Kafkas Univ Vet Fak Derg
22 (3): 457-459, 2016
DOI: 10.9775/kvfd.2015.14566

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Short Communication

# The Effect of Different Storage Temperatures and Times on the Viability of *Helicobacter pullorum* [1]

Beren BAŞARAN KAHRAMAN [1] Kemal METİNER [1] Belgi DİREN SIĞIRCI [1]
Baran ÇELİK [1] M. Cemal ADIGÜZEL [1] A. Funda BAĞCIGİL [1]
Serkan İKİZ [1] N. Yakut ÖZGÜR [1] Seyyal AK [1]

[1] *This work was supported by Scientific Research Projects Coordination Unit of Istanbul University. Project number: 22384*

[1] Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University, TR-34320 Avcılar, Istanbul - TURKEY

**ArticleCode: KVFD-2015-14566 Received: 26.12.2015 Accepted: 12.01.2016 Published Online: 12.01.2016**

## Abstract

In the experimental study, four chicks were inoculated orally with 1x10$^9$ cfu/ml of *H. pullorum*, ATCC 51801 strain. Stool samples from each animal were collected and a pool of faecal content has been achieved. Totally 77 swabs taken from the sample pool were stored at -18°C, +4°C, + 25°C, respectively and were examined by culture every hour within first 24 h. In conclusion, the survival time of *H. pullorum* was determined as 11 hours at +25°C, 14 hours at +4°C and 16 hours at -18°C.

***Keywords:*** *H. pullorum, Viability, Chicks, Feces*

# *Helicobacter pullorum*'un Değişik Isılarda ve Sürelerde Dayanıklılığının Belirlenmesi

## Özet

Bu deneysel çalışmada, 4 adet civcive 1x10$^9$ cfu/ml *H. pullorum*, ATCC 51801 suşu oral yolla inokule edildi. Dışkı örnekleri toplandı ve dışkı havuzu oluşturuldu. Toplamda dışkı havuzundan alınan 77 svab -18°C, +4°C ve + 20-25°C'de saklandı ve 24 saat içinde her saat başı kültürel metot ile incelendi. Sonuç olarak, *H. pullorum*'un dayanıklılığı +25°C'de 11 saat, +4°C'de 14 saat ve -18°C'de 16 saat olarak belirlendi.

***Anahtar sözcükler:*** *H. pullorum, Dayanıklılık, Civciv, Dışkı*

## INTRODUCTION

*Helicobacter pullorum*, first described by Stanley *et al.*[1], has been associated with hepatobiliary and gastro-intestinal diseases in chickens and in human beings. This putative enterohepatic pathogen, or its DNA, has been detected in the intestinal contents of chickens [2-4], guinea fowl [5], turkeys [6] and a psittacine bird [7]. In humans, *H. pullorum* has been isolated from feces [8,9]. Although it has not yet been clearly proven that *H. pullorum* has zoonotic potential. As poultry carcasses can be contaminated by *H. pullorum* [2] during slaughtering, the potential role of these bacteria as an emerging foodborne human pathogen needs to be considered [4].

Like other *Helicobacter* species, *H. pullorum* is fastidious bacterial pathogen difficult to isolate [10,11]. Although it has been mentioned by many researchers that samples for isolation procedures should be send to the laboratories within minimum time period and with cold chain [3,4], there is no reported study about transportation procedures or limitations of the samples. Therefore, examining the viability of *H. pullorum* in different temperatures and time periods was decided. Several authors reported that different atmospheric environments, incubation period and incubation temperatures had been examined for the isolation of *H. pullorum* [1,3,4,10]. Culture is time consuming and difficult, and fresh samples are needed for this purpose, therefore PCR is also recommended to use along with culture [1,12].

The aim of this study is to examine the viability of *Helicobacter pullorum* in different temperatures and time periods.

