University Hospital Prague, Department of Otorhinolaryngology and Maxillofacial Surgery, U vojenské nemocnice 1200, 169 02 Prague 6, Czech Republic; e-mail: jan.rotnagl@uvn.cz
http://doi.org/10.32725/jab.2024.018
Submitted: 2023-09-10 • Accepted: 2024-08-05 • Prepublished online: 2024-09-06
J Appl Biomed 22/3: 141–148 • EISSN 1214-0287 • ISSN 1214-021X

With the serious declining trend in the success rate of Hp eradication from the stomach, coupled with the evidence of the growing antibiotic resistance in established treatment protocols, alternative causes of this gradually increasing lack of success have been explored (Yee, 2016). Many studies have focused on identifying extragastric reservoirs as possible sources of persistence and reinfection after successful Hp eradication from the stomach (Dos Santos and Carvalho, 2015; Iwańczak and Iwańczak, 2012; Papastergiou et al., 2014; Seo et al., 2014; Shiota and Yamaoka, 2014; Vakil and Vaira, 2013; Yee, 2016). Over the course of years, evidence of Hp has been found in the oral cavity, particularly in dental plaque and saliva (Ahmed et al., 2006; Amiri et al., 2015; Ismail et al., 2016; Krajden et al., 1989; Liu et al., 2009; Navabi et al., 2011; Payão and Rasmussen, 2016; Rasmussen et al., 2010; Yee, 2016; Zou and Li, 2011). In addition to its effect on gastric reinfection, the presence of Hp in this locality has been associated with pathological processes such as halitosis, pyrosis, or aphthous stomatitis (Adler et al., 2014; López-Valverde et al., 2022). Furthermore, an increased rate of periodontal pathology has been observed in Hp-positive patients (al Sayed et al., 2014; Flores-Treviño et al., 2019). Some studies have also focused on Hp detection and the determination of possible hypotheses for Hp activity in the adenotonsillar tissue (Lukeš et al., 2012, 2014; Nártová et al., 2014; Payão and Rasmussen, 2016). Most of these studies, including identical ones, are characterized by an extreme diversity of results, depending on the detection method and specific locality (Adler et al., 2014; Cammarota et al., 1996; López-Valverde et al., 2022; Luman et al., 1996; Román-Román et al., 2013; Von Recklinghausen et al., 1994; Wang et al., 2002). Similarly, continued discussion revolves around whether the presence of Hp in these localities may only be the consequence of gastric reflux, or if the oral cavity may only serve as transient locality of Hp presence after an assumed oro-oral transmission (Kurtaran et al., 2008; Yee, 2016, 2017). The lack of success of Hp culture testing in this locality is probably due to the following: the Hp colonies are not large; Hp is difficult to collect and preserve; and Hp competes with other bacteria present in the oropharyngeal flora (Yee, 2016, 2017). The capture rate of positive cultures in the oral cavity that approaches the actual occurrence rate only increased after the introduction of special culture media with the addition of an "artificial ammonia cloud" (Yee, 2016). Additionally, as demonstrated in some studies, the oral biofilm may have an impact on the morphology of the present Hp, which can exist in its coccoid form under unfavorable conditions. This form cannot be cultured successfully, although its pathological potential is preserved (Bakhti and Latifi-Navid, 2021; Krzyżek and Gościniak, 2018). However, multiple studies have demonstrated that an extragastric reservoir of Hp exists in this locality – in the form of viable bacteria whose pathogenic potential is not yet fully understood (Ahmed et al., 2006; Amiri et al., 2015; Ismail et al., 2016; Liu et al., 2009; Payão and Rasmussen, 2016; Rasmussen et al., 2010; Yee, 2016). According to these studies, Hp can persist in the extragastric reservoirs even after systemic treatment or eradication of the stomach (Adler et al., 2014; al Sayed et al., 2014; Andersen and Rasmussen, 2009; Assumpção et al., 2010; Bakhti and Latifi-Navid, 2021; Flores-Treviño et al., 2019; Gao et al., 2011; Jia et al., 2012; Krzyżek and Gościniak, 2018; López-Valverde et al., 2022; Navabi et al., 2011; Yee, 2016; Zaric et al., 2009; Zou and Li, 2011). This is probably due to the specific characteristics in some localities in oral cavity which are poorly accessible to antibiotics, similar to dental plaque. Additionally, Hp has demonstrated the ability to survive adverse situations in its inactive coccoid form (Adler et al., 2014; Bakhti and Latifi-Navid, 2021; Krzyżek and Gościniak, 2018). The results of numerous studies clearly indicate a relationship between the presence of Hp in the oral cavity and reduced success rates of its eradication from the stomach, as well as increased success rates of eradication with concomitant eradication from the dental plaque when using local periodontal therapy (Abadi et al., 2014; Hirsch et al., 2012; Krzyżek and Gościniak, 2018; Li et al., 2021; Wang et al., 2014; Yee, 2016).

The etiology of sialolithiasis remains unclear. Inflammation, sialomicrolith theory, pH changes, and, in recent years, the role of the local biofilm have been considered in the theory of its formation (Kao et al., 2020; Marchal et al., 2001). The origin of most salivary gland tumors is unknown, although continued genetic research has been conducted to uncover it (Guzzo et al., 2010; Horn-Ross et al., 1997; Kao et al., 2020; Lewis et al., 2016; Seethala, 2017; Young and Okuyemi, 2022).

