Serotyping of Helicobacter Pylori Antibody Reflected on Human Health

Valon Morina¹, Rrahman Ferizi²*, Fatmir Cakaj³, Mohamed Fawzy Ramadan⁴

1. College Heimerer, Department of Technical Laboratory, Prishtina, Kosovo.
2. University of Prishtina, Paramedical Department, Faculty of Medicine Prishtina, Kosovo.
3. University of Prishtina, Biology Department, Faculty of Mathematics - Natural Sciences, Prishtina, Kosovo.
4. Zagazig University, Department of Agricultural Biochemistry, Faculty of Agriculture, 44519 Zagazig, Egypt.

Abstract
It is well-known that Helicobacter pylori is diagnosed as a carcinogenic bacterium among the scientific community, and it is also accounted as one of the human opportunistic pathogens since it is estimated that 50% of the world's population is colonized with this gram-negative bacterium. If this infection doesn't treat professionally, colonization of this pathogens usually continues throughout life. Analysis of H. pylori bacteria is a relevant diagnostic tool in the etiology of alternative gastrointestinal disorders, such as chronic active gastritis with non-clinical symptoms, peptic ulcers, gastric adenocarcinoma as well as lymphoid intercourse with inflammation of the gastric mucous membrane. Predispositions to this infections are mainly based on the aspect of polymorphism of immune determination genes as well as stomach acid secretion processes, as a determinant factor for H. Pylori's bacterial affinity to colonize the host gastric environment.

The objective of this study was to analyze the antibody of Helicobacter pylori in several fish species from three Kosovo rivers. Fish samples were collected during March-October 2017-2018 from Sitnica, Lepenci, and Lumbardhi i Prizrenit rivers by applying the electrofishing method. The bacteriological analyses included the following fish species Salmo trutta fario, Squalius cephalus, Rutilus rutilus and Carassius gibelio.

The analyses of anti. H. pylori in the blood of fish samples was seroprevalence negative. The variability of H. pylori reflected for research, risk human consumption, and public health.

Keywords: Fish, river pollution, Helicobacter pylori, antibody.

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Introduction

Helicobacter pylori previously named Campylobacter pylori, is a Gram-negative, microaerophilic bacterium found in the stomach, and may be present in other parts of the body, such as the eye.¹,²,³ It is estimated that 50% of the world's population is infected by this bacteria.⁴,⁵ It was identified in 1982 by Australian scientists Barry Marshall and Robin Warren with further research led by British scientist Stewart Goodwin, who found that it was present in patients with chronic gastritis and gastric ulcers, conditions not previously believed to have a microbial cause.

It is also linked to the development of duodenal ulcers and stomach cancer. However, over 80% of individuals infected with the bacterium are asymptomatic and it may play an important role in the natural stomach ecology.⁶ More than 50% of the world's population harbor H. pylori in their upper gastrointestinal tract.

Recently, the possible association between Helicobacter pylori infection and chronic urticaria (CU) is discussed in some investigations. Since the prevalence of infected persons with H. pylori and CU in Kosovo is high, the aim of this study was to evaluate correlation between the concentrations of anti-H. Pylori antibodies (IgG and IgA) and CU. The study population included 105 persons (18 – 65 Years old). With CU were diagnosed 62 of them.⁷

Although actual data indicate that continued efforts are needed to improve the overall educational statuses of women, it seems that a practical short- to medium-term solution

*Corresponding author:
Rrahman Ferizi
University of Prishtina, Paramedical Department, Faculty of Medicine, Prishtina, Kosovo.
E-mail: rahman.ferizi@uni-pr.edu

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might be to improve exposure in the various information media, such as radio, television, pamphlet, etc.\textsuperscript{9} Quality assurance method for services can be used by the health program managers to define clinical guidelines and standard operating procedures, as well as for assessments of performance using appropriate standards.\textsuperscript{9}

Basic knowledge about vitamin D also it should be improved about its sources, health effects and risk factors of its inadequacy or deficiency through the development of an appropriate education program for the public.\textsuperscript{10}