**İletişim (Correspondence)**
+90 212 4737070/17360
beren@istanbul.edu.tr

## MATERIAL and METHODS

This study was approved by the Animal Care Committee of Istanbul University, Faculty of Veterinary Medicine, Approval no: 2012/49.

In the experimental study, four 19-day-old chicks, which were *determined H. pullorum* negative by cultural methods and PCR, were *used*. The chicks were inoculated orally with $1x10^9$ cfu/ml of *Helicobacter pullorum*, ATCC 51801 strain and clinically examined on an hourly basis. After 24 h, stool samples from each animal were collected and examined by culture and PCR analyses for confirmation of the experimental infection. It was confirmed with detection of *H. pullorum* by culture described by Ceelen *et al.*[3] and Zanoni *et al.*[4] and by PCR according to Stanley *et al.*[1] and Ceelen *et al.*[3].

After confirmation of the experimental infection, cecal and colon contents of each animal were collected followed after the necropsy of the animals and a pool of faecal content have been achieved [13].

Totally 77 swab samples were collected with Stuart agar gel medium transport swabs (Copan Diagnostics) from the sample pool. This phase regarded as hour "0". Two of the swab samples were used for isolation and confirmation of *H. pullorum* occurrence by PCR and the remaining swabs were divided into three groups consisting of 25 swabs. Sample groups were stored at -18°C, +4°C, +25°C, respectively and those samples were examined by culture every hour within first 24 h and the last inoculation onto medium was performed at the 48$^{th}$ h.

The swabs were inoculated into Brain Heart Infusion Broth (HiMedia) supplemented with inactivated horse serum and glucose (Sigma Chemical Co). Dilutions were inoculated onto Blood Agar Base No 2 (Oxoid) with 5% sheep blood using the modified filter technique of Steele and McDermott [14]. 100 µl of dilutions was spread onto 47 mm large in diameter and 0.65 µm pore size sterile filter (Sartorius) that has been previously placed on the Blood Agar Base No 2 surface. The plate was incubated at 37°C for 1 h in a microaerobic atmosphere. After incubation, the filter was removed and the agar surface streaked with a sterilized loop. Then plates were incubated under microaerophilic condition using gas generating kit (CampyGen, Oxoid) at 37°C for 7 days and examined daily for microbial growth. Very small, greyish-white, alpha hemolytic colonies were selected and purified on a Blood Agar Base No 2 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 3.5% (w/v) NaCl, 1% (w/v) glycine and 1% (w/v) bile, growth at 25°C, 42°C and on MacConkey Agar ((BBL, Becton, Dickinson and Company), susceptibility to nalidixic acid and cephalotin (BBL, Becton, Dickinson and Company) [3,4].

## RESULTS

Growth and biochemical characteristics of *H. pullorum* were shown in *Table 1*.

*H. pullorum* was isolated from the swab samples taken at hour "0" and occurrence of the agent confirmed by PCR from the sample taken at the same hour. The survival duration of the microorganism were 11 h, 14 h and 16 h at 25°C, +4°C and -18°C, respectively.

## DISCUSSION

In this study, isolation medium, atmospheric conditions, incubation time, and incubation temperature provided in optimal conditions for recovery of *H. pullorum* from the experimentally infected chicks' cecal and colon contents. The effects of different storage temperatures and times on the viability of *H. pullorum* which were stored in their natural environment such as feces were determined. The survival time of *H. pullorum* was determined as 11 h at +25°C, 14 h at +4°C and 16 h at -18°C.