However, Hp's ability to enter the salivary gland environment has never been studied. Our study aimed to determine whether Hp could be delivered to the duct system of the salivary gland. This environment is relatively separate from the assumed oro-oral transmission route to the gastrointestinal tract. Therefore, the occurrence of Hp in this locality cannot be considered as a consequence of contamination. And further more, whether HP is also able to penetrate the salivary gland parenchyma.

The confirmation of Hp presence in this locality could be a first step in further research of its possible pathological potential in this new site of its occurrence. Subsequent confirmation of its ability to cause local infection could determine possible hypotheses of its role in sialopathies suitable for further research.

We decided to use real time PCR as a highly specific and sensitive tool for detecting Hp. This fully automated method that skips the difficulties of other HP detection methods is well established in our cooperating laboratory. Although programs for quantification were used, quantification was not the task of this work. Only semi-quantitative analysis (positive/negative) was chosen as most valuable for the purpose of this study. Simultaneously, this approach enabled us to perform genotyping of Hp strains. The decision to analyze PCR Hp positivity in ductal system using concrements instead of saliva expressed from the duct was based on two reasons. Firstly, the concrement had been present for several years and had been in contact with saliva in the same region during that time. Secondly, material from non-viable bacteria could be detected using PCR. Therefore, salivary stones can be considered a good "time-lapse" material, reflecting not only its Hp positive or negative current status but also the previous conditions throughout the entire period of the concrement's creation.

Furthermore, based on knowledge of Hp's ability to invade the intercellular space, we decided to analyze tissues from certain types of salivary gland tumors to find out whether HP can penetrate the parenchyma of the salivary gland.

This study provides compelling evidence of the Hp presence in salivary ductal system and in salivary parenchyma. The pathologic potential in this newly discovered site of Hp occurrence has never been studied yet.

Materials and methods

Selection of study subjects

All samples were collected at the Department of Otorhinolaryngology and Maxillofacial Surgery of the 3rd Faculty of

Medicine, Charles University, and Military University Hospital Prague.

This study included patients with chronic obstructive salivary disease due to lithiasis, as well as patients with salivary gland tumors who required surgery. Consent to treatment and subsequent examination of biological material was obtained from each patient. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Central Military Hospital Prague. Reference number: 108/16-80/2021.

First, 53 sialoconcrements were collected during the following surgical procedures: sialoendoscopic sialolithectomy, combined mini-invasive sialolithectomy, and submandibular salivary gland extirpation. Second, individual tissue samples from 7 salivary gland tumors were collected during salivary gland tumor resection and tested for Hp. In addition, we separated the group of 50 patients with submandibular salivary gland concrements from the control group. The control group included 40 patients from the same department with no clinical symptoms or a history of sialopathy symptoms. Saliva from the submandibular gland was collected from these patients. Due to the diversity of the examined samples saliva/concrement the statistic evaluation for Hp possible causal association with lithiasis was not possible. In our opinion, unfortunately no other feasible control material is available to compare the situation in patients with and without sialolithiasis. Using "freely expressed" saliva for Hp detection in patients with lithiasis may not be suitable for possible false negative results due to an insufficient amount of captured bacteria for PCR detection (Šeligová et al., 2020).

Principles of sample collection

Concrements – 53 patients

Sialoendoscopy

Surgery was performed under local anesthesia, and the concrement was removed intraorally via the natural path of the duct using endoscopy.

Combined mini-invasive sialolithectomy

Surgery was performed under local or general anesthesia, and the concrement was removed intraorally through a discission of the duct with endoscopic assistance (submandibular/parotid salivary gland) or through an external mini-incision (parotid salivary gland).

Submandibular salivary gland extirpation

Surgery under general anesthesia: Excision of the submandibular salivary gland using an external approach, with subsequent removal of the concrement from the gland.

Salivary tumor tissue – 7 patients

Salivary gland tumor resection

Extracapsular dissection, superficial parotidectomy (segmental parotidectomy), and total parotidectomy with neck dissection were performed under general anesthesia in accordance with the size and histopathological characteristics of the lesion.

Individual tumors' histopathological characteristics included low-grade mucoepidermoid carcinoma of the parotid gland (GP) once, spinocellular carcinoma of the GP once, adenoid cystic carcinoma of the submandibular gland (*Glandula submandibularis*; GSM) once, cystadenolymphoma of the GP once, and pleomorphic adenoma of the GP three times.

Given the low number, tumors were considered one unit for the needs of the study, irrespective of their histopathological characteristics.

All the surgeries were performed using sterile techniques. To exclude any possible contamination with oropharyngeal flora, special care was paid to minimizing the contact of the collected sample with the oral mucosa in cases where the concrements were collected intraorally. Samples collected externally were aseptic in accordance with the principles of surgery.

Control group – 40 patients

The saliva sample was collected by expressing saliva from the submandibular gland duct while establishing close contact with the natural duct opening. Extra caution was exercised to exclude any possible contact between the saliva and oral mucosa.

Handling of the samples

Each collected sample was immediately placed in the special transport medium, Remel MicrotestR M4RT Collection and Transport Medium (Remel Inc., USA). The samples were then transported to the laboratory of the Department of Microbiology and Immunology, 1st Faculty of Medicine, Charles University, Prague, for further processing.