Infection is more prevalent in developing countries, and incidence is decreasing in Western countries. \textit{H. pylori}'s helical shape (from which the generic name is derived) is thought to have evolved to penetrate the mucoid lining of the stomach.\textsuperscript{11,12} The discovery of \textit{Helicobacter pylori} in 1982 was the starting point of a revolution concerning the concepts and management of gastro duodenal diseases.\textsuperscript{13} To avoid the acidic environment of the interior of the stomach (lumen), \textit{H. pylori} uses its flagella to burrow into the mucus lining of the stomach to reach the epithelial cells near, where the pH is more neutral.\textsuperscript{14}

\textit{H. pylori} is able to sense the pH gradient in the mucus and move towards the less acidic region (chemotaxis). This also keeps the bacteria from being swept away into the lumen with the bacteria’s mucus environment, which is constantly moving from its site of creation at the epithelium to its dissolution at the lumen interface.\textsuperscript{11}

It is now well accepted that the most common stomach disease, peptic ulcer disease, is an infectious disease, and all consensus conferences agree that the causative agent, \textit{H. pylori}, must be treated with antibiotics.\textsuperscript{16,17} Furthermore, the possibility emerged that this bacterium could be the trigger of various malignant diseases of the stomach and it is now a model for chronic bacterial infections causing cancer. The rare gastric mucosa-associated lymphoid tissue (MALT) lymphoma is the best example for which most of Bradford Hill's criteria of causality have been fulfilled, including remission of the cancer after a successful eradication of \textit{H. pylori}.\textsuperscript{18} While sufficient proof is lacking for the most common gastric cancer, i.e., gastric adenocarcinoma, numerous data have highlighted the essential role of the villain.\textsuperscript{19} The public health importance of the discovery of \textit{H. pylori} and its role in stomach diseases was recognized in 2005 by the attribution of the Nobel Prize in Physiology or Medicine to B. Marshall and R. Warren. In the history of Nobel prizes, this is only the third time that the discovery of a bacterium has been acknowledged.\textsuperscript{20}

Data regarding risk factors and modes of transmission are still controversial; it appears that there could be several modes of transmission.\textsuperscript{21,22,23,24} It is important to point that there have not been studies about the \textit{H. pylori} infection in Kosovo’s rivers fish population. \textit{H. pylori} were analyzed using indirect immunofluorescence for the detection of a prevalence of \textit{H. pylori} antibody, which is a specific test called enzyme-linked immunosorbent assay (ELISA or EIA). This research would reflect the epidemiology of this bacterial infection in the fish population and it would indicate the potential risk factors especially for human consumers.

**Growth conditions**

\textit{H. pylori} only grow at temperatures of 30-37°C. All the required growth conditions are met in the gastrointestinal tract of all warm-blooded animals. At temperatures below 30°C, \textit{H. pylori} could survive in some foods, such as fresh fruit and vegetables, fresh poultry or fish, fresh meats, and some dairy products. \textit{H. pylori} survived at 30°C in laboratory media water and milk and survived longer at lower temperatures.\textsuperscript{25,26,27,28}

**Tests for detection and identification of \textit{H. pylori}**

Several tests are available to detect \textit{H. pylori}. In an infected individual’s stomach, \textit{H. pylori} is the only organism that expresses urease: thus \textit{H. pylori} can be detected indirectly by identifying urease in a biopsy specimen.\textsuperscript{29} Foods and faeces are not routinely tested for \textit{H. pylori}. When they are tested, isolation and detection of the pathogen can be obscured by many factors (described below), leading to false negative results. Furthermore, the incubation time of the infection might be too long to allow a connection to be made between the source of infection and apparent clinical disease. \textit{H. pylori} can enter a viable but nonculturable state under adverse conditions, such as those present in faeces, under fully aerobic conditions, and in low water activity environments. Using specific and sensitive polymerase chain reaction techniques for detecting \textit{H. pylori} might solve this problem, but sensitivity values have been shown to vary.\textsuperscript{30}
Risk factors for infection

