

Investigation of *Helicobacter pullorum* Occurrence in Chicken in the Marmara Region of Turkey[#]

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Geliş Tarihi / Received: 30.11.2011

ABSTRACT

The present study was to investigate the incidence of *Helicobacter pullorum* in caecum and colon of 96 broiler chickens. All samples were obtained from different commercial broiler slaughtering facilities in Marmara region of Turkey. The presence of *H. pullorum* was assessed by conventional culture method and specific polymerase chain reaction (PCR) test. *H. pullorum* were not isolated from any of the samples. In contrast, *H. pullorum* DNA was detected by PCR in 55.21% (53/96) of the samples. Also, it was indicated that caecum was commonly infected than colon. The results showed that *H. pullorum* is present in Marmara region of Turkey. Further studies are needed to identify the risk factors for the presence of *H. pullorum* for human health.

Key Words: *Helicobacter pullorum*, chicken, intestine, isolation, PCR

ÖZET

MARMARA BÖLGESİNDE TAVUKLARDA *HELICOBACTER PULLORUM*'UN VARLIĞININ ARAŞTIRILMASI

Bu çalışmada 96 etçi tavuğa ait sekum ve kolon örneklerinde *Helicobacter pullorum*'un insidensi araştırıldı. Örnekler Türkiye'nin Marmara bölgesindeki farklı ticari etçi kesimhanelerden toplandı. *H. pullorum* varlığı konvansiyonel kültürel metotlar ve spesifik Polimeraz zincir reaksiyonu (PCR) ile araştırıldı. Örneklerden *H. pullorum* izole edilmedi. Ancak *H. pullorum* DNA'sı PCR ile örneklerin %55,21 (53/96)'inde saptandı. Etken sekumda kolondan daha yüksek oranda belirlendi. Bu çalışmada sonuçlar Marmara bölgesinde *H. pullorum* varlığını ortaya koydu. İnsan sağlığı için *H. pullorum*'un risk faktörü olup olmadığının saptanması için konu ile ilgili araştırmaların sürdürülmesinin gerekli olduğu sonucuna varıldı.

Anahtar Kelimeler: *Helicobacter pullorum*, tavuk, bağırsak, izolasyon, PCR

Introduction

The genus *Helicobacter* is a member of the *Campylobacteriales* in the *Epsilonproteobacteria* subphylum (Miller et al., 2006). The genus can roughly be divided into gastric and

enterohepatic *Helicobacter* species (EHS) such as *H. pullorum*. It is Gram negative, non-spore forming, spiral, curved or fusiform rods that is sensitive to nalidixic acid, mostly catalase positive and a urease-negative organism with unsheathed flagella (Stanley et al., 1994).

[#] This article was summarised from the first author's PhD thesis.

H. pullorum has been identified in the intestinal tract and the liver of humans, mammals and birds (Fox and Lee, 1997). It was commonly found in the caecum and large intestine of broiler chickens (Atabay et al., 1998). *H. pullorum* can contaminate poultry carcasses at the abattoir during slaughtering; therefore some authors consider this bacterial species to be a foodborne human pathogen (Burnens et al., 1996). It has been suggested that *H. pullorum* may play a role in gastroenteritis, primary sclerosing cholangitis, cirrhosis, hepatocellular carcinoma (Rocha et al., 2004; Stanley et al., 1994; Steinbrueckner et al., 1997) and Crohn's disease (Andersen., 2001).

There have been some reports on the incidence of *H. pullorum* in Switzerland (Burnens et al., 1996); in Denmark (Atabay et al., 1998), in Belgium (Ceelen et al., 2006), in Italy (Zanoni et al., 2007), in France (Pilon et al., 2005). In Turkey, the prevalence of this pathogen in broiler has not been investigated.

The present study was undertaken to determine the occurrence of *H. pullorum* in chickens in Marmara region of Turkey.

Materials and Methods

Sample origin

The gastrointestinal tracts of broiler chickens were collected at intervals between April 2009 and February 2010. A total of 96 caecum and colon were investigated for the occurrence of *H. pullorum*. Samples were collected from 96 broilers from 12 flocks in Marmara region of Turkey (Kirkclareli, Edirne, Tekirdag, Istanbul, Kocaeli, Yalova, Sakarya, Bursa, Bilecik, Balikesir, Canakkale). Samples were taken from the caecum and colon following after evisceration and processed avoiding cross-contamination. The samples were packed into a separate sterile plastic bag using fresh disposable gloves, kept cool and examined within 6 hours after sampling and stored at -20°C. The presence of *H. pullorum* was examined by conventional culture method and polymerase chain reaction (PCR).

Control strain

Helicobacter pullorum type strain 51801 obtained from the American Type Culture Collection were used in the culture and PCRs as positive control.

