

PCR Amplification of *Helicobacter pullorum* 16S rRNA Gene in Cecal Content of Pet Birds

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Abstract

For evaluation of pet birds as a resource of *Helicobacter pullorum*, 100 cecal content samples were collected from 50 finches and 50 Australian parrots, with no clinical signs suggestive of systemic and/or enteric disease, in Iran. DNA was extracted from cecal samples and PCR was carry out for identification of *H. pullorum* 16S rRNA gene. Results showed, *H. pullorum* DNA was detected in 3 of the 50 (6%) cecal samples of finch and 6 out of 50 (12%) cecal samples of Australian parrot. This study represents that finch and Australian parrot can be a source of *H. pullorum* infection and serve as reservoirs for the *H. pullorum*.

Keywords: Australian Parrot, Finch, *Helicobacter pullorum*

Evcil Kuşların Sekum İçeriklerinden *Helicobacter pullorum* 16S rRNA Geninin Amplifikasyonu

Özet

Helicobacter pullorum kaynağı olarak evcil kuşların değerlendirilmesi amacıyla İran'da sistemik veya enterik klinik belirtileri göstermeyen 50 ispinoz ve 50 Avustralya papağanından toplam 100 sekum içeriği örneği toplandı. Sekal örneklerden DNA ekstraksiyonu yapıldı ve *H. pullorum* 16S rRNA geninin identifikasyonu amacıyla PCR gerçekleştirildi. *H. pullorum* geni 50 ispinozun 3'ünde (%6) ve 50 Avustralya papağanının 6'sında (%12) belirlendi. Çalışmanın sonuçları ispinoz ve Avustralya papağanının *H. pullorum* enfeksiyonu kaynağı ve bu etken için rezervuar olabileceğini göstermiştir.

Anahtar sözcükler: Avustralya papağanı, İspinoz, *Helicobacter pullorum*

INTRODUCTION

Helicobacter pullorum is a gram-negative species, slightly curved rod with monopolar, nonsheathed flagella, classified in ureas-negative enterohepatic group of Helicobacters ^[1] which naturally colonize the gastrointestinal tract surface ^[2]. *H. pullorum* has been linked with enteritis and hepatitis in broiler chickens and laying hens, and diarrhea, gastroenteritis, and liver disease in humans ^[1]. This bacterium can be considered a foodborne human pathogen ^[1]. Although there are some studies on the prevalence of *H. pullorum* in poultry ^[1,3,4] limited information is present in other birds ^[5]. Until now, there are no report on the presence of *H. pullorum* in pet birds. To reduce contamination and illness caused by *H. pullorum*, the

finding of *Helicobacter* resources is very important. Hence, the aim of this study was to investigate the frequency of *Helicobacter* in cecal content of finch and Australian parrot in Chaharmahal-va-Bakhtiyari and Mazandaran Provinces, West and North of Iran. The aim of this study was to determine the occurrence of *H. pullorum* in pet birds by using polymerase chain reaction (PCR) to clarify the role of pet birds as reservoir of this bacterium in Iran.

MATERIAL and METHODS

Fifty cecal content samples from 50 finches and Australian parrots (each of them) was collected from 20 pet markets in Chahrmahal-va-Bakhtiyari and Mazandaran province, west and north of Iran. The sampled birds had



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no clinical signs suggestive of systemic and/or enteric disease. DNA was extracted from cecal content samples using a commercial DNA extraction kit (AccuPrep, Bioneer co., South Korea).

PCR was carried out to amplify a fragment of 447 bp of the 16S rRNA gene of *H. pullorum*. The sequence of the primers was as follows: forward primer: 5'-ATGAA TGCTAGTTGTTGTCAG; reverse primer: 5'-GATTGGCTCC ACTTCACA [6]. PCR amplification was performed in PCR buffer containing 1.5 mM MgCl₂, 200 μM each dNTPs, 10 pM each primer, and 1.0 unit of *Taq* polymerase (Fermentas, Germany) in a 25 μL total reaction volume. The gene ruler 100-bp DNA ladder plus (Fermentas, Germany) was used. The amplification was carried out in a thermal cycler (Mastercycler Gradient, Eppendorf, Germany) under the following conditions: an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation, annealing, and extension at 94°C for 1 min, 60°C for 90 sec, and 72°C for 90 sec, respectively, and a final extension at 72°C for 5 min. The PCR product was then analyzed by electrophoresis in 1.5% agarose gel and visualized under UV light after staining with ethidium bromide.