Processing of the samples

DNA isolation

Every sample, with the exception of saliva, was first mechanically homogenized. A tube containing the sample and transport medium was thoroughly vortexed, and 400 µl pipetted into a 2 ml ependorf tube. Nucleic acids were isolated using an automatic isolation kit (MagNA Pure Compact; Roche, Tegimenta AG, Switzerland) in the ratio of 400 µl of sample to 100 µl of eluent and fourfold concentration. Tubes with eluted nucleic acid isolates were thus prepared for further processing.

Real-time PCR test

Hp was detected using RIDA GENE *Helicobacter pylori* IVD kit (R-Biopharm AG, Darmstadt) according to the manufacturer's instructions on a Rotor-Gene 6000 (Corbett/Qiagen) instrument.

Hp genotyping

Three real-time PCR assays using the LightCycler TaqMan MasterMix (Roche Diagnostics, Mannheim, Germany) were used for genotyping. The primers and probes (sequences according to Van Doorn et al., 1997) *CagA*, *VacA* S1a, *VacA* S1b, *VacA* S2, *VacA* M1, and *VacA* M2 were optimized in cooperation with TIB Molbiol (Berlin, Germanny) (Table 1, 2). TaqMan assays were run on LightCycler 2.0 (Roche Diagnostics, Mannheim, Germanny) according to the user's manual. Semi-quantitative analysis (positive/negative) was performed using the LightCycler 480 software.

Table 1. Overview of used probes

Gene	Type	No. of nucleotides	Sequence	Detection n/m
cagA	Cag TM	28	6FAM-ATA ACG CTG TCG CTT CAT ACG ATC CTG A-BBQ	530
vacA S	S1a LC	21	LC Red610-GCR TTR GTC AGC ATC ACA CCG-PH	610
vacA S	S1b LC	21	LC Red640-GCG TTG ATT AGY KCC ATA CCG-PH	640
vacA S	S2 LC	21	LC Red705-GCT AAY ACG CCA AAY GAT CCC-PH	705
vacA M	M1 TM	30	6FAM-ACC ACC ATT ACC CGT ATC AAT ACC TTT AAA-BBQ	530
vacA M	M2 TM	26	HEX-CTA GTG TTT AGC CCG TTA TCG CTC TT-BBQ	560

Table 2. Overview of used primers

Gene	Primer	No. of nucleotides	Sequence
cagA	*cagA* F	24	5-TTG ACC AAC AAC CAC AAA CCG AAG-3
cagA	*cagA* R	22	5-CTT CCC TTA ATT GCG AGA TTC C-3
vacA S	VA1F	21	5-ATG GAA ATA CAA CAA ACA CAC-3
vacA S	VA1R	19	5-CTG CTT GAA TGC GCC AAA C-3
vacA M	HPMGF	21	5-CAG AGC CAC TTT CAA TAA CGA-3
vacA M	HPMGR	21	5-CGT CCA AAT AAT TCC AAG GG-3

Results

Fifty-three concrements and 7 tissue samples from the salivary tumors were collected from 60 patients who underwent surgery for sialolithiasis or various types of salivary tumors. The presence of Hp was demonstrated in 41 of 60 patients (68.3%) in the study group. In a separate group of patients with various salivary tumors, Hp was demonstrated in 6 of the 7 patients, representing 85.7% of the sample. In a separate group of 50 patients with submandibular salivary gland concrements, Hp were observed in 33 of the 50 patients (66%). In addition, saliva samples from the submandibular duct were collected from 40 patients with no clinical symptoms or history of sialopathy as the control group. In contrast, Hp was detected in only 7 of the 40 patients in the control group, that is, in 17.5% of the samples (Fig. 1A, B). The remaining 3 patients with GP lithiasis were Hp positive in 2 of 3 cases.

The *CagA*-positive genotype was found in 14 of the 41 Hp-positive samples. *VacA* S1AM1 was the most common genotype. S1A was found in 20 of the 41 Hp-positive samples, and M1 in 28 of the 41 Hp-positive samples. M2 was detected in 13 positive samples, S1B in 14, and S2 in 7. However, genotyping revealed no statistically significant differences between the individual genotypes in separate groups (Table 3).

The 68.3% Hp positivity rate in the assessed group confirmed the presence of Hp inside the salivary glands. *VacA* S1AM1 was the most common genotype. *CagA*-positive samples accounted for only 34% of all Hp-positive samples. On the other hand, Hp was detected in only 7 of the 40 patients in the control group, that is, in 17.5% of the sample.