*H. pylori* is found in all parts of the world, although the prevalence is higher in developing countries. Almost all infections occur before the age of 10 years. In industrialized countries *H. pylori* seroprevalence in children younger than 5 years of age is 1–10%, whereas in developing countries rates of more than 50% are common in children of the same age group.31 In industrialized countries a decrease in the risk of infection is observed in successive generations (a cohort effect).32 The acquisition of infection does not appear to be seasonal. Infection seems to occur equally commonly among men and women, although one study found a higher risk in men and another found a higher risk in boys aged 3–9 years.33

In industrialized countries, individuals of high socioeconomic status are often less likely to be infected, with the exception of those in some ethnic subgroups. Interfamilial clustering of infection is common, and, especially in industrialized countries, infection occurs more often in individuals who live in crowded environments.34

Materials and methods

**Subjects**

The samples were obtained from March-October 2017-2018. An average of 60 live fish samples (Salmo trutta fario, Squalius cephalus, Rutilus rutilus and Carassius gibelio) were used from rivers located in three different Kosovo Country. Then samples were transported to analyze in the National Institute of Public Health of Kosovo, Molecular Microbiology Laboratory.

**Experimental procedure**

Evaluations of Anti. *H. pylori* in the fish species: Salmo trutta m. Fario, Squalius cephalus, Rutilus rutilus, and Carassius gibelio have been studied to predict the prevalence of *H. pylori* and possible transmission to consumption of untreated fish. This idea may be due in part to core community values that recognize the interconnectedness of human and environmental health.25, 26 For the analysis of this microbiological parameter venous puncture was used and then blood was collected from anesthetized fish using ether. The blood was taken by caudal vein with cardiac puncture using 2 ml sterile plastic disposable syringes fitted with 0.8x38-mm hypodermic needles, then 2 ml blood was taken and the serum was stored at -20°C. The ELISA tests with Anti- H.pylori antibodies were assayed by the Cobas Core anti- H. pylori IgG EIA (Roche Diagnostics Division, Basel-Switzerland), a second-generation EIA. The test was performed essentially according to manufacturer's instructions. This method has been previously validated in the Brazilian human population. The sensitivity, specificity and positive and negative predictive values of the test are 95.4, 100, 100 and 91.4%, respectively.27

**Statistical analysis**

The sample size was obtained as previously described. We considered a relatively average number of fish samples of 60 (for each river) analyzing the prevalence of *H. pylori* infection, which resulted in 0 % frequency of the Anti. *H. pylori* infection.

**Results**

This research determined a comparison between the control group of fish samples (Iber Lepenci and Lumbarathi I Prizrenit river) and experimental group (Sitnica) that have not shown any significant differences (Table 1,2,3). Among all of 60 blood fish samples, 19 (32%) were Salmo trutta m Fario, 20 (33%) Squalius cephalus and 21 (35%) Rutilus rutilus and Carassius gibelio. The average age of fishes was 0.1 ± 1.4 years. There were also no significant differences between water quality and environmental conditions at the present time (Table 4,5,6).

Also, water has been considered a possible transmission route of *H. pylori* infection, especially in developing countries.28 The fact of these conditions occurred in accordance with previous studies that showed a greater probability of acquiring the infection of *H. pylori* infection.29, 30

**Discussion**

During our analyses, we did not find a correlation between Anti. *H. pylori* infection and fish samples income from different rivers. It is important to highlight that this is the first study in our country which gives a possible potential source of Anti. *H. pylori* infection and transmission through human consumption. Furthermore, another similar study-based research is
necessary to give a better evaluation of potential sources of transmissions of H. pylori. Rivers that we studied have a lot of fish and the consumption of river fish continues especially by children.