Although it has been considered that detection of the number of bacteria in the each inoculated medium would

***Table 1.*** *Growth and biochemical characteristics of H. pullorum*
***Tablo 1.*** *H. pullorum'un üreme ve biyokimyasal özellikleri*

| **Growth and Biochemical Characteristics** | ***H. pullorum*** |
|---|---|
| Gram-negative | + |
| Oxidase | + |
| Catalase | + |
| Urease | - |
| Hippurate hydrolysis | + |
| Indoxyl acetate hydrolysis | - |
| Nitrate reduction | + |
| Trace $H_2S$ in TSI | + |
| **Growth on Media Containing** | |
| 1% (w/v) glycine | - |
| 1% (w/v) bile | + |
| 3.5% (w/v) NaCl | - |
| **Growth at** | |
| 42°C ($mO^2$) | + |
| 25°C ($mO^2$) | - |
| **Antimicrobial Susceptibility** | |
| Nalidixic acid (30 µg) | S |
| Cephalotin (30 µg) | R |

***TSI*** *= Triple sugar iron,* ***NaCl*** *= Sodium chloride,* ***mO²*** *= microaerobic atmosphere;* ***S*** *= Susceptible,* ***R*** *= Resistant*

contribute the results of the study, due to the difficulties of isolation procedures this step has not been performed. However as a result of this study, it is revealed that the samples should be processed without any storage time, and in a case if they need to be stored, -18°C would be better instead of higher temperatures like +4°C or +25°C.

Different ratios were determined in the studies on the presence and prevalence of *H. pullorum* even few studies have been conducted worldwide [2-4,8,12,15,16]. The authors suggested that it is difficult to compare those results due to the different diagnostic methods and different kinds of samples, such as frozen versus fresh samples that have been used in each study [12]. Ceelen *et al.*[3] examined the occurrence of *H. pullorum* in broilers from samples stored at -20°C and -70°C, by using both polymerase chain reaction (PCR) and isolation, respectively. The researchers suggested that their low isolation rate of *H. pullorum* from cecal samples might have been the result of examining frozen, as opposed to fresh, samples. Manfreda *et al.*[12] reported that the relatively high isolation rate (78.47%) in their study compared with other studies [2] might have been due to the usage of fresh materials instead of frozen samples.

*H. pullorum* were not isolated from any of the samples while *H. pullorum* DNA was detected in 55.21% (53/96) by PCR in a study in which the presence of *H. pullorum* in caecum and colon of 96 broiler chickens from different commercial slaughtering facilities were examined [15]. The authors indicated that, some of the samples could be collected only by authorized veterinarians instead of themselves, therefore the delivery time of the samples to the veterinarians were not clear, and those samples were transferred to the laboratory within 6 hours on 4°C. In addition to this situation the processing of the sample has taken approximately one hour in the laboratory. The researchers suggest that the reason of lack of isolation in the study might have been due to the time period passed between sampling and culture.

When the survival time of the bacteria in feces is considered, it can be suggested that the time period for the transmission of the agent via contaminated poultry meat is relatively limited. It has been revealed that, studies such as detailed examination of occurrence of *H. pullorum* on the retail poultry products, determination of viability period of the agent on poultry carcasses would be helpful for understanding the transmission of the bacteria through human and designating the bacteria as a food-borne pathogen. The role of *H. pullorum* among the food-borne infections will be better understood by further epidemiological studies based on the current study.

## REFERENCES

**1. Stanley J, Linton D, Burnens AP, Dewhirst FE, On SLW, Porter A:** *Helicobacter pullorum* sp. nov.-genotype and phenotype of a new species isolated from poultry and from human patients with gastroenteritis. *Microbiology,* 140, 3441-3449, 1994. DOI: 10.1099/13500872-140-12-3441

**2. Atabay HI, Corry JEL, On SLW:** Identification of unusual Campylobacter-like isolates from poultry products as *Helicobacter pullorum*. *J Appl Microbiol*, 84, 1017-1024, 1998. DOI: 10.1046/j.1365-2672.1998.00438.x

**3. Ceelen LM, Decostere A, Van den Bulck K, Stephen LW, Margo B, Ducatelle R, Haesebrouck F:** *Helicobacter pullorum* in chickens, Belgium. *Emerg Infect Dis,* 12, 263-267, 2006.