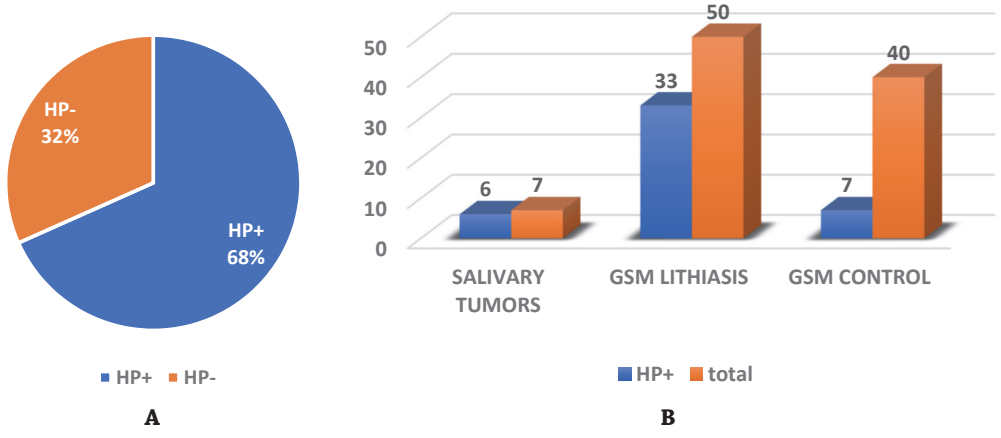

Fig. 1. (**A**) Total HP positivity in study group of 60 patients (concrements + tumor tissue); (**B**) HP positivity in individual groups (tumor tissue, lithiasis, control)

Table 3. *Helicobacter pylori* positivity results and genotyping

Study HP+			Sm	*n*	*CagA*+	*CagA*–	*VacA* S1A	*VacA* S1B	*VacA* S2	*VacA* M1	*VacA* M2	%
41/60	GSM	34/51	SE	13	5	8	4	7	2	8	5	66.7
			Ext	20	5	15	11	5	4	15	5	
			Tu	1		1		1		1		
	GP	7/9	SE	2		2	2			1	1	77.8
			Ext									
			Tu	5	4	1	3	1	1	3	2	
Total S		41/60		41	14	27	20	14	7	28	13	68.3
Control HP+												
Total C	GSM	7/40	Sal.	7	2	5	3	3	1	3	4	17.5

Note: Sal. – Saliva, Sm – Sample, *n* – Count, GSM – Submandibular gland, GP – Parotid gland, HP – *Helicobacter pylori*, SE – Concrement from sialoendoscopy, Ext – Concrement from gland exstirpation, Tu – Tumor.

Discussion

Considering the rapidly decreasing success rate of Hp eradication in the stomach using ATB established treatment protocols, other possible causes of this gradually increasing lack of success are being explored in addition to growing resistance to ATB. In recent years, extragastric reservoirs have been considered a possible source of persistence and subsequent reinfection after seemingly successful eradication from the stomach (Gao et al., 2011; Liu et al., 2009; Wang et al., 2002, 2014; Yee, 2016, 2017).

Molecular biology methods have clearly confirmed this increase in ATB resistance, particularly to clarithromycin (Dos Santos and Carvalho, 2015; Iwańczak and Iwańczak, 2012; Murata et al., 2020; Papastergiou et al., 2014; Seo et al., 2014; Shiota and Yamaoka, 2014; Vakil and Vaira, 2013). This confirms the necessity of escalating therapy. Treatment cycles have been extended, and new antibiotics such as levofloxacin have been introduced into treatment protocols. Treatment invasiveness has also been growing, as is the case with individualized therapy using endoscopically guided ATB susceptibility pre-treatment testing, which seems very promising for the future (Dos Santos and Carvalho, 2015; Iwańczak and Iwańczak, 2012; Liu et al., 2015; Papastergiou et al., 2014; Seo et al., 2014; Shiota and Yamaoka, 2014; Vakil and Vaira, 2013; Yee, 2016). However, all these procedures increase the burden on the patient and are also reflected in increased costs. Furthermore, many authors suggest that to focus solely on increasing ATB resistance while dismissing the role of extragastric reservoirs is incorrect.

The biofilm characteristics and the specific anatomical nature of the studied oral cavity localities probably explain the ineffectiveness of systemic therapy in this area; thus, the ability of Hp to persist in these extragastric reservoirs even after therapy (Adler et al., 2014; al Sayed et al., 2014; Andersen and Rasmussen, 2009; Assumpção et al., 2010; Bakhti and Latifi-Navid, 2021; Flores-Treviño et al., 2019; Gao et al., 2011; Jia et al., 2012; Krzyżek and Gościniak, 2018; López-Valverde et al., 2022; Navabi et al., 2011; Yee, 2016; Zaric et al., 2009; Zou and Li, 2011). Several studies have clearly indicated that the reduced success rate of Hp eradication from the stomach depends on the Hp presence in the oral cavity. Similarly, an increased success rate of Hp eradication was demonstrated with the concomitant eradication of dental plaque using local periodontal therapy (Abadi et al., 2014; Hirsch et al., 2012; Krzyżek and Gościniak, 2018; Li et al., 2021; Wang et al., 2014; Yee, 2016). These studies may have provided an answer to the ongoing discussion on the viability of Hp detected in the oral cavity, with the above-mentioned difficulty of its culturability and, on the contrary, the possibility of detecting fragments of non-viable bacteria using PCR (Yee, 2016). However, determination of viability of the identified bacteria was not substantial because the primary purpose of our study was to reveal the presence of Hp in a new locality.

Additionally, some authors have raised doubts about the existence of extragastric reservoirs by suggesting that Hp detected in the oral cavity may simply be captured during reflux from the stomach, and it may be detected transiently during its passage to the stomach, or due to potential contamination from food (Krzyżek and Gościniak, 2018; Yee, 2016, 2017).