This research study showed a lack of prevalence of *H. pylori* infection in these fish rivers. However, there have not been any differences in the prevalence of *H. pylori* among the diverse rivers mentioned. The lack of prevalence in our studies it would not be an overall window of *H. Pylori* infection, because transmission could transmit from person to person after eating infected fish especially when there is not a good care during the cooking. The human stomach is the only reservoir of *H. pylori* as the areas and growth of *H. Pylori* are in the gastrointestinal tract and warm-blooded animals. Other relevant potential transmissions of *H. Pylori* are; fresh fruit and vegetables, fresh poultry or fish and fresh meats.

## Conclusions

Lack of Anti.*H. Pylori* on the blood of fish samples could justify the water purity of these rivers according to the microbiological aspect, even though there was the presence of sewage waste collector. In the future molecular techniques, such as ribotyping or restriction fragment length polymorphism can help to clarify transmission of *H. pylori*, to evaluate correlation between risk factors and *H. pylori* infection. The best model to prevent the possibility of fish infection by *H. pylori* bacteria is regular and professional supervision of river pollution, biosecurity measures as well as the improvement of the quality of the fish at the national level.

## Acknowledgements

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## Declaration of Interest

The authors report no conflict of interest.

<table>
<thead>
<tr>
<th>Municipalit y</th>
<th>Fish (0.1 ± 1.4 year)</th>
<th>Number of examples</th>
<th>Sample</th>
<th>Anti. <em>H. pylori</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIZREN</td>
<td><em>Salmo trutta m Fario</em></td>
<td>19 (32%)</td>
<td>2 (mL/blood)</td>
<td>Neg (-)</td>
</tr>
<tr>
<td></td>
<td><em>Squalius cephalus</em></td>
<td>20 (33%)</td>
<td>2 (mL/blood)</td>
<td>Neg (-)</td>
</tr>
<tr>
<td></td>
<td><em>Rutilus rutilus and Carassius gibelio</em></td>
<td>21 (35%)</td>
<td>2 (mL/blood)</td>
<td>Neg (-)</td>
</tr>
</tbody>
</table>

**Table 1.** Isolation and serotyping of anti. *H. pylori* on fish species in Lumbardhi Prizrenit river.

<table>
<thead>
<tr>
<th>Municipality</th>
<th>Fish (0.1 ± 1.4 years)</th>
<th>Number of examples</th>
<th>Sample</th>
<th>Anti. <em>H. pylori</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>SHTERPCA</td>
<td><em>Salmo trutta m Fario</em></td>
<td>19 (32 %)</td>
<td>2 (mL/blood)</td>
<td>Neg (-)</td>
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<tr>
<td></td>
<td><em>Squalius cephalus</em></td>
<td>20 (33%)</td>
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<td>Neg (-)</td>
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<tr>
<td></td>
<td><em>Rutilus rutilus and Carassius gibelio</em></td>
<td>21 (35%)</td>
<td>2 (mL/blood)</td>
<td>Neg (-)</td>
</tr>
</tbody>
</table>

**Table 2.** Isolation and serotyping of anti. *H. pylori* on fish species in Lepenci river.
Table 3. Isolation and serotyping of anti. *H. pylori* on fish species in Sitnica river.

Table 4. Heavy metal concentrations in the water samples of Lumbardhi i Prizrenit river.

Table 5. Heavy metal concentrations in the water samples of Lepenci river.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Standard method</th>
<th>Permissible limit</th>
<th>March-October 2017-2018</th>
</tr>
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<tr>
<td>Zink (Zn)</td>
<td>APHA 3111B</td>
<td>mg/L</td>
<td>0.041</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>APHA 3111B</td>
<td>mg/L</td>
<td>≤0.022</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>APHA 3111B</td>
<td>mg/L</td>
<td>≤0.023</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>APHA 3111B</td>
<td>mg/L</td>
<td>≤0.053</td>
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<tr>
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<td>mg/L</td>
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<td>Nickel (Ni)</td>
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<td>mg/L</td>
<td>≤0.021</td>
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<td>Potassium (K)</td>
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<td>mg/L</td>
<td>1.73</td>
</tr>
</tbody>
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Table 6. Heavy metal concentrations in the water samples of Sitinca river.

References