Isolation of *H. pullorum*

Approximately 0.5 g of caecal contents were squeezed into 0.5 ml of sterile saline and shaken using a vortex mixer. An aliquot of 100 µl of each sample was diluted in 400 µl of a sterile mixture containing 25 ml of Brain Heart Infusion Broth (HiMedia, M210), 75 ml of inactivated horse serum and 7.5 g of glucose (Sigma Chemical Co, MO 63178). Dilutions were inoculated on Brucella Agar (HiMedia M074) with 5% sheep blood using the modified filter technique of Steele and McDermott (1984). 100 µl of dilutions was spread on a 47 mm large in diameter and 0.65 µm pore size sterile filter (Sartorius 11105-47N) that has been previously placed on the Brucella Agar surface. The plate was incubated at 37°C for 1 hour in a microaerobic atmosphere. After incubation, the filter was removed and the agar surface streaked with a sterilized loop. Then plates were incubated anaerobically (CampyGen CNO25A, Oxoid) at 37°C for 7 days and examined daily for microbial growth. Very small, greyish-white, alpha haemolytic colonies were selected and purified on a Brucella Agar plate. The biochemical characterization of the isolates was performed using the following tests: catalase, cytochrome oxidase, urease, hippurate and indoxyl acetate hydrolysis, nitrate reduction, hydrogen sulphide production in triple sugar iron agar, growth in the presence of 1% (w/v) NaCl, 1% (w/v) glycine and 1% (w/v) bile, at 25°C, 42°C and on MacConkey Agar (BD), growth at 37°C in aerobic and anaerobic conditions and under microaerobic atmosphere with and without hydrogen, susceptibility to nalidixic acid, cephoperazone and cephalotin (BBL, Becton, Dickinson and Company) (Ceelen et al., 2006).

PCR and gel electrophoresis

DNA was extracted from approximately 25 mg caecum, colon, jejunum and liver tissue applying a commercial tissue kit (DNeasy®

Tissue Kit, Qiagen, Venlo, The Netherlands). The PCR assay amplifying a 447 bp fragment of the 16S rRNA gene of *H. pullorum* (Stanley et al., 1994). The primers used in this PCR assay were primers 5' ATGAATGCTAGTTGTTGTCAG 3' and 5'GATTGGCTCCACTTCACA 3'. From each sample, 2 µl of the template was added to 8 µl of the PCR mixture, containing 0.03 U/µl Taq polymerase Platinum (Takara- RR001A), 10 x PCR Buffer (Takara- RR001A), 3 mM MgCl₂, 40 µM of each deoxynucleoside triphosphate (Takara- RR001A), a final primer concentration of 0.5 µM and sterile distilled water. The conditions used for the amplifications were the following: an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 90 seconds and elongation at 72°C for 90 s, and a final elongation at 72°C for 5 min. Five µl of the PCR products of each sample were mixed with 1 µl of loading buffer 6 x (TAKARA-A125) and were electrophoresed through an agarose gel containing 1.5% Multi Purpose Agarose (Sigma E8751) and 1 x TBE Buffer containing 50 ng/ml ethidium bromide (BioBasic A0026). As molecular size marker, the Gene Ruler 100 bp DNA ladder plus (BioBasic M1020-1/ M1020-2) was used. Electrophoresis was performed at a constant voltage of 90 V in 0.5 x TBE buffer for 60 min. The gels were visualized using UV Transilluminator.

Results

H. pullorum were not isolated from any of the samples using conventional culture method. In contrast, *H. pullorum* DNA detected with PCR in 55.21% (53/96) of the samples. *H. pullorum* DNA was detected from 31 (32.29%) of the caeca and 7 (10.15%) of the colon samples and 15 (15.63%) both of them.

Discussion

The studies on the prevalence of *H. pullorum* in poultry are rare (Atabay et al., 1998; Burnens et al., 1996; Ceelen et al., 2006; Manfreda et al., 2006; Zaroni et al., 2007).

Atabay et al. (1998) collected 15 fresh carcasses and 60.0% were found to be positive for *H. pullorum*. Our findings showed similarities with their results. Contrary to this, the studies which had higher results (100.0%, 78.47% and 100.0%) than ours were reported by Manfreda et al. (2006), Pilon et al. (2005) and Zaroni et al. (2007), respectively. In another study, the prevalence of *H. pullorum* was found to be 4.0% in caecum contents of 150 healthy broiler chickens (Burnens et al., 1996). These differences may be originated from detection methods, sampling procedures, regions and the geographical conditions.

In this study, the highest rate of *H. pullorum* was isolated mostly from caecum (47.92%). The caecum in chickens was considered the preferred colonization site by this agent. Similar results were obtained by Ceelen et al. (2006). The authors collected samples from 100 broilers from 11 flocks and *H. pullorum* DNA was detected in only five liver (4.6%) and 11 jejunal (10.9%) samples as opposed to 35 colonic (31.8%) and 37 caecal (33.6%) samples. In a previous study, Miller et al. (2006) stated the presence of *H. pullorum* in 43 out of 120 (35.83%) caecum samples.

Helicobacter species are fastidious bacterial pathogens that are difficult to isolate. According to the results of this study, samples tested positive in PCR while no *H. pullorum* was detected using culture isolation. In a previous study, Neubauer and Hess (2006) reported that specified pathogen-free chickens was infected with the ATCC *H. pullorum* reference isolate 51801 originating from chickens. Starting on day 7 of life birds from each group were euthanized at different time points up to 35 days. Various organ samples were processed by culture and a *H. pullorum* specific PCR then no live bacteria were reisolated at any time from birds infected with the avian isolate but bacterial nucleic acid was detected in the caeca of 5 birds and one in the gallbladder. Therefore Neubauer and Hess (2006) point out that PCR method is more helpful for detection of these fastidious bacteria.

In conclusion, *H. pullorum* was detected in Marmara Region of Turkey. The results indicated that poultry carcasses present a potential hazard for public health. Therefore, it is essential to identify the risk factors for the presence of *H. pullorum* for human health.

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