We attempted to avoid these controversies by selecting the locality of the salivary glands, which are anatomically different from the area of the assumed oro-oral transmission or gastric reflux routes.

According to most authors, PCR is considered suitable for Hp detection in the oral cavity. In addition to its ability to detect coccoid forms of Hp, PCR can also be used for genotyping, determining the virulence of the detected bacteria (Duś et al., 2013; Liu et al., 2015; Wang et al., 2014; Yee, 2016). On the other hand, Šeligová et al. (2020) mentioned that 1–5 × 10^3 cells/g or ml of specimen was required as a detection limit for successful PCR detection. Given the non-constant occurrence and small amounts of bacteria in saliva and stool, this study questioned whether PCR detection from saliva is a relevant screening method.

Concrements are ideal materials for detecting Hp in saliva. The fact that concrements develop and persist in the salivary gland for many years, combined with the ability of PCR to detect even fragments of non-viable bacteria, means that concrements represent a perfect time-lapse material. The detected concentration of Hp in concrement allows for retrogradation of any possible Hp in saliva inside salivary gland over the course of many years. For this reason, it is necessary to keep in mind that the statistically significant difference between the Hp positivity rate in the material from submandibular gland sialoliths compared to that from "free" saliva expressed from the submandibular duct in the control group can be also caused

by the varying amount of bacteria in these materials. For this possible controversy, we made no statistical conclusion of the association between the presence of Hp and lithiasis. Unfortunately, no other feasible control material is available to compare the situation in patients with and without obstructive pathology. In our opinion, using "freely expressed" saliva for Hp detection in patients with lithiasis may not be suitable. A negative result from saliva detection may only reflect the possible limits of PCR-based Hp detection from saliva, and it does not change the fact that Hp is present inside the ductal system obtained from sialoliths. Further research is thus needed to establish this possible hypothesis.

Hp positivity in salivary gland tumors is a remarkable and interesting finding. Although a high percentage of Hp was detected in 6 out of the 7 tumors examined (85.7% of the sample), the limited size of this group hinders us from formulating any clear conclusions based solely on this finding. However, this provides sufficient stimulus to continue research in this direction. Hp detection from the tissue surrounding the tumor and from the salivary glands of healthy individuals is suggested. Because obtaining salivary gland tissue samples from healthy individuals is difficult, we propose tissue sampling during neck dissection in patients treated for non-salivary malignancies.

The complete etiology of sialolithiasis remains unclear. The role of the local biofilm, as well as the pH changes in its formation, is considered (Kao et al., 2020; Marchal et al., 2001). On the other hand, it is well known that pH changes are a natural part of the behavior of Hp (Wang et al., 2014; Yee, 2016). The origin of most salivary gland tumors is unknown, although continued genetic research has been conducted to uncover it. In contrast, the carcinogenic potential of Hp in the stomach has been well mapped (Adler et al., 2014). Similarly, Sjögren syndrome, a chronic autoimmune disease that causes salivary obstruction, is associated with an increased risk of malignant salivary gland lymphoma (Theander et al., 2006). Now that we have demonstrated the presence of Hp in the salivary glands, could similar factors play any role in the development of obstructive or tumoral sialopathies?

According to the published literature, a lower number of virulent strains are found in the oral cavity than in the stomach (Payão and Rasmussen, 2016; Rasmussen et al., 2010). We observed similar results in the salivary glands, where only 34% of the *CagA*-positive samples were found among the total positive samples. Thus, it can be concluded that if Hp is involved in any of the sialopathies examined, the *CagA* virulence factor most likely does not play a role.

Conclusion

Hp was detected in 68.3% of the evaluated sialopathy samples. The results of our study clearly confirm the presence of Hp inside the salivary glands' ductal system, as well as its supposed ability to penetrate the parenchyma of the gland. This area can thus be considered as another possible extragastric reservoir with unstudied pathological potential. The question of its viability in this locality, ability to cause local infection, and thus its potential relationship with gastric infection or reinfection should be the subject of further study.

We have observed significant difference in the Hp occurrence rate in the study group with submandibular sialolithiasis compared to the control group, and the surprising result of Hp positivity in salivary tumors. This represent strong impulses for further research to confirm or disprove the potential role of Hp in chronic obstructive pathologies of salivary glands, as well as any possible role of Hp in the etiology of salivary gland tumors. *CagA*-positive samples accounted for only 34% of all Hp-positive samples. As a result, if Hp is involved in any of the examined sialopathies, the *CagA* virulence factor is unlikely to play a role.

Authors' contributions

JR: conceptualization; *JR:* writing and preparing the original draft; *JR, EP:* methodology; *JR, JH:* investigation; *JA, RH:* supervision; *JA:* funding acquisition; *DK, EP:* data curation; *JR:* project administration.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Central Military Hospital Prague. Reference number: 108/16-80/2021. Date of EC session: 18.10.2021.

Informed consent statement

Informed consent was obtained from all participants involved in the study.

Funding

This research was funded by the Ministry of Defence of the Czech Republic, Project MO 1012. It was supported by the Cooperatio Program, research area SURG.

Ethical aspects and conflict of interest

The authors have no conflict of interest to declare.

References

Abadi ATB, Mobarez AM, Teymournejad O, Karbalaei M (2014). Concomitant Colonization of *Helicobacter pylori* in Dental Plaque and Gastric Biopsy. J Pathog 2014: 871601. DOI: 10.1155/2014/871601.