RESULTS

In the inspection of sampled birds no clinical signs were observed. In PCR, a 447 bp fragment of *H. Pullorum* 16S rRNA gene was amplified in 3 out of 50 finches (6%) and 6 out of 50 Australian parrot (12%) (Fig. 1). The positive samples were collected from 5 different pet markets in both Chahrmahal-va-Bakhtiyari and Mazandaran provinces in Iran.

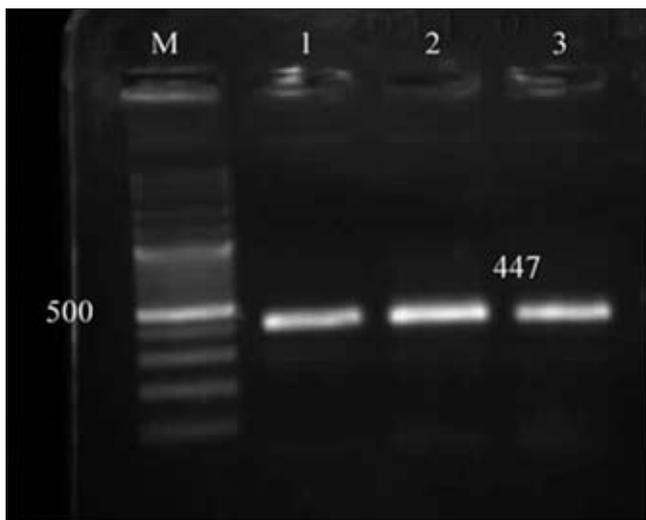


Fig 1. PCR amplification of the 16S rRNA gene of *Helicobacter pullorum* in cecal content of finch and Australian parrot (M: DNA ladder marker; lanes 1, 2 and 3: positive samples)

Şekil 1. İspinoz ve Avustralya papağanının sekum içeriklerinden *Helicobacter pullorum* 16S rRNA geninin PCR amplifikasyonu (M: DNA merdiven markırı; şerit 1, 2 ve 3: pozitif örnekler)

DISCUSSION

This study showed the infection of digestive contents of Australian parrot and finch by *H. pullorum* and demonstrated that 6% cecal content samples of finch and 12% cecal content samples of Australian parrot were positive for the 16SrRNA gene of *H. pullorum*. Presence of *H. pullorum* in the cecal contents of these bird species indicates its ability to colonize in digestive system of these birds. Previous reports showed that *H. Pullorum* could infect the digestive system of human and avian species. This bacterium was isolated from digestive contents of broiler chicken, laying hens [1,7], guinea fowl [8] and in a psittacin bird [5]. Moreover, *H. pullorum* has been isolated in fecal samples from humans with gastroenteritis in the UK, Canada, Germany and Switzerland [9] and its DNA was detected from gallbladder of a woman suffering from chronic cholecystitis and from livers of patients suffering from cirrhosis and/or hepatocarcinoma [10,11]. However, lack of knowledge about pathogenic mechanisms of the bacterium has made it difficult to relation of bacteria with disease signs. In infected persons, no history of human contact with birds was reported so far. Nevertheless, recent studies demonstrated that *H. pullorum* could be an initiator of the intestinal inflammation, because of the releasing cytolethal distending toxin, leading to activation of kB-necrozing factor pathway in the mucous intestine cells [12]. However, Infection of apparently healthy finch and Australian parrots with *H. pullorum* indicated that these birds might be referred as a source enterohepatic pathogenic bacteria in humans and causing digestive problems to humans.

Several studies have investigated the infection status of the birds with *H. pullorum* [9,13], which their comparisons are more complex because of using different samples and techniques, like culture or PCR. Several epidemiologic studies demonstrated prevalence of this micro-organism in cecal content of birds, especially layer and broiler chicken, ranging from 4% [9] to 100% [13] that depending on the bird species, diet type, keeping and growing system, different samples and techniques for the bacteria detection. This is the first report refer to infection rate of *H. pullorum* from cecal contents of the pet birds (finch and Australian parrot) in Iran. It is necessary to conduct more studies on carriage of the *H. pullorum* in different bird species and its pathogenicity in humans.

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