**4. Zanoni RG, Rossi M, Giacomucci D, Sanguinetti V, Manfreda G:** Occurence and antibiotic susceptibility of *Helicobacter pullorum* from broiler chickens and commercial laying hens in Italy. *Int J Food Microbiol*, 116, 168-173, 2007. DOI: 10.1016/j.ijfoodmicro.2006.12.007

**5. Nebbia P, Tramuta C, Ortoffi M, Bert E, Cerruti Sola S, Robino P:** Identification of enteric *Helicobacter* in avian species. *Schweizer Archiv für Tierheilkunde*, 149, 403-407, 2007. DOI: 10.1024/0036-7281.149.9.403

**6. Zanoni RG, Piva S, Rossi M, Pasquali F, Lucchi A, De Cesare A, Manfreda G:** Occurrence of *Helicobacter pullorum* in turkeys. *Vet Microbiol*, 149, 492-496, 2011. DOI: 10.1016/j.vetmic.2010.11.013

**7. Ceelen LM, Decostere A, Martel A, Pasmans F, Haesebrouck F:** First report of *Helicobacter pullorum* in the faece of a diarrhoeic psittacine bird *(Psephotus haematogaster). Vet Rec*, 159, 389-390, 2006. DOI: 10.1136/vr.159.12.389

**8. Burnens AP, Stanley J, Nicolet J:** Possible association of *Helicobacter pullorum* with lesions of vibrionic hepatitis in poultry. **In,** Campylobacters, Helicobacters and Related Organism. 291-293, Springer US, 1996.

**9. Steinbrueckner BG, Hearter K, Pelz S, Weiner JA, Rump W, Deissler S, Bereswill M:** Isolation of *Helicobacter pullorum* from patients with enteritis. *Scand J Infect Dis*, 29, 315-318, 1997. DOI: 10.3109/00365549709019053

**10. Andersen LP:** New *Helicobacter* species in humans. *Dig Dis,* 19, 112-115, 2001. DOI: 10.1159/000050664

**11. Taneera J, Moran AP, Hynes SO, Nilsson HO, Al-Soud W, Wadstrom T:** Influence of activated charcoal, porcine gastric mucin and beta-cyclodextrin on the morphology and growth of intestinal and gastric *Helicobacter* spp.. *Microbiology,* 148, 677-684, 2002. DOI: 10.1099/00221287-148-3-677

**12. Manfreda G, Rossi M, Sanguinetti V, Gavioli R, Lozito P, De Cesare A, Zanoni RG:** Prevalence of *Helicobacter pullorum* in broiler chickens reared in intensive and extensive farms. *World's Poultry Sci J,* 62, 551-552, 2006.

**13. Eleroğlu H, Yıldırım A, Şekeroğlu A, Duman M:** Comparison of the growth performance and carcass characteristics of two slow-growing broiler genotypes fed diets supplemented with dry oregano (*Origanum vulgare* L.) or lemon balm (*Melissa officinalis* L.) leaves under the organic system. *Kafkas Univ Vet Fak Derg*, *20*, 49-58, 2014. DOI: 10.9775/kvfd.2013.9444

**14. Steele TW, McDermott SN:** The use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from feces. *Pathology*, 16, 263-265, 1984.

**15. Basaran-Kahraman B, Ak S:** Investigation of *Helicobacter pullorum* occurence in chicken in Marmara region of Turkey. *J Fac Vet Med Istanbul Univ*, 39, 63-66, 2012.

**16. Borges V, Santos A, Correia CB, Saraiva M, Ménard A, Vieira L, Sampaio DA, Pinheiro M, Gomes JP, Oleastro M:** *Helicobacter pullorum* isolated from fresh chicken meat: Antibiotic resistance and genomic traits of an emerging foodborne pathogen. *Appl Environ Microbiol*, 81, 8155-8163, 2015. DOI: 10.1128/AEM.02394-15