Adler I, Muiño A, Aguas S, Harada L, Diaz M, Lence A, et al. (2014). *Helicobacter pylori* and oral pathology: relationship with the gastric infection. World J Gastroenterol 20(29): 9922–9935. DOI: 10.3748/WJG.V20.I29.9922.

Ahmed KS, Khan AA, Ahmed I, Tiwari SK, Habeeb MA, Ali SM, et al. (2006). Prevalence study to elucidate the transmission pathways of *Helicobacter pylori* at oral and gastroduodenal sites of a South Indian population. Singapore Med J 47: 291–296.

al Sayed A, Anand PS, Kamath KP, Patil S, Preethanath RS, Anil S (2014). Oral Cavity as an Extragastric Reservoir of *Helicobacter pylori*. ISRN Gastroenterol 2014: 261369. DOI: 10.1155/2014/261369.

Amiri N, Abiri R, Eyvazi M, Zolfaghari MR, Alvandi A (2015). The frequency of *Helicobacter pylori* in dental plaque is possibly underestimated. Arch Oral Biol 60(5): 782–788. DOI: 10.1016/j.archoralbio.2015.02.006.

Andersen LP, Rasmussen L (2009). *Helicobacter pylori*-coccoid forms and biofilm formation. FEMS Immunol Med Microbiol 56(2): 112–115. DOI: 10.1111/j.1574-695X.2009.00556.x.

Assumpção MB, Martins LC, Barbosa HPM, dos Santos Barile KA, de Almeida SS, Assumpção PP, de Oliveira Corvelo TC (2010). *Helicobacter pylori* in dental plaque and stomach of patients from Northern Brazil. World J Gastroenterol 16(24): 3033–3039. DOI: 10.3748/WJG.V16.I24.3033.

Bakhti SZ, Latifi-Navid S (2021). Oral microbiota and *Helicobacter pylori* in gastric carcinogenesis: what do we know and where next? BMC Microbiol 21(71). DOI: 10.1186/s12866-021-02130-4.

Cammarota G, Tursi A, Montalto M, Papa A, Veneto G, Bernardi S, et al. (1996). Role of dental plaque in the transmission of

Helicobacter pylori infection. J Clin Gastroenterol 22(3): 174–177. DOI: 10.1097/00004836-199604000-00004.

Dos Santos AA, Carvalho AA (2015). Pharmacological therapy used in the elimination of *Helicobacter pylori* infection: A review. World J Gastroenterol 21(1): 139–154. DOI: 10.3748/wjg.v21.i1.139.

Duś I, Dobosz T, Manzin A, Loi G, Serra C, Radwan-Oczko M (2013). Role of PCR in *Helicobacter pylori* diagnostics and research – new approaches for study of coccoid and spiral forms of the bacteria. Postepy Hig Med Dosw (Online) 67: 261–268. DOI: 10.5604/17322693.1044005.

Flores-Treviño CE, Urrutia-Baca VH, Gómez-Flores R, de La Garza-Ramos MA, Sánchez-Chaparro MM, Garza-Elizondo MA (2019). Molecular detection of *Helicobacter pylori* based on the presence of *cagA* and *vacA* virulence genes in dental plaque from patients with periodontitis. J Dent Sci 14(2): 163–170. DOI: 10.1016/J.JDS.2019.01.010.

Gao J, Li Y, Wang Q, Qi C, Zhu S (2011). Correlation between distribution of *Helicobacter pylori* in oral cavity and chronic stomach conditions. J Huazhong Univ Sci Technolog Med Sci 31(3): 409–412. DOI: 10.1007/S11596-011-0391-6.

Guzzo M, Locati LD, Prott FJ, Gatta G, McGurk M, Licitra L (2010). Major and minor salivary gland tumors. Crit Rev Oncol Hematol 74(2): 134–148. DOI: 10.1016/j.critrevonc.2009.10.004.

Hirsch C, Tegtmeyer N, Rohde M, Rowland M, Oyarzabal OA, Backert S (2012). Live *Helicobacter pylori* in the root canal of endodontic-infected deciduous teeth. J Gastroenterol 47(8): 936–940. DOI: 10.1007/S00535-012-0618-8.

Horn-Ross PL, Ljung BM, Morrow M (1997). Environmental factors and the risk of salivary gland cancer. Epidemiology 8(4): 414–419. DOI: 10.1097/00001648-199707000-00011.

Ismail H, Morgan C, Griffiths P, Williams J, Jenkins G (2016). A Newly Developed Nested PCR Assay for the Detection of *Helicobacter pylori* in the Oral Cavity. J Clin Gastroenterol 50(1): 17–22. DOI: 10.1097/MCG.0000000000000310.

Iwańczak F, Iwańczak B (2012). Treatment of *Helicobacter pylori* infection in the aspect of increasing antibiotic resistance. Advances in Clinical and Experimental Medicine 21(5): 671–680.

Jia CL, Jiang GS, Li CH, Li CR (2012). Effect of dental plaque control on infection of *Helicobacter pylori* in gastric mucosa. J Periodontol 80(10): 1606–1609. DOI: 10.1902/jop.2009.090029.

