



**Національний  
університет  
біоресурсів і  
природокористування  
України**

**Факультет  
ветеринарної  
медицини**

**НДІ Здоров'я тварин**



**«ЄДИНЕ ЗДОРОВ'Я – 2022»  
Матеріали Міжнародної наукової конференції**



**22-24 вересня 2022 р.  
НУБіП України, м. Київ**

**UDC 636.7.09:616.98-074**

**APPLICATION OF THE PCR METHOD FOR THE DETECTION OF  
GASTRIC *HELICOBACTER* SPP. IN THE SALIVA OF DOGS**

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Following the discovery of *Helicobacter pylori* in humans, several research teams have studied the existence of spiral bacteria in the stomachs of dogs and cats. This led to the isolation of the following *Helicobacter* species: *Helicobacter heilmannii*, *Helicobacter felis*, *Helicobacter salomonis* and *Helicobacter bizzozeronii*. However, the role of these species in the pathogenesis of gastric disease in companion

animals remains unknown. The *Helicobacter* spp. is widely distributed in dogs. Despite the high prevalence, its transmission, including human-human, animal-human, human-animal and animal-animal paths, remains unclear.

The aim of research

The aim of this study was to identify the species and determine the prevalence of gastric *Helicobacter* in the saliva of dogs.

Materials and Methods

The study was carried out on 30 dogs of different breeds, age and of both genders, from 1 to 15 years old. Saliva samples were obtained using sterile oral swabs, which were then placed in sterile tubes and frozen at -20 C° for assessment using the PCR method.

Saliva DNA was prepared using the Omega Bio-tek, Inc. “Forensic DNA kit”.

Nested-PCR method was used to detect *Helicobacter* microorganisms and to identify their species. It consists of performing two subsequent PCR reactions. In the first reaction, the DNA retrieved from samples is used as the matrix, and a pair of external F and R primers is applied. Together with polymerase and a pair of WF and WR primers, the product of the first reaction forms the matrix of the second reaction. This increases the sensitivity of the method. At the same time, the use of two different sets of primers ensures a high specificity of the method and eliminates false-positive results.

Thermo Scientific™ DreamTaq DNA Polymerase™ was used to synthesize DNA.

Based on nested-PCR method, the presence of *Helicobacter* spp. was found in saliva samples from 23 (76.6%) dogs. This gastric bacterium was not found in the saliva samples of seven (23.4%) dogs. Twenty-one (70.0%) animals were infected with a single species, while nine were infected with two *Helicobacter* species (30.0%).

*Helicobacter heilmannii* was the most commonly identified species and was found in 22 (95.7%) cases. Other species were less common: *Helicobacter felis* – 1 (4.4%) case, *Helicobacter salomonis* – 4 (17.4%) cases, *Helicobacter pylori* – 2 (8.7%) cases and *Helicobacter bizzozeronii* – 3 (13.0%) cases. The following combinations were found in animals infected with two *Helicobacter* species: *Helicobacter heilmannii* + *Helicobacter pylori* – 2 (22.2%) cases, *Helicobacter heilmannii* + *Helicobacter salomonis* – 3 (33.3%) cases, *Helicobacter felis* + *Helicobacter salomonis* – 1 (11.2%) cases and *Helicobacter heilmannii* + *Helicobacter bizzozeronii* – 3 (33.3%) cases.

Conclusion

Gastric *Helicobacter* spp. occurs relatively frequently in the saliva of dogs with gastritis. The most commonly identified species is *Helicobacter heilmannii*. PCR is a very good method for the detection and identification of the species *Helicobacter* spp. The obtained results indicate that canine saliva may be a potential source of *Helicobacter* spp. infection for other animals and humans.

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