Kao WK, Chole RA, Ogden MA (2020). Evidence of a microbial etiology for sialoliths. Laryngoscope 130(1): 69–74. DOI: 10.1002/lary.27860.

Krajden S, Fuksa M, Anderson J, Kempston J, Boccia A, Petrea C, et al., 1989. Examination of human stomach biopsies, saliva, and dental plaque for *Campylobacter pylori*. J Clin Microbiol 27(6): 1397–1398. DOI: 10.1128/JCM.27.6.1397-1398.1989.

Krzyżek P, Gościniak G (2018). Oral *Helicobacter pylori*: Interactions with host and microbial flora of the oral cavity. Dent Med Probl 55(1): 75–82. DOI: 10.17219/DMP/81259.

Kurtaran H, Uyar ME, Kasapoglu B, Turkay C, Yilmaz T, Akcay A, Kanbay M (2008). Role of *Helicobacter pylori* in pathogenesis of upper respiratory system diseases. J Natl Med Assoc 100(10): 1224–1230. DOI: 10.1016/S0027-9684(15)31471-1.

Lewis AG, Tong T, Maghami E (2016). Diagnosis and Management of Malignant Salivary Gland Tumors of the Parotid Gland. Otolaryngol Clin North Am 49(2): 343–380. DOI: 10.1016/j.otc.2015.11.001.

Li X, Chaouhan HS, Li CH, Yu TM, Wang IK, Lin CL, et al. (2021). Higher Risk of Gastric *Helicobacter pylori* Infection in Patients with Periodontitis: A Nationwide Population-Based Retrospective Cohort Study in Taiwan. Int J Environ Res Public Health 18(21): 11678. DOI: 10.3390/ijerph182111678.

Liu Q, Qi D, Kang J, Jin Y, Liu W, Gao W, et al. (2015). Efficacy of real-time PCR-based detection of *Helicobacter pylori* infection and genotypic resistance-guided quadruple therapy as the first-line treatment for functional dyspepsia with *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol 27(3): 221–225. DOI: 10.1097/MEG.0000000000000186.

Liu Y, Yue H, Li A, Wang J, Jiang B, Zhang Y, Bai Y (2009). An epidemiologic study on the correlation between oral *Helicobacter pylori* and gastric *H. pylori*. Curr Microbiol 58(5): 449–453. DOI: 10.1007/S00284-008-9341-3.

López-Valverde N, Macedo de Sousa B, López-Valverde A, Suárez A, Rodríguez C, Aragoneses JM (2022). Possible Association of Periodontal Diseases With *Helicobacter pylori* Gastric Infection: A Systematic Review and Meta-Analysis. Front Med (Lausanne) 9: 822194. DOI: 10.3389/fmed.2022.822194.

Lukeš P, Pavlík E, Potuznikova B, Nartova E, Foltynova E, Plzak J, et al. (2014). Detection of *Helicobacter pylori* in oropharyngeal lymphatic tissue with real-time PCR and assessment of its carcinogenic potential. Eur Arch of Otorhinolaryngol 271(2): 399–405. DOI: 10.1007/s00405-013-2574-1.

Lukeš P, Pavlík E, Potužníková B, Plzák J, Nártová E, Doseděl J, et al. (2012). Comparison of *Helicobacter pylori* genotypes obtained from the oropharynx and stomach of the same individuals – a pilot study. Prague Med Rep 113(3): 231–239. DOI: 10.14712/23362936.2015.21.

Luman W, Alkout AM, Blackwell CC, Weir DM, Palmer KR (1996). *Helicobacter pylori* in the mouth – negative isolation from dental plaque and saliva. Eur J Gastroenterol Hepatol 8(1): 11–14. DOI: 10.1097/00042737-199601000-00004.

Marchal F, Kurt AM, Dulguerov P, Lehmann W (2001). Retrograde theory in sialolithiasis formation. Arch Otolaryngol Head Neck Surg 127(1): 66–68. DOI: 10.1001/archotol.127.1.66.

Mendoza-Cantú A, Urrutia-Baca VH, Urbina-Ríos CS, de La Garza-Ramos MA, García-Martínez ME, Torre-Martínez HHH (2017). Prevalence of *Helicobacter pylori vacA* Genotypes and *cagA* Gene in Dental Plaque of Asymptomatic Mexican Children. Biomed Res Int 2017: 4923640. DOI: 10.1155/2017/4923640.

Murata M, Sugimoto M, Mizuno H, Kanno T, Satoh K (2020). Clarithromycin Versus Metronidazole in First-Line *Helicobacter Pylori* Triple Eradication Therapy Based on Resistance to Antimicrobial Agents: Meta-Analysis. J Clin Med 9(2): 543. DOI: 10.3390/jcm9020543.

Nártová E, Kraus J, Pavlík E, Lukeš P, Katra R, Plzák J, et al. (2014). Presence of different genotypes of *Helicobacter pylori* in patients with chronic tonsillitis and sleep apnoea syndrome. Eur Arch Otorhinolaryngol 271(3): 607–613. DOI: 10.1007/s00405-013-2607-9.

Navabi N, Mirzazadeh A, Aramon M (2011). Does the presence of the *Helicobacter pylori* in the dental plaque associate with its gastric infection? A meta-analysis and systematic review. Dent Res J (Isfahan) 8(4): 178–182. DOI: 10.4103/1735-3327.86033.

Papastergiou V, Georgopoulos SD, Karatapanis S (2014). Treatment of *Helicobacter pylori* infection: Past, present and future. World J Gastrointest Pathophysiol 5(5): 392–399. DOI: 10.4291/wjgp.v5.i4.392.

Payão SL, Rasmussen LT (2016). *Helicobacter pylori* and its reservoirs: A correlation with the gastric infection. World J Gastrointest Pharmacol Ther 7(1): 126–132. DOI: 10.4292/wjgpt.v7.i1.126.

Rasmussen LT, Labio RW, Gatti LL, Silva LC, Queiroz VF, Smith Mde A, Payão SL (2010). *Helicobacter pylori* detection in gastric biopsies, saliva and dental plaque of Brazilian dyspeptic patients. Mem Inst Oswaldo Cruz 105(3): 326–330. DOI: 10.1590/S0074-02762010000300015.

Román-Román A, Giono-Cerezo S, Camorlinga-Ponce M, Martínez-Carrillo DN, Loaiza-Loeza S, Fernández-Tilapa G (2013). *vacA* genotypes of *Helicobacter pylori* in the oral cavity and stomach of patients with chronic gastritis and gastric ulcer. Enferm Infecc Microbiol Clin 31(3): 130–135. DOI: 10.1016/J.EIMC.2012.09.002.

Seethala RR (2017). Salivary Gland Tumors: Current Concepts and Controversies. Surg Pathol Clin 10(1): 155–176. DOI: 10.1016/j.path.2016.11.004.

Šeligová B, Lukáč Ľ, Bábelová M, Vávrová S, Sulo P (2020). Diagnostic reliability of nested PCR depends on the primer design and threshold abundance of *Helicobacter pylori* in biopsy, stool, and saliva samples. Helicobacter 25(2): e12680. DOI: 10.1111/HEL.12680.

Seo JH, Woo HO, Youn HS, Rhee KH (2014). Antibiotics resistance of *Helicobacter pylori* and treatment modalities in children with *H. pylori* infection. Korean J Pediatr 57(2): 67–71. DOI: 10.3345/kjp.2014.57.2.67.

Shiota S, Yamaoka Y (2014). Strategy for the treatment of *Helicobacter pylori* infection. Curr Pharm Des 20(8): 4489–4500. DOI: 10.2174/13816128113196660731.

Theander E, Henriksson G, Ljungberg O, Mandl T, Manthorpe R, Jacobsson LTH (2006). Lymphoma and other malignancies in primary Sjögren's syndrome: a cohort study on cancer incidence and lymphoma predictors. Ann Rheum Dis 65(6): 796–803. DOI: 10.1136/ARD.2005.041186.

Vakil N, Vaira D (2013). Treatment for *H. pylori* infection: New challenges with antimicrobial resistance. J Clin Gastroenterol 47(5): 383–388. DOI: 10.1097/MCG.0b013e318277577b.

van Doorn LJ, Giesendorf BA, Bax R, van der Zeijst BA, Vandamme P, Quint WG (1997). Molecular discrimination between *Campylobacter jejuni, Campylobacter coli, Campylobacter lari* and *Campylobacter upsaliensis* by polymerase chain reaction based on a novel putative GTPase gene. Mol Cell Probes 11(3): 177185. DOI: 10.1006/mcpr.1997.0100.

Von Recklinghausen G, Weischer T, Ansorg R, Mohr C (1994). No cultural detection of *Helicobacter pylori* in dental plaque. Zentralbl Bakteriol 281(1): 102–106. DOI: 10.1016/S0934-8840(11) 80643-2.

Wang J, Chi DS, Laffan JJ, Li C, Ferguson DA, Litchfield P, Thomas E (2002). Comparison of cytotoxin genotypes of *Helicobacter pylori* in stomach and saliva. Dig Dis Sci 47(8): 1850–1856. DOI: 10.1023/A:1016417200611.

Wang XM, Yee KC, Hazeki-Taylor N, Li J, Fu HY, Huang ML, Zhang GY (2014). Oral *Helicobacter pylori*, its relationship to successful eradication of gastric *H. pylori* and saliva culture confirmation. J Physiol Pharmacol 65(4): 559–566.

Yee JKC (2016). *Helicobacter pylori* colonization of the oral cavity: A milestone discovery. World J Gastroenterol 22(2): 641–648. DOI: 10.3748/WJG.V22.I2.641.

Yee JKC (2017). Are the view of *Helicobacter pylori* colonized in the oral cavity an illusion? Exp Mol Med 49(11): e397. DOI: 10.1038/emm.2017.225.

Young A, Okuyemi OT (2022). Benign Salivary Gland Tumors. National Library of Medicine 1: 1–15.

Zaric S, Bojic B, Jankovic L, Dapcevi B, Popovic B, Cakic S, Milasin J (2009). Periodontal therapy improves gastric *Helicobacter pylori* eradication. J Dent Res 88(10): 946–950. DOI: 10.1177/0022034509344559.

Zou QH, Li RQ (2011). *Helicobacter pylori* in the oral cavity and gastric mucosa: a meta-analysis. J Oral Pathol Med 40(4): 317–324. DOI: 10.1111/J.1600-0714.2011.